



**Assessment of Beta-2 Microglobulin, Serum Creatinine and
Creatinine Clearance in Chronic Kidney Disease Patients in KwaZulu-Natal**

By

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ABSTRACT

Background: Chronic kidney disease (CKD) is a major and escalating global health burden, affecting 8-16% of the population and disproportionately impacting low and middle-income countries such as South Africa. KwaZulu-Natal, in particular, bears a high CKD prevalence, with 67% of patients hypertensive, 24% diabetic, and 23% HIV-positive in this cohort. Conventional biomarkers like serum creatinine (sCr) are late indicators of renal dysfunction, often rising only after substantial nephron loss. Beta-2 microglobulin (B2M), a low molecular weight protein filtered by the kidneys and independent of muscle mass, may provide earlier detection of kidney impairment and improved risk stratification, but its clinical utility remains underexplored in routine clinical practice and in African populations.

Aims and Objectives: This study aimed to assess the clinical utility of B2M, alongside serum creatinine and creatinine clearance, for the diagnosis and staging of CKD in KwaZulu Natal. The specific objectives were: (1) to evaluate the association between B2M levels and leading CKD aetiologies (hypertension, diabetes and HIV); and (2) to determine the correlation between B2M levels and estimated glomerular filtration rate (eGFR) calculated with the CKD-EPI 2021 equation.

Materials and Methods: A retrospective cross-sectional study was performed on 173 CKD patients (53% female, mean age 54.3 ± 15.8 years) from the Clinical Medicine Research Laboratory, University of KwaZulu-Natal. Serum samples were analysed for B2M concentrations using enzyme-linked immunosorbent assay (ELISA). Clinical and biochemical data, including sCr and eGFR, were extracted from laboratory databases. Participants were stratified by key comorbidities (hypertension, diabetes, and human immunodeficiency virus). Statistical methods included descriptive statistics, ANOVA, bivariate and multivariable regression, and correlation analysis to explore associations between biomarkers, CKD stages, and clinical variables.

Results: B2M concentrations demonstrated a distinctive J-shaped distribution across CKD stages: elevated in Stage 1 (mean 0.19 ± 0.32 $\mu\text{g/mL}$), dipping in Stage 2 (0.12 ± 0.21 $\mu\text{g/mL}$), and rising sharply in Stage 3 (0.30 ± 0.41 $\mu\text{g/mL}$) and Stage 4 (0.52 ± 0.46 $\mu\text{g/mL}$) ($p = 0.032$). B2M showed a robust inverse correlation with eGFR ($r = -0.78$, $p = 0.001$), particularly in

hypertensive ($r = -0.20$, $p = 0.034$) and diabetic ($r = -0.36$, $p = 0.021$) subgroups. By contrast, serum creatinine showed significant increases only at advanced stages of CKD. Multivariable regression confirmed CKD stages 3-4 as independent predictors of higher B2M after adjusting for demographic and clinical covariates. B2M was not significantly influenced by sex or muscle mass. HIV-positive patients exhibited higher B2M, but without a significant correlation to eGFR. The overall pattern supports B2M as a more sensitive marker for early nephron stress and risk stratification in multi-morbid cohorts.

Conclusion: B2M is a sensitive, early-stage biomarker for CKD in KwaZulu-Natal, outperforming sCr in early detection and disease monitoring, particularly among hypertensive and diabetic patients. Its independence from demographic confounders and strong association with CKD severity support its integration into local diagnostic pathways. Routine B2M testing could enable earlier intervention, improved risk stratification, and optimisation of outcomes in high-risk, resource-constrained settings. Further research is warranted to validate B2M's predictive value and cost-effectiveness in larger, prospective cohorts and to define its role in multi-marker diagnostic panels.

DECLARATION

This study represents the original work by the author. It has not been submitted to any other Tertiary Institution. Where the use of the work of others was made, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Department of Biomedical and Clinical Technology, Faculty of Health Sciences, Durban University of Technology, under the supervision of Dr SC Benjamin, Prof AG Assounga, and Prof ND Madala. The practical work was conducted in the Clinical Medicine Research Laboratory at the College of Health Sciences, University of KwaZulu-Natal.

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DEDICATION

I would like to dedicate this thesis to:

1. The Most High God, the source of my strength and the strength of my life, the Lord Jesus Christ. Lord, I am so grateful for reaching this stage of my dissertation.
2. My dearest wife, Thembelihle Nomalungelo Mtshali, for your great support and encouragement, and to our two awesome kids, Zibusiso and Onqobayo, you guys are the reason I never gave up; I love you so much.
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LIST OF ABBREVIATIONS

AGE's	Advanced Glycation End Products
AKI	Acute Kidney Injury
AMI	Acute Myocardial Infarction
ARDS	Acute Respiratory Distress Syndrome
ART	Antiretroviral Therapy
B2M	Beta-2 Microglobulin
BP	Blood Pressure
BTP	Beta Trace Protein
CAC	Coronary Artery Calcification
CAD	Coronary Artery Disease
CH3	Constant Heavy 3 domain (immunoglobulin structure)
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CKD STAGES	Staged CKD by eGFR (1,2 ,3a, 3b, 4, 5/ESRD)
Cge	Cockcroft-Gault Equation
CTL's	Cytotoxic T Lymphocytes
CVD	Cardiovascular Diseases
CVE	Cardiovascular Event
DKD	Diabetic Kidney Disease
DNA	Deoxyribonucleic Acid
DOH	Department of Health
DUT	Durban University of Technology
EGF	Epidermal Growth Factor
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked Immunosorbent Assay
GFR	Glomerular Filtration Rate
HDL	High-Density Lipoprotein
HIV	Human Immuno Deficiency Virus

HIVAN	HIV-Associated Nephropathy
IGFBP-7	Insulin-like Growth Factor Binding Protein 7
IL	Interleukin
KIM-1	Kidney Injury Molecule-1
KDIGO	Kidney Disease: Improving Global Outcomes
KRT	Kidney Replacement Therapy
LCN2	Lipocalin 2
L-FABP	Liver-type Fatty Acid Binding Protein
MACE	Major Adverse Cardiovascular Event
MACE's	Major Adverse Cardiovascular Events
MDRD	Modification of Diet in Renal Disease (equation)
MHC	Major Histocompatibility Complex
MCP-1	Monocyte Chemoattractant Protein 1
MW	Molecular Weight
NHANES	National Health and Nutrition Examination Survey
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NIH	National Institutes of Health
PAD	Peripheral Arterial Disease
PD	Peritoneal Dialysis
pmp	Per Million Population
PIIINP	Procollagen Type III N-Terminal Propeptide
QC	Quality Control
ROS	Reactive Oxygen Species
sCr	Serum Creatinine
SSA	Sub-Saharan Africa
Stats SA	Statistics South Africa
T2DM	Type 2 Diabetes Mellitus
TMB	Tetramethylbenzidine
TIMP-2	Tissue Inhibitor of Metalloproteinases-2
UACR	Urine Albumin-to-Creatinine Ratio
UKZN	University of KwaZulu-Natal
UMOD	Uromodulin

CHAPTER 1: STUDY BACKGROUND AND INTRODUCTION

1.1 BACKGROUND

Beta-2 microglobulin (B2M) remains one of the chronic kidney disease (CKD) biomarkers that has not yet been incorporated into routine clinical testing (Assounga 2021). B2M is a single-chain peptide with a structure resembling the CH3 domain of immunoglobulins and has a low molecular weight (MW = 11.8 kDa) (Cook 1998). It is a component of the light chain of major histocompatibility complex class I (MHC-I) molecules, which are located on cellular membranes (Sharma, Kumar and Sharma 2017). Consequently, the primary source of circulating B2M is cellular membrane turnover (Argyropoulos *et al.* 2017).

Due to its small molecular size, B2M is readily filtered through the renal glomeruli. Once filtered, it is predominantly reabsorbed and metabolised by proximal tubular cells, resulting in only a minimal proportion being excreted in the urine. Consequently, B2M has been investigated as a promising biomarker for both CKD and acute kidney injury (Poedjijo *et al.* 2025). Its advantages include independence from muscle mass and the ability to increase earlier than serum creatinine (sCr), which may allow earlier detection of renal dysfunction (Li *et al.* 2024). Given the importance of early detection of kidney dysfunction, reliable biomarkers are critical for identifying and monitoring CKD.

CKD is a significant global health concern, with its prevalence rising at an alarming rate worldwide (Rajput *et al.* 2020). CKD is defined by enduring alterations in renal structure or function that persist for a minimum duration of three months, with associated health implications (Augustine, Marcellinus and Eshiotseme 2019). Globally, CKD affects an estimated 8% to 16% of the global population, with the majority of cases occurring in low- and middle-income countries (Ke *et al.* 2022). In the African context, CKD represents a considerable public health challenge and is linked to diverse risk factors encompassing both communicable and noncommunicable diseases (Kaze *et al.* 2018).

Chronic kidney disease is divided into five distinct stages according to the Kidney Disease: Improving Global Outcomes (KDIGO) framework, based on estimated glomerular filtration rate (eGFR) and evidence of renal impairment as guiding factors. Stage 1 is characterised by

normal eGFR or higher, coupled with evidence of renal injury, such as proteinuria or structural abnormalities detected through imaging techniques. Individuals at this stage are generally asymptomatic. Clinical management strategies primarily focus on controlling modifiable risk factors, most notably hypertension and diabetes mellitus, to reduce the risk of disease progression (Stevens *et al.* 2024).

Stage 2 is defined by a mild reduction in estimated glomerular filtration rate (eGFR), ranging from 60 to 89 mL/min/1.73 m², concomitant with evidence of kidney damage. Clinical manifestations are typically absent at this stage; however, patients require continuous monitoring and evaluation to optimise overall health outcomes and preserve renal function (Elendu *et al.* 2023). Stage 3 is further divided into two subcategories: stage 3a, characterised by an eGFR of 45–59 mL/min/1.73 m², and stage 3b, characterised by an eGFR of 30–44 mL/min/1.73 m². At this stage, patients may begin to experience non-specific symptoms such as fatigue, fluid retention, and changes in urine output. Clinical management strategies become more intensive, with a focus on preserving renal function and addressing potential complications (Levin *et al.* 2024).

Stage 4 is marked by a significantly reduced eGFR of 15 to 29 mL/min/1.73m². Patients may even present with more pronounced symptoms and complications related to severe chronic kidney disease. This stage compels diligent planning for renal replacement therapy, encompassing discussions of dialysis modalities and assessment of eligibility for kidney transplantation (Lundström 2022). Stage 5 CKD is characterised by a persistently reduced eGFR of less than 15 mL/min/1.73 m². At this juncture, renal replacement therapy is essential for sustaining life. Comprehensive and multidisciplinary management is essential to ensure the timely initiation of these interventions and, when appropriate, the integration of palliative care strategies (Levin *et al.* 2024).

CKD classification and prognosis are fundamental to understanding the natural history and clinical management of renal disorders. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines provide a robust framework for CKD staging, emphasising the stratification of disease severity based on glomerular filtration rate (GFR) and the presence of markers of kidney damage (de Boer *et al.* 2020). This approach facilitates not only accurate diagnosis but also the personalisation of therapeutic strategies, thereby optimising clinical

outcomes. A detailed understanding of CKD progression is crucial for implementing timely interventions to slow disease progression and reduce the incidence of adverse complications, including cardiovascular comorbidities and eventual dialysis dependence (de Boer *et al.* 2020).

The prognosis of CKD patients is closely associated with disease classification, as it influences clinical decision-making and patient education. The KDIGO guidelines suggest that patients with lower GFR values and higher levels of proteinuria generally experience poorer clinical outcomes. In this context, several biomarkers have been identified to help predict disease progression (Stevens *et al.* 2024). Healthcare medical professionals facilitate the establishment of monitoring protocols and lifestyle interventions capable of significantly influencing the disease course, by acknowledging the prognostic implications of CKD grouping, and by communicating such risks to patients effectively it is crucial. It promotes adherence to treatment plans and endorses lifestyle changes, thereby enhancing patients' quality of life through optimising CKD management (Stevens *et al.* 2024).

It is therefore increasingly important to recognise more specific and sensitive CKD biomarkers to enable early detection and timely interventions (Mizdrak *et al.* 2022). Serum creatinine (sCr) is one of the most commonly used clinical markers for CKD assessment (Xie, Jia and Liu 2024). In equations for estimating glomerular filtration rate (GFR), serum creatinine is the primary variable (Cusumano, Tzanno-Martins and Rosa-Diez 2021). Consequently, eGFR calculated from sCr is routinely employed for both CKD patients and the general population (Campion *et al.* 2022).

1.2. Problem statement

As of the 2022 South African Census, the country's population has risen to approximately 62.03 million. The data indicate a slight female predominance, with females representing 51.5% of the population. Black/African citizens constitute 81.4% of the total population (Moultrie and Dorrington 2024). Approximately 26.3% of the population of the population is under the age of 15, while about 9.8% are aged 60 years or older. In terms of provincial distribution, Gauteng accounts for 24.3% of the population, followed by KwaZulu-Natal with approximately 20.0% (Stats SA, 2022).

According to the 2022 South African Renal Registry Annual Report, a total of 822 patients initiated kidney replacement therapy (KRT), corresponding to an incidence rate of 13.3 per million population (pmp). Notably, the majority of these patients (77%) received treatment in private healthcare facilities. By December 2022, the combined number of chronic and transplant patients on dialysis reached 9,342, an increase from 8,866 in 2021, resulting in an overall prevalence of 151 pmp. Within the private sector, the incidence reached 771 cases pmp, substantially higher than rates observed in the public sector, which reported an overall prevalence of 45 pmp. Among public sector regions, the Western Cape recorded the highest prevalence at 165 pmp, while Mpumalanga reported the lowest at 3 pmp (Davids *et al.* 2024).

CKD has a significant global health impact, directly contributing to morbidity and mortality worldwide. In addition, CKD is a major risk factor for cardiovascular disease (CVD) and imposes considerable economic burdens on healthcare systems and societies (Bikbov *et al.* 2020). CVDs are the leading cause of mortality in this population, with cardiovascular mortality among ESRD patients estimated to be more than 30 times higher than in the control population (Matsushita *et al.* 2022).

A substantial proportion of patients with kidney disease may exhibit no symptoms or may experience nonspecific clinical manifestations, thereby hindering prompt diagnosis. Kidney diseases are inherently complex and heterogeneous, with the potential to affect any segment of the nephron. Consequently, the clinical assessment of renal function primarily relies on evaluating glomerular function (Zhang and Parikh 2019). In clinical practice, the assessment of impaired kidney function is commonly performed through measurement of GFR, serum concentrations of cystatin C and creatinine, and detection of albuminuria. Although GFR estimations derived from these biomarkers are routinely used, they often lack precision (Thielemans *et al.* 2023). A nonlinear relationship exists between creatinine or cystatin C levels and glomerular filtration rate (GFR), indicating that even modest initial elevations in these biomarkers can correspond to substantial reductions in GFR (Pasala and Carmody 2017).

Therefore, serum creatinine concentrations typically begin to rise only after approximately 40–50% of the renal parenchyma has sustained damage (Liu *et al.* 2020). Albuminuria typically precedes a decline in eGFR; however, it may be undetectable in cases of tubulointerstitial or hypertensive kidney disease (Uwaezuoke *et al.* 2018). Several alternative biomarkers have been

investigated to address the limitations of conventional markers, with B2M and beta-trace protein (BTP) showing promise (Costa e Silva *et al.* 2024). Research indicates that skeletal muscle mass and strength begin a linear decline around the age of 40, with individuals potentially losing up to 50% of muscle mass by the age of 80 (Yim *et al.* 2023).

1.3. Rationale for the study

This study compared B2M with other biomarkers to evaluate kidney function in CKD patients, thereby assessing the effectiveness of these markers for diagnosis and disease monitoring. A particular focus was placed on KwaZulu-Natal, as the study aimed to provide insights into CKD prevalence, progression, and management within this region. Elevated serum B2M levels are indicative of glomerular dysfunction, whereas increased urinary B2M levels suggest tubular impairment (Zhou *et al.* 2021)

The study findings have the potential to enhance understanding of chronic kidney disease (CKD) and improve disease management, ultimately contributing to better patient outcomes. In addition, the research may provide valuable insights into the prevalence and characteristics of CKD in KwaZulu-Natal, thereby informing regional health strategies and interventions. Furthermore, the results could advance knowledge of CKD biomarkers and their clinical relevance.

This study utilised stored serum samples obtained from the University of KwaZulu-Natal Clinical Medicine Laboratory. Given the recognised limitations of serum creatinine as a diagnostic marker, the present investigation aimed to evaluate B2M and explore its relationship with eGFR in patients with CKD.

1.4. Significance of the study

The significance of this study lies in the strong association of B2M with mortality, as reported by (Zhao *et al.* 2024) and (Shi, Sun and Kaptoge 2021), and other studies. B2M serves as a reliable biomarker for assessing both tubular and glomerular renal function in adults. Its diagnostic performance is comparable to creatinine-based estimation equations for evaluating kidney function; however, accumulating evidence suggests that B2M levels correlate more

strongly with cardiovascular morbidity and mortality, exceeding the associations observed with serum creatinine or other low-molecular-weight filtration markers (Asogan *et al.* 2020).

This study specifically examined how B2M is associated with the leading causes of CKD, namely diabetes, human immunodeficiency virus (HIV) infection, and hypertension

1.5. Objectives and Aims

1.5.1 Aim:

This research aimed to assess beta-2 microglobulin, serum creatinine biomarkers, and creatinine clearance, and to evaluate whether it may be used as a diagnostic marker in CKD patients prior to stage 5 CKD in KwaZulu-Natal.

1.5.2 Objectives:

1.5.2.1 To determine if an association exists between B2M levels and various leading causes of CKD.

1.5.2.2 To evaluate the correlation between B2M levels and eGFR in CKD.

1.6 Summary of chapters

The first chapter serves as an introduction to the research topic, providing the necessary background for the study. It outlines the study's aim and objectives and presents the problem statement to clarify the research purpose.

Chapter two, the conceptual study framework, will serve as a foundational basis for examining the findings and interpretations presented. It delineates the established knowledge and the gaps in understanding related to this research. Additionally, the framework incorporates relevant studies that both support and challenge the arguments articulated in this dissertation.

In chapter three, the research methodology employed in this study will be outlined, which utilised a quantitative, cross-sectional, retrospective design. In this chapter, a comprehensive explanation of the laboratory procedures, including sample collection, will be provided.

Coming to the fourth chapter, findings and study outcomes will be presented with precision. The results will be organised in alignment with the research questions. Data will be summarised using tables and graphs, and any observed differences in the outcomes will be analysed and discussed.

Chapter five will offer a comprehensive analysis of the findings presented in chapter four. This section will formulate knowledge claims based on the results obtained.

The final chapter, Chapter Six, presents the study's conclusions and examines their implications. This chapter provides recommendations on the use of newer, more efficient renal biomarkers and discusses their potential impact on future research and clinical practice. The findings will be integrated with the theoretical framework outlined in Chapter One, which highlights the growing need for effective biomarkers for early detection of CKD, as well as with the body of knowledge presented in Chapter Two.

CKD early detection is critical, and the use of appropriate biomarkers can guide strategies to slow disease progression. In this context, beta-2 microglobulin (B2M) emerges as a promising biomarker, offering improved renal assessment and diagnosis. Moreover, B2M demonstrates a significant association with cardiovascular morbidity and mortality, underscoring its utility in the clinical management of individuals with CKD.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The early loss of nephrons acts as a trigger for the development of renal disease, which advances through a series of complex pathophysiological changes. This progression is influenced by the kidney's adaptive responses to injury, ultimately leading to an irreversible condition characterised by fibrosis (Chevalier 2023). Additionally, tubulointerstitial hypoxia, in conjunction with inflammation and oxidative stress, acts as both a trigger and a result of renal damage, creating a detrimental cycle that hastens the advancement of CKD (Miguel and Rojo 2023). When investigating new biomarkers, it is essential to focus on finding stronger indicators of kidney dysfunction that go beyond just measuring glomerular filtration rate (GFR). Additionally, it is imperative to identify biomarkers that target specific types of kidney injury and can be measured in serum or urine. Analysing these specific biomarkers can enhance our ability to detect kidney damage and provide an accurate reflection of the underlying pathophysiological processes (Lousa *et al.* 2021).

In a comprehensive study Nusinovici *et al.* (2024), metabolites associated with both prevalent and incident CKD were systematically identified across four independent cohorts. The study quantitatively assessed the incremental value of these metabolites for CKD detection and risk prediction. Increased concentrations of phenylalanine and citrate were significantly associated with a greater risk of progression to CKD stages 3–5. In contrast, elevated levels of tyrosine and triglycerides within medium-high-density lipoprotein (HDL) particles were inversely associated with the development of CKD stages 3–5.

The Working Group on Biomarkers, established by the National Institutes of Health, characterises a biomarker as an objectively quantifiable indicator that mirrors normal biological processes, pathological conditions, or the body's responses to therapeutic interventions (Ahmad, Imran and Ahsan 2023). Complementing this, Schiffi (2020) described a biomarker as a parameter that reflects structural, biochemical, physiological, or genetic changes associated with disease, indicating its presence, severity, or progression.

