

**SALIVARY CREATININE AS A DIAGNOSTIC TOOL FOR
EVALUATING PATIENTS WITH CHRONIC KIDNEY
DISEASE**



UNIVERSITY *of the*
WESTERN CAPE

**A mini-thesis submitted in partial fulfillment of the requirements
for the degree of MSc (Dent) in Oral Medicine.**

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KEYWORDS

Salivary creatinine

Serum creatinine

Chronic kidney disease



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ABSTRACT

Background: Preliminary studies have shown the potential use of saliva in the diagnosis of chronic kidney disease (CKD). For saliva to completely replace serum as a diagnostic and monitoring tool for CKD, studies must be done to determine its effectiveness as a substitute in diagnosing chronic kidney disease, at each stage of the disease.

Aim: The aim of the study was to evaluate the role of saliva as a safe and non-invasive alternative to serum, for creatinine estimation, in all stages of chronic kidney disease.

Method: A cross sectional study was conducted at the Renal Unit of Tygerberg Hospital, on 230 patients at all stages of CKD. Informed consent was obtained; thereafter saliva and serum samples were collected for creatinine analysis. Correlation between serum and salivary creatinine was determined using Spearman's correlation test. Receiver Operating Characteristics (ROC) analysis was used to determine the diagnostic ability of salivary creatinine and a cut-off value for sensitivity and specificity of salivary creatinine to diagnose CKD with GFR < 60ml/min was obtained.

Results: Serum creatinine values ranged from 46 μ mol/L to 1581 μ mol/L with a median value of 134 μ mol/L. Salivary creatinine values ranged from 3 μ mol/L to 400 μ mol/L with a median of 11 μ mol/L. Spearman's correlation analysis showed a strong positive correlation ($r = 0.82$) between serum and salivary creatinine values for all CKD stages. Linear regression analysis of serum and salivary creatinine for CKD patients was significant in all CKD stages, except for stage 1. Area under the curve for salivary creatinine was 0.839. A cut-off value of 8.50 μ mol/L showed a sensitivity of 78.3% and specificity of 74.0% at eGFR < 60ml/min, for classifying patients as having CKD.

Conclusion: The results support the potential of salivary creatinine as a non-invasive diagnostic tool for estimating serum creatinine in patients with chronic kidney disease.



DECLARATION

I, the undersigned, Dada Oluwaseyi Temilola, hereby declare that the work contained in this dissertation titled; “Salivary Creatinine as a Diagnostic Tool for Evaluating Patients with Chronic Kidney Disease” is my original work and has not been previously in its entirety or in any part submitted at any university for any degree or examination.



Dada Oluwaseyi Temilola

October 2017



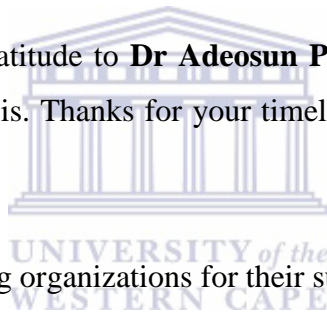
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DEDICATION

This dissertation is dedicated to my **God**, my *Strength*, my *Refuge* and my *Fortress*. Thank you Lord for your faithfulness always.

To my parents; *Mr and Mrs Temilola Eromioyewon*, thank you so much for your love, support and understanding.

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LIST OF ABBREVIATIONS

CKD: Chronic kidney disease

Pmp : Per million population

GFR : Glomerular filtration rate

eGFR : Estimated glomerular filtration rate

KDIGO : Kidney Disease Improving Global Outcomes

CHC : Community health center

ROC : Receiver Operator Characteristics

EDD : Endothelium-dependent dilation

CKD-EPI : Chronic Kidney Disease Epidemiology Collaboration

MDRD : Modification of Diet in Renal Disease

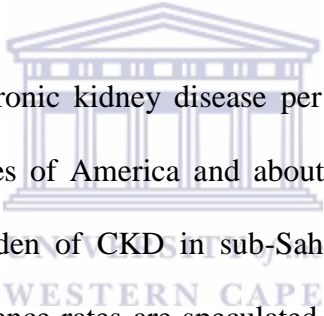
PPV : Positive predictive value

NPV : Negative predictive value



CHAPTER 1: INTRODUCTION

Chronic kidney disease (CKD) has been defined as abnormalities of kidney structure or function, present for greater than 3 months, with implications for health (Levin et al., 2013). CKD is an important chronic disease worldwide (Ruggenenti et al., 2001) and one major reason for this, is the increase in new cases of diabetes mellitus (Wild et al. 2004) and hypertension (Gupta, 2004). CKD has a big impact on the quality of life of people suffering from the disease (Levey et al., 2003) and is a major risk factor for the development of heart disease and stroke.



The number of new cases of chronic kidney disease per annum is about 337 per million population (pmp) in United States of America and about 95 pmp in the United Kingdom (Proctor et al., 2005). The burden of CKD in sub-Saharan Africa countries is sparsely documented, although their incidence rates are speculated to be 3-4 times higher than those reported in developed countries (Naicker et al. 2009). Studies on the prevalence of CKD in sub-Saharan Africa reported a CKD prevalence range of 2% to 30% (Stanifer et al., 2014; Adeniyi et al., 2017). A prevalence of 17.3% was reported in a suburban South African population of mixed ancestry (Matsha et al., 2013).

A diagnosis of CKD is usually established using serum creatinine levels to calculate the glomerular filtration rate (GFR) and the urinary albumin/creatinine ratio or urinary protein/creatinine ratio, to detect proteinuria. To assess the severity of CKD and ensure appropriate patient management, the Kidney Disease Improving Global Outcomes (KDIGO)

group recommended the classification system below (Andrassy, 2013). It is based on the cause, GFR, albuminuria levels and links the disease severity to risks of adverse outcomes.

Table 1: Definition and classification of chronic kidney disease. Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2012.

				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30mg/mmol
GFR categories (ml/min/1.73 m ²) Description and range	G1	Normal or high	≥90	1 if CKD	1	2
	G2	Mildly decreased	60–89	1 if CKD	1	2
	G3a	Mildly to moderately decreased	45–59	1	2	3
	G3b	Moderately to severely decreased	30–44	2	3	3
	G4	Severely decreased	15–29	3	3	4+
	G5	Kidney failure	<15	4+	4+	4+

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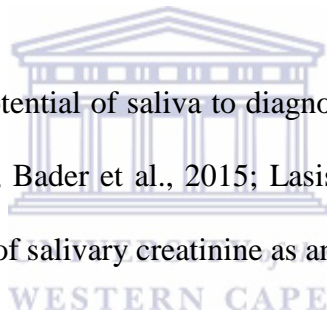
The procedure for collection of blood for serum analysis is invasive and needs a trained professional. A simple accurate diagnostic test that provides a reliable evaluation of disease status and stages would be beneficial to both patients and clinicians.

Saliva contains various analytes that can be used to detect systemic diseases or provide evidence of exposure to harmful substances. Due to the application of new scientific approaches such as bioinformatics, metabolomics, genomics and proteomics, the saliva research field is growing fast (Ahmadi et al., 2010). The advantage of saliva is that sample collection is non-invasive and can be performed by the patient, with little involvement from

the health care provider (Kaufman and Lamster 2002). It may be a cost-effective procedure for the screening of a large number of people (Lee et al., 2009). Using saliva will be particularly advantageous to patients suffering from clotting disorders and those with difficult venous access (Bayraktar et al., 2009; Kaufman and Lamster, 2002).

The potential use of saliva to detect various local diseases including oral, head and neck cancers (St John et al., 2004) as well as systemic diseases such as type 2 diabetes, lung, pancreatic, breast and ovarian cancers, has been established (Rao et al, 2009; Gao et al, 2009; Zhang et al., 2010; Lee et al., 2012). Further studies are needed to standardize the diagnostic use of saliva (Pfaffe et al. 2011; Gröschl, 2008).

Few studies have explored the potential of saliva to diagnose and monitor the progression of CKD (Venkatapathy et al., 2014; Bader et al., 2015; Lasisi et al., 2016). The present study investigated the diagnostic value of salivary creatinine as an alternative to serum creatinine.



CHAPTER 2: LITERATURE REVIEW

Chronic kidney disease is one of the major chronic diseases worldwide (Ruggenti et al., 2001) and one major reason is the daily global increase in new cases of diabetes-mellitus (Wild et al., 2004) and hypertension (Gupta, 2004). Chronic kidney disease is a problem of high public health concern because it affects quality of life (Levey et al., 2003). A recent systematic review reported global prevalence values of CKD stages 1 to 5 as being 13.4% and 10.6% in stages 3 to 5 (Hill et al., 2016). The burden of chronic kidney diseases in sub-Saharan Africa is sparsely documented and Naicker (2009) suggested that the incidence rates in sub-Saharan Africa may triple the number reported in developed countries. Prevalence values ranging from 6.1% to 17.3% have been reported in a mixed ancestry cohort in suburban Western Cape, South Africa (Adeniyi et al., 2017; Matsha et al., 2013).

Chronic kidney disease needs frequent serum analysis of venous blood for diagnosis, monitoring drug outcomes during management and to determine patient prognosis. The kidneys primarily excrete creatinine, a waste product of muscle metabolism. Its serum level is used to estimate the glomerular filtration rate. The procedure for collecting blood for serum analysis is an invasive one, causing both discomfort and anxiety to patients. Thus, a diagnostic test that provides a reliable evaluation of disease status and stages, with less risk, would be of value to both the patients and health care providers.

Saliva has numerous analytes to help detect various systemic diseases and determine disease severity. Saliva has the advantage over serum because the procedure for salivary collection is non-invasive, easy to do, economical and its collection requires little participation from the health care provider (Kaufman and Lamster, 2002). When required, a repeat sample can be easily accessed. Salivary samples can also be used for the screening large numbers of people

with less cost implications than haematological sampling (Lee et al., 2009). Saliva as a diagnostic medium could be valuable to patients with clotting disorders and those with compromised venous access (Bayraktar et al., 2009; Kaufman and Lamster, 2002).

Several studies have shown that saliva can be used to detect disease conditions such as pancreatic, lung and breast cancer, renal disease and type II diabetes (Malamud, 2011). However, for these findings to be generally accepted, further scientific studies for each disease is needed, to standardize the diagnostic value of saliva with other body fluids (Pfaffe et al., 2011; Gröschl, 2008).

For saliva to replace blood as a diagnostic and monitoring tool for patients with CKD, studies must be designed to determine the effectiveness of saliva as a substitute to blood in diagnosing chronic kidney disease at the various stages. A few studies (Venkatapathy et al., 2014; Lasisi et al., 2016; Bader et al., 2015) have explored the possibility but none have been established its diagnostic role of saliva for all stages of CKD, nor its role in monitoring disease progression from one stage to another.

Detection and Classification of CKD patients

The Kidney Disease Outcomes Quality Initiative (KDOQI) developed guidelines for the detection and evaluation of chronic kidney disease (Levin, et al., 2013; Andrassy, 2013). These guidelines (Levin et al. 2013) defined CKD and outlined treatment goals for each stage.

KDOQI recommended that CKD classification should be based on cause, GFR estimation (eGFR) and albuminuria categories. This recommendation is important because including

cause and severity in the classification, links to risks of adverse outcomes such as mortality and kidney status, which will guide management of CKD.

Causes of CKD

CKD occurs as a result of primary kidney disease or underlying systemic diseases secondarily affecting the kidney (Table 3). The guidelines recommend that all patients be assessed for risk factors associated with kidney disease, where after patients with identifiable risk factors receive further screening. The high-risk conditions for CKD include diabetes, hypertension, family history of CKD, recurrent urinary tract infections or systemic conditions affecting the kidneys (Andrassy, 2013).

Estimated Glomerular Filtration Rate (eGFR)

Glomerular filtration, the first step in urine formation, is the passive ultrafiltration of plasma across the glomerular capillaries into Bowman's space. Glomerular filtration rate (GFR) is an indication of kidney function. GFR cannot be measured directly, but assessed from clearance measurements or estimated from serum levels of endogenous filtration markers, such as creatinine or cystatin C (Snyder and Pendergraph, 2005). Creatinine clearance rate is the volume of blood plasma that is cleared of creatinine per unit time and is a useful measure for approximating the GFR. However, creatinine clearance overestimate GFR due to active secretion of creatinine in the proximal tubules of the kidneys. Glomerular filtration rate estimated from endogenous makers is known as estimated GFR (eGFR) and is used in clinical practice to diagnose and monitor CKD patients.

Current KDOQI guidelines (Levin, et al., 2013; Andrassy, 2013) for screening of CKD use serum creatinine and albumin. The latter is obtained from random urine samples.

The serum creatinine level is used to calculate an estimated glomerular filtration rate (eGFR). The two most commonly used equations used to calculate eGFR are Modification of Diet in Renal Disease (MDRD) Study equation and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Table 2). In general, eGFR estimating equations include age, sex, race, and body size as surrogates for creatinine generation by muscle. The CKD-EPI equation was developed in 2009 and uses the same four variables as the MDRD Study equation (Levey et al. 2009). MDRD and CKD-EPI both use the same variables, but differ in the values used in their calculation. The CKD-EPI equation is less biased than the MDRD study equation, especially at GFR 60 ml/min/1.73 m², a small improvement in precision and greater accuracy. (Gonwa et al., 2004). An ethnicity factor of 1.212, adopted for African American for the four variable MDRD study equation was found to overestimate mGFR in black South Africans (Hendrick et al., 2008). Hendrick et al. (2008) found that the four variable MDRD equation without the use of the ethnicity factor minimally overestimated mGFR and therefore suggested that the ethnicity factor of 1.212 should not be used MDRD equation in black South African.

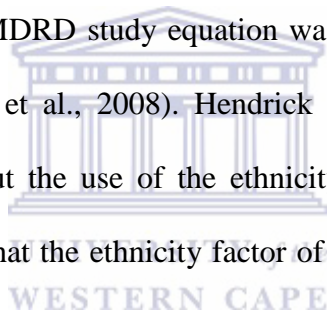


Table 2: Common equations used for the calculation of eGFR

MRDR equation	CKD-EPI equation
$eGFR = 175 \times (S_{Cr})^{-1.54} \times (age)^{-0.203} \times 0.742 \text{ [if female]} \times 1.212 \text{ [if Black]}$	$eGFR = 141 \times \min(S_{Cr}/k, 1)^{\alpha} \times \max(S_{Cr}/k, 1)^{-1.209} \times 0.993^{\beta} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if Black]}$
<p>Abbreviations / Units</p> <p>eGFR (estimated glomerular filtration rate) = mL/min/1.73</p> <p>S_{Cr} (standardized serum creatinine) = mg/dL</p> <p>age = years</p>	<p>Abbreviations / Units</p> <p>eGFR (estimated glomerular filtration rate) = mL/min/1.73</p> <p>S_{Cr} (standardized serum creatinine) = mg/dL</p> <p>$k = 0.7$ (females) or 0.9 (males)</p> <p>$\alpha = -0.329$ (females) or -0.411 (males)</p> <p>min = indicates the minimum of S_{Cr}/k or 1</p> <p>max = indicates the maximum of S_{Cr}/k or 1</p> <p>age = years</p>

Albuminuria

Albuminuria, which is the presence of protein albumin in urine, has also been used to classify CKD. An albumin:creatinine ratio < 30mg/g is considered normal, while albumin:creatinine ratios > 30mg/g in urine is considered diagnostic for CKD (Baumgarten and Gehr, 2011) (Table 1).

Table 3: Classification of CKD based on presence or absence of systemic disease and location within kidney of pathologic-anatomic findings

	Examples of systemic diseases affecting the kidney	Examples of primary kidney diseases (absence of systemic diseases affecting the kidney)
Glomerular diseases	Diabetes, systemic autoimmune diseases, systemic infections, drugs, neoplasia (including amyloidosis)	Diffuse, focal or crescentic proliferative GN; focal and segmental glomerulosclerosis, membranous nephropathy, minimal change disease
Tubulointerstitial diseases	Systemic infections, autoimmune, sarcoidosis, drugs, urate, environmental toxins (lead, aristocholic acid), neoplasia (myeloma)	Urinary-tract: infections, stones, obstruction
Vascular diseases	Atherosclerosis, hypertension, ischemia, cholesterol emboli, systemic vasculitis, thrombotic microangiopathy, systemic sclerosis	ANCA-associated renal limited vasculitis, fibromuscular dysplasia
Cystic and congenital diseases	Polycystic kidney disease, Alport syndrome, Fabry disease	Renal dysplasia, medullary cystic disease, podocytopathies

Abbreviations: ANCA, antineutrophil cytoplasmic antibody; CKD, chronic kidney disease; GN, glomerulonephritis

Genetic diseases are not considered separately because some diseases in each category are now recognized as having genetic determinants.

*Note that there are many different ways in which to classify CKD. This method of separating systemic diseases and primary kidney diseases is only one, proposed by the Work Group, to aid in the conceptual approach.