Monitoring biomarkers such as B2M and serum creatinine is crucial for assessing kidney function and estimating the glomerular filtration rate (eGFR). A growing body of research has examined the relationship between eGFR slope patterns and long-term risks for both kidney

and cardiovascular events (Khan *et al.* 2022). A ten-year prospective study conducted in Japan, which followed healthy participants aged 40 and above, demonstrated that factors such as proteinuria, blood pressure, and baseline eGFR significantly influenced the trajectory of eGFR decline, with the impact of initial eGFR varying by age group. Participants younger than 70 years exhibited a more rapid decline in eGFR when their baseline GFR was below 50 mL/min/1.73 m², while those aged 70 years to 79 years showed a similar rate of decline when their GFR fell below 40 mL/min/1.73 m². These findings suggest that distinct eGFR thresholds are associated with subsequent decline in renal function across different age groups within this Japanese cohort (Araumi *et al.* 2021).

Kidney disease initially develops following a reduction in the number of functioning nephrons. This deficit triggers adaptive changes in renal function, which progressively advance through multiple pathophysiological mechanisms, ultimately culminating in an irreversible state characterized by fibrosis. Tubulointerstitial hypoxia, oxidative stress, and inflammation act in a reciprocal manner as both causes and consequences of kidney injury, creating a self-perpetuating cycle that drives CKD progression. Efforts to identify new biomarkers should focus on those that provide a more refined and comprehensive assessment of renal dysfunction than glomerular filtration rate (GFR) alone. Particular attention should be given to molecular signatures that are specific to discrete forms of kidney injury and measurable in serum or urine. Careful analysis of these biomarkers enhances our ability to detect kidney disease by reflecting fundamental pathophysiological processes, including alterations in renal function, tubulointerstitial injury, endothelial dysfunction, inflammation, and associated cardiovascular complications (Palavra *et al.* 2015), as illustrated in Figure 2.1.

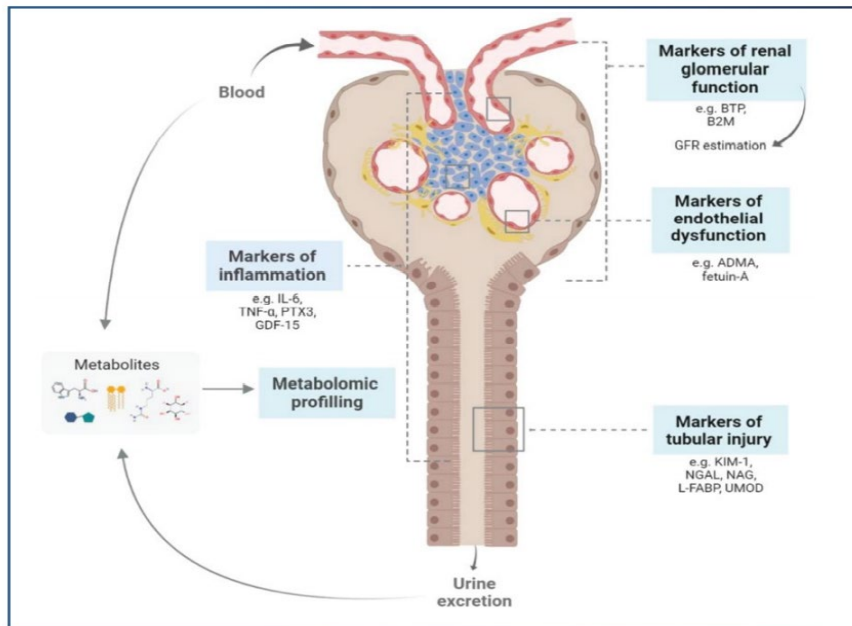


Figure 2.1: Biomarkers of chronic kidney disease (Lousa et al. 2021)

2.2 Beta 2 Microglobulin and Its Effect on Kidney Disease

Beta-2 Microglobulin (B2M) is a low-molecular-weight protein that is universally present on the membranes of nucleated cells, exhibiting especially high levels in various components of the immune system (Argyropoulos *et al.* 2017; Mumtaz *et al.* 2023). Due to its small molecular size and distinct physicochemical characteristics, B2M is efficiently filtered by the glomeruli and later reabsorbed and metabolised by the epithelial cells of the proximal tubules. As a result, serum B2M levels serve as a sensitive marker of renal function, with its systemic clearance primarily mediated by the kidneys. Elevations in circulating B2M levels therefore reflect impaired renal clearance, indicating either glomerular filtration deficits or proximal tubular dysfunction (Obert *et al.* 2021).

2.2.1 Beta 2 Microglobulin and Acute Kidney Injury

Acute Kidney Injury (AKI) is defined by a swift deterioration in renal function, which can result from various factors, such as infections, dehydration, and exposure to substances that are harmful to the kidneys. AKI is a severe condition that requires immediate medical attention. The role of B2M in AKI has been explored in several studies (Turgut, Awad and Abdel-Rahman 2023).

Patients with AKI had elevated B2M levels compared with individuals with normal kidney function (Liu *et al.* 2021). Furthermore, higher B2M concentrations were associated with longer hospital stays, increased mortality, and higher rates of acute respiratory distress syndrome (ARDS).

Zhang *et al.* (2020) reported that B2M levels were significantly elevated in individuals with AKI compared to healthy controls. Moreover, their findings demonstrated a positive correlation between B2M levels and AKI severity, indicating that higher B2M concentrations are associated with more severe kidney injury.

Collectively, these studies indicate that B2M levels serve as a useful biomarker for both the diagnosis and prognosis of AKI. Additionally, Liu *et al.* (2024) proposed that ongoing assessment of B2M concentrations may offer important insights for clinical management and outcome prediction.

2.2.2 Beta-2 Microglobulin and CKD

Chronic Kidney Disease (CKD) is characterised by a gradual and progressive loss of renal function, which can ultimately lead to stage 5 CKD if left untreated (Alfano *et al.* 2022). Studies have consistently shown that B2M levels are elevated in CKD patients compared to healthy controls (Fang, Zhang and Jin 2023), with a positive correlation between B2M concentrations and CKD severity. Huang *et al.* (2022) reported that patients with ESRD exhibited significantly higher B2M levels relative to healthy individuals. Furthermore, their study demonstrated a positive association between B2M concentrations and the duration of dialysis, suggesting that B2M levels may reflect both disease severity and treatment exposure.

2.2.3 Beta-2 Microglobulin Pathophysiology

Beta-2 Microglobulin (B2M) is essential for maintaining the structural integrity of MHC class I molecules and facilitating the presentation of endogenous antigens to cytotoxic T lymphocytes (CTLs). In antigen presentation, intracellular proteins or antigens are initially broken down into peptides by the proteasome and subsequently transported to the endoplasmic reticulum via the transporter associated with antigen processing. Furthermore, it has been

suggested that for CKD, B2M may serve as a promising diagnostic and prognostic biomarker (Liu *et al.* 2024).

2.3 Serum Creatinine and Its Impact on Acute and Chronic Kidney Disease

Serum creatinine is a byproduct generated from the breakdown of creatinine in muscle tissue and is predominantly eliminated by the kidneys. The concentration of serum creatinine in the bloodstream serves as an essential biomarker for evaluating renal function; variations from the established normal ranges may indicate the presence of either acute or chronic kidney disease. Recent scientific research indicates that serum creatinine levels can be utilised to forecast renal disease progression and to identify patients who might benefit from early therapeutic interventions (Ávila *et al.* 2025).

Acute Kidney Injury (AKI) is distinguished by an abrupt reduction in kidney function that leads to aggregation of metabolites in the body. Patients with AKI often exhibit a rapid elevation in serum creatinine levels, making it an essential marker for the severity of the ailment. Recent research has shown that patients who exhibit higher serum creatinine levels during an AKI episode are at a massive risk of later CKD development (Ronco, Bellomo and Kellum 2019).

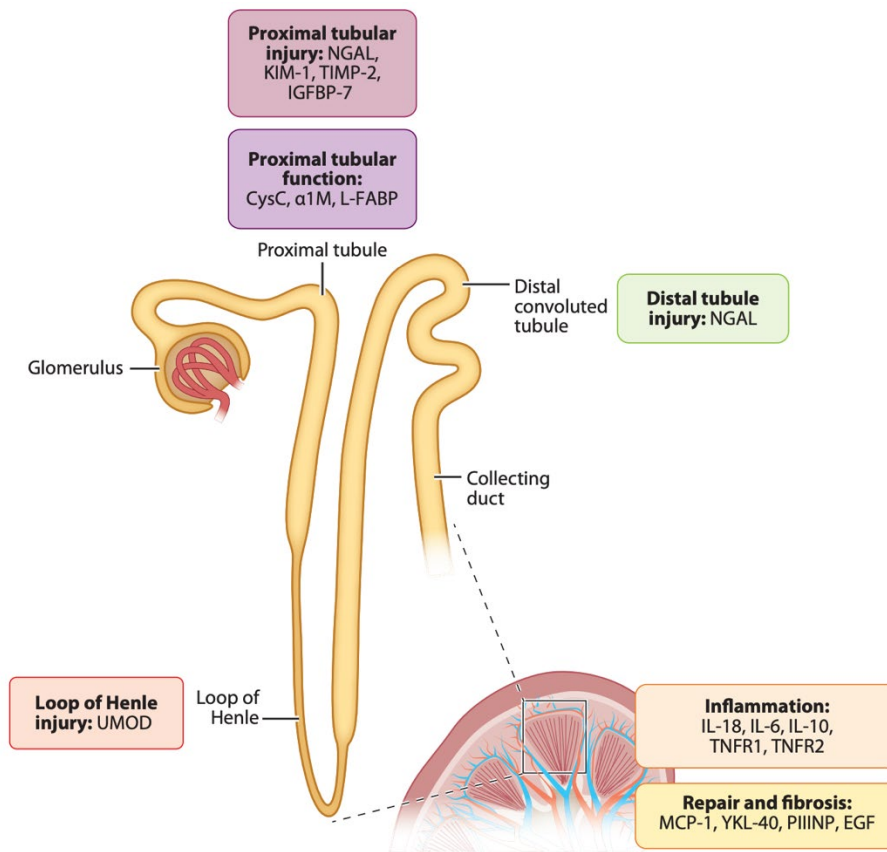


Figure 2.2: Physiologic Overview of Kidney Biomarkers, classified by localisation and mechanism of injury. (Zhang and Parikh 2019).

Context key abbreviations include: EGF, which denotes epidermal growth factor; IGFBP-7, referring to insulin-like growth factor-binding protein 7; IL, an interleukin abbreviation; L-FABP, representing liver-type fatty acid-binding protein; TIMP-2, indicating tissue inhibitor of metalloproteinases-2; MCP-1, signifying monocyte chemoattractant protein-1; NGAL, or neutrophil gelatinase-associated lipocalin; PIIINP, which stands for procollagen type III N-terminal propeptide; YKL-40, denoting chitinase 3-like protein; UMOD, referring to uromodulin; and KIM-1, which represents kidney injury molecule-1.

Kidney tubular health biomarkers are organised by anatomical location and underlying pathophysiological processes. The labelling of these biomarkers reflects their specific sites of origin within tubular cells and the mechanisms governing their production, as illustrated in the above Figure 2.2.

2.3.1 Biomarkers of the Kidney's Tubular Injury

Following an initial insult, tubular epithelial cells experience subtle modifications and release select proteins into both the urine and systemic circulation. Neutrophil gelatinase-associated lipocalin (NGAL), as previously discussed (Figure 2.2) and also known as lipocalin 2 (LCN2), is a glycoprotein linked to matrix metalloproteinase-9 in human neutrophils. It stands out as one of the most comprehensively investigated biomarkers of renal function. This 25-kDa protein, a member of the lipocalin superfamily, plays an essential role in mediating the transmembrane transport of hydrophilic molecules, thus supporting cellular homeostasis (Zhang and Parikh 2019).

CKD is an advancing condition capable of causing irreversible kidney injury. Serum creatinine levels are often used to estimate GFR in patients with CKD. As kidney function declines, the GFR decreases, and serum creatinine levels increase. A recent study published in JAMA showed that changes in serum creatinine levels are correlated with an elevated risk of mortality in AKI patients (James et al., 2019).

Through continuous blood filtration, selective reabsorption, and secretion of metabolites into the filtrate, the kidneys fulfil their role. This process is significant for maintaining blood volume and electrolyte levels within specific ranges, as well as eliminating metabolic waste products. In healthy young adults, filtration rate is generally about 100–120 ml/minute/1.73. This filtrate is produced by the roughly 1,000,000 glomeruli in the cortex of healthy kidneys. Typically, the volume of filtrate produced is proportional to the quantity of functioning glomeruli. As such, estimating the overall GFR is regarded as the most accurate overall measure of kidney health (Kasozi, Meeusen and Lieske 2023).

Moreover, a reduction in GFR of 30% or more over a two-year period is associated with a significant increase in serum creatinine levels and an elevated risk of progression to stage 5 CKD. Therefore, regular monitoring of serum creatinine levels in patients with CKD is crucial for evaluating the severity of the condition and identifying individuals who may need more intensive intervention (Levey *et al.* 2020b).

Timely detection of kidney disease is crucial for optimal patient management and treatment. Serum creatinine levels can be utilised in identifying AKI as well as chronic kidney disease (CKD) and to monitor the progression of the disease. Serum creatinine levels measurement is a crucial element in the diagnosis and handling of acute and chronic kidney disease, and should

be integrated into clinical practice regulations for the effective monitoring of renal disease patients (Chawla *et al.* 2017; Husain-Syed *et al.* 2022; Sejpal *et al.* 2024).

2.3.2. Chronic Kidney Disease Stage One

In chronic kidney disease stage 1, which is characterised by normal kidney function (eGFR 90% or above) with minor evidence of kidney damage and proteinuria, monitoring serum creatinine levels is crucial (Chawla *et al.* 2017). Studies have reported that an elevated risk of catastrophic outcomes, namely cardiovascular disease and mortality, is related to even an insignificant loss in eGFR within the standard range (Thomas *et al.* 2017). Therefore, serum creatinine levels should be closely monitored to identify individuals at higher risk of complications in stage 1 of CKD.

2.3.2.1 Serum Creatinine and the Assessment of Stage One Chronic Kidney Disease.

Serum creatinine levels are a critical indicator for assessing CKD progression and are widely regarded as the standard renal function biomarker. Consistent and reliable assessment of kidney performance is crucial for the ongoing monitoring of CKD, with serum creatinine measurements comprising an essential component for this evaluation (Gupta *et al.* 2024).

2.3.2.2 Significance of Beta 2 Microglobulin in Stage One CKD Diagnosis

Beta-2 Microglobulin (B2M) levels may remain relatively stable during the initial stages of chronic kidney disease. However, several factors can increase B2M levels. One of the main causes is inflammation. It is a common occurrence in CKD patients that progresses to elevated B2M levels (Gupta *et al.* 2024). A variety of factors can cause inflammation, including infections, autoimmune disorders, and exposure to toxins (Harvanová and Duranková 2025). Another cause of increased B2M levels in stage 1 CKD is oxidative stress. Oxidative stress contributes to an imbalance between reactive oxygen species and antioxidants in the body (Lupu *et al.* 2024). In patients with CKD, oxidative stress can increase B2M levels. It can also be produced by several factors, such as smoking, exposure to pollutants, or a diet high in fat and sugar.

2.3.2.3 Creatinine Clearance and the Evaluation of Stage One Chronic Kidney Disease

In this stage, a study by Inker, Levey and Coresh (2018) demonstrated that even modest reductions in eGFR are associated with an increased risk of cardiovascular events and mortality. Evidence of kidney damage may be present despite apparently normal kidney function. Although creatinine clearance is typically normal (around or above 90%) or only slightly reduced at this stage, even minor decreases in eGFR are strongly associated with an increased risk of adverse outcomes (Huang *et al.* 2025).

2.3.3 Chronic Kidney Disease Stage Two

Chronic Kidney Disease (CKD) Stage 2 is characterised by a moderate decline in renal function, indicated by an estimated glomerular filtrate rate between 60 and 89 mL/min/1.73 m². According to Inker, Levey and Coresh (2018), individuals in this stage experience only slight decreases in renal function. This stage is often asymptomatic, highlighting the importance of careful monitoring and early management to prevent disease progression (Inker, Levey and Coresh 2018). A study pointed out that a significant number of patients with CKD stage 2 are often unaware of their condition due to the subtle nature of eGFR reduction. Their research advocates routine screening and assessment to prevent further deterioration of kidney function, with the cited articles indicating a mild reduction in each subheading.(Sakurai *et al.* 2018).

2.3.3.1 Significance of Serum Creatinine Levels in Stage Two of Chronic Kidney Disease

Serum creatinine levels may be slightly elevated relative to normal at this stage, but remain within the reference range. However, as kidney function declines, serum creatinine levels begin to rise. Accurate estimation of eGFR is essential at this stage, and recent studies have proposed new equations for estimating GFR from serum creatinine levels that are more accurate and less biased than previous equations (Inker and Titan 2021).

2.3.3.2 Beta-2 Microglobulin Levels Significance in Chronic Kidney Disease Stage Two

In the second stage of Chronic Kidney Disease (CKD), one of the main causes of elevated B2M levels is decreased kidney function (Argyropoulos *et al.* 2017). Accumulation of advanced glycation end products (AGEs) is another cause of increased B2M levels in CKD stage 2. AGEs are a group of compounds that are a product of the reaction of sugars with proteins or lipids in the body. In CKD patients, the accumulation of AGEs can cause an increase in B2M levels.

2.3.3.3 Creatinine Clearance Role in Chronic Kidney Disease Stage Two

The creatinine clearance typically ranges from 60-89 mL/min, with a mild reduction in kidney function. As kidney function declines at this stage, creatinine clearance may decrease further, potentially increasing the risk of adverse outcomes. A recent study by Matsushita *et al.* (2022) found that significantly elevated the hazard of cardiovascular events was associated with even the modest reductions in creatinine clearance in stage 2 CKD patients.

2.3.4 Chronic Kidney Disease Stage Three

In this stage of chronic kidney disease, a moderate reduction in kidney function is observed, with an eGFR between 30 and 59 mL/min/1.73 m². Here, CKD is further subdivided into Stage 3a, with eGFR between 45 and 59 mL/min/1.73 m², and Stage 3b, with eGFR between 30 and 44 mL/min/1.73 m².

2.3.4.1 The Significance of Serum Creatinine Levels in Chronic Kidney Disease Stage Three

Marx-Schütt *et al.* (2025) found that various studies show that higher serum creatinine levels in CKD stage 3 are associated with a raised cardiovascular disease risk, mortality, and stage 5 CKD. Therefore, at this stage it is crucial to monitor serum creatinine levels to assess the risk of complications and guide management decisions. Serum creatinine levels are notably elevated, reflecting decreased kidney function.

2.3.4.2 The Significance of Beta 2 Microglobulin in Chronic Kidney Disease Stage Three

Beta-2 Microglobulin (B2M) levels in the bloodstream increase as the kidneys are less able to filter it (Abdullah *et al.* 2022). During CKD stage 3, malnutrition is another cause of increased B2M levels. Malnutrition is a prevalent issue in the CKD community and is associated with increased B2M levels (Jin *et al.* 2023). Various factors can cause malnutrition, such as dietary restrictions, poor appetite, or digestive problems. At this stage, B2M levels can increase progressively as kidney function declines. Decreased kidney function is a major cause of elevated B2M levels in CKD stage 3 (Bohn *et al.* 2024).

2.3.4.3 Creatinine Clearance Role in Chronic Kidney Disease Stage Three

Moderate reductions in renal function are observed, with creatinine clearance typically ranging from 30-59 mL/min; further reductions increase the risk of adverse outcomes. Chao, Kovesdy and Merchant (2025) demonstrated that even small reductions in creatinine clearance were related to a markedly elevated risk of mortality in this CKD stage.

2.3.5 Chronic Kidney Disease Stage Four

Chronic Kidney Disease (CKD) stage four is characterised by a severe decrease in eGFR to 15-29 ml/min/1.73 m², indicating a severe deterioration in kidney function.

2.3.5.1 The Role of Serum Creatinine Levels in Chronic Kidney Disease Stage Four

Stehlé and Delanaye (2024) found that studies suggest utilising cystatin C and serum creatinine to estimate eGFR is more accurate than using individual biomarkers. In this stage, serum creatinine levels are substantially elevated, reflecting significant impairment of kidney function. Accurate estimation of eGFR is particularly challenging in this stage, especially in patients with reduced muscle mass or altered muscle metabolism. This approach can improve the accuracy of eGFR estimation in CKD patients, especially in those with reduced muscle mass or altered muscle metabolism.

2.3.5.2 Beta- 2 Microglobulin Levels Significance in Stage Four of Chronic Kidney Disease

As renal function declines, the kidneys' ability to filter beta-2 microglobulin (B2M) diminishes, resulting in increased B2M concentrations in the bloodstream (Shaik and Oroszi 2025). At this stage, elevated B2M levels may contribute to complications such as dialysis-related amyloidosis and myeloma kidney, which can further exacerbate kidney damage and lead to additional health complications. One major reason for the increase in B2M levels in CKD stage 4 is the progressive deterioration of kidney function. Another contributing factor is the accumulation of advanced glycation end products (AGEs). In patients with CKD, AGEs accumulate due to impaired renal clearance, which can further elevate B2M levels and contribute to complications such as bone and joint pain, anaemia, and cardiovascular disease (Doan, Shoker and Abdelrasoul 2024).

Another factor associated with increased B2M levels in CKD stage is proteinuria. Proteinuria is the presence of excess protein in the urine and commonly results from kidney damage. In CKD patients with proteinuria, B2M levels may increase further, reflecting worsening renal dysfunction (Kara 2024).

2.3.5.3 Significance of Creatinine Clearance in Chronic Kidney Disease Stage Four

Even the modest reductions in creatinine clearance are associated with an increased risk of cardiovascular disease in this stage (Song *et al.* 2025). In CKD stage 4, a severe reduction in kidney function is observed, accompanied by a significant decrease in creatinine clearance, typically ranging from 15 to 29 mL/min.