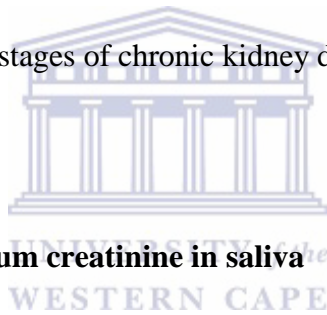
Saliva as a diagnostic tool for CKD

The use of human saliva as a diagnostic and prognostic indicator of CKD, has only recently received attention (Venkatapathy et al. 2014; Lasisi et al., 2016)

Studies (Venkatapathy et al. 2014; Lasisi et al., 2016; Bader et al., 2015; Xia et al., 2012) showed conflicting evidence when comparing salivary and serum creatinine levels of CKD patients and healthy people. Venkatapathy et al. (2014) only found a positive correlation ($r=0.731$) between salivary and serum creatinine levels in patients with chronic kidney disease and a negative correlation in healthy control ($r = -0.326$). However, Xia et al. (2012) found a positive correlation in both their cases ($r = 0.971$) and healthy control ($r = 0.932$). The positive correlation found in CKD patients may be due to the concentration gradient created by the increased serum creatinine levels in the CKD patients' saliva (Nakahari et al., 1996) and alteration in the permeability of the salivary gland cells, allowing creatinine to diffuse easily through the tight intercellular junction of the salivary gland. Previous studies reported a negative correlation in healthy controls (Venkatapathy et al., 2014; Lasisi et al.,

2016; Bader et al., 2015). The high molecular weight and low lipid solubility of creatinine make it difficult to cross the tight intercellular junction of the salivary gland in health (Lamb et al., 2006). However, Xia et al. (2012) found a positive correlation between salivary and serum creatinine levels in both their chronic kidney disease patients and healthy controls. Seethalakshmi et al. (2014) reported a significant correlation between salivary and serum creatinine levels in late stages of CKD. Bader et al (2015) found that the salivary creatinine concentrations of patients in the middle and late stages of chronic kidney disease were higher than those of healthy people and early-stage CKD patients.

Though studies have been done to correlate the salivary and serum creatinine levels (Venkatapathy et al., 2014; Lasisi et al., 2016; Bader et al., 2015), no known study has evaluated its diagnostic use in all stages of chronic kidney disease.



Mechanism of expression of serum creatinine in saliva

Creatinine is a metabolic waste product, primarily excreted by the kidneys. Virtually all the creatinine filtered by the glomerulus is excreted without reabsorption in the renal tubules and its level in the blood is used as an index of renal function (Guyton and Hall, 2006).

However, as renal function decreases, GFR decreases leading to an increase tubular secretion of creatinine. Increasing tubular creatinine secretion is usually considered to be due to greater interstitial creatinine concentration to which tubular cells are exposed as CKD progresses (Rose et al., 2001). Salivary glands are surrounded by many capillaries and are highly permeable, facilitating the free exchange of blood-based molecules into the salivary gland acini. The transport of molecules into salivary gland occurs via either transcellular (passive and active transport) or paracellular (extra cellular ultrafiltration) diffusion mechanisms

(Yoshizawa et al., 2013). Diffusion of molecules is considered to be the common route for movement of molecules from blood to saliva and the ability of molecules to diffuse depends on the size and the electric charge of the molecules (Nagarathinam et al., 2017).

Salivary creatinine is about 10-15% of the serum levels (Nagarathinam et al., 2017). The normal range of serum creatinine in females is 45-84 $\mu\text{mol/L}$ while the normal range in males is 59-104 $\mu\text{mol/L}$ (Mazzachi et al., 2000)) and salivary creatinine is 4.42 $\mu\text{mol/L}$ - 17.68 $\mu\text{mol/L}$ (Venkatapathy et al., 2014). The expression of serum creatinine in saliva is due to the ultrafiltration of creatinine into saliva. Ultra-filtration is an extra cellular mechanism for transport of blood substances into saliva by filtration through the spaces between the acinus and the ductal cells. Only very small sized molecules can be transported through ultra-filtration and filtration may also occur through the gap junctions between cells of secretory units.

When a molecule's concentration increases in blood, a corresponding increase in diffusion of these molecules occurs into the saliva, with an associated increased concentration of the salivary markers (Yoshizawa et al., 2013). Similarly, when serum creatinine levels are increased, so too is their concentration in saliva. The increase in salivary creatinine due to concentration gradient diffusion makes saliva a potential tool for measuring renal function.

Gap in literature

Although the previous studies suggest that saliva may be useful as a substitute for serum creatinine, most of these studies only focused on the level of salivary creatinine in the end stages of chronic kidney disease (Venkatapathy et al. 2014). None of these studies assessed the diagnostic value of salivary creatinine at each stage of chronic kidney disease in order to

provide evidence for substituting saliva for serum to diagnose and monitor chronic kidney disease. Venkatapathy et al. (2014) concluded that more scientific studies are needed to investigate the diagnostic value of salivary creatinine at all stages of chronic kidney disease.

Methods of saliva collection

Sample collection can be performed by either unstimulated (spitting directly into a tube) or stimulated methods. The former is preferred because most analytes (such as proteins) can be quantified without any changes in their usual quantification (Hansen et al. 2003; Turpeinen et al. 2009). However, the volume of saliva collected by this method is low (Burtis and Bruns 2014). Stimulated saliva is obtained by stimulating the salivary glands using substances such as citric acid or by mastication (Kaufman and Lamster 2002). Saliva collection from individual glands can be done by cannulation of the salivary gland duct or by connecting specific collecting devices to the glandular duct openings into the mouth. These procedures are slow, complex, uncomfortable, technique sensitive and not the preferred method of collection (Chianeh and Prabhu 2014).

The spitting method was the preferred one in most studies (Venkatapathy et al. 2014; Bader et al., 2015; Lasisi et al., 2016). Timing of the sample collection is also important and influences the specific constituents available for analysis. Venkatapathy et al. (2014) in their study, regarded 9am to 11am as optimum collection time. Smoking, food or liquid ingestion (except water) should be avoided an hour before collection of unstimulated saliva because it can affect its constituents. Rinsing of the mouth with water before collection is important, as it helps to remove any residues that may interfere with the analyses (Nunes et al. 2011)

After sample collection, the saliva should be preserved at around 4 °C, if not processed immediately and can be kept for a maximum of 6 hours. Sample storage at –20°C to –80 °C is recommended if the sample needs to be preserved for a longer duration, to prevent further protein degradation (de Jong et al., 2011).

Analysis of Saliva

The different components of saliva can be used to evaluate, diagnose and monitor most common systemic conditions.

Jaffe's reaction is the most widely used for creatinine measurement (Krishnegowda et al. 2013). However, this method is deficient in sensitivity, reproducibility, and precision in the presence of interfering substances, which could be endogenous or exogenous (Weber and Zanten, 1991). These include Bilirubin, creatine, dopamine, ascorbic acid and cephalosporines. Jaffe's method has since been modified to eliminate some of the drawbacks, such as the specific adsorption of creatinine, removal of interfering compounds, dialysis, varying the pH, and kinetic measurements. None of these modifications have successfully removed the interferants present in biological samples.

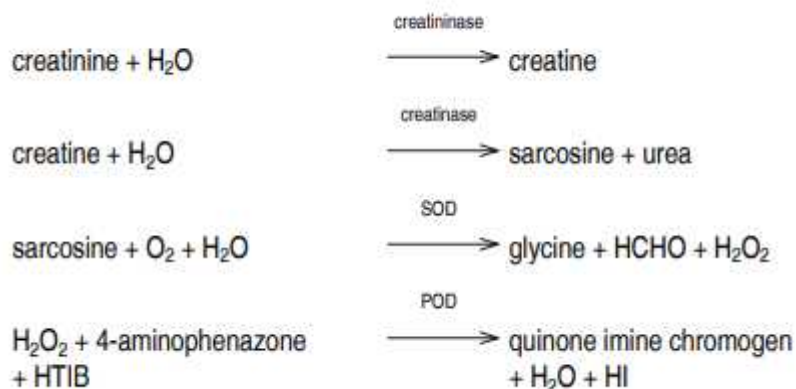
Jaffe's method has more recently been modified using some multi-enzyme systems to improve its specificity for detecting creatinine in biological samples. The use of multi-enzyme systems however, requires caution, as there is an increased risk of interference between the enzymes (Krishnegowda et al. 2013).

Table 4: Positive and negative interference of Jaffe's reaction

Positive Interference	Negative interference
Acetone	Bilirubin
Cefazolin	Lipids
Cefoxitin	Acetoacetic acid
Glucose	Phenacemide

Other methods available for creatinine analysis include the use of reagents such as 3,5-dinitrobenzoic acid, a mixture of dimethyl sulfoxide, methanol and tetramethyl ammonium hydroxide, 3,5-dinitrobenzoyl chloride, 1,4-naphthoquinone-2-sulfonate, Sakaguchi's color reaction of creatinine with o-nitrobenzaldehyde and mass fragmentography (Krishnegowda et al. 2013; Peake and Whiting, 2006). Estimation of creatinine by direct spectrophotometric procedure has recently been attempted (Krishnegowda et al. 2013).

The only alternative methods that have been widely adopted for routine clinical laboratory use are enzymatic creatinine methods (Myers et al., 2006). This enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. Catalyzed by peroxidase the liberated hydrogen peroxide reacts with 4-aminophenazone and HTIB to form a quinone imine chromogen.



Creatinine measurement has recently be Standardized by NKDEP's Laboratory Working Group in conjunction with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the European Communities Confederation of Clinical Chemistry to reduce inter-laboratory variation in calibration of creatinine assay (Piéroni et al., 2017). The National Institute for Standards and Technology (NIST) has released a standard reference material useful in establishing calibrations for routine creatinine measurement procedures, with demonstrated commutability with native clinical specimens in routine methods. The standard reference materials were value-assigned with the gas chromatography (GC) -isotope dilution mass spectrometry (IDMS) and liquid chromatography (LC)-IDMS reference measurement procedures (Dodder et al., 2007).

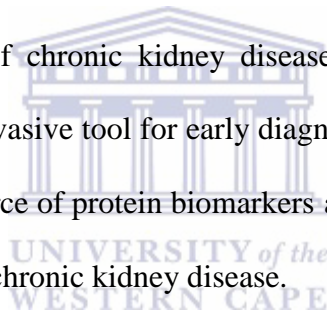
Since the standardization of creatinine, manufacturers have been asked to standardize their creatinine assays to an IDMS reference measurement procedure. This has helped to ensure that the same sample give the same result irrespective of the laboratory and the method used (Jaffe or enzymatic) (Carobene et al., 1997).

Clinical Benefit of this study

Saliva has the advantage over serum because its collection is non-invasive, economical and can be done with minimal assistance from the health care provider (Kaufman and Lamster, 2002). When needed, a repeat salivary sample can be collected, with less patient discomfort. Saliva as a diagnostic medium would be valuable to patients suffering from clotting disorders and for those with compromised venous access (Bayraktar et al., 2009; Kaufman and Lamster 2002). Thus, a simple diagnostic test that provides a reliable evaluation of disease status at various stages would benefit both patients and health care providers.

Conclusion

The incidence and prevalence of chronic kidney disease is increasing globally, thus the development of a reliable, less invasive tool for early diagnosis and patient monitoring would be beneficial. Saliva is a rich source of protein biomarkers and has potential as a point of care method to diagnose and monitor chronic kidney disease.



CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

This chapter discusses the aims and objectives of the study as well as its research design and methodology. Tygerberg hospital is a tertiary referral facility. The clinical management of patients with chronic kidney disease is based in the Renal Unit at Tygerberg Hospital, which has a well-equipped ward for in-patient management and two weekly morning outpatient clinics. Serum creatinine measurement is the internationally accepted method to diagnose and monitor chronic kidney disease. The present study investigated the use of saliva as an alternative for the diagnosis of chronic kidney disease.

Patients recruited for this study were classified according to the guidelines of KDOQI. The CKD classification was based on GFR, which is calculated using CKD-EPI equations estimated by serum creatinine level. Patients were grouped into five stages using the CKD-EPI equations for estimating GFR. Variables included in the CKD-EPI equation for estimating GFR are log serum creatinine, sex, race and age on the natural scale.

Table 5: The CKD-EPI equations for estimating GFR on the natural scale

Race and Sex	Serum Creatinine $\mu\text{mol/L}$ (mg/dL)	Equation
Black		
Female	≤ 62 (≤ 0.7)	$\text{GFR} = 166 \times (\text{Scr}/0.7)^{-0.329} \times (0.993)^{\text{Age}}$
	> 62 (> 0.7)	$\text{GFR} = 166 \times (\text{Scr}/0.7)^{-1.209} \times (0.993)^{\text{Age}}$
Male	≤ 80 (≤ 0.9)	$\text{GFR} = 163 \times (\text{Scr}/0.9)^{-0.411} \times (0.993)^{\text{Age}}$
	> 80 (> 0.9)	$\text{GFR} = 163 \times (\text{Scr}/0.9)^{-1.209} \times (0.993)^{\text{Age}}$
White or other		
Female	≤ 62 (≤ 0.7)	$\text{GFR} = 144 \times (\text{Scr}/0.7)^{-0.329} \times (0.993)^{\text{Age}}$
	> 62 (> 0.7)	$\text{GFR} = 144 \times (\text{Scr}/0.7)^{-1.209} \times (0.993)^{\text{Age}}$
Male	≤ 80 (≤ 0.9)	$\text{GFR} = 141 \times (\text{Scr}/0.9)^{-0.411} \times (0.993)^{\text{Age}}$
	> 80 (> 0.9)	$\text{GFR} = 141 \times (\text{Scr}/0.9)^{-1.209} \times (0.993)^{\text{Age}}$

CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; GFR = glomerular filtration rate.



3.2 Aims and Objectives

The aim of the study was to evaluate the role of saliva as a safe and non-invasive alternative to serum, for creatinine estimation, in patients with chronic kidney disease.

The objectives of this study were:

- To determine the average diagnostic value of salivary creatinine at all stages of chronic kidney disease.
- To correlate the levels of salivary creatinine with serum creatinine at each stage of chronic kidney disease.

3.3 Research design

In this cross sectional study, data (saliva and serum samples) was collected from patients in the various stages of chronic kidney disease at a single visit.

3.4 Sampling criteria

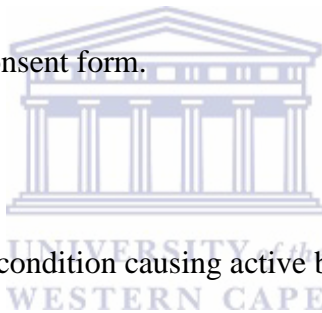
A convenient sampling method was employed to choose participants who met the selection criteria. Consecutive patients, who presented to the Renal Unit outpatient clinic on Wednesday and Friday mornings, were recruited until the required sample size was obtained. The CKD category into which patients' were enrolled was based on their CKD stage as determined by the previous laboratory measurements of serum creatinine not older than three months.

3.4.1 Inclusion criteria

- Patients 18 years and above with a confirmed diagnosis of CKD.
- Patients who signed the consent form.

3.4.2 Exclusion criteria

Participants with any oral condition causing active bleeding into the oral cavity.



3.5 Sample size

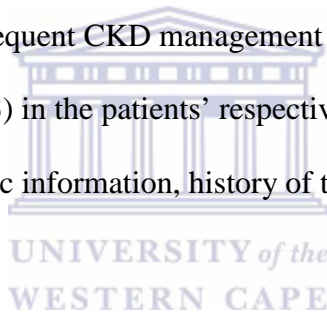
The minimum sample size required was calculated using the estimated means of salivary creatinine in a known test and control (Tomás et al., 2008), with the standard normal values set at 0.05 and a power of 90 %. The calculated minimum sample size for each stage was rounded up to 40 participants. A sample size of 50 patients was included for stages one, two and three because these three stages formed the largest group of CKD patients and are the stages in which CKD is usually diagnosed. Forty patients each were included for stages 4 and 5 and a total of 230 patients were included in the study.

3.6 Data collection

A structured questionnaire was used for data collection. Saliva collection was done using graduated sterile bottles, while blood samples were collected using serum separation tubes (SST) sample bottles specific for analyzing serum creatinine and other blood biochemistry tests.

3.6.1 Study information Sheet and Questionnaire

A study information sheet (Appendix 5) was given to all the patients and they were allowed to ask questions about the study. Consenting patients signed the consent form to participate in the study (Appendix 4) and were advised that they could withdraw from the study at any stage without affecting their subsequent CKD management at the Renal Unit. Questionnaires were completed (Appendix 1, 2, 3) in the patients' respective language. The data captured included patient sociodemographic information, history of tobacco and alcohol use, medical history and medication used.



3.6.2 Sample collection

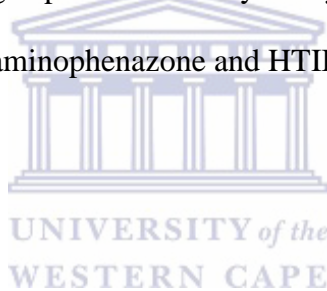
The study participants were recruited in the morning before the commencement of the clinic and were instructed to refrain from eating and drinking at least 90 minutes before collection. They were asked to rinse their mouths with water prior to the sample collection, to void the mouth of contaminants.

The saliva collection procedure was done once the nephrologist had seen the patient and was only obtained from those patients for whom serum creatinine sampling was indicated. The patient was given a graduated sterile tube and instructed to sit in a comfortable position with their eyes open, head tilted slightly forward and to avoid swallowing or making oral movements during collection. They were asked to pool the saliva in the floor of the mouth

and collect it in the graduated tube every 60 seconds or when they experienced an urge to swallow. This process was repeated until 2 mL of whole saliva was obtained. The saliva collection was carried out between 9:00 am and 12:30 pm, to minimize the effect of diurnal variation. The patients' went immediately thereafter to the blood room for venous blood collection for serum analysis.