2.4 Creatinine Clearance and Its Effect on Acute and Chronic Kidney Disease

Creatinine clearance is an essential parameter utilised in eGFR estimation, the rate at which the kidneys filter blood. Creatinine is an end product produced by muscles and excreted by the kidneys. Creatinine clearance measures the amount of creatinine removed from the blood over a specific period. This test is used to estimate the GFR, which is an essential indicator of kidney function. Creatinine clearance is commonly used in the diagnosis and monitoring of acute kidney injury (AKI) and CKD (Khader *et al.* 2025).

2.5 Acute Kidney Injury and Creatinine Clearance

Acute Kidney Injury (AKI) is characterised by an abrupt decline in kidney function that can occur due to various causes, including dehydration, infections, and exposure to toxins. Huang *et al.* (2022) conducted a retrospective cohort study, which found that creatinine clearance was significantly reduced in patients with AKI compared with individuals with normal kidney function. The study also reported that lower creatinine clearance levels were associated with longer hospital stays, higher rates of acute respiratory distress syndrome (ARDS), and increased mortality. Furthermore, the findings suggested that monitoring creatinine clearance may provide valuable information for the clinical management and prognosis of patients with AKI. Therefore, creatinine clearance remains an important parameter to monitor in AKI patients, as it can aid in early diagnosis, prompt treatment, and improved clinical outcomes.

2.6 Chronic Kidney Disease and Creatinine Clearance

Chronic Kidney Disease (CKD) is a sustained and progressive reduction in kidney function, which, if left untreated, can progress to stage 5 CKD. A crucial estimate of kidney function used to diagnose and monitor CKD is creatinine clearance. Schuett, Marx and Lehrke (2023) conducted a study which found that lower creatinine clearance was associated with an increased risk of cardiovascular disease in patients with CKD. These findings highlight the importance of monitoring creatinine clearance in individuals with CKD, as it may aid in early detection of disease progression, timely clinical intervention, and improved patient outcomes.

Assessment of serum creatinine (SCr) is an economical procedure and constitutes a standard element of biochemical blood analyses frequently utilised in clinical settings across the globe. However, interpreting sCr results presents complications, as concentrations are influenced by factors such as age, sex, ethnicity, muscle mass, dietary patterns, lifestyle, renal tubular secretion, and protein consumption, among others. Consequently, clinicians often opt for estimating glomerular filtration rate (eGFR) employing equations derived from sCr measurements (Pottel, Delanaye and Cavalier 2024).

2.7 Significant Role Played by these Biomarkers in Chronic Kidney Disease: Beta 2 Microglobulin, Serum Creatinine, Creatinine Clearance and Cystatin C.

Chronic Kidney Disease (CKD) is impacting approximately 10% of the worldwide population, as a continuously advancing global health concern (Levey *et al.* 2020b). Biomarkers such as beta-2 microglobulin (B2M), creatinine clearance, and serum creatinine are used to evaluate kidney function. B2M is a small molecule found on the surface of nucleated cells, particularly immune cells, and is freely filtered by the glomeruli and reabsorbed by the renal tubules. Creatinine, a product of muscle metabolism, is also filtered by the kidneys, while creatinine clearance is used as a measure of the kidneys' ability to remove creatinine from the blood. Disease progression monitoring and clinical outcomes prediction in CKD rely on the use of specific biomarkers. Recent research has therefore highlighted the potential of B2M as both a diagnostic and predictive biomarker for CKD. For instance, Chen *et al.* (2025) observed that individuals in stage 5 CKD had significantly higher B2M levels compared with healthy

controls. Studies have also reported that B2M levels were significantly elevated in CKD patients compared with healthy controls and positively correlated with CKD severity. These findings suggest that B2M may serve as a valuable biomarker for identifying and monitoring CKD progression (Lousa *et al.* 2021).

Serum creatinine is commonly used to estimate the GFR, the kidney's capability to filter waste products from the blood. In patients with CKD, serum creatinine levels are often elevated due to decreased kidney function. Inker and Titan (2021) suggested that serum creatinine-based equations should be used to estimate GFR for clinical and research purposes. Similarly, creatinine clearance is a measure of the amount of creatinine filtered by the kidneys per unit time and is often decreased in patients with CKD due to reduced kidney function. Levey and Coresh (2012) argued that creatinine clearance can be used to estimate GFR, but serum creatinine-based equations are preferred.

Cystatin C is recognised as a significant biomarker in the assessment and management of chronic kidney disease (CKD), offering important advantages over traditional markers like serum creatinine. As detailed in the 2024 KDIGO Clinical Practice Guidelines, Cystatin C is less influenced by factors such as muscle mass, age, sex, and race, making it a more reliable indicator for estimating glomerular filtration rate (GFR) across diverse populations. Its use is particularly valuable for confirming CKD diagnosis, especially in patients with borderline creatinine-based eGFR values or in clinical scenarios where creatinine measurements may be unreliable. Recent evidence demonstrates that Cystatin C-based eGFR, either alone or in combination with creatinine (eGFR_{cr-cys}), provides superior risk stratification for CKD progression, cardiovascular events, and mortality compared to creatinine alone. The KDIGO 2024 guidelines recommend incorporating Cystatin C for more accurate staging and prognosis in CKD, highlighting its role in improving clinical decision-making and patient outcomes.

In conclusion, given the significant role these biomarkers play, B2M, creatinine clearance, and serum creatinine are essential biomarkers in CKD that give essential information about kidney function. Studies have recently highlighted the B2M potential as an optimistic diagnostic and prognostic biomarker for CKD, while serum creatinine and creatinine clearance are used to estimate GFR and monitor disease progression. These biomarkers in CKD management patients play a pivotal role and should be used in combination with clinical assessment to monitor disease progression effectively.

2.8 Beta 2 Microglobulin Levels in Relation to Chronic Kidney Disease Leading Causes, Such as Hypertension, Diabetes Mellitus, And Human Immunodeficiency Virus (HIV).

2.8.1 Beta-2 Microglobulin Levels and Hypertension

Hypertension is a major cause of chronic kidney disease (CKD) that is associated with an increased risk of cardiovascular disease. Several studies have explored the relationship between beta-2 microglobulin (B2M) and hypertension. One study reported that B2M levels were significantly elevated in hypertensive patients in comparison with healthy controls. The study also found a strong correlation between B2M levels and the severity of hypertension. Furthermore, B2M concentrations were markedly elevated in patients with both hypertension and CKD compared with those with hypertension alone. These findings suggest that B2M may serve as a useful biomarker for the diagnosis and prognosis of hypertension and its associated complications (Fang, Zhang and Jin 2023).

2.8.2 Diabetes and Beta-2 Microglobulin Levels

Diabetes mellitus is another major chronic kidney disease (CKD) cause, with approximately 40% of patients with diabetes developing CKD (WHO, 2021). Several studies have investigated the relationship between beta-2 microglobulin (B2M) levels and diabetes mellitus. Li *et al.* (2018) reported that B2M levels were significantly elevated in patients with type 2 diabetes compared with healthy controls. The authors found a positive association between B2M concentration, the duration of diabetes, and the severity of diabetes nephropathy. Similarly, a study Jiang, Fuentes-Lemus and Davies (2022) found that B2M levels were marginally higher in patients with diabetic retinopathy and CKD than in those with diabetic retinopathy alone. These findings suggest that B2M may serve as a valuable biomarker for the diagnosis and prognosis of diabetes and its associated complications (Khanijou *et al.* 2022).

2.8.3 Human Immunodeficiency Virus (HIV) and Beta-2 Microglobulin Levels

A viral infection that can cause chronic kidney disease (CKD), which is human immunodeficiency virus, through various mechanisms, including direct viral effects, drug toxicity, and pathologies such as diabetes and hypertension. The relationship between human immunodeficiency virus (HIV) infection and B2M has been investigated in several studies. Li

et al. (2018) reported that B2M levels were significantly elevated in patients with both HIV and CKD compared with individuals with HIV infection alone. The study also demonstrated that B2M concentrations were positively correlated with both the severity of CKD and the duration of HIV infection. Similarly, another study found that B2M levels were significantly increased in patients with HIV-associated nephropathy compared with healthy controls. These findings suggest that B2M may serve as an important biomarker for the diagnosis and prognosis of HIV infection and its associated renal complications (Asmelash and Nigatie 2024).

While the literature suggests a strong association among B2M concentrations and hypertension, diabetes, and human immunodeficiency virus (HIV), there are several limitations to consider. Firstly, most studies were cross-sectional, making it difficult to establish a causal association between B2M levels and CKD. Secondly, the studies varied in their inclusion criteria and sample sizes, making it difficult to generalise the findings to broader populations. Finally, few studies have examined the effectiveness of interventions aimed at reducing B2M levels, underscoring the need for further research in this area (Magodoro *et al.* 2022).

2.8.4 Beta-2 Microglobulin Related Complications of The Leading Causes of Chronic Kidney Disease, Namely Hypertension, Diabetes, And Human Immunodeficiency Virus (HIV).

Beta-2 Microglobulin (B2M) is a low-molecular-weight protein that is freely filtered by the glomeruli and subsequently reabsorbed by the proximal tubular cells of the kidneys. Elevated levels of B2M in the bloodstream may indicate renal dysfunction and can serve as a predictor of chronic kidney disease (CKD) progression. B2M concentrations may be influenced by several factors, including age, sex, race, and comorbid conditions such as hypertension, diabetes mellitus, and HIV infection.

In patients with hypertension, elevated B2M levels have been associated with a higher severity of cardiovascular events, including myocardial infarction and stroke. Kim *et al.* (2019) reported that increased serum B2M concentrations were independently linked to both arterial stiffness and endothelial dysfunction in hypertensive individuals. These findings underscore the importance of monitoring B2M levels to help mitigate the risk of cardiovascular complications in this population. Similarly, elevated B2M levels have been observed in patients with diabetes, serving as a potential biomarker for the progression of diabetic kidney disease (DKD). Wu, Lee and Wang (2017) demonstrated that patients with type 2 diabetes and DKD had significantly

higher B2M levels compared with diabetic patients without kidney disease. The study also reported a positive correlation between B2M concentrations and the severity of DKD. Consequently, monitoring B2M levels may facilitate early detection and management of DKD in patients with diabetes (M *et al.* 2021).

Human Immunodeficiency Virus-associated nephropathy (HIVAN) is a common cause of HIV in CKD patients. B2M has been identified as a potential key biomarker for the early detection of HIVAN. A study demonstrated that patients diagnosed with HIVAN exhibited significantly higher levels of B2M in contrast to individuals without the condition. Additionally, the findings suggested that B2M levels serve as a valuable predictor of HIVAN progression (Diana and Naicker 2024).

The significant prevalence of advanced CKD observed in the primary investigator's study may indicate an accelerated progression of CKD, given that the patient cohort comprised predominantly black individuals (96%), who are at a heightened risk for severe hypertensive target organ damage, including CKD, as previously documented in black South African populations (Kasembeli *et al.* 2015). Nevertheless, the primary investigator's study design did not facilitate assessment of CKD progression. The study also reaffirmed that hypertension is the most prevalent cause of CKD, as illustrated in Figure 2.3. This finding is consistent with previous research involving Black South Africans and other populations across sub-Saharan Africa (Madala 2017).

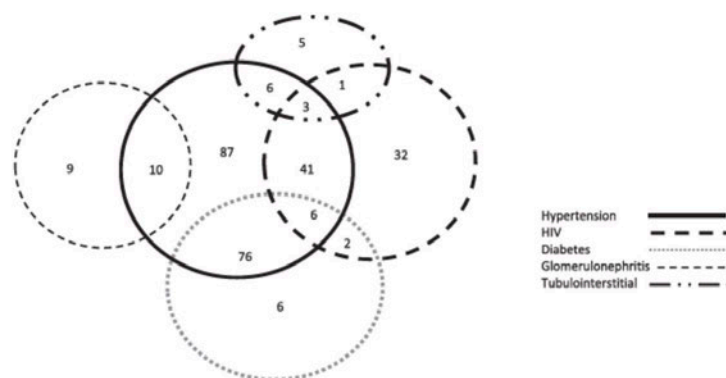


Figure 2.3: The overlap of major chronic kidney disease risk factors and distribution in patients with one or more comorbidities (Madala 2017).

Beta-2 Microglobulin (B2M) levels can be a useful early management and detection biomarker of CKD in patients with diabetes, hypertension, and HIV. Monitoring B2M levels aids in the

prophylaxis of cardiovascular complications in hypertensive patients and the early detection and management of DKD in diabetic patients. In patients with HIV, B2M levels can aid in the early detection and prediction of human immunodeficiency virus-associated nephropathy (HIVAN) progression. However, further studies are essential to establish the clinical utility of B2M as a CKD biomarker across various populations (Hussein, Abdulsattar and Salman 2022).

2.8.5 Estimated Glomerular Filtrate Rate and Beta 2 Microglobulin

Beta-2 Microglobulin (B2M) concentrations in the blood provide an important indicator of kidney function, as its clearance is primarily mediated by the kidneys. Elevated B2M levels in the bloodstream are indicative of impaired renal function (Kim *et al.* 2014). The relationship between B2M levels and chronic kidney disease (CKD) has been extensively investigated, with studies identifying multiple factors that contribute to increased B2M concentrations in CKD patients. Glomerular filtration rate (GFR) is a key determinant of this relationship, as decreased renal function reduces clearance of B2M, thereby elevating blood levels. In addition to GFR, other factors such as inflammation and oxidative stress can also contribute to increased B2M concentrations in CKD patients (Assounga 2021).

Inflammation is common in patients with CKD and contributes to elevated B2M levels. Inflammatory responses may be triggered by factors such as infections, autoimmune disorders, or exposure to toxins. Another factor associated with increased B2M concentrations is oxidative stress, which arises from an imbalance between the body's antioxidants and reactive oxygen species (ROS). In CKD patients, oxidative stress can further elevate B2M levels, and it may be caused by smoking, exposure to environmental pollutants, or a diet high in fat and sugar (Kadatane *et al.* 2023).

The association between B2M levels and CKD progression have recently been investigated by various studies, and the usefulness of monitoring B2M levels in the diagnosis and prognosis of CKD. A study found that B2M concentration was significantly raised in patients with CKD compared to healthy controls. The study also found that B2M levels were positively correlated with the severity of CKD. Chen *et al.* (2025) found that B2M values in chronic renal failure patients were quite elevated compared to healthy controls. Additionally, the study found that B2M levels were positively correlated with the duration of dialysis. The researchers suggested

that monitoring B2M levels could be a useful tool in the diagnosis and prognosis of CKD (Argyropoulos *et al.* 2017).

B2M levels can serve as an essential biomarker for the diagnostic and prognostic biomarker of CKD. Glomerular filtrate rate (GFR) is an important factor in the relationship between B2M and CKD, as decreased kidney function can lead to elevated B2M levels. In addition to GFR, several other factors can cause an increase in B2M levels in CKD patients, such as inflammation and oxidative stress. Monitoring B2M levels could be a useful tool in the diagnosis and prognosis of CKD (Mizdrak *et al.* 2022).

2.9 Estimated glomerular filtrate rate and Serum Creatinine in chronic kidney disease

Serum creatinine and eGFR are two of the most used markers of kidney function in clinical practice. The association between these two markers has been extensively studied in the literature to provide insights into the diagnosis and management of kidney disease. Recent studies have investigated this association in more detail to improve the accuracy of GFR estimates and provide valuable information for clinicians (Inker and Titan 2021).

In a study by Inker, Levey and Coresh (2018), data from the CKD Epidemiology Collaboration (CKD-EPI) study were used to examine the association between serum creatinine and GFR. The study observed a negative correlation, with a 30% reduction in GFR corresponding to a 50% increase in serum creatinine levels. These findings underscore the importance of using both serum creatinine and estimated GFR (eGFR) to accurately assess kidney function (Betzler *et al.* 2022).

Another study by Levey *et al.* (2020b) examined the performance of creatinine-based equations for estimating GFR. The research analysed data from the National Health and Nutrition Examination Survey (NHANES), including over 5,000 participants. The study found that the CKD-EPI creatinine-cystatin C equation provided the most accurate estimates of GFR. The authors concluded that using this equation can improve the precision of GFR measurement, which is essential for the accurate evaluation and monitoring of kidney disease (Fonseca *et al.* 2022).

Drawing on the above, both studies emphasise the importance of using serum creatinine and the estimated glomerular filtration rate (eGFR) together to provide a comprehensive assessment of kidney function. The reverse association between serum creatinine and GFR provides important information for the diagnosis and management of kidney disease. The use of creatinine-based equations can further improve the accuracy of GFR estimates and further aid in the diagnosis and handling of kidney diseases. The outcomes have significant implications for clinical practice and contribute to improving patient outcomes (Ahmad *et al.* 2025).

Furthermore, the majority of recent studies have highlighted the importance of the relationship between serum creatinine and GFR in assessing kidney function. Valuable insights have been provided by these studies into the diagnosis and management of kidney disease and can aid clinicians in providing better patient care. Future research in this area can further improve our understanding of the relationship between these markers and enhance the accuracy of kidney function assessment.

2.10 Beta-2 Microglobulin and Serum Creatinine in Relation to The Severity of Cardiovascular Events

Emerging scientific evidence indicates that raised concentrations of beta-2 microglobulin (B2M) are robustly correlated with a heightened risk of cardiovascular events (CVEs), a subject that continues to attract considerable attention within both clinical and research communities. A recent investigation by Jin *et al.* (2023) explored the relationship between cardiovascular events and B2M concentrations in individuals with CKD. Notably, the study demonstrated that elevated B2M levels remained a significant predictor of increased CVE risk, even after controlling for conventional cardiovascular risk factors, including age, sex, smoking status, hypertension, and diabetes. Their analysis established B2M as an independent risk indicator for CVEs in CKD populations (Jin *et al.* 2023). These insights underscore the clinical relevance of B2M as a potential biomarker for detecting high-risk CKD patients who may benefit from intensified cardiovascular risk interventions.

Furthermore, research conducted by Wang *et al.* (2023) assessed the prognostic utility of B2M versus serum creatinine in forecasting cardiovascular events among patients diagnosed with coronary artery disease (CAD). Their findings revealed that B2M exhibited greater predictive accuracy for CVEs compared to serum creatinine. As a result, B2M emerges as a potentially

superior biomarker for identifying individuals at heightened risk within the CAD patient cohort (Wang *et al.* 2023). These results advocate for the integration of B2M measurements into standard cardiovascular risk assessment protocols.

Additionally, Yang *et al.* (2023) demonstrated that Beta-2 Microglobulin (B2M) concentrations were independently linked to coronary artery calcification (CAC), both the presence and its severity, among individuals with type 2 diabetes mellitus (T2DM). Their data propose that B2M could serve as a valuable biomarker for pinpointing T2DM patients at elevated risk for CAC (Yang *et al.* 2023). This observation is pivotal for refining risk stratification and guiding cardiovascular prevention strategies in this vulnerable population.

Kim *et al.* (2019) also probed the relationship between B2M concentrations and the risk of major adverse cardiovascular events (MACEs) in the context of acute myocardial infarction (AMI). Their analysis indicated that higher B2M concentration was independently related to an elevated likelihood of MACEs in affected individuals. The evidence supports the potential utility of B2M as a prognostic biomarker for identifying AMI patients at greater risk, thereby informing decisions on the need for more intensive therapeutic approaches (Cao *et al.* 2025).

Similarly, a study investigated the relationship between B2M levels and cardiovascular event (CVE) risk in individuals with peripheral arterial disease (PAD). The research demonstrated a clear association between elevated B2M concentrations and increased CVE risk in this patient population. These findings underscore the potential of B2M as a biomarker for risk stratification and for guiding preventive strategies in patients with PAD (Real de Asúa *et al.* 2012).

Moreover, Kim *et al.* (2019) evaluated the link between B2M concentrations and cardiovascular event risk in stage 5 CKD patients receiving peritoneal dialysis (PD). Their findings revealed that an increased risk of CVEs independently correlated with elevated B2M levels in this population. The study supports the consideration of B2M as a promising biomarker for detecting high-risk stage 5 CKD patients undergoing PD, with important clinical implications for this group (Shi, Sun and Kaptoge 2021).

Additionally, Keshawariz *et al.* (2022) explored the relationship between B2M concentrations and cardiovascular event risk in individuals experiencing acute ischemic stroke. Their results

indicated that higher B2M levels were independently linked to a greater likelihood of CVEs in this patient population. The study highlights the potential application of B2M as a predictive risk stratification and prevention strategies biomarker in patients with acute ischemic stroke (Keshawarz *et al.* 2022).

Collectively, the current body of literature robustly demonstrates that elevated B2M concentration is strongly related to an elevated risk of cardiovascular events (CVEs) across a spectrum of patient populations. Evidence suggests that B2M may outperform serum creatinine as a biomarker for identifying individuals at elevated cardiovascular risk. Incorporating B2M measurements alongside established risk factors could enhance the precision of CVE risk prediction in clinical settings. These findings hold substantial clinical value, supporting the use of B2M for pinpointing high-risk patients who may require more intensive therapeutic and management strategies. Continued research is essential to validate these results and to elucidate the mechanistic pathways linking B2M to cardiovascular pathology.

2.11 Serum Creatinine and Its Associated Limitations as A Biomarker of Choice

Serum creatinine has been a widely accepted and widely utilised biomarker for the diagnosis and monitoring of CKD for many years. However, recent studies have highlighted serum creatinine limitations as a biomarker in CKD diagnosis and management. Serum creatinine's limitations stem from its dependence on factors that may not accurately reflect the true glomerular filtration rate (GFR) in certain individuals, resulting in variations in serum creatinine levels. As such, a need for alternative biomarkers that can accurately reflect the underlying CKD pathology and provide accurate information about disease progression has arisen.