3.6.3 Sample analysis

Serum and saliva samples collected were processed and analyzed by enzymatic method for the determination of serum and salivary creatinine level. Enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. Catalyzed by peroxidase the liberated hydrogen peroxide reacts with 4-aminophenazone and HTIBa to form a quinone imine chromogen



3.7 Pilot Study

A pilot study was carried out on 5 patients in each of the five stages at the commencement of the study. The purpose of this process was to test the feasibility of the questionnaire and the adequacy of the data-capturing sheet. This session also provided an estimate of the time for salivary sample collection from the patient. The questionnaire was restructured where necessary.

3.8 Ethical considerations

Approval to conduct the study was granted by the University of the Western Cape Biomedical Research Ethics Committee (approval number: BM/16/5/4, Appendix 6). Permission to carry out the research was also obtained from the Tygerberg Hospital Research Committee (Appendix 7). Patients were made aware of the study and given all the relevant

information both verbally and in writing. Participation in the study was entirely voluntary and participants had the right to withdraw at any stage.

Patient anonymity was ensured by assigning study numbers, which were captured on the data capture sheet and sample tubes. The data capture sheets for each patient enrolled in this study were kept in a locked drawer.

3.9 Data analysis

The data obtained was entered into an MS Excel sheet and data analysis was done using SPSS v17.0. Pearson's correlation coefficient (r) is a measure of the strength of the association between the two variables and was used to test the correlation between serum and salivary creatinine levels. Linear regression equations were derived to estimate the plasma level of creatinine and urea from the salivary levels. Receiver Operating Characteristic (ROC) analysis was used to evaluate the diagnostic potential of salivary creatinine compared to blood and to determine if salivary creatinine could distinguish patients with CKD from healthy individuals. The ROC curve graphically displays the trade-off between sensitivity and specificity and chooses the best cut-offs for clinical use. The overall performance was assessed by the Total area under the curve and the cut-off values were determined based on the best trade-off between the sensitivity and specificity.

Chapter 4: Results

This chapter shows the results generated from this present study. Table 6 shows the frequency distribution of patients with CKD stages 1-5 by gender. Table 7 shows the range of patients' serum and salivary creatinine with the mean values. The Pearson correlation analysis is presented in Table 8, while Table 9 shows the linear regression analysis of serum and salivary creatinine for CKD patients in stages 1 to 5. The results from the Receiver Operator Characteristics (ROC) curve analysis of serum and salivary creatinine levels are presented in Tables 10 and 11.



Frequency distribution

The study population comprised a total of 230 CKD patients, of which 50 patients were in stage 1, 50 patients in stage 2, 50 patients in stage 3, 40 patients in stage 4 and 40 patients in stage 5.

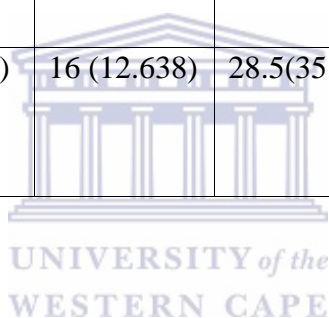
Table 6: The frequency distribution of patients with CKD stages 1-5 by gender.

Gender	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Total
Male	16	17	23	19	14	89 (38.7%)
Female	34	33	27	21	26	141 (61.3%)
	50	50	50	40	40	230 (100%)

Serum creatinine values (Table 7) for all the patients ranged from 46 μ mol/L to 1581 μ mol/L with a median of 134 μ mol/L (SD 229.10). Salivary creatinine values ranged from 3 μ mol/L to 400 μ mol/L with a median of 11 μ mol/L (SD 50.99).

Table 7: Serum and salivary creatinine values for patients in CKD stages 1-5.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Combined
Serum creatinine range (μ mol/L)	46 -93	65- 133	102-232	161- 462	312- 1581	46-1581
Median serum creatinine (μ mol/L)	66 (14.549)	91 (14.759)	149 (32.167)	276 (50.929)	518 (284.64)	134 (229.10)
Salivary creatinine range (μ mol/L)	3 -19	3- 18	4-63	5- 222	72 – 400	3 -400
Median salivary creatinine (μ mol/L)	6.2 (5.103)	9 (5.153)	16 (12.638)	28.5(35.72)	67.5 (86.50)	11 (50.99)



Spearman correlation analysis (Table 8) was done to determine the association between serum and salivary creatinine and showed a positive correlation for all CKD stages.

Table 8: Correlation analysis for serum and salivary combined using Spearman correlation.

	Combined	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
R	0.82	0.16	0.31	0.38	0.42	0.55
P	0.00**	0.25	0.03*	0.005**	0.007**	0.00**

Correlation is strong at (r=1.0 to 0.5), moderate at (r=0.3 to 0.5), weak at (r=0.1 to 0.3), no correlation at (r=0.1)

Figure 1: Correlation between serum and salivary creatinine levels for all CKD patients combined.

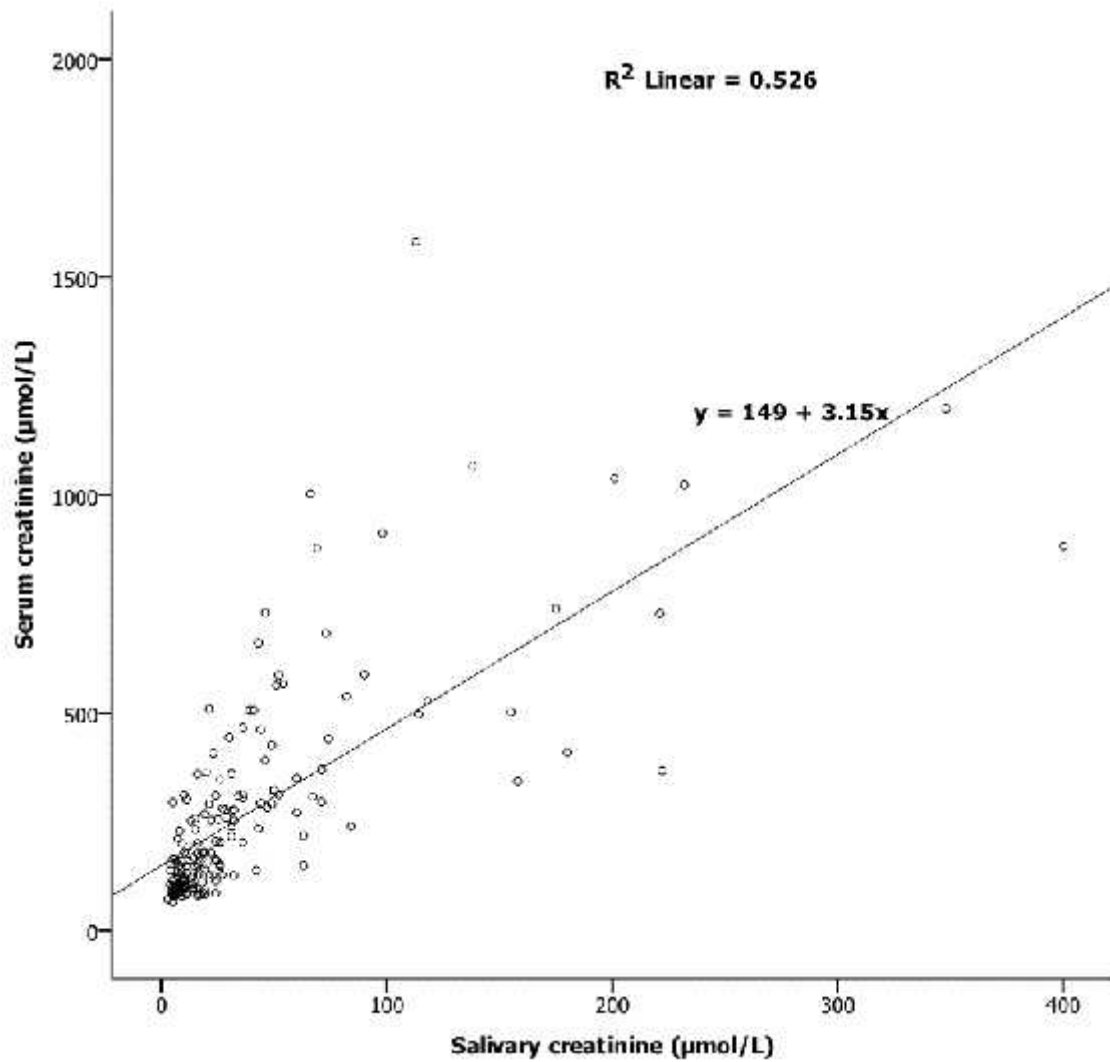


Figure 2: Correlation between serum and salivary creatinine levels for patients with CKD stage 1

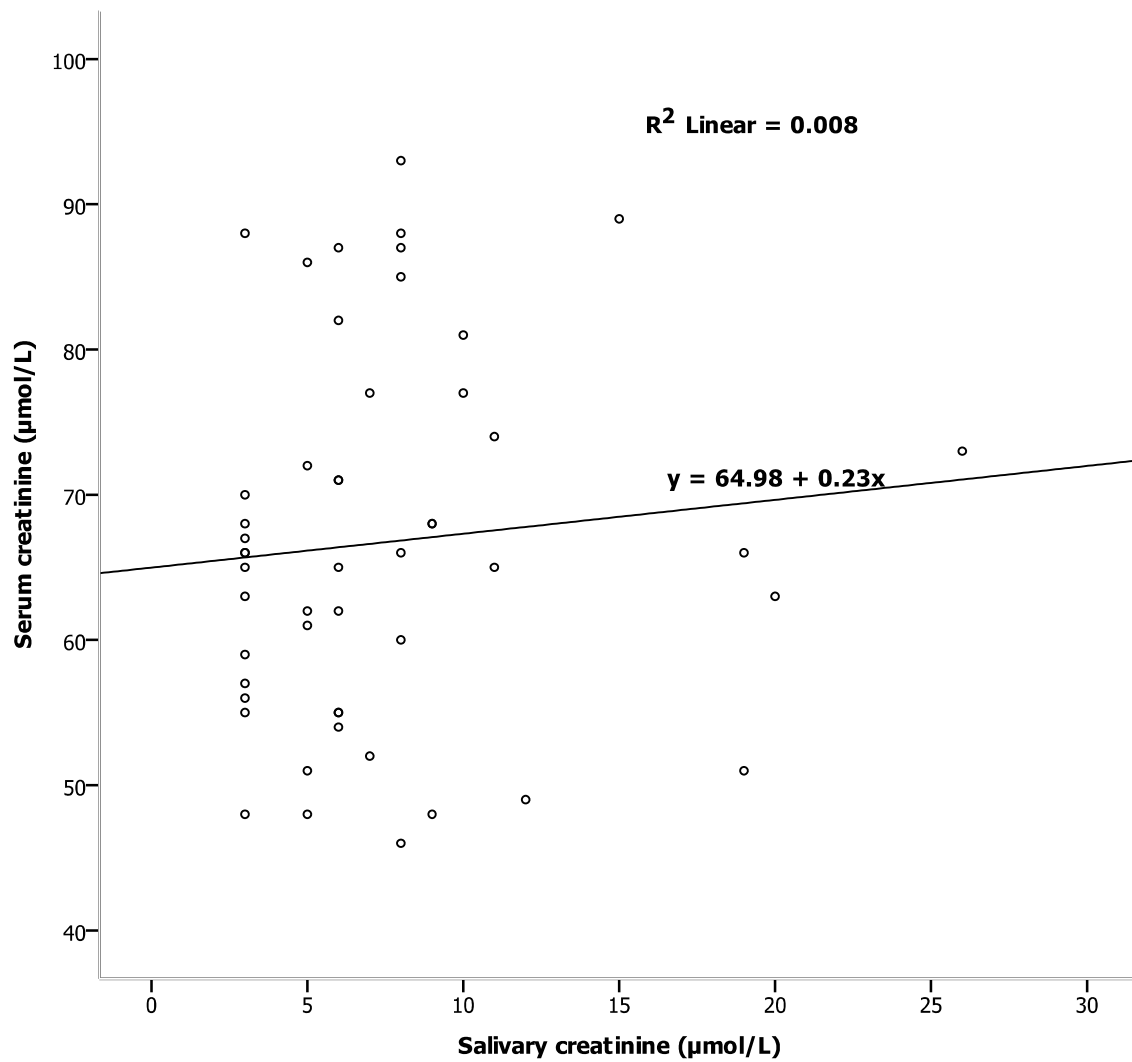


Figure 3: Correlation between serum and salivary creatinine level for patients with stage 2 CKD.

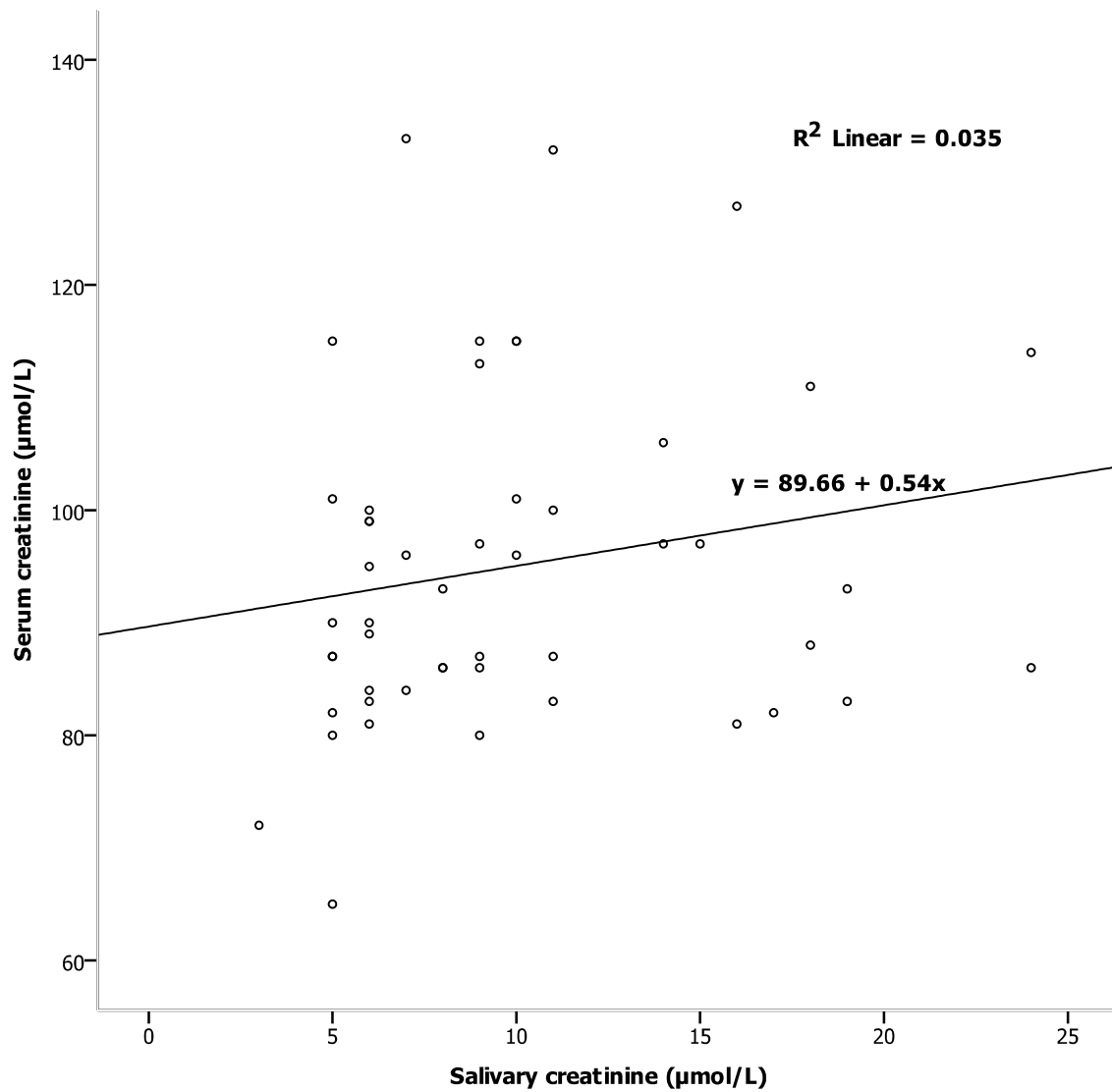


Figure 4: Correlation between serum and salivary creatinine levels for patients with stage 3 CKD.