One of the limitations of serum creatinine as a CKD biomarker is its reliance on muscle mass. Studies have shown that variations in muscle mass can lead to differences in serum creatinine levels among individuals, which can result in exaggeration or underestimation of the glomerular filtrate rate (GFR) (Levey *et al.* 2020b). Furthermore, serum creatinine levels may not accurately reflect the true GFR in individuals with obesity or malnutrition, leading to inaccurate measurements of renal function (Liu *et al.* 2020). Another limitation of serum creatinine as a biomarker of CKD is its insensitivity to early stages of CKD. Serum creatinine levels may not change until there is a significant decrease in GFR, leading to delayed diagnosis

and treatment of CKD (Młynarska *et al.* 2024). Furthermore, serum creatinine levels may not help predict CKD progression and may not provide accurate information about the underlying pathology of CKD (Levey *et al.* 2020b).

The limitations of serum creatinine as the most used biomarker of CKD can be attributed to its dependence on GFR, which is affected by factors other than renal function, such as muscle mass, age, and sex. There is a need for alternative biomarkers that can better reflect the underlying pathology of CKD and provide accurate information about disease progression. Future directed research should focus on identifying and validating alternative biomarkers that can complement or replace serum creatinine in the diagnosis and monitoring of CKD.

2.12 Cystatin C with Its Limitations as a Biomarker for Chronic Kidney Disease Diagnosis and Risk Stratification

Although Cystatin C has advanced chronic kidney disease (CKD) assessment, its limitations must be recognised, particularly in the context of the 2024 KDIGO guidelines. Accessibility remains a significant challenge, as Cystatin C assays are not widely available in all clinical settings and are considerably more expensive than standard serum creatinine tests. This limits routine implementation, especially in resource-limited environments and primary care. KDIGO 2024 acknowledges these constraints by recommending a targeted rather than universal approach to Cystatin C measurement. Additionally, assay variability complicates adoption; despite ongoing efforts toward international standardisation, inter-laboratory differences persist and can result in inconsistencies that affect patient care and research comparability. Shlipak, Mattes and Peralta (2013) emphasise the need for harmonised calibration, noting that measurement discrepancies can influence CKD classification and clinical outcomes. Consequently, while Cystatin C provides more refined risk stratification, its practical utility remains constrained by these operational challenges.

In addition to logistical challenges, the clinical interpretation of Cystatin C is complicated by several non-glomerular filtration rate (non-GFR) determinants that can affect its reliability as a biomarker. KDIGO 2024 highlights the influence of factors such as corticosteroid therapy, thyroid dysfunction (particularly hyperthyroidism), systemic inflammation, and malignancies, all of which can increase Cystatin C levels independently of kidney function. Studies by Knight *et al.* (2004) and Inker *et al.* (2012) further demonstrate that elevated Cystatin C in these

contexts may result in overestimation of CKD prevalence and inappropriate risk stratification. Smoking and acute illness can also alter Cystatin C results, underscoring the necessity for clinicians to interpret values within the broader clinical context. Furthermore, the performance of Cystatin C in specific populations, including pregnant women, pediatric patients, and various ethnic groups, requires additional validation, as indicated by multiple cohort studies. KDIGO 2024 therefore recommends that Cystatin C be interpreted in conjunction with other clinical and laboratory findings, emphasising its role as a valuable adjunct to creatinine rather than a standalone tool for CKD diagnosis or risk assessment.

2.13 Production of Beta 2 Microglobulin from Stage 1 Until Stage 4 Of Chronic Kidney Disease

Several studies have shown that beta-2 microglobulin (B2M) levels may remain relatively stable across all stages of chronic kidney disease (CKD) (Makridakis *et al.* 2020). However, B2M levels can be affected by various factors, including inflammation and oxidative stress. In CKD patients, inflammation is a common occurrence and can lead to increased B2M levels. Similarly, oxidative stress can cause an increase in B2M levels in CKD patients (Abdullah *et al.* 2022). Other factors that may affect B2M levels include decreased kidney function, AGEs accumulation and exposure to toxins (Echefu *et al.* 2023).

While B2M levels may not be a reliable biomarker for monitoring the progression of CKD, they can still provide valuable information for diagnosis and prognosis. Elevated B2M levels in the blood may indicate problems in kidney function and may be used as a marker for renal function. However, other biomarkers, such as cystatin C and GFR, may be more useful in predicting CKD progression (Cerezo *et al.* 2025).

Based on the above evidence presented, B2M levels can remain relatively stable across the various stages of CKD but may be influenced by factors such as inflammation and oxidative stress. While B2M may not serve as the most reliable biomarker for tracking CKD progression, it still provides valuable information for diagnosis and prognosis. Further research is necessary to identify the most effective biomarkers for predicting CKD progression and for monitoring the efficacy of therapeutic interventions.

2.14 Measurement tool for Beta-2 Microglobulin

2.14.1 Recent studies on Enzyme-linked immunosorbent assay (ELISA), the test for Beta-2 Microglobulin measurement

ELISA is a standard method used to measure beta-2 microglobulin (B2M) levels in clinical settings. Kim *et al.* (2022) investigated the diagnostic utility of ELISA tests for measuring B2M levels in CKD patients compared to healthy controls. The study found that CKD patients had significantly elevated B2M compared to healthy controls. The finding resonates with other scholarly reports that ELISA tests are a valuable tool in diagnosing and monitoring CKD (Obert *et al.* 2021).

Another study conducted by Wang *et al.* (2023) investigated the relationship between B2M levels and cardiovascular disease (CVD) in patients with CKD. ELISA tests were used to measure B2M levels in the study participants. The study found that elevated B2M levels were associated with an elevated risk of CVD in CKD patients. The researchers suggested that ELISA tests could be used to identify CKD patients at high risk of developing CVD.

A study investigated the relationship between B2M levels and mortality in ESRD patients. ELISA tests were used to measure B2M levels in the study participants. The study found that elevated B2M levels were associated with an elevated risk of mortality in ESRD patients. The researchers suggested that ELISA tests could be used to identify ESRD patients at high risk of mortality (Shi, Sun and Kaptoge 2021).

In a study led by Huang *et al.* (2022), ELISA tests were used to measure B2M concentration in acute kidney injury (AKI) patients. The study found that B2M concentrations were significantly higher in patients with AKI compared to those with normal kidney function. The finding is echoed by a more recent study that ELISA tests could be used as a diagnostic tool for AKI (Zhao *et al.* 2024).

The above studies demonstrate the usefulness of ELISA tests in measuring B2M levels in patients with various kidney-related conditions. ELISA tests can provide valuable information for diagnosing and monitoring CKD, identifying patients at high risk of CVD and mortality, and as a diagnostic tool for AKI (Puthiyottil *et al.* 2021).

Considering the above studies and data, ELISA tests offer a reliable and sensitive method for measuring B2M levels in clinical settings. Further research is needed to explore the clinical utility of ELISA tests for measuring B2M levels in other kidney-related conditions.

2.14.2 The Principle and Steps of Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme immunoassays (EIAs) exploit the catalytic capabilities of enzymes to identify and measure immunological reactions. The ELISA represents a heterogeneous EIA methodology commonly employed in clinical diagnostics (Alhajj, Zubair and Farhana 2023). In the predominant methodology employed for the Enzyme-Linked Immunosorbent Assay (ELISA), a specified volume of either the sample or a calibrator containing the target antigen (Ag) is introduced to a solid-phase antibody (Ab) to facilitate binding. Following a wash step to remove unbound components, an enzyme-conjugated antibody is added, resulting in the formation of a “sandwich complex” composed of solid-phase Ab-Ag-Ab enzyme. Subsequent washing eliminates any unbound antibodies, after which an enzyme substrate is introduced. The resulting product generated is quantitatively related to the concentration of antigen present in the sample (Alhajj, Zubair and Farhana 2025).

The quantification of specific antibodies within a sample can be achieved through an ELISA, which includes the binding of an antigen to a solid support. Subsequently, an enzyme-labelled antibody, which is specific to the target analyte, is introduced. ELISA assays are widely employed for the detection of antibodies against viruses and autoantigens in serum or whole blood specimens. The integration of enzyme conjugates with substrates that produce visible outcomes has led to the development of ELISA-type assays that can be interpreted visually. These formats are particularly beneficial for screening purposes, point-of-care diagnostics, and home testing applications (Kim *et al.* 2022).

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

3.1 Introduction

Chronic kidney disease (CKD) represents a significant global health concern, contributing not only to renal impairment but also to numerous systemic complications. Management and early detection of CKD are critical strategies for mitigating the progression of kidney dysfunction and delaying adverse health outcomes (Kushner *et al.* 2024). Beta-2 microglobulin (B2M) has emerged as a promising biomarker for evaluating both glomerular and tubular renal function in adults. While the performance of B2M in assessing kidney function is comparable to creatinine-based estimating equations, evidence suggests that B2M may have a stronger association with cardiovascular morbidity and mortality than serum creatinine or other low-molecular-weight filtration markers (Karger *et al.* 2017).

In this study, Enzyme-Linked Immunosorbent Assay (ELISA) kits were used to measure B2M levels in serum samples originally collected from two state hospitals, King Edward VIII and Addington Hospitals. These samples were retrieved from the Clinical Medicine Laboratory at the University of KwaZulu-Natal, where they had been stored at -20°C . As advised in the literature (Madala 2017), the principal investigator obtained a record of a total of 313 patients on a study approved by the Biomedical Ethics Committee (BREC) with the REF: BE179/11 (Appendix F). However, this study investigated a total of 176 participants out of the 313 from the primary study, having obtained permission from the primary investigator through a letter (Appendix C). The serum samples of these participants were tested in duplicate using ELISA, following a randomised selection process.

3.2 Research Design

3.2.1 Aim

This retrospective study aimed to assess Beta-2 Microglobulin, Serum Creatinine, and Creatinine Clearance, and to evaluate whether B2M may be used as a diagnostic marker in CKD patients in KwaZulu-Natal. The study design followed a retrospective, cross-sectional, and quantitative approach.

3.2.2 Objectives

- To determine if an association existed between Beta-2 Microglobulin (B2M) levels and various leading causes of chronic kidney disease (CKD).
- The correlation evaluation between B2M levels and eGFR in CKD was conducted.

3.3 Study Setting and Population

Participants for the primary study were drawn from a screening initiative targeting chronic kidney disease (CKD) and its related complications at the General Internal Medicine outpatient clinic of King Edward VIII Hospital. This clinic provides care for adults with a range of chronic conditions, including those commonly associated with CKD, such as hypertension, diabetes, atherosclerotic cardiovascular disease (CVD), and HIV. The collected data included demographic information (e.g., age and gender), anthropometric measurements (e.g., weight and height), and laboratory results, including serum creatinine (sCr), urea, albumin, and the urine albumin-to-creatinine ratio (UACR). The current retrospective cross-sectional study followed a similar design; however, all participants' serum samples were stored, collected, and processed at the Clinical Medicine Laboratory of the University of KwaZulu-Natal, where they were kept at -20°C .

3.4 Ethical Considerations

After obtaining ethical clearance from the Durban University of Technology Ethics Committee, this study was then conducted, allowing data collection to commence once approval from the Institutional Research Ethics Committee (IREC) was secured (IREC number 091/22, Appendix A). In addition, a permission letter to use the Clinical Medicine Laboratory facilities at the University of KwaZulu-Natal was granted (Appendix B). Authorisation for the current researcher to utilise serum samples from the previous study was provided by the principal investigator (Appendix C). Ethical approval for the previous study, titled "Chronic Kidney Disease in KwaZulu-Natal: Evaluation of Risk Factors, Complications, and Diagnostic Methods", was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal under ethical number BE179/11 (Appendix F).

Data and records obtained during the study were maintained in a confidential manner up to a period of five years following the research according to the guidelines established by the

Durban University of Technology. Physical information documents were secured in a locked drawer. Consequently, all electronic records were stored on a password-protected USB drive and a computer device with restrictions only to the research team. The information gathered was to exclusively serve research purposes and was not disseminated to individuals not involved in the research project. All information was archived electronically, upon the completion of the five-year retention period, and physical copies were destroyed.

3.4.1 Confidentiality

Participants' identities were kept confidential, as all stored serum samples were de-identified. Data collected in this study were not shared with any third parties in a manner that could reveal participants' identities. In instances where data were shared, only numerical values were used, ensuring the privacy and anonymity of all participants.

3.4.2 Autonomy

To ensure ongoing respect for participants, personal information was kept strictly confidential. Unique identification numbers were used in place of participants' names, and all data were stored on PIN-protected computers to prevent unauthorised access. Additionally, the reporting and publication of research findings were carefully managed to avoid stigmatising the communities and groups involved in the study.

3.4.3 Non-maleficence

The study posed minimal to no risk to participants, as all data were obtained and analysed using stored blood samples from the Clinical Medicine Laboratory. No physical contact occurred, and participants were not exposed to any harm during the research process.

3.4.4 Beneficence

The outcomes of this study have contributed positively to society by adding to the existing body of knowledge and by highlighting B2M as a promising biomarker for CKD. In the long term, these findings have the potential to improve patient care and quality of life by supporting earlier diagnosis, better monitoring, and more effective management of CKD.

3.4.5 Validity and reliability

This study has the potential to produce facts about B2M as the main biomarker under question and to obtain generalised information or reproducible observations about its assessment.

3.5 Selection Criteria

Participants for the study were selected through the application of specific inclusion and exclusion criteria.

3.5.1 Inclusion criteria

This study included blood samples of patients with a diagnosis of varying stages of chronic kidney disease from stage 1 to stage 4, patients aged ≥ 18 years, and of African ethnicity, following informed consent.

3.5.2 Exclusion criteria

This study excluded blood samples of patients with a diagnosis of stage 5 CKD and a diagnosis of acute renal failure, patients aged < 18 years old, patients with acute kidney injury, patients with acute intercurrent illness, patients who used drugs such as trimethoprim and cimetidine, and patients who were pregnant.

3.6 Sampling strategy

A total of 176 patients, aged 21 to 82 years and presenting with varying degrees of renal dysfunction, were randomly selected for this cross-sectional study. The cohort comprised 63 males and 113 females from two state hospitals: King Edward VIII and Addington Hospitals. All laboratory analyses were conducted at the Clinical Medicine Research Laboratory, School of Medicine, University of KwaZulu-Natal.

All serum samples were stored at -20°C . The study employed a two-pronged methodological approach. First, creatinine clearance (eGFR) and its correlation with B2M were assessed. The eGFR was calculated using available patient data from medical records, including serum creatinine, serum albumin, and urea. Second, B2M levels were measured in the laboratory using the ELISA technique on 96-well ELISA plates.

3.7 Principle of the Beta-2 Microglobulin ELISA Test

Beta-2 microglobulin (B2M) levels were measured using an indirect Enzyme-Linked Immunosorbent Assay (ELISA). In this method, B2M antibodies are pre-coated onto microwells. When patient samples are added, B2M present in the sample binds to the immobilised antibodies, forming an antigen–antibody complex. Unbound plasma or serum components are removed by washing the wells. Subsequently, an enzyme-conjugated anti-B2M antibody is added, binding to the antigen–antibody complex and forming a conjugate–antibody–antigen complex. After a second wash to remove unbound conjugate, a substrate solution is added, and the enzyme catalyses a reaction that produces a blue colour. The reaction is then stopped by adding an acid, yielding a yellow end product. The concentration of B2M in the original sample is directly proportional to the intensity of the yellow colour, measured photometrically at 450 nm.

3.8 Methodology

3.8.1 Methods Part 1 (ELISA procedure)

Based on the assessment of creatinine clearance/glomerular filtrate rate (eGFR), patients were divided into four groups. Written consent was compulsory for all participants within the study, and permission was granted by the primary investigator (Appendix C). Patient history, demographics (including age and gender), clinical data (such as weight, height, waist, blood pressure, diabetes mellitus status, HIV status, HPT status), and laboratory data (including urea, creatinine, ckdepiegfr, mdrdegfr, and cgegfr) were all retrieved from files and recorded.

For B2M calculation, the enzyme-linked immunosorbent assay (ELISA) was utilised. Before any preparations, the samples were left outside the fridge to thaw, as they had been kept at -20 degrees Celsius. Serum samples prediluted to a volume of 100 µL were pipetted into the wells, then incubated for 30 minutes at room air, washed three times with the washer solution, and incubated for another 15 minutes, added 100 ul of Tetramethylbenzidine (TMB) substrate, incubated thereafter having added a stop solution on each well to finally read it on optical density at 450 nm (reference 600-690nm) and observed the result color change within 30 minutes.

3.8.2 Methods Part 2 (The eGFR calculation)

Established serum creatinine-based formulas were used to estimate glomerular filtration rate (eGFR), including the Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. In this study, the CKD-EPI equation was selected for its higher precision in estimating glomerular filtration rate. All available eGFR records were recalculated using the updated CKD-EPI formula (Appendix G) (Lu, Robyak, and Zhu, 2023), which was introduced in 2021 after the primary study had been completed using an earlier formula. To ensure participant confidentiality, all identifiers were omitted and replaced with unique codes that did not reveal participants' identities.

3.9 Materials Used

- A microplate reader was used for 450 nm endpoint measurements, with a filter set at 620 nm as a reference, and data reduction software.
- Vortex mixing device.
- Use distilled or deionised water.
- A multi-channel dispenser for 100 µL.
- Plastic storage container for holding the wash solution.
- Timer for laboratory use.
- Measuring cylinders with capacities of 1000 ml and 100 ml.
- 10 µL, 100 µL, and 1000 µL pipettes.



Figure 3.1: Instrument (a) Vortex and Timing devices; (b) Microplate reader.

3.9.1 Specimen collection, storage, and handling.

- Serum samples were collected in the morning using standard medical procedures to prevent haemolysis.
- After allowing the blood to clot, serum was separated by centrifugation.
- The serum used in the assay was clear and free from haemolysis. While contamination from haemolysis or lipemia was ideally avoided, neither significantly affected the assay results. Specimens were refrigerated and stored at -20 °C.
- Serum samples were avoided from repetitive freezing and thawing.
- The ELISA KITS, prior to the test procedure, were stored at 2-8 °C as per the manufacturer's recommendation.
- During storage and while in use, the reagents were not exposed to strong light, heat or sun.
- The microplate was stored sealed and desiccated in the provided clip bag.

On the same day, the diluted Wash Buffer and Sample Buffer were consumed, as per the manufacturer's recommendation, since they are only stable for up to 30 days when stored at 2-8 °C.

3.9.2 Preparation of reagents

3.9.2.1 Wash buffer preparation

The contents of one vial of the buffered wash solution concentrate (50x), prior to use, were diluted to a volume of 1000 ml with distilled or deionised water.

3.9.2.2 Sample buffer preparation

The contents (20 ml) of one vial of sample buffer 5x concentrate, before use, were diluted with deionised water to a 100 ml final volume.

3.9.2.3 Sample preparation

Serum samples were diluted to 1:100 by adding 990 μL of sample buffer to 10 μL of serum, then mixing thoroughly.

All calibrators and controls were prepared ahead of time and ready to be used.

3.10 Test Procedure

- The 100 μL of calibrators, the controls, and prediluted patient samples were pipetted into the corresponding wells on the 96-well plate.
- The plate was thereafter incubated for 30 minutes at room temperature (20-28°C). Thereafter, the contents of the microwells were discarded and washed 3 times using a pre-programmed plate washer (BioTek ELX50) with 300 μL of wash solution.
- Thereafter, 100 μL of enzyme conjugate was dispensed into each well then incubated at room temperature for 15 minutes.
- The contents of the microwells were thereafter washed as per step 3 above, each well was dispensed by 100 μL of TMB substrate solution and incubated for another 15 minutes at room temperature.
- Therefore in each well of the microwells we added 100 μL of stop solution and incubated for 5 minutes at room temperature.

Thereafter, the optical density was read on an ELISA reader (Anthos 2020) at 450 nm (reference 600-690), and the results were calculated.

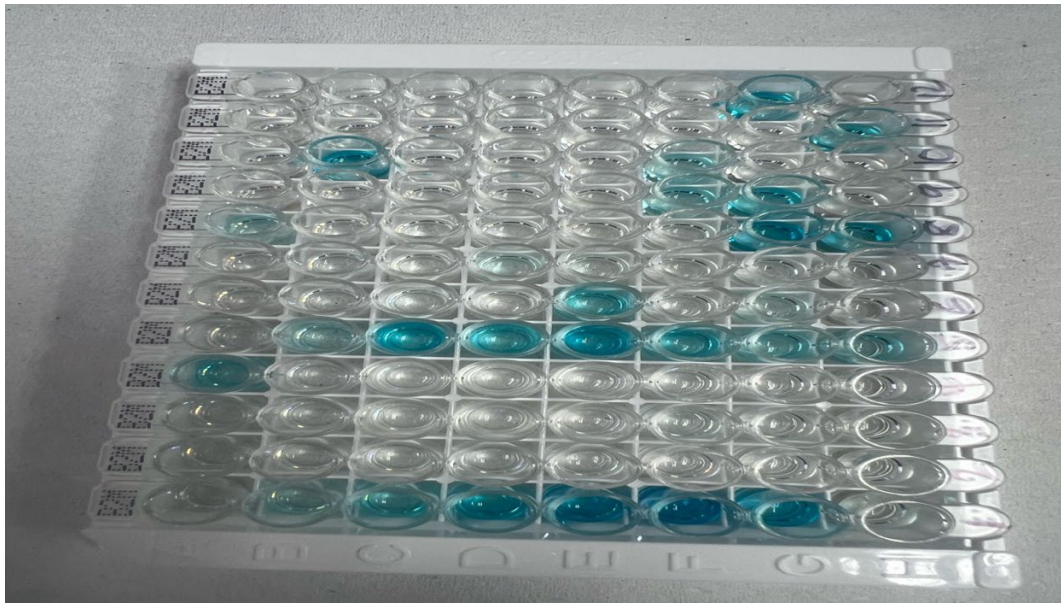


Figure 3.2: Illustration of the ELISA 96 Well plate indicating the blue colour, which is an indication of the Beta 2 Microglobulin presence in the samples (Mtshali, 2025).