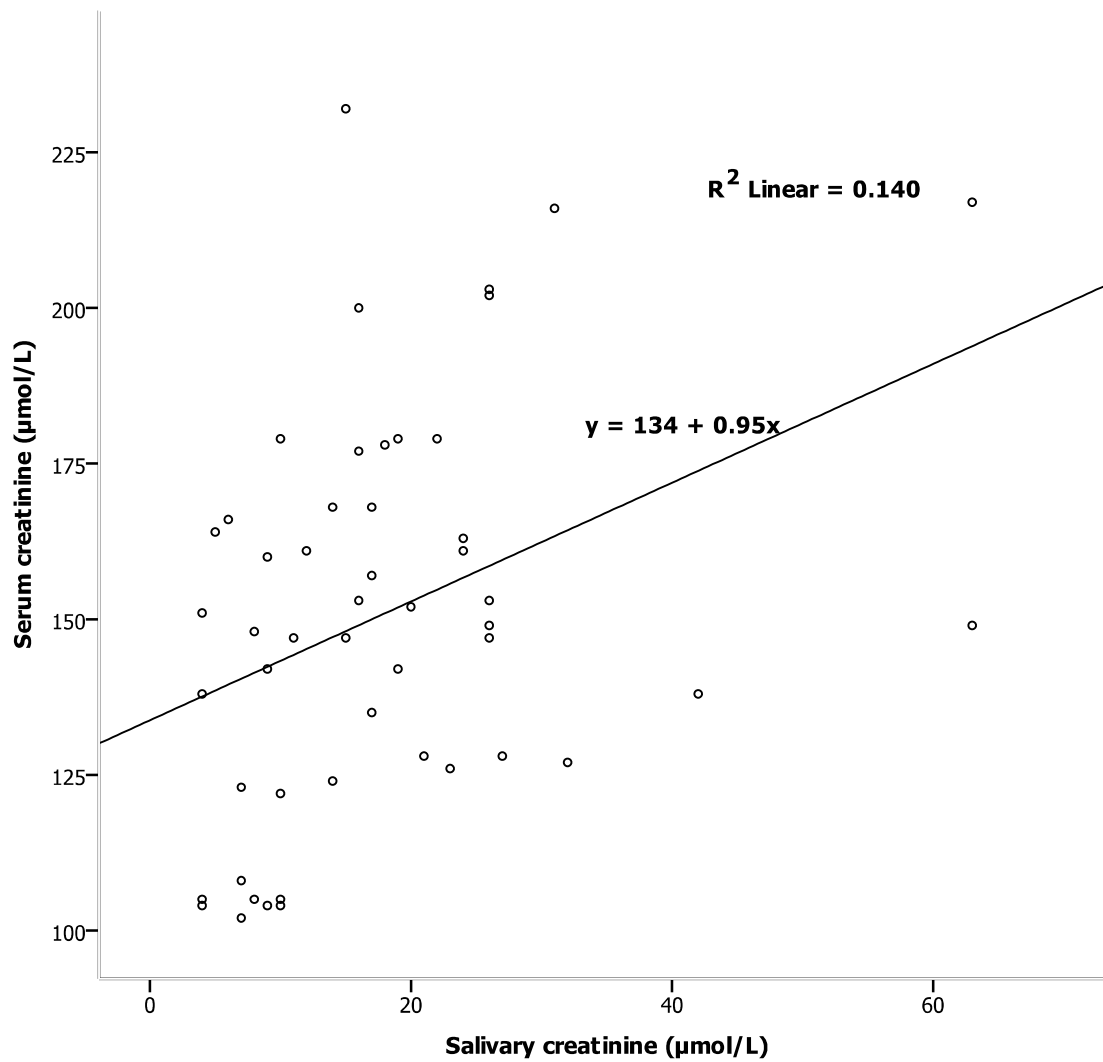


Figure 5: Correlation between serum and salivary creatinine levels for patients with stage 4 CKD.

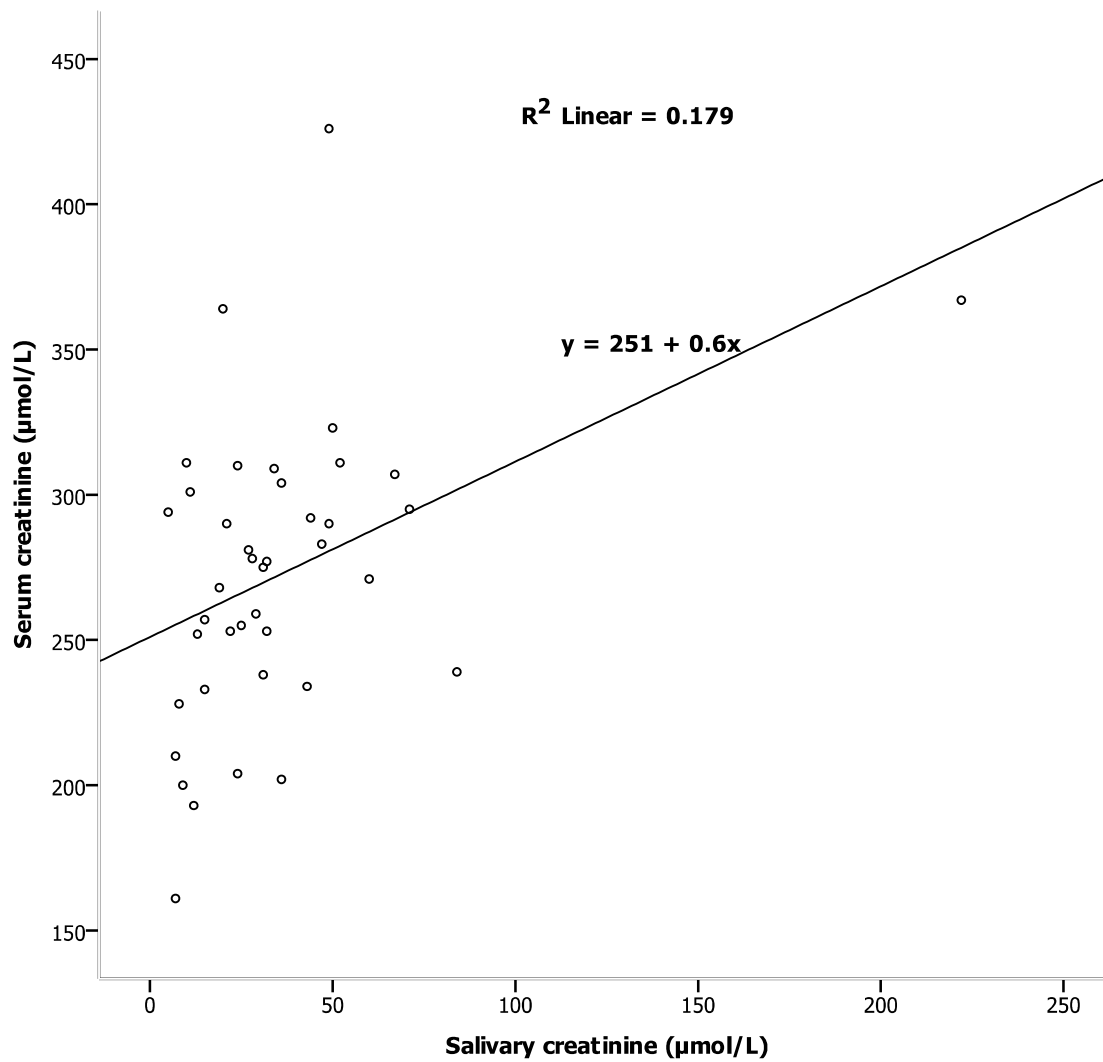


Figure 6: Correlation between serum and salivary creatinine levels for patients with stage 5 CKD.