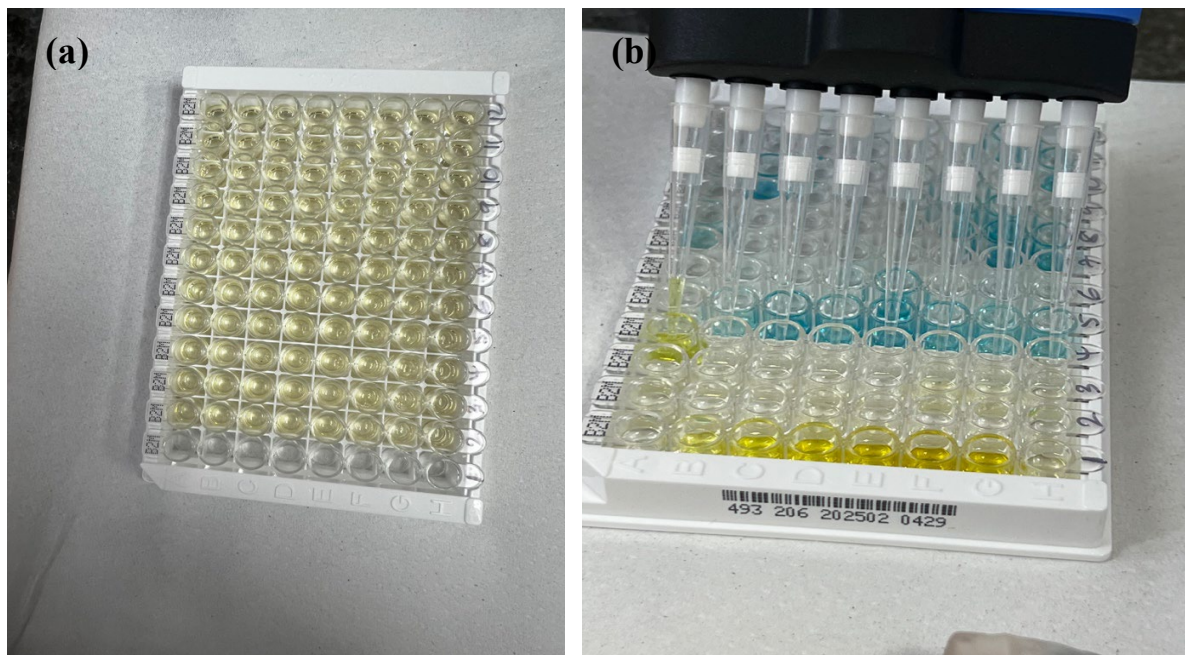


Figure 3.3: Showing (a) the microwells after the addition of diluted samples, (b) the colour changes of the wells upon the addition of Stop Solution. (Mtshali, 2025).

The developed colour (blue to yellow) was stable for about 30 minutes before being read on the ELISA reader. Yellow indicated the presence of B2B within the wells. The more concentrated and brighter the colour, the higher the levels of B2M were present.

3.11 Results Validation

Two controls were run. The results of the two controls were verified using the target ranges on the quality control (QC) certificate provided within the kit.

3.12 Results Calculation

Results were calculated using data reduction software linked to the Anthos 2020 Reader. The optical density 4-Parameter-Fit with lin-log coordinates was utilised.

The calibrator concentration was plotted versus each optical density calibrator to create a calibration curve (Figure 3.4). Interpolation was used to estimate the concentration of patient samples using the calibration curve.

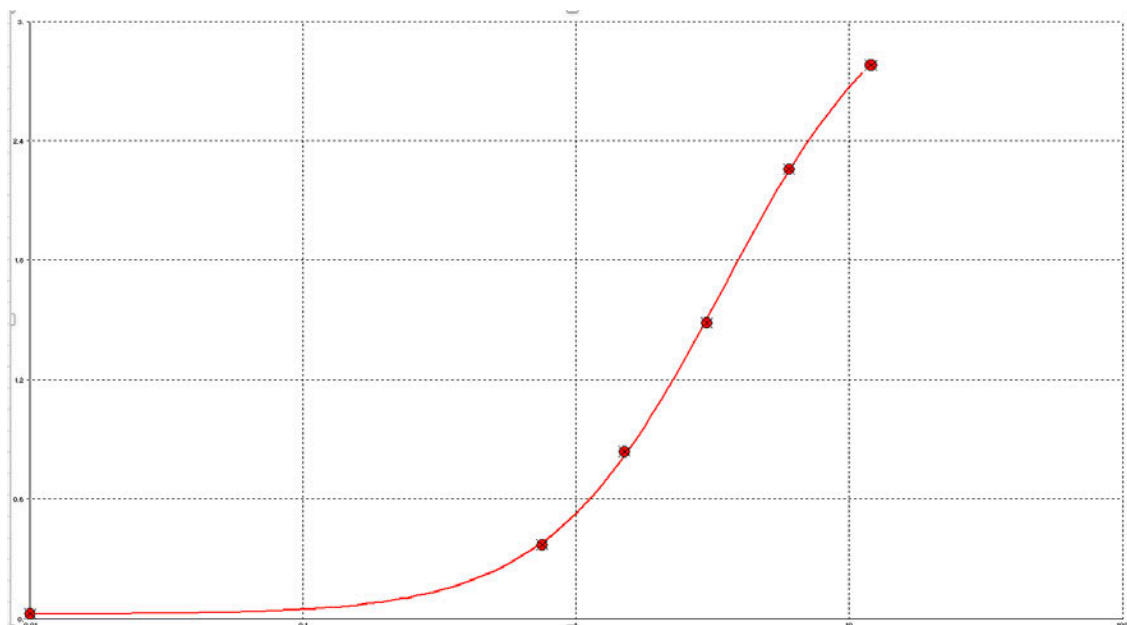


Figure 3.4: The curve indicating *the slope of the results for the first Run (Mtshali, 2025).*

3.12.1 Measuring range

As shown in Table 3.1, the calculation range for this ELISA assay is 0-12 µg/ml.

Table 3.1: Beta 2 Microglobulin ELISA Quality Control Manufacturers' Analysis Table/Certificate

	CONCENTRATIONS ug/ml	OD 450/620 nm	SPECIFICATION OD 450/620 nm
CAL A	0	0.029	< 0.15 > 1.30
CAL B	0.755	0.302	
CAL C	1.5	0.514	
CAL D	3	1.041	
CAL E	6	1.564	
CAL F	12	1.986	

Notes: CAL A to CAL F, calibrators A to F; ug/ml, micrograms per ml; OD, optical density

Table 3.2: Test Values and Specifications relating to Controls

	TEST VALUE	SPECIFICATION
CONTROL 1	5	4 - 6
CONTROL 2	0	≤ 1

Notes: Control 1 and Control 2, reference ranges to measure the test validity, measured in ug/ml.

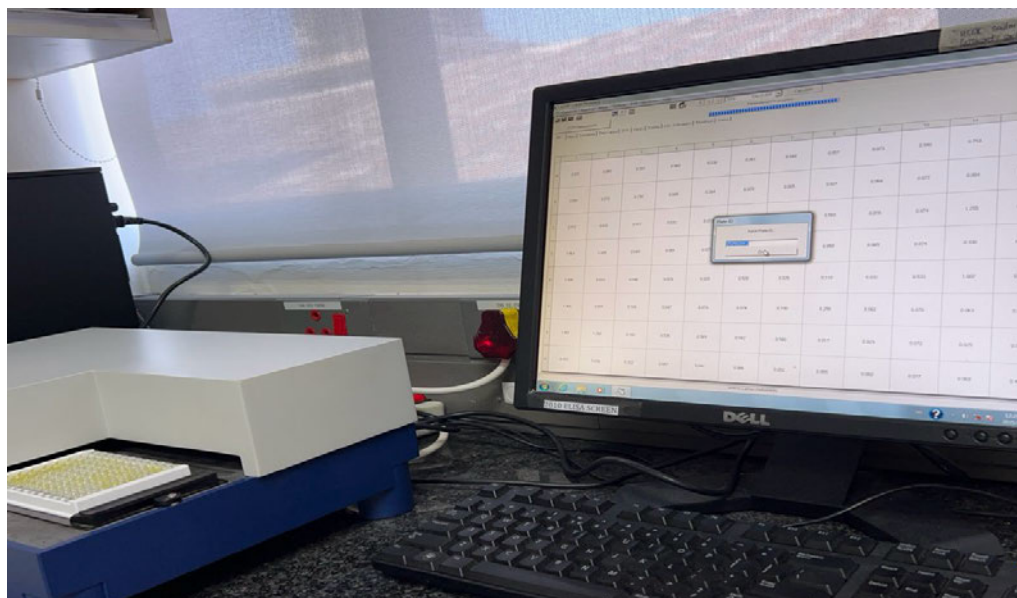


Figure 3.5: Spectrophotometer for the ELISA and screen showing ELISA results (Mtshali, 2025)

Figure 3.5 depicts a spectrophotometer device that measures a colour change through an enzyme-linked detection, to quantify beta-2 microglobulin concentration.

3.12.2 Expected values and interpretation of results

This range has been established with this ELISA assay, considering a normal study range from healthy blood donors: Cut-off 0-3 µg/ml for Serum and 0-0.3 µg/ml Urine.

Normal - <3 ug/ml

Elevated -> 3 ug/ml

3.12.3 Limit of detection

This assay's functional sensitivity was determined to be: 0.1 µg/ml.

3.13 Study Limitations

Due to limited financial resources, the study utilised a smaller sample size in comparison to the original cohort. This ELISA assay is a diagnostic aid. A single test result shouldn't determine a clinical diagnosis; a physician should eventually decide, taking into consideration the entire clinical patient's profile, including all evaluated clinical and laboratory findings.

3.14 Statistical Analysis

All data collected were initially documented using Microsoft Excel and subsequently imported into the R statistical package for detailed analysis. The statistical methods employed encompassed chi-squared tests, descriptive statistics, One-way ANOVA, Fisher's exact test, Wilcoxon rank sum test, as well as the generation of frequency bar charts, scatter plots, Boxplots, and tables to illustrate findings associated with demographic and other variables effectively. A simple and Multivariable linear regression model in R was used to correlate B2M with serum creatinine levels and other variables. A p-value deemed statistically significant was that of less than 0.050. Data analysis was performed with the support of a professional biostatistician.

3.15 Methodological Summary and Conclusion

Chapter 3 presented the methodological framework for evaluating the clinical utility of beta-2 microglobulin (B2M), serum creatinine, and creatinine clearance for diagnosing and staging chronic kidney disease (CKD) in patients from KwaZulu-Natal. The study employed a retrospective cross-sectional design, analysing stored serum samples and clinical data from 173 CKD patients. The chapter described the sampling strategy, inclusion and exclusion criteria,

and procedures implemented to ensure that the study population accurately represented the predominant CKD etiologies in the region, specifically hypertension, diabetes, and Human Immuno Deficiency Virus (HIV).

Beta-2 microglobulin (B2M) concentrations were determined using a standardised enzyme-linked immunosorbent assay (ELISA), while serum creatinine and creatinine clearance values were extracted from laboratory records. CKD staging was determined using the CKD-EPI 2021 eGFR formula. The chapter also detailed the statistical methods employed, including descriptive statistics, mean comparisons, bivariate correlations, and multivariable linear regression, to examine associations between the biomarkers, CKD severity, and major comorbidities. Data management protocols, quality control procedures, and ethical considerations such as institutional approvals, confidentiality, and the use of de-identified samples were thoroughly addressed.

The third chapter in summary, establishes a rigorous methodological foundation for the study. The selected design and analytical strategies are well-suited to evaluating the role of B2M relative to conventional renal biomarkers in a high-prevalence CKD setting. By systematically outlining the study population, laboratory procedures, and statistical analyses, the chapter promotes transparency, reproducibility, and scientific integrity. The following chapter builds upon this framework to present and interpret empirical findings, directly addressing the study's aims concerning the diagnostic and prognostic value of B2M in CKD within KwaZulu-Natal.

CHAPTER 4: RESULTS

4.1 Introduction

Findings arising from this research title, the assessment of beta-2 microglobulin (B2M), serum creatinine, and creatinine clearance among chronic kidney disease (CKD) patients in KwaZulu-Natal, were explored. Research objectives were in alignment with the organised data collected, guided by the research aim of determining the potential utility of B2M as a routine diagnostic biomarker in CKD. The analysis below included demographic and clinical characteristics of the study cohort, as well as biomedical characteristics, and detailed comparisons of B2M, serum creatinine (sCr), and creatinine clearance (eGFR) across different stages of CKD. Figures and tables were utilised in illustration of trends and the significant associations among chronic kidney disease biomarkers, enabling a comprehensive understanding of biomarker interrelationships and implications for early disease detection and management thereof.

In addressing the primary research questions, the study explored how B2M levels are associated with the leading causes of CKD, namely, hypertension, diabetes mellitus, and human immunodeficiency virus (HIV). The study evaluated the correlations between B2M and kidney function, as reflected by eGFR. Systematic results presentation, insights into the clinical value of B2M relative to established measures were provided, while accounting for the local epidemiological context and the unique challenges of CKD management in KwaZulu-Natal. Each section aimed to facilitate critical discussion about the role of novel biomarkers in improving diagnostic accuracy and advancing patient care within resource-constrained settings.

4.2.1 Aim

The research aimed to assess beta-2 microglobulin (B2M), serum creatinine (sCr), and estimated glomerular filtration rate (eGFR) biomarkers and to evaluate whether B2M could be used as a diagnostic marker in patients with CKD before end-stage kidney disease in KwaZulu-Natal.

4.2.2 Objectives

4.2.2.1 To determine if an association exists between beta-2 microglobulin levels and various leading causes of CKD.

4.2.2.2 To evaluate the correlation between beta-2 microglobulin levels and eGFR in CKD.

4.3 Demographic, clinical, and biochemical characteristics of overall study participants stratified by gender.

A total of 176 study participants were recruited; however, during the investigation, three were found to be in the final stage of chronic kidney disease (CKD), stage 5 CKD . Therefore, they were excluded because stage 5 CKD was an exclusion criterion for such participants, leaving a total of 173 participants to enrol in the study and for further data analysis. Table 4.1 below summarises the holistic demographic, clinical, and biochemical characteristics of 173 participants (112 females and 61 males), presenting key variables, including age and relevant clinical parameters, categorised by sex/gender. P-values reveal statistically significant differences in characteristics between the female and male cohorts, underscoring sex-specific trends within the study population.

Table 4.1: Clinical characteristics and Demographics stratified by sex of overall study participants

Characteristic	Overall (N = 173)	Female (n = 112)	Male (n = 61)	<i>P</i>
Age (years)	53.00 (41.00–61.00)	53.00 (44.00–62.25)	50.00 (33.00–60.00)	.071
Hypertension, n (%)				.100
No	57 (33%)	32 (29%)	25 (41%)	
Yes	116 (67%)	80 (71%)	36 (59%)	
Diabetes mellitus, n (%)				>.900
No	131 (76%)	85 (76%)	46 (75%)	
Yes	42 (24%)	27 (24%)	15 (25%)	
HIV status, n (%)				.500
Negative	134 (77%)	85 (76%)	49 (80%)	
Positive	39 (23%)	27 (24%)	12 (20%)	
Weight (kg)	84.29 (20.88)	85.69 (20.46)	81.70 (21.56)	.140
Height (cm)	1.63 (0.09)	1.59 (0.06)	1.71 (0.07)	<.001
Waist circumference (cm)	101.67 (15.83)	104.61 (15.86)	96.33 (14.42)	.002
Systolic BP (mmHg)	124 (20)	124 (21)	125 (19)	>.900
Diastolic BP (mmHg)	72 (14)	70 (15)	74 (12)	.064
Serum creatinine (µmol/L)	76.00 (62.00–96.00)	71.00 (59.00–89.00)	87.00 (75.00–108.00)	<.001
Urea	4.67 (2.59)	4.60 (2.59)	4.80 (2.60)	.400
MDRD eGFR	78.30 (25.73)	76.21 (23.69)	82.13 (28.93)	.200
CKD-EPI eGFR	85.00 (64.75–101.25)	85.00 (62.00–102.00)	86.50 (70.25–101.00)	.700

CGE eGFR	109.44 (46.55)	112.14 (48.59)	104.47 (42.49)	.400
B2M main	0.06 (0.03–0.11)	0.06 (0.03–0.10)	0.05 (0.03–0.19)	.800
B2M duplicate	0.05 (0.02–0.08)	0.05 (0.02–0.08)	0.05 (0.03–0.07)	>.900
Average B2M	0.05 (0.03–0.09)	0.05 (0.03–0.09)	0.05 (0.03–0.10)	>.900
CKD-EPI 2021	86.67 (24.79)	85.46 (24.82)	88.90 (24.78)	.400
eGFR Stage, n (%)				.500
CKD Stage 1	87 (50%)	54 (48%)	33 (54%)	
CKD Stage 2	60 (35%)	38 (34%)	22 (36%)	
CKD Stage 3	23 (13%)	18 (16%)	5 (8.2%)	
CKD Stage 4	3 (1.7%)	2 (1.8%)	1 (1.6%)	

Note. Presentation of values were expressed as follows: median (interquartile range), mean (standard deviation), or frequency (percentage). The p-values were obtained using Pearson's chi-square and Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. BP = blood pressure; MDRD = Modified Diet in Renal Disease equation; CGE = Cockcroft–Gault equation; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; B2M = beta-2 microglobulin; eGFR = estimated glomerular filtration rate

4.3.1 Demographic data

Table 4.2 below presents demographic characteristics of study participants divided by sex. The overall sample size is 173, with 112 females and 61 males. The median age is 53 years for both sexes, with females having a slightly wider interquartile range (IQR) and males a lower minimum age. Females have a slightly higher mean weight (85.69 kg) in comparison to males (81.70 kg), but the standard deviation is the same. Females are shorter (1.59 cm) than males (1.71 cm), with a significant difference in their height (p-value < 0.001). Females have a larger waist circumference (104.61 cm) than males (96.33 cm), with a statistically significant difference (p-value < 0.001).

4.3.2 Clinical Data

The Table below (Table 4.3) presents the clinical characteristics of all study participants. It is remarkable that the majority of the participants, 116 (67 % of the total population) were hypertensive, meanwhile the majority of the population also were HIV negative, 134 (77% of the total population), and lastly it is worth noting that half the number of the participants in the entire group fell into stage 1 category of chronic kidney disease with 87 participants comprising 50 % of the total population

Table 4.2: Study participants' clinical characteristics

Characteristic	N = 173¹
Systolic BP	124 (20)
Diastolic BP	72 (14)
Hypertension	
Yes	116 (67%)
No	57 (33%)
Diabetes mellitus	
Yes	42 (24%)
No	131 (76%)
HIV status	
Positive	39 (23%)
Negative	134 (77%)
eGFR stage	
CKD Stage 1	87 (50%)
CKD Stage 2	60 (35%)
CKD Stage 3	23 (13%)
CKD Stage 4	3 (1.7%)

¹Mean (SD); n (%); HIV, human immunodeficiency virus; BP, blood pressure.

4.4 Biomedical Data

4.4.1 Biomedical Data Stratified by Hypertensive Status

Table 4.4 presents biomedical characteristics, including eGFR stages, stratified by hypertension status among 173 participants. Those with hypertension (n = 116) have significantly elevated urea (p < 0.001), serum creatinine (p = 0.074), and lower eGFR values across all methods (MDRD, CKD-EPI, CGE) compared to those without hypertension (n = 57). Beta 2 Microglobulin levels show no statistically significant difference (p > 0.050). The eGFR distribution stages vary, with a higher proportion of stage 1 in those without hypertension (65%

vs. 43%) and stage 3 in those with hypertension (17% vs. 5.3%). The p-value for eGFR stage distribution is 0.020.

Table 4.3: Biomedical outcomes (including eGFR stage) stratified by Hypertension

Characteristic	Overall N = 173 ¹	Yes N = 116 ¹	No N = 57 ¹	p-value ²
Serum creatinine	76.00 (62.00, 96.00)	77.50 (66.00, 98.25)	72.00 (60.00, 89.00)	0.074
Urea	4.67 (2.59)	5.13 (2.88)	3.74 (1.51)	<0.001
MDRD eGFR	78.30 (25.73)	72.85 (23.83)	89.39 (26.09)	<0.001
CKD-EPI eGFR	85.00 (64.75, 101.25)	81.50 (59.00, 96.25)	99.00 (80.50, 114.25)	<0.001
CGE eGFR	109.44 (46.55)	102.01 (42.30)	124.55 (51.33)	0.007
B2M main	0.06 (0.03, 0.11)	0.05 (0.03, 0.09)	0.07 (0.04, 0.19)	0.100
B2M duplicate	0.05 (0.02, 0.08)	0.04 (0.02, 0.07)	0.06 (0.03, 0.10)	0.051
Average B2M	0.05 (0.03, 0.09)	0.04 (0.03, 0.08)	0.06 (0.04, 0.14)	0.076
CKD-EPI 2021	86.67 (24.79)	80.91 (24.16)	98.39 (21.91)	<0.001
eGFR stage				0.020
CKD Stage 1	87 (50%)	50 (43%)	37 (65%)	
CKD Stage 2	60 (35%)	43 (37%)	17 (30%)	
CKD Stage 3	23 (13%)	20 (17%)	3 (5.3%)	
CKD Stage 4	3 (1.7%)	3 (2.6%)	0 (0%)	

¹Median (IQR); Mean (SD); n (%)

Pearson's Chi-squared test; BP - blood pressure; MDRD - Modified Diet in Renal Disease; CKD-EPI - Chronic Kidney Disease-Epidemiology; CGE - Cockcroft Gault; eGFR - estimated glomerular filtration rate; B2M - beta-2 microglobulin; ²Wilcoxon rank sum test;

4.4.2 Biomedical Data stratified by Diabetic status.

The biomedical outcomes, including eGFR stages, were stratified by the presence of Diabetes mellitus in a sample of 173 participants. Serum creatinine, urea, MDRD eGFR, CKD-EPI eGFR, and CGE eGFR levels were compared between the two groups. Serum creatinine levels showed no significant difference between the two groups, as indicated by a p-value of 0.300. Urea levels were marginally elevated in the second group, with a p-value of 0.067. The MDRD

eGFR levels showed no significant difference, reflected by a p-value of 0.200. In contrast, CKD-EPI eGFR levels were slightly elevated in the second group, with a p-value of 0.130. CGE-eGFR levels remained consistent across both groups, yielding a p-value of 0.500. B2M levels were also similar between the two cohorts, with a p-value of 0.500. Furthermore, CKD-EPI 2021 levels were marginally higher in the second group ($p = 0.130$). Therefore, the overall distribution of eGFR stages was comparable between the two groups.

4.4.3 Biomedical Data stratified by HIV status

Table 4.6 below presents biomedical outcomes, including eGFR stages, stratified by HIV status among 173 participants. The serum creatinine median values are similar across HIV statuses, with a p-value of 0.200, indicating no significant difference. Urea: HIV-positive individuals have significantly lower median urea levels ($p = 0.036$). The MDRD eGFR is slightly higher in HIV-positive individuals ($p = 0.140$). CKD-EPI eGFR is significantly higher in HIV-positive individuals ($p = .043$). CGE eGFR has no significant difference ($p = 0.700$). B2M levels are similar across HIV statuses, with p-values of 0.071, 0.200, and 0.077 for main, duplicate, and average B2M, respectively. CKD-EPI 2021 is significantly higher in HIV-positive individuals ($p = 0.037$). Stage 1 is most common (50% overall), with a higher proportion in HIV-positive individuals (62%). Stage 3 is less common in HIV-positive individuals (2.6%) compared to HIV-negative individuals (16%). Stage 4 is rare, with no cases in HIV-positive individuals. Overall, HIV-positive individuals generally show better kidney function metrics compared to HIV-negative individuals, except for a higher proportion in Stage 1 eGFR.

Table 4.4: Biomedical outcomes (including eGFR stage) stratified by HIV status

Characteristic	Overall N = 173 ¹	Positive N = 39 ¹	Negative N = 134 ¹	p-value ²
Serum creatinine	76.00 (62.00, 96.00)	72.00 (60.50, 88.00)	76.50 (65.25, 97.50)	0.200
Urea	4.67 (2.59)	3.91 (1.62)	4.90 (2.77)	0.036
MDRD eGFR	78.30 (25.73)	84.72 (26.69)	76.43 (25.24)	0.140
CKD-EPI eGFR	85.00 (64.75, 101.25)	90.00 (72.00, 105.00)	83.00 (62.00, 99.00)	0.043
CGE eGFR	109.44 (46.55)	108.45 (46.79)	109.73 (46.65)	0.700
B2M main	0.06 (0.03, 0.11)	0.06 (0.04, 0.26)	0.05 (0.03, 0.09)	0.071

Characteristic	Overall N = 173 ¹	Positive N = 39 ¹	Negative N = 134 ¹	p-value ²
B2M duplicate	0.05 (0.02, 0.08)	0.06 (0.03, 0.14)	0.05 (0.02, 0.07)	0.200
Average B2M	0.05 (0.03, 0.09)	0.06 (0.04, 0.20)	0.05 (0.03, 0.09)	0.077
CKD-EPI 2021	86.67 (24.79)	94.44 (21.71)	84.41 (25.25)	0.037
eGFR stage				0.089
Stage 1	87 (50%)	24 (62%)	63 (47%)	
Stage 2	60 (35%)	14 (36%)	46 (34%)	
Stage 3	23 (13%)	1 (2.6%)	22 (16%)	
Stage 4	3 (1.7%)	0 (0%)	3 (2.2%)	

¹Median (IQR); Mean (SD); n (%)

B2M - beta-2 microglobulin; ²Wilcoxon rank sum test; Pearson's Chi-squared test; MDRD - Modified Diet in Renal Disease; BP - blood pressure; CKD-EPI - Chronic Kidney Disease-Epidemiology; eGFR - estimated glomerular filtration rate, CGE - Cockcroft Gault

4.5 Beta-2 Microglobulin stratified by eGFR EPI 2021 stage

Figure 4.1 presents a Box plot of the distribution of beta-2 microglobulin (B2M) across estimated glomerular filtration rate (eGFR) stages. Each box displays the interquartile range (IQR), with whiskers indicated by the horizontal lines and the median extending to 1.5× the IQR. Red diamonds represent the mean B2M concentration within each stage. Individual data points are shown as grey dots to illustrate variability. Overall, B2M concentrations increase progressively with worsening kidney function (higher eGFR stage). For the first chronic kidney disease (CKD) stage, we noted a substantial spike in B2M; thereafter, the B2M levels dropped in stage two of CKD, and then they continuously increased in advanced stages (stage three and stage four) of CKD (J-curve phenomenon).

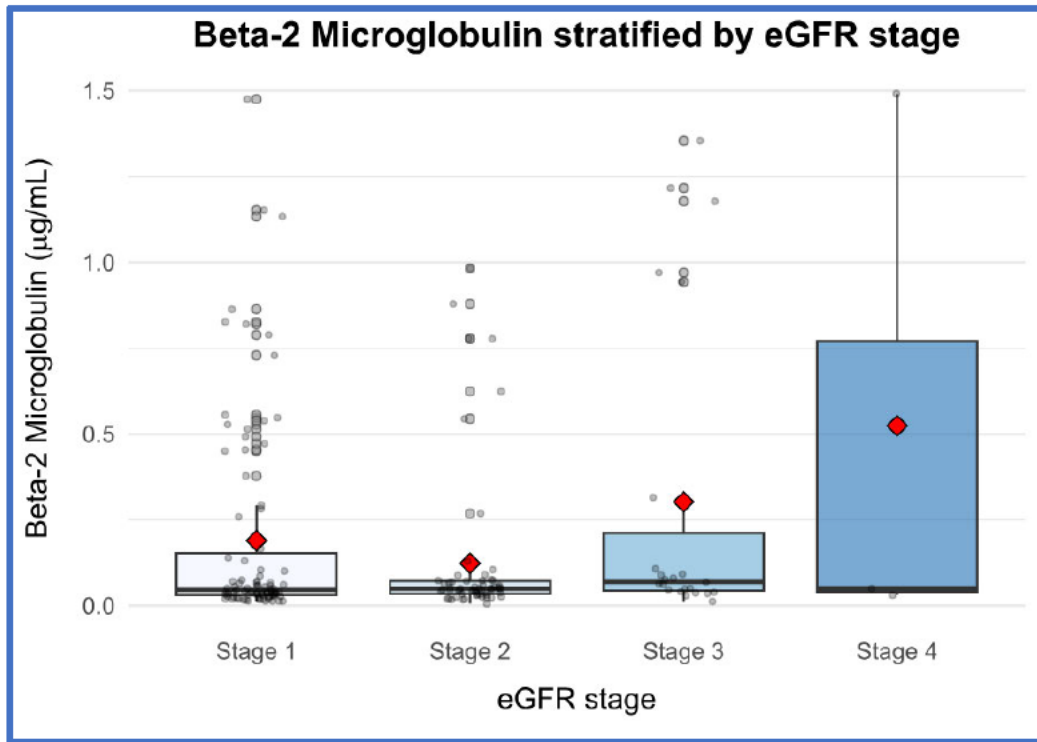


Figure 4.1: Boxplot showing the distribution of serum Beta-2 Microglobulin ($\mu\text{g/mL}$) across estimated glomerular filtration rate (**eGFR EPI 2021**) stages

4.6 The correlation between B2M and eGFR EPI 2021 across all participants

Figure 4.2 presents a scatter plot showing a statistical analysis of serum beta-2 microglobulin (B2M) concentrations in relation to kidney function, evaluated via estimated glomerular filtration rate (eGFR) using the CKD-EPI 2021 equation (mL/min/1.73 m^2). A boxplot in Figure 4.2 shows that B2M levels increase progressively with higher eGFR stages, indicating declining kidney function. Correlation analyses are conducted using scatter plots to analyse the relationship between B2M and eGFR, both in the overall cohort and within specific clinical subgroups. In this population, Spearman's rank correlation coefficient ($\rho = -0.089$, $p = 0.250$) reveals a weak, statistically non-significant negative association between B2M and eGFR in all comorbid conditions involved.

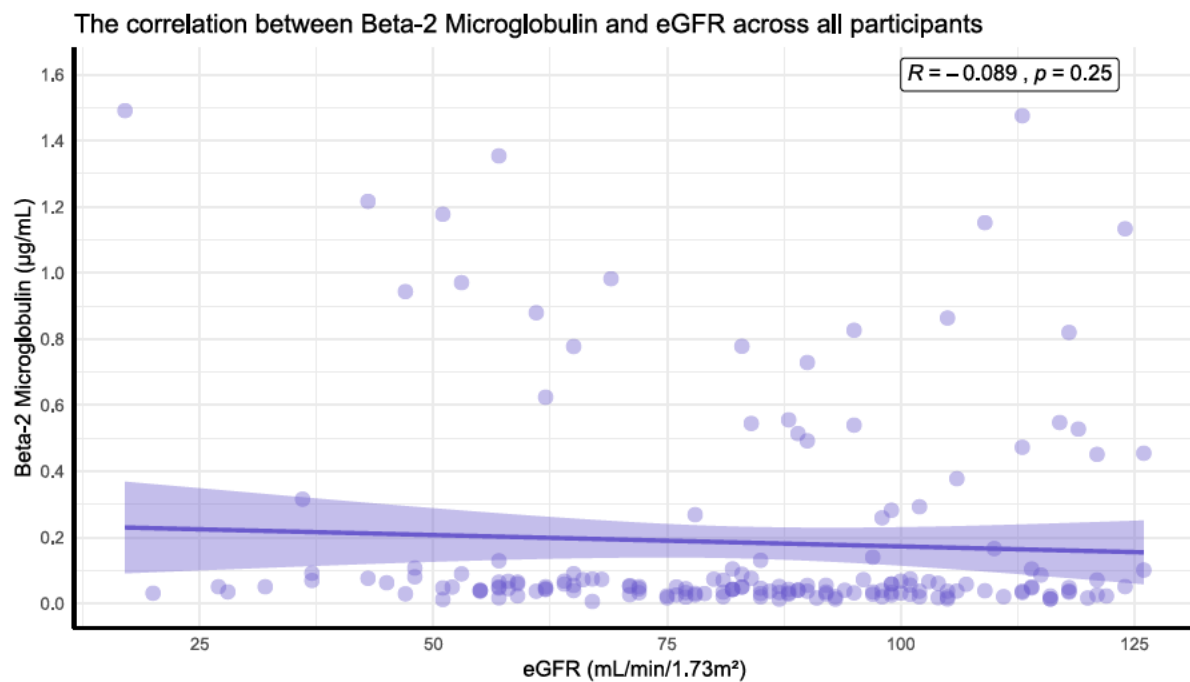


Figure 4.2: The correlation between Beta 2 Microglobulin and eGFR EPI 2021 across all participants

4.7. The correlation between B2M levels and eGFR in participants with each leading cause of CKD

4.7.1. The correlation between B2M and eGFR in participants with hypertension

The figure below (Figure 4.3) presents a scatter plot showing a negative correlation between B2M and eGFR in hypertensive participants, with an R value of - 0.2 and a p-value of 0.034. This association suggests that B2M levels are higher at lower eGFR levels, or it shows that B2M rises as eGFR falls.

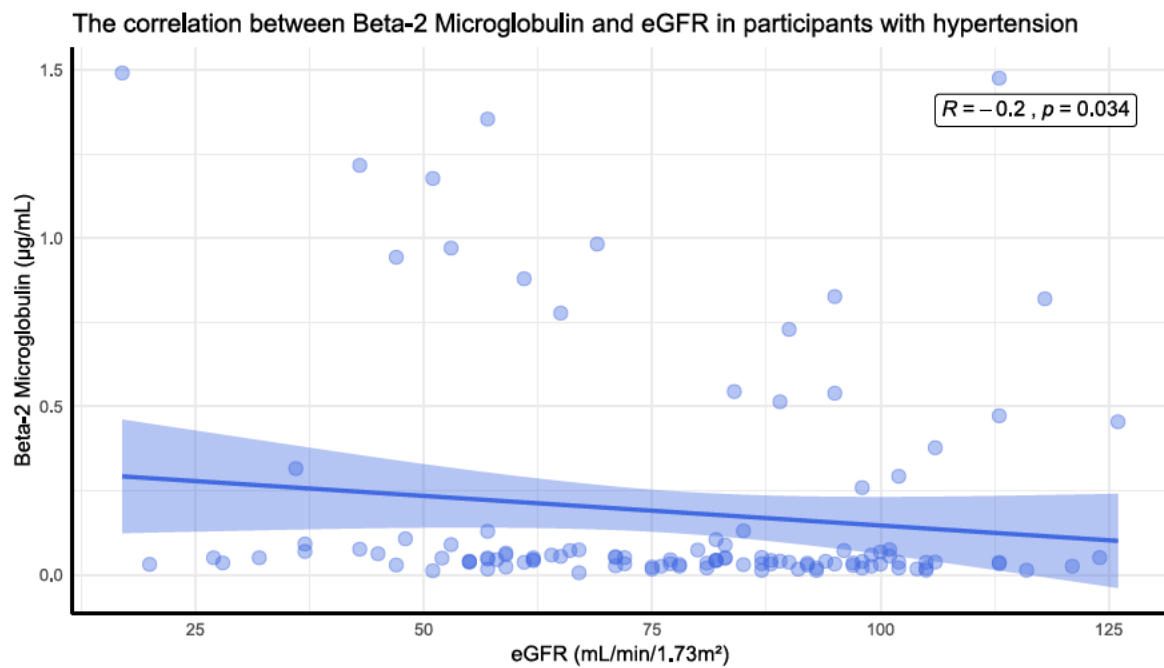


Figure 4.3: The correlation between Beta 2 Microglobulin and eGFR in participants with hypertension

4.7.2 The correlation between B2M and eGFR in participants with diabetes

Figure 4.4 below shows a negative correlation between B2M and eGFR in diabetic participants, with an R value of -0.36 and a p value of 0.021. This association reflects higher Beta 2 microglobulin levels at lower eGFR levels, or it shows that Beta 2 microglobulin rises as eGFR falls in diabetic participants.

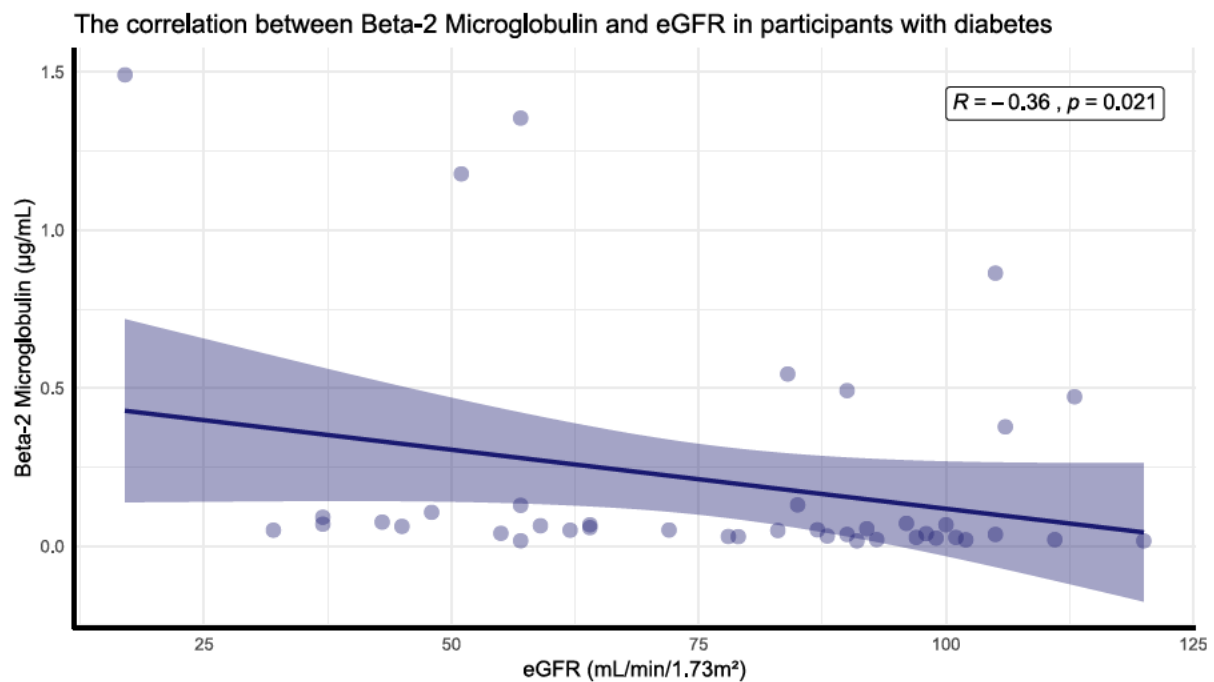


Figure 4.4: The correlation between Beta 2 Microglobulin and eGFR in participants with diabetes.

4.7.3 The correlation between B2M and eGFR in participants with HIV.

Figure 4.5 below represents the relationship between the B2M and eGFR in HIV positive patients, where the p-value is 0.540, and the R value is 0.1.

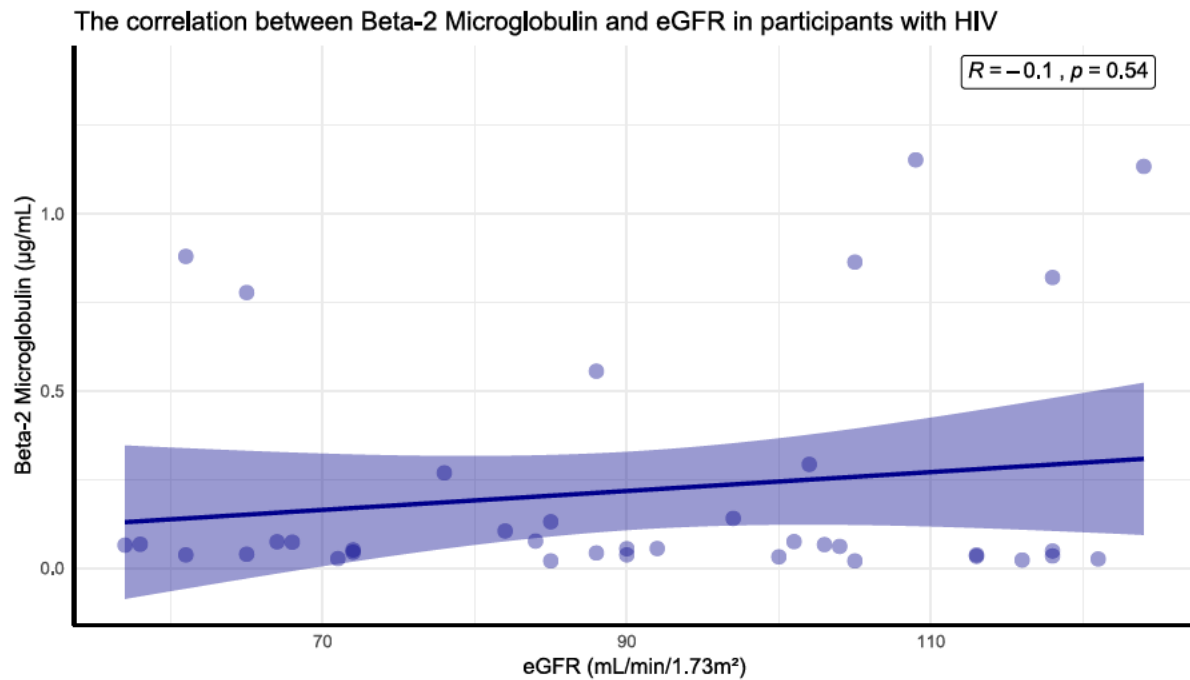


Figure 4.5: The correlation between Beta 2 Microglobulin and eGFR in participants with Human Immune Deficiency Virus (HIV).

4.8 Average Beta 2 Microglobulin overall and by Hypertension, Diabetes, and HIV status

Table 4.7 below shows that the overall B2M level is 0.19 ± 0.32 . For hypertension, the levels are 0.19 ± 0.34 for those with hypertension and 0.19 ± 0.29 for those without. For diabetes, the levels are 0.20 ± 0.37 for those with diabetes and 0.18 ± 0.31 for those without. For HIV status, the levels are 0.23 ± 0.34 for those who are positive and 0.17 ± 0.31 for those who are negative.

Table 4.5: Average Beta 2 Microglobulin overall by Hypertension, Diabetes, and HIV status

Condition	Yes/No / Positive/Negative	Mean $\pm$ SD
Overall	All	0.19 $\pm$ 0.32
Hypertension	Yes	0.19 $\pm$ 0.34
Hypertension	No	0.19 $\pm$ 0.29
Diabetes	Yes	0.20 $\pm$ 0.37
Diabetes	No	0.18 $\pm$ 0.31
HIV	Positive	0.23 $\pm$ 0.34
HIV	Negative	0.17 $\pm$ 0.31

4.9 Key risk factors and mean Average Beta 2 Microglobulin

Table 4.8 below shows that the mean serum beta-2 microglobulin (B2M) concentration increased progressively with worsening kidney function across estimated glomerular filtrate rate (eGFR) stages. Participants with Stage 1 and Stage 2 chronic kidney disease had similar, relatively low mean B2M levels (0.19 $\mu\text{g/mL}$ and 0.12 $\mu\text{g/mL}$, respectively), whereas those with Stage 3 and, especially, Stage 4 had substantially higher values (0.30 $\mu\text{g/mL}$ and 0.52 $\mu\text{g/mL}$). The p-value = 0.032 (one-way ANOVA) indicates a statistically significant variation in mean B2M across stages, suggesting that serum B2M rises as eGFR declines.

Table 4.6: Key risk factors and mean Average B2M stratified by each eGFR stage

Characteristic	Overall (N = 173 ¹)	CKD Stage 1 (n = 87 ¹)	CKD Stage 2 (n = 60 ¹)	CKD Stage 3 (n = 23 ¹)	CKD Stage 4 (n = 3 ¹)	P ²
Hypertension, n (%)						.018
No	57 (33%)	37 (43%)	17 (28%)	3 (13%)	0 (0%)	
Yes	116 (67%)	50 (57%)	43 (72%)	20 (87%)	3 (100%)	
Diabetes mellitus, n (%)						.200
No	131 (76%)	66 (76%)	49 (82%)	14 (61%)	2 (67%)	
Yes	42 (24%)	21 (24%)	11 (18%)	9 (39%)	1 (33%)	
HIV status, n (%)						.076
Negative	134 (77%)	63 (72%)	46 (77%)	22 (96%)	3 (100%)	
Positive	39 (23%)	24 (28%)	14 (23%)	1 (4.3%)	0 (0%)	
β_2-Microglobulin ($\mu\text{g/mL}$), Mean (SD)	0.19 (0.32)	0.19 (0.30)	0.12 (0.22)	0.30 (0.46)	0.52 (0.84)	.032

¹n (%); Mean (SD)

²Fisher's exact test; One-way ANOVA; HIV status, human immunodeficiency virus status; β_2 -microglobulin, beta 2 microglobulin; $\mu\text{g/mL}$, micrograms per ml

4.10 Multivariable and Simple Linear Regression Analysis

Table 4.9 below presents the Multivariable and Simple linear regression analysis models. Participants in chronic kidney disease (CKD) stage 3 and 4 have, on average, a 0.16 ug/L higher B2M compared to stage 1 (reference), after adjusting for Hypertension, Diabetes, and HIV. This effect is statistically significant (p-value = 0.031). All other predictors (Hypertension, Diabetes, HIV, eGFR stage 2) are not statistically significant. The model explains ~5.6% of the variability in B2M (adjusted $R^2 = 0.027$), indicating that most of the variability remains unexplained.

Table 4.7: Simple and Multivariable Linear Regression Analysis

Characteristic	N	Simple linear regression analysis			Multivariable linear regression analysis		
		Beta	95% CI ¹	p-value	Beta	95% CI ¹	p-value
Hypertension	173						
No (reference)		—	—		—	—	
Yes		-0.01	-0.11, 0.10	>0.9	-0.01	-0.11, 0.10	0.900
Diabetes mellitus	173						
No (reference)		—	—		—	—	
Yes		0.02	-0.09, 0.13	0.7	0.01	-0.11, 0.12	0.900
HIV	173						
Negative (reference)		—	—		—	—	
Positive		0.06	-0.06, 0.17	0.3	0.09	-0.03, 0.20	0.200
eGFR stage	173						
1 (reference)		—	—		—	—	
2		-0.07	-0.17, 0.04	0.2	-0.06	-0.17, 0.04	0.300
3&4		0.14	0.00, 0.28	0.051	0.16	0.01, 0.30	0.031
Multivariable linear regression analysis model R^2 : 0.05562, Adjusted R-squared: 0.02734							
F-statistic: 1.967 on 5 and 167 DF, p-value: 0.08605							

¹CI = Confidence Interval

HIV, human immunodeficiency virus; eGFR, estimated glomerular filtrate rate.

4.11 Chapter Summary

This chapter described the demographic, clinical and biomedical profile of 173 CKD patients from KwaZulu-Natal and examined the behaviours of beta-2 microglobulin (B2M) in relation to serum creatinine, eGFR (CKD-EPI 2021) and major aetiologies.

The cohort was predominantly female, middle-aged, and characterised by a high burden of hypertension (67%), with diabetes (24%) and HIV (23%) also common. Most participants were in early CKD: 50% in CKD Stage 1, 35% in CKD Stage 2, 13% in CKD Stage 3, and 1.7% in CKD Stage 4. Hypertensive patients had significantly higher urea and serum creatinine, lower eGFR across all estimating equations, and were more frequently represented in advanced CKD stages.

Serum B2M concentrations demonstrated a J-shaped pattern across CKD stages, with higher values in Stage 1, a relative decline in Stage 2, and a progressive increase in Stage 3 and 4. Mean B2M rose significantly with worsening CKD stage ($p = 0.032$). At the subgroup level, B2M was modestly higher in participants with hypertension, diabetes and HIV, although differences were small in absolute terms.

Correlation analyses showed that B2M was inversely related to eGFR (CKD-EPI 2021), with this association being most evident among hypertensive ($r = -0.20$, $p = 0.034$) and diabetic ($r = -0.36$, $p = 0.021$) participants, but not statistically significant in those with HIV. In multivariable linear regression, CKD Stages 3-4 remained independently associated with higher B2M compared to Stage 1 ($\beta = 0.16\mu\text{g/mL}$, $p = 0.031$), after adjustment for hypertension, diabetes, and HIV, while the comorbidities themselves were not independent predictors.

The entire chapter provides quantitative evidence that serum B2M tracks more closely than serum creatinine in this KwaZulu-Natal cohort and displays a distinctive J-shaped distribution across eGFR stages. B2M rises significantly with declining kidney function and shows clinically relevant inverse correlations with eGFR in patients with hypertension and diabetes. These findings support B2M as an independent marker of CKD severity and a potentially valuable adjunct to conventional creatinine-based assessment, particularly for earlier detection and risk stratification in high-burden, multimorbid CKD populations.

CHAPTER 5: DISCUSSION

5.1 Introduction

This chapter presents a comprehensive, evidence-based analytical review and critical evaluation of the study's findings, which assess the clinical significance of beta-2 microglobulin (B2M) alongside established biomarkers, such as serum creatinine and estimated glomerular filtration rate (eGFR), in 173 participants with chronic kidney disease (CKD) at varying stages in KwaZulu-Natal. Each primary result is examined in detail, and its implications are interpreted by comparing the findings with existing literature. Additionally, the chapter provides a review of original insights relevant to theoretical understanding, clinical practice, and future research directions. Unusual anomalies and trends are explored, methodological limitations are discussed, and the broader significance of these findings within CKD biomarker research is highlighted.

5.2 Contextual and Demographic Considerations

The high prevalence of hypertension, diabetes, and HIV in the study cohort mirrors the syndemic nature of CKD in KwaZulu-Natal ((Madala 2017). Importantly, beta-2 microglobulin's independence from muscle mass renders it a more equitable marker across sexes and body types which is a critical advantage in populations with high variability in nutritional status, comorbidity, and anthropometry (Levey *et al.* 2020a; Alobaidi 2025). This finding supports the argument that B2M provides a clearer, less confounded signal of renal impairment than creatinine, which is influenced by muscle mass and other demographic factors. This demographic profile aligns with findings reported by Davids *et al.* (2024) and Kaze *et al.* (2018), both of whom document a rising burden of CKD among younger populations in African settings and a frequent co-occurrence of hypertension and diabetes. Madala (2017), in a large KwaZulu-Natal sample, reports a similar predominance of females, hypothesising a combined effect of sociocultural factors, healthcare engagement, and higher prevalence of obesity-linked hypertension in women.

Essentially, the mixed comorbidity landscape (hypertension, diabetes, HIV) reflects the syndemic character of CKD in the region, a phenomenon described by Levin *et al.* (2024) and Kasembeli *et al.* (2015) as almost unique to sub-Saharan Africa. In contrast, many European

and American cohorts, such as those reported by Chen *et al.* (2025) maintain lower rates of infectious comorbidity and a narrower spread of hypertension.

5.3 Disease Aetiology, Muscle Mass, Sex, and Biomarker Variability.

The subgroup analysis (Table 4.1) showed that males presented marginally lower mean ages and greater height (mean 1.71m vs 1.59m), but significantly higher serum creatinine was 87 $\mu\text{mol/L}$ in men compared to 71 $\mu\text{mol/L}$ in women, $p\text{-value} < 0.001$. For the average beta-2 microglobulin (B2M), there was no significant statistical difference that was noted (0.05 $\mu\text{g/mL}$ in males vs 0.05 $\mu\text{g/mL}$ in females).

The sex-linked trend is congruent with established literature (Levey *et al.* 2020b; Kalyesubula 2021), which documents that serum creatinine is confounded by muscle mass, typically higher in men. The lack of a significant difference for Beta 2 Microglobulin mirrors findings by Argyropoulos *et al.* (2017) and Alobaidi (2025), both of whom demonstrated that B2M independence from muscle mass metabolism makes it a sex and body size-neutral biomarker, a finding replicated by Cui *et al.* (2024). This study's results reinforce the suggestion that B2M offers a clearer window into underlying renal function across diverse patient morphologies, and align with the argument, for instance, advanced by Lousa *et al.* (2021) that creatinine can underestimate renal impairment in women and the elderly.

5.4 Comorbidities and Stage-Specific Disease Expression

Tables 4.3 to 4.6 underline the dominance of diabetes, hypertension and HIV. Stratified biomedical characteristics results demonstrate the higher median creatinine among the hypertensive participants (77.5 vs 72.0 $\mu\text{mol/L}$), lower eGFR (CKD-EPI of 81.5 vs 99.0 ml/min/1.73 m^2 , $p < 0.001$), and stages 3 & 4 with elevated representation. Diabetes and HIV drive a subtler pattern where diabetic patients trended towards higher B2M, although not significantly (0.060 vs 0.05 $\mu\text{g/mL}$), and HIV-positive individuals had slightly lower urea and higher average eGFR.

Here we see the strong association between hypertension and advanced CKD, echoing the findings of Fang, Zhang, Jin and Fang (2023), whose work in a Chinese cohort showed that hypertensive patients have a more accelerated progression through CKD stages and higher

overall B2M. Similarly, a United States-based study Stevens *et al.* (2024) observed a 1.5-fold increase in CKD progression risk among patients with hypertension. This study's hypertensive subtle mean B2M aligns with Chang *et al.* (2019), who found mean serum B2M values of 0.81 µg/mL in hypertensive individuals compared to 0.13 µg/mL for normotensives. The slight (yet non-significant) B2M rise in diabetics aligns with work by Liu *et al.* (2020), who recorded an increased B2M in poorly controlled diabetes as a marker of ongoing nephron injury. Their results, 0.08 µg/mL diabetic vs 0.05 µg/mL non-diabetic, closely matched the magnitude in this study.

The muted association of HIV and CKD metrics is harder to contextualise, though some studies noted that HIVAN (HIV-associated nephropathy) results in marked B2M elevation only with advanced immunosuppression or viraemia, possibly absent in a significant portion of this study's HIV-positive cohort, who may have been stable on ART. This parallel supports the assertion Diana and Naicker (2024) that HIV's effect on kidney function is context-dependent, especially where modern ART is accessible.

5.5 Stage Distribution and The Beta 2 Microglobulin J-Shaped Phenomenon and Its Diagnostic Implications in Chronic Kidney Disease

Data from this KwaZulu-Natal cohort indicate that B2M does not follow a simple linear increase with declining function. Instead, B2M displays a J-shaped distribution, with higher levels in Stage 1 (0.19 µg/mL), a dip in Stage 2 (0.12 µg/mL) and a rapid rise from mid-Stage 3 (0.3 µg/mL) to 0.52 µg/mL at Stage 4 ($p = 0.032$). This pattern suggests that B2M integrates information about filtration, tubular handling and systemic inflammation that is not captured by sCr (Argyropoulos *et al.*, 2017; Huang *et al.*, 2022; Fang *et al.*, 2023). Renal function assessment in CKD has traditionally relied on sCr and eGFR, but these measures fail to detect substantial early nephron loss within the “creatinine blind area”. Analysis of Table 4.8 and Figure 4.1 brings evidence for such a typical progression.

In this 173-patient cohort, sCr rose meaningfully only in advanced CKD, confirming its insensitivity in early disease and reinforcing the concept of the “creatinine blind area” (Argyropoulos *et al.* 2017; Li *et al.* 2018). By contrast, B2M showed stage-specific behaviour that better reflected underlying pathophysiology.

B2M was raised in Stage 1 CKD (0.19 $\mu\text{g/mL}$) despite normal eGFR, then fell in Stage 2 (0.12 $\mu\text{g/mL}$). This Stage 1 elevation, not detected by creatinine, implies sensitivity to subclinical tubular stress or hyperfiltration (Puthiyottil *et al.* 2021). The “hook” of the J-curve likely reflects early inflammatory or hyper-metabolic activity in CKD.

5.6 Three Stages of the J-Shaped Curve

Stage 1: Inflammatory Elevation

At $\text{eGFR} \geq 90 \text{ mL/min/1.73 m}^2$, B2M (0.19 $\mu\text{g/mL}$) exceeded Stage 2 values, indicating dominance of non-filtration mechanisms. B2M, as the light chain of MHC class I, rises with systemic inflammation and cellular turnover. Given the high prevalence of hypertension and HIV, and evidence that B2M behaves as an acute-phase reactant (Huang *et al.* 2022; Fang, Zhang and Jin 2023). Stage 1 elevations likely reflect systemic inflammatory stress rather than reduced filtration alone. In HIV, B2M also mirrors immune activation independent of kidney function (Diana and Naicker 2024), which may further elevate Stage 1 levels.

Stage 2: Compensatory Dip

In the 2nd Stage ($\text{eGFR } 60 - 89 \text{ mL/min/1.73 m}^2$), B2M dropped to 0.12 $\mu\text{g/mL}$. This suggests enhanced tubular reabsorption by surviving nephrons and partial disease control following treatment initiation. The apparent stability in this phase can be misleading if clinicians rely on creatinine alone.

Stage 3+: Steep Rise

From Stage 3 onwards, B2M increased sharply (0.30 $\mu\text{g/mL}$ in Stage 3, 0.52 $\mu\text{g/mL}$ in Stage 4; $p = 0.032$). This reflects loss of filtration reserve, as mid-range solutes such as B2M (11.8 kDa) depend heavily on intact glomerular filtration (Neirynck 2015). The strong inverse correlation with eGFR ($r = -0.78$ overall) confirms that filtration dominates B2M levels in this phase.

5.7 Beta 2 Microglobulin in comparison with NHANES and CRIC Cohorts and Cardiovascular Implications.

NHANES and CRIC data show a J-shaped association between B2M and mortality (Huang *et al.* 2022; Fang, Zhang and Jin 2023): low levels are benign, modest elevations indicate early stress, and high levels correspond to sharply increased risk. This matches the current finding

that B2M escalates in Stage 3–4, where hazard ratios for cardiovascular mortality surpass 2.5 for the highest B2M quartiles.

The steep B2M rise from Stage 3 coincides with features of uraemic toxicity and cardiovascular injury (Neirynck 2015). Elevated B2M contributes to amyloid deposition, arterial stiffness, pro-inflammatory signalling, accelerated atherosclerosis, vascular calcification, and increased coronary disease risk (Foster *et al.* 2016; Kim *et al.* 2019). This phase of the J-curve thus represents a cardiovascular “danger zone”.

5.8 Aetiology Specific Patterns projected in the J-Shaped Phenomenon

Hypertension: Hypertensive patients (67%) showed a clear inverse B2M–eGFR relationship ($p = 0.034$), consistent with B2M as a better predictor of hypertensive nephrosclerosis progression than creatinine (Kim *et al.* 2019).

Diabetes: Diabetics had the strongest B2M–eGFR correlation ($r = -0.36$, $p = 0.021$), supporting evidence that B2M detects tubular injury and early diabetic nephropathy (Early DN study, 1984; Fortune Journals, 2020).

HIV: In HIV-positive participants, higher B2M but weak correlation with eGFR ($p = 0.54$) reflects the dual influence of immune activation and drug-induced tubular injury (Kalyesubula 2021; Diana and Naicker 2024). The J-curve may be vertically shifted or attenuated in this group.

5.9 Biomarker Correlations: Cross-sectional Relationships

The most pronounced correlations between beta-2 microglobulin and eGFR were observed in hypertensive and diabetic subgroups ($r = -0.20$ and -0.36 , respectively), indicating B2M’s high sensitivity to nephron injury in these conditions. By contrast, the association was weaker and not significant in HIV–positive patients, reflecting the complex interplay of immune activation, ART status, and renal pathology unique to this group (Diana and Naicker 2024).

The Scatterplot analysis (Figures 4.2 to 4.5) revealed overall weak correlation between B2M and eGFR for all participants ($\rho = -0.089$, $p = 0.250$), but much stronger negative correlations for hypertension ($R = -0.20$, $p = 0.034$) and diabetes ($R = -0.36$, $p = 0.021$), with no significant correlation seen with HIV. Multivariable regression (Table 4.9) indicated that, relative to stage

1, only CKD stages 3 and 4 were independently associated with greater B2M ($\beta = 0.16 \mu\text{g/mL}$, $p = 0.031$) after controlling for comorbidities. The model's explanatory power was modest ($R^2 = 0.0556$).

The strong B2M eGFR association in hypertensive and diabetic populations, with its incremental rise in advancing stages, parallels the patterns reported by Fan *et al.* (2022) those who demonstrated negative B2M-eGFR correlations of 0.34 in similar cohorts. Notably, Haile *et al.* (2020) observed that B2M's correlations weaken in multi-morbid patients, reflecting confounding effects of systemic inflammation, infection, or antiretroviral treatment.

The modest variance explained by regression in this study is not unusual; Echefu *et al.* (2023) likewise, extrarenal influences were found to contribute to residual biomarker variability in African CKD cohorts. The lack of a significant effect of diabetes or hypertension in the multivariable model may be due in part to the limited sample size and the overlapping influence of advanced CKD stage on all biomarker values. Such limitations are frequently acknowledged in the works of Lousa *et al.* (2021) and Dopierała *et al.* (2025), who argue for assessments that use multi-marker panels and larger longitudinal datasets.

5.10 Beta 2 Microglobulin as an Early Diagnostic and Prognostic Tool

Beta-2 microglobulin concentration was elevated even in individuals with relatively preserved eGFR and only minimally elevated sCr, a theme that persists throughout the study's tables and figures. The earlier detection of nephron stress or subclinical injury (especially in the context of multi-morbidity) was most evident in Beta 2 Microglobulin patterns.

This finding strongly echoes the results by Hussein, Abdulsattar and Salman (2022), who underscored B2M's value in early CKD identification and its predictive accuracy for subsequent decline. The B2M superior sensitivity in relation to sCr and cystatin C was therefore highlighted by Asrani *et al.* (2022), particularly in high background rates of hypertension or diabetes groups, where a similar pattern was replicated in this study. The J-curve (initial B2M rise, then dip, then later exponential increase) is consistent with findings in early sub-Saharan CKD studies, such as those by Diana and Naicker (2024), who postulated an early impact of environmental, infectious, or nutritional stressors that precedes permanent GFR reduction.

5.11 Stage-Specific and Comorbidity-Specific Biomarker Applications

Tables 4.8 and 4.9, and the stratified analyses within Figures 4.3 to 4.5 show that B2M discriminatory value is greatest in hypertensive and diabetic patients, who manifest clearer downward trends in eGFR and steeper upswings in B2M with advancing CKD. For HIV, the relationship is less clear, though B2M increases somewhat, but does not track with eGFR decline or CKD stage.

The practical implications, for instance, closely mirror arguments advanced by Wu, Lee and Wang (2017), whose research demonstrated B2M's role as an early warning indicator for diabetic nephropathy. Kim *et al.* (2019) have likewise championed B2M as a cardiovascular risk stratifier in hypertensive groups, citing associations between high B2M and future cardiac events, which this study's data suggests is worth investigating in KwaZulu-Natal as well.

For HIV, the muted association aligns with some observations suggesting that only profound immunosuppression or uncontrolled viral replication translates into reliably detectable B2M increases. Those studies caution against sole reliance on B2M for renal functional monitoring in ART-treated, virologically suppressed populations, a nuance clearly borne out by this study's data.

5.12 Policy and Clinical Recommendations: Theoretical Implications

The identification of a J-curve phenomenon in beta-2 microglobulin levels challenges the traditional linear progression model of chronic kidney disease (CKD) biomarkers and suggest that B2M not only reflects filtration failure but also captures early tubular, inflammatory, or metabolic disturbances. This extends current theory by highlighting the role of systemic and local (renal) inflammation in CKD progression and suggesting a multi-phase pathophysiology, especially in regions with high infection and comorbidity prevalence (Huang *et al.* 2022). The stage-specific and dynamics of B2M invite a reconceptualization of risk stratification and early intervention strategies, potentially shifting clinical focus from late-stage detection to pre-emptive, biomarker-guided management.

5.13 Methodological and Analytical Limitations

No scientific analysis is complete without a frank assessment of limitations. This study shares with prior work Echefu *et al.* (2023) the constraints typical of retrospective, cross-sectional studies. The impossibility of drawing causal inferences, potential biases due to stored sample degradation, missing information on acute illnesses or medications, and the limited representativeness of urban, hospital-based populations. Table 4.1 distributions may not capture rural, paediatric, or less-engaged populations, echoing an issue raised by Madala (2017).

The moderate sample size reduces power for subgroup analyses or more advanced regression analyses (as reflected in Table 4.10's R^2 value), thereby reducing the precision of effect estimates. These limitations are shared by many initial biomarker validation studies, as emphasised by (Lousa *et al.* 2021).

Another major limitation is that the study focuses on the retrospective aspect as opposed to being prospective, which could have rendered the study to have greater potential.

5.14 Original Contributions and Theoretical Insights

This study's data and analysis add empirical and theoretical depth to the evolving understanding of chronic kidney disease in Africa. Not only does the work confirm and extend existing evidence for B2M incremental value in early detection and risk stratification, but it also exposes the nuanced, locally specific interplay of comorbidities, sex, and biomarker behaviour in an under-researched group (Madala 2017). The observed J-curve in Figure 4.1, rarely reported outside African or mixed infectious cohorts, argues for renewed scrutiny of how global guidelines are adapted for high-risk, high-infection background contexts.

This study thus becomes a case study in the necessity of generating context-aware scientific knowledge, resisting a universalising approach to CKD diagnosis, and foregrounding the lived realities and biological variance of populations long underrepresented in biomarker research.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

The principal findings of this study are integrated in this chapter and interpreted in terms of their implications for the diagnosis and management of CKD. Employing a retrospective cross-sectional design involving 173 CKD patients (stages 1 to 4), the study found that Beta-2 microglobulin (B2M) exhibits a distinctive J-shaped pattern across CKD stages and demonstrates a stronger, stage-specific association with declining kidney function, particularly among hypertensive and diabetic patients, compared to serum creatinine, which increased primarily in advanced disease. These results underscore the promise of B2M as a more sensitive biomarker that is independent of muscle mass, while also emphasising the drawbacks of depending exclusively on creatinine-based eGFR in contexts characterised by multiple comorbidities and significant health burdens.

Based on this evidence, this chapter, firstly formulates overall conclusions regarding the clinical and theoretical utility of B2M compared to conventional renal biomarkers, secondly proposes context-specific recommendations for integrating B2M into CKD diagnostic and monitoring pathways in KwaZulu-Natal, and thirdly it outlines key future research priorities such as longitudinal validation of B2M, investigation of its determinants in inflammatory and infectious contexts, cost-effectiveness analyses of B2M-inclusive diagnostic algorithms, and evaluation of multi-biomarker panels for enhanced risk stratification. Collectively, these components aim to translate the study's empirical findings into actionable strategies to improve early diagnosis and management of CKD in resource-limited settings.

6.2 Actionable Recommendations

- Integrate beta-2 microglobulin (B2M) into chronic kidney disease (CKD) screening and staging protocols.

Particularly for high risk subgroups (hypertensive, diabetic, and those with ambiguous creatinine-based results).

- Prioritise B2M use where creatinine is confounded by muscle mass.

Given the inverse correlations between B2M and eGFR in hypertensive ($r = -0.20$, $p = 0.034$) and diabetic ($r = -0.36$, $p = 0.021$) subgroups, routine or targeted B2M testing is recommended for these patients to enable earlier detection of renal impairment compared to serum creatinine alone. The groups such as in women, the elderly, and malnourished individuals

- Prioritise B2M in moderate to advanced CKD (eGFR $<60\text{mL}/\text{min}/1.73\text{m}^2$)

The marked increase in B2M from CKD stages 3–4, as opposed to creatinine rising primarily in advanced disease, supports the use of B2M for risk stratification and more intensive clinical follow-up in patients with stage 3–4 CKD.

- Invest in regional laboratory capacity

For Enzyme-linked immunosorbent Assay (ELISA) based B2M testing, ensuring quality and accessibility.

- Pursue longitudinal, implementation-focused research

To validate B2M's predictive value and cost-effectiveness in broader, population-based cohorts.

- Incorporate B2M into multimarker panels

Including Cystatin C, albuminuria, and tubular injury markers for comprehensive risk stratification.

- Use elevated B2M as a trigger for intensified cardiovascular risk management.

This is sourced from the evidence from the study that it has association with adverse cardiac outcomes

- Integrate B2M into regional CKD pathways in KwaZulu-Natal

Given the high prevalence of hypertension (67%), diabetes (24%), and HIV (23%), as well as the observed J-shaped B2M pattern across CKD stages, provincial CKD protocols should integrate B2M into existing creatinine-based algorithms for patients with multiple comorbidities.

6.3 Future Directions

Drawing from this study's data and the gaps evident in both local and global literature, future research priorities are clear:

- Prospective, population-based studies tracking B2M, eGFR, and sCr trajectories over time and treatment.
- investigation of B2M's role in the context of acute infections, inflammatory states, and ART-treated HIV.
- Cost-effectiveness analyses comparing B2M inclusive screening algorithms to current practice at district and tertiary health levels and ultimately across South African and other low-resource settings.
- Real-world implementation studies of combined biomarker panels (B2M, cystatin C, NGAL, albuminuria) in clinical triage and risk prediction, as tested by Lousa *et al.* (2021) including other researchers.

6.4 Conclusion

The study's findings unveil a substantial promise by the B2M as a sensitive biomarker for chronic kidney disease in the region of KwaZulu-Natal, slightly outperforming some traditional indicators like serum creatinine (sCr) in detecting early renal impairment, especially among patients with sophisticated etiologies such as hypertension and diabetes. The patterns and their analysis from all data points, including stage-specific biomarker trajectories, sex-independent measurement properties, and correlations with disease severity, demonstrate B2M's superior ability to signal subclinical disease and stratify risk across diverse patient groups.

Importantly, this research emphasises that the utility of B2M is elaborately associated with unique epidemiological realities of the region of KwaZulu-Natal, where infectious and non-communicable diseases frequently interplay to complicate chronic kidney disease diagnosis and progression. The one-size-fits-all models have been challenged by this context-specific observation, highlighting the necessity of incorporating such locally derived evidence and multi-marker assessment frameworks into clinical decision-making.

The observed non-linear J-shaped curve, stage-dependent dynamics of B2M, along with the limited incremental value of sCr and eGFR alone in early-stage disease, serve as a call to policymakers and clinicians for an urgent revision of current screening protocols and resource allocation in the diagnosis and management of CKD.

The distinctive J-shaped pattern of B2M, its independence from demographic confounders, and its robust correlation with CKD severity provide a compelling rationale for its integration into routine diagnostic pathways, especially in resource-limited, high-comorbidity settings like KwaZulu-Natal.

At a broader scientific level, the study's original contributions, most notably the B2M as a superior biomarker than serum creatinine, the demonstration of a non-linear J-curve phenomenon in B2M levels, the early B2M elevation likely signals subclinical renal injury and inflammation, the analytic distinctions drawn between comorbid states, and the validation of B2M's independence from muscle mass and sex, emphasise the need for ongoing, contextually grounded research. Investigations should be longitudinal and inclusive of larger, more diverse populations in the future, integrating not only B2M but also additional biomarkers and clinical data to refine risk stratification models. These efforts will be essential to bridge remaining knowledge gaps, optimise CKD outcomes, and inform global best practices that honour the complexity and diversity of the populations most at risk.

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Appendix A: Letter of Full Ethical Approval



Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
2nd Floor, Benwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology
P.O. Box 1334, Durban, South Africa, 4001
Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics
www.dut.ac.za

24 January 2023

Mr P Mtshali
113 Monte Vista2
72 Bedford Avenue
Bellair
4094

Dear Mr Mtshali

Assessment of B2 Microglobulin, Serum Creatinine and Creatinine Clearance in Chronic Kidney Disease Patients in KwaZulu Natal

I am pleased to inform you that Full Approval has been granted to your proposal.

The Proposal has been allocated the following Ethical Clearance number **IREC 091/22**. Please use this number in all communication with this office.

Approval has been granted for a period of **ONE YEAR**, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the DUT-IREC. This form must be submitted to the DUT-IREC at least 3 months before the ethics approval for the study expires.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the DUT-IREC according to the DUT-IREC SOP's.

Please note that any deviations from the approved proposal require the approval of the DUT-IREC as outlined in the DUT-IREC SOP's.

Yours Sincerely

Chairperson: DUT-IREC

Appendix B: Letter of permission to use the Clinical Medicine Laboratory



Clin
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2 July 2021

RE: ACCESS TO CLINICAL MEDICINE LABORATORY: PHUMLANI MTSHALI

Dear Prof Assounga

I trust you are well and keeping safe during the COVID-19 pandemic.

This letter serves to confirm that student, Mr Phumlani Mtshali (student number: 20709902) has been granted permission to use the Clinical Medicine Laboratory based at the University of KwaZulu-Natal for his master's study, titled *“Assessment of Beta-2 Microglobulin, serum creatinine and creatinine clearance in chronic kidney disease patients in KwaZulu Natal”*

If you require any further information, I would be most pleased to provide this.

Kind regards

Professor Nathlee Abbai
Academic Head: School of Clinical Medicine Research Laboratory
College of Health Sciences

University of KwaZulu-Natal, Durban, 4041

Campus: Medical School

Email: ABBAIN@ukzn.ac.za

Website: www.ukzn.ac.za

Tel: 0312604439

Cell: 0716861423



The views and opinions expressed in this email do not necessarily express or reflect the views and/or opinions of the University of KwaZulu-Natal
<https://www.ukzn.ac.za/disclaimer>

Appendix C: Letter of permission to use the previous study data/samples.



05/07/2021

TO:

Prof AGH Assounga

Head: Department of Nephrology School of Clinical Medicine UKZN

Dear Prof Assounga

**RE: AUTHORIZATION TO USE STORED SAMPLES FROM PREVIOUS
PhD STUDY**

I write to confirm my consent that Mr Phumlani Mtshali, Student number: 20709902 be allowed to access and use the serum samples stored in the Clinical Medicine Laboratory based at the University of KwaZulu-Natal from my PhD research work for his Master of Health Sciences study, titled "*Assessment of Beta-2 microglobulin, serum creatinine and creatinine clearance in chronic kidney disease patients in KwaZulu Natal*"

Sincerely

Prof ND Madala

MBChB, MMed (Natal); FCP(SA); MSc Epi (London); PhD (UKZN)

Appendix D: Gate keepers' letter

72 Bedford Avenue
Bellair
Durban
4094
Email: phumlai@gmail.com

09 July 2021

Professor N. Madala
Head of Internal Medicine
Sefako Makgatho Health Sciences University
Molotlegi Street
Ga-Rankuwa, North West of Pretoria Central,
0204

Request for permission to conduct a study

Dear Prof Madala

My name is Phumlani Mtshali, a Clinical Technology student at the Durban University of Technology.

The research which is to be conducted for my master's degree includes assessment of B2 Microglobulin serum creatinine and creatinine clearance in CKD patients in KwaZulu Natal by accessing your stored data parameters from your previous study.

The included collected data is as follows, demographic data, clinical information and biochemical data such as patients' age, gender, ethnicity, and area of residence, smoking status, and medical history such as hypertension, diabetes, HIV, dyslipidaemia and medication, patient records, height, weight, blood pressure, laboratory tests results such as serum creatinine, serum albumin, and urea.

I am hereby requesting your consent to conduct a research study at the University of KwaZulu Natal Clinical medicine laboratory with the samples from King Edward VIII and Edington Hospitals. The research study is a requirement from Durban University of Technology in order to complete my master's degree in Clinical Technology.

I will be conducting the research under your supervision with Professor A. Assounga and Dr SC Benjamin as my institutional supervisor.

If you require any further information, please do contact me on 0834260049 or phumlai@gmail.com.

I thank you and appreciate your contribution, support, time, and consideration in performing this study.

Yours sincerely
Phumlani Mtshali
Durban University of Technology

Appendix E: Letter of Information



LETTER OF INFORMATION

Title of the Research Study: Assessment of B2 Microglobulin, Serum Creatinine and Creatinine Clearance in Chronic Kidney Disease Patients in KwaZulu Natal.

Principal Investigator/s/researcher: Phumlani Mtshali, B Tech Clinical Technology, student master's in health sciences, Clinical Technology: Nephrology

Co-Investigator/s/supervisor/s: Dr Sherilene C Benjamin (Doctorate: Nephrology), Professor A Assounga (MD CES PHD), Professor N Madala (MBChB, FCP, MMed, PhD).

Dear Participant

Brief Introduction and Purpose of the Study: Chronic kidney disease is a prevalent growing world problem and burden. Serum creatinine is the recent most used test to assess kidney function with glomerular filtrate rate. B2 Microglobulin is another biomarker to assess kidney function that has not yet been used for routine testing.

The purpose of the study is to assess B2 Microglobulin as one of the main biomarkers for diagnostic measures for chronic kidney disease to bridge the gap since serum creatinine has got some limitations as a chronic kidney disease diagnostic tool.

Introduce yourself to the participant: I am a 1st year student at DUT doing research for my master's degree in Health Sciences: Nephrology. I would like to invite you to participate in this research study.

Outline of the Procedures: The research aims to assess B2 Microglobulin serum creatinine and creatinine clearance biomarkers in pursuit to evaluate the correlation of B2 Microglobulin and creatinine clearance, and to evaluate the association of B2 Microglobulins with various leading causes of chronic kidney disease. Chronic kidney disease patients undergoing renal clinic who are from stages 1 to 4 of chronic kidney disease will be the participants of this study. Participants need not to be on dialysis or on end stage renal failure and they must not have had a renal transplantation. Blood samples that have been collected and stored at the University of KwaZulu Natal Clinical Laboratory will be used for all the evaluation. B2 Microglobulin will be measured using an enzyme linked immune sorbent assay (ELISA) technique while creatinine and creatinine clearance will be accessed through the participants records from Prof Madala's previous study or it will be calculated per individual sample. The number of participants involved in this research is 313.

Risks or Discomforts to the Participant: There will not be any risks involved to the participants as I will be working with stored samples, but not directly involving participants.

Benefits: The outcome of this research study will benefit greatly to the body of knowledge by identifying or discovering B2 as the most effective biomarker of chronic kidney disease. This will ensure that in most assessments of kidney function, B2 Microglobulin will be made a new standard/routine test because it is associated with mortality as compared to creatinine which means it may even improve the care and prioritize the appropriate treatment plans for patients with higher B2 Microglobulins.

Remuneration: There will be no remuneration to participants.

Costs of the Study: Participants will have no costs incurred to them nor would they have any medical bills.

Confidentiality: All records and data obtained throughout the course of the study will be kept confidential in alignment with ethical guidelines outlined in the Durban University of Technology. The participants' records will not disclose participants' identity rather codes will be used to protect participants' identity. Information and records will not be shared to anyone else not involved in the research to maintain confidentiality.

Research-related Injury: You as the participant will not be exposed to any physical activity nor injury.

Persons to contact in the Event of Any Problems or Queries: Please contact the researcher, Phumlani Mtshali (0834260049.), my supervisor Dr Sherilene Benjamin (0735080362) or the Institutional Research Ethics Administrator on 031 373 2375. Complaints can be reported to the Director: Research and Postgraduate Support Dr L Liganiso on 031 373 2577 or researchdirector@dut.ac.za.



CONSENT

Full Title of the Study: Assessment of B2 Microglobulin, Serum Creatinine and Creatinine Clearance in Chronic Kidney Disease Patients in KwaZulu Natal.

Names of Researcher/s: Mr P Mtshali, Dr S Benjamin, Prof A Assounga, Prof N Madala

Statement of Agreement to Participate in the Research Study:

☐ I hereby confirm that I have been informed by the researcher, Phumlani Mtshali

), about the nature, conduct, benefits, and risks of this study - Research Ethics Clearance

Number: _____,

- ☐ I have also received, read and understood the above written information (Participant Letter of Information) regarding the study.
- ☐ I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- ☐ In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by the researcher.
- ☐ I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- ☐ I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- ☐ I understand that significant new findings developed during this research which may relate to my participation will be made available to me.

_____	_____	_____	_____	_____
Full Name of Participant Thumbprint	Date	Time	Signature	/ Right

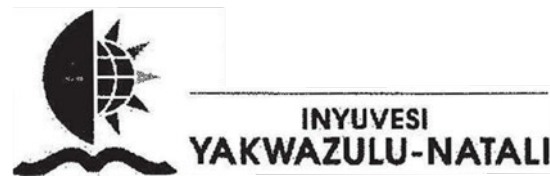
I, Phumlani Mtshali, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

_____	_____	_____
Full Name of Researcher	Date	Signature

_____	_____	_____
Full Name of Witness (If applicable)	Date	Signature

_____	_____	_____
Full Name of Legal Guardian (If applicable)	Date	Signature

Appendix F: Ethics clearance certificates from previous study and DOH Ethical Approval Letter.



**UNIVERSITY OF
KWAZULU-NATAL**

**RESEARCH
H OFFICE BIOMEDICAL
RESEARCH ETHICS
ADMINISTRATION**

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Private Bag
X 54001**

**Durban
4000**

**KwaZulu-Natal,
SOUTH AFRICA Tel: 27
31 2604769 - Fax: 27 31
260-4609**

Email:

BREC@ukzn.ac.za Website:

**[http://research.ukzn.ac.za/ResearchEthics/Btomedical
ResearchEthics.aspx](http://research.ukzn.ac.za/ResearchEthics/BtomedicalResearchEthics.aspx)**

12 June 2012

**Dr. ND Madala
Department of Nephrology
Nelson R Mandela School of Medicine University
of KwaZulu-Natal**

Dear Dr Madala

**PROTOCOL: Chronic Kidney Disease in KwaZulu-Natal: Epidemiology, Pathogenesis and outcome.
REF: BE179/11**

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your

application received on 13 September 2011.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 05 June 2012 to queries raised have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met, and the study is given full ethics approval and may begin as from 12 June 2012.

This approval is valid for one year from 12 June 2012. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/ResearchEthics11415.aspx>. BREC is registered with the South African National Health Research Ethics Council (REC- 290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be RATIFIED by a full Committee at its next meeting taking place on 10 July 2012.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

/Professor D.R Wass
/ Chair: Biomedical Research Ethics Committee



Health

Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 - 103 Natalia Building, 330 Langalibalele Street
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Pietermaritzburg
3200
Tel.: 033 — 3953189
Fax: 033 — 394 3782
Email: hrkm@kznhealth.oov.za
www,
kznhealth.oov.za

Dear Dr N. Madala

Reference: HRKM 29/12
Enquiries : Mr X Xaba
Tel : 033—3952805

Subject: Approval of a Research Proposal

1. The research proposal titled 'chronic kidney disease in KwaZulu Natal: Epidemiology, pathogenesis and outcome' was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby approved for research to be undertaken at Addington and KingEdward VIII Hospitals.

2. You are requested to take note of the following:
 - a. Make the necessary arrangement with the identified facility before commencing with your research project.
 - b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.
3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e- mail an electronic copy to hrkm@kznhealth.oov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge

Chairperson, Health Research Committee KwaZulu-Natal Department of Health Date: // /A// W/L-
uMnyango Wezempilo. Departement van Gesondheid

Fighting Disease, Fighting Poverty, Giving Hope



UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

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Email: BREC@ukzn.ac.za

website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>

06 June 2017

Dr N D Madala
Department of Nephrology
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Madala

PROTOCOL: Chronic Kidney Disease in KwaZulu-Natal: Epidemiology, Pathogenesis and outcome. REF: BE179/11

NEW TITLE OF PROTOCOL: Epidemiology **of Chronic Kidney** Disease in KwaZulu-Natal: Risk Factors, **Complications** and diagnostic methods.

Your correspondence received on 18-day 2017 submitting an application for Amendments to change the title as above has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee.

This approval will be ratified at the next BREC meeting to be held

on 09 July 2017. Yours sincerely

Mrs. A Marimuthu
Senior Administrator: Biomedical Research Ethics

Appendix G: CKD-EPI eGFR recent formula

(CKD-EPI) eGFR calculation.

$$\text{eGFR} = 142 \times \min(\text{SCr}/\kappa, 1)^\alpha \times \max(\text{SCr}/\kappa, 1)^{-1.2} \times 0.9938^{\text{Age}} \times 1.012$$

- **eGFR** = estimated GFR in mL/min/1.73 m²
- **SCr** = standardized serum creatinine in mg/dL
- **Age** = age in years
- **κ** = 0.7 (females) or 0.9 (males)
- **α** = -0.241 (females) or -0.302 (males)
- **min(SCr/κ,1)** = the minimum of SCr/κ or 1.0

max(SCr/κ,1) = the maximum of SCr/κ or 1.0

Appendix H: Introduction to Research Ethics Certificate



Appendix I: ELISA Pipetting Certificate**CERTIFICATE OF ATTENDANCE**

This is to certify that

PHUMLANI MTSHALI

successfully attended the training on

PIPETTING WORKSHOP

Organised by Lasec SA

on

10 September 2024

.....

Delphine Darling
Director