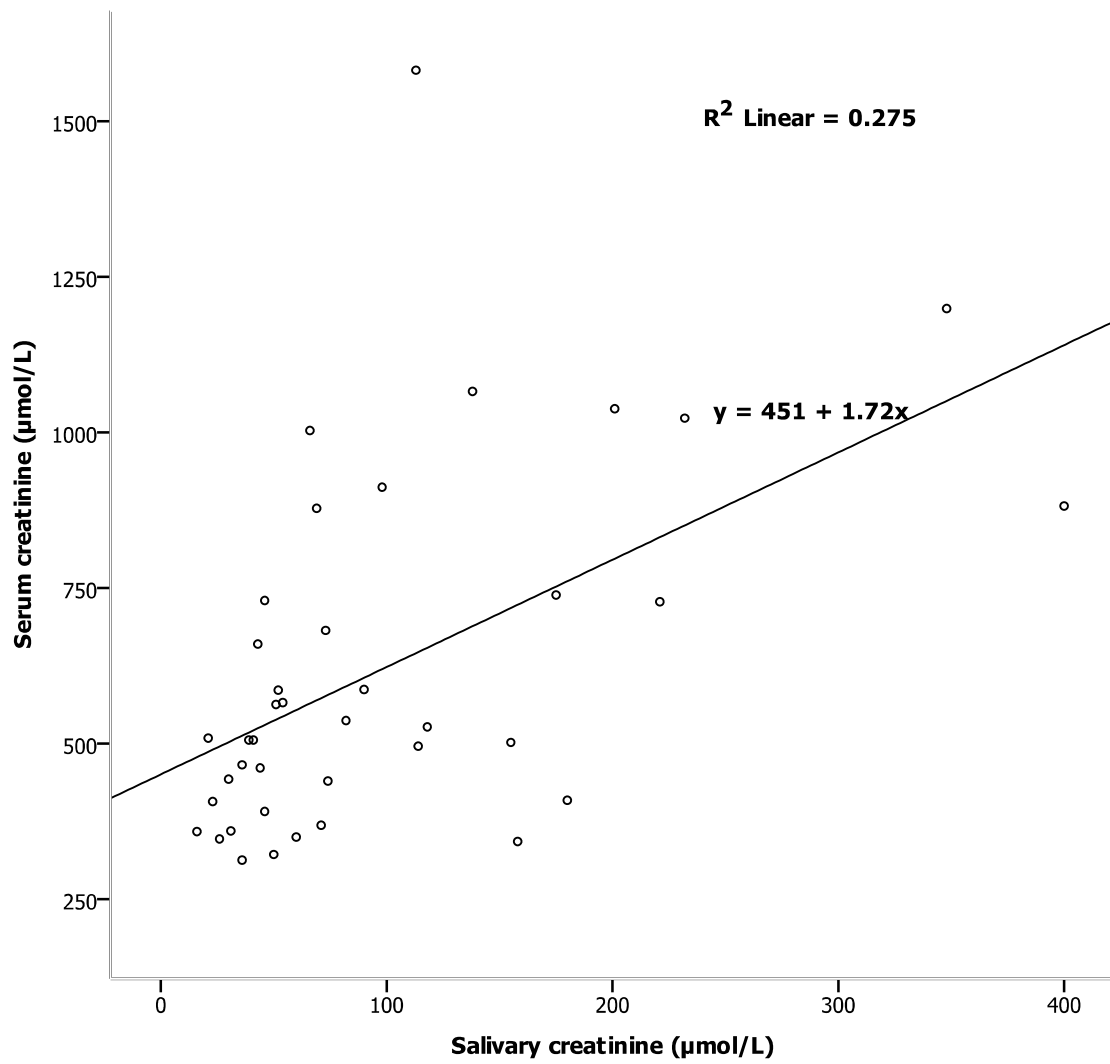


Table 9 shows linear regression analysis of serum and salivary creatinine for CKD patients. Linear regression was significant in all stages except for stage 1.

Table 9: Linear regression analysis of serum and salivary creatinine for CKD patients in stages 1 to 5.

Stage	p-value	Linear regression equation	R ²	R ² (%)	R
Stage 1	0.465	$Y = 64.9 + (0.23) \times (\text{Salivary Cr})$	0.008	0.80%	0.894
Stage 2	0.030	$Y = 89.7 + (0.54) \times (\text{Salivary Cr})$	0.035	3.50%	0.187
Stage 3	0.008	$Y = 134 + (0.95) \times (\text{Salivary Cr})$	0.140	14.0%	0.374
Stage 4	0.007	$Y = 251 + (0.6) \times (\text{Salivary Cr})$	0.179	17.9%	0.423
Stage 5	0.001	$Y = 451 + (1.72) \times (\text{Salivary Cr})$	0.275	27.5%	0.524

Dependent Variable: Serum Creatinine



ROC curve

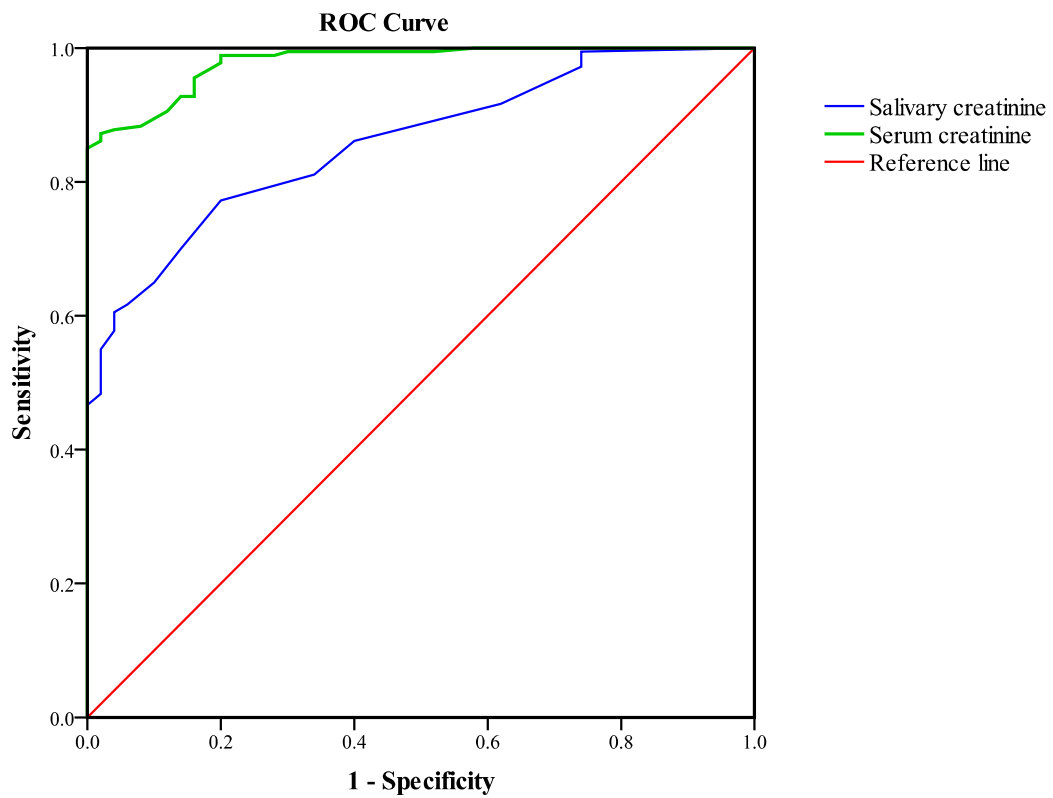


Figure 7: Receiver Operator Characteristics (ROC) curve of serum and salivary creatinine levels.

Table 10 shows the area under the curve of ROC curve. The area under the curve for salivary creatinine is considered good by interpretation considering the value 0.839.

Table 10: Interpretation of area under the curve ROC curve.

Test result variables	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Serum creatinine (µmol/L)	0.979	0.008	< 0.01	0.964	0.994
Salivary creatinine (µmol/L)	0.839	0.028	< 0.001	0.784	0.894

AUC values- Excellent (0.90-1); Good (0.80-0.90); Fair (0.70-0.80); Poor (0.60-0.70); Fail (<0.60)

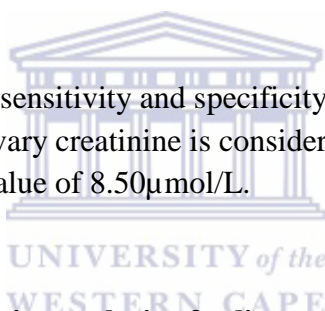


Table 11 shows cut off values for sensitivity and specificity of salivary creatinine to diagnose CKD with GFR < 60ml/min. Salivary creatinine is considered to have the best specificity and sensitivity at salivary creatinine value of 8.50µmol/L.

Table 11: Sensitivity and specificity analysis of salivary creatinine for different cut-off values considering serum creatinine as the gold standard.

Salivary creatinine (µmol/L)	Sensitivity (%)	Specificity (%)
3.50	99.4	26.0
4.50	97.2	26.0
6.50	86.1	56.0
8.50	78.3	74.0
11.50	64.4	88.0
14.5	60.6	90.0
15.5	57.8	92.0

Chapter 5: Discussion

The present study investigated the role of salivary creatinine as a diagnostic tool for all five stages of chronic kidney disease (CKD stages 1-5). Previous studies have only evaluated its potential in patients with CKD stages 4 and 5 (Venkatapathy et al., 2014; Lasisi et al., 2016).

A total of 230 patients with chronic kidney disease were recruited, with 50 patients each in stages one to three and 40 patients in stages four and five. The participants were stratified this way as stages 1 to 3 have the highest prevalence in the general population, while stages 4 and 5 have the least (Moorman et al., 2007). The age range of patients was 18 - 82 years. The mean age was 39.5 years (SD 13.05) and was comparable to other studies by Lasisi et al., 2016 and Venkatapathy et al., 2014, who reported a mean age of 39.5 and 47.5 respectively

Studies (Coresh et al., 2007; Prakash et al., 2009) have found CKD to be more prevalent in persons aged 60 years and older. This higher prevalence in older patients may reflect the different risk factors for CKD, such as diabetes and hypertension, which are more common in older individuals. In addition, high rates of CKD in older patients may also occur due to an age-associated decline in kidney function (Prakash and O'Hare, 2009).

There were more females (61.3%) than males in the study population. This is in accordance with others studies, which found a higher prevalence of CKD among females in United States (Coresh et al., 2003; Coresh et al., 2005) and Western Cape, South Africa (Matsha et al., 2013). This increased female prevalence may be due to fact that diabetes mellitus (DM), the most common cause of chronic kidney disease is more prevalent amongst females (Hilawe et al., 2013; Mbanya et al., 1997).

Serum creatinine median value for all the patients was 134 μ mol/L (range=46 μ mol/L-1581 μ mol/L) while salivary creatinine median value was 11 μ mol/L (range= 3 μ mol/L-400 μ mol/L). The range of serum and salivary creatinine for patients in stage 1 fell within the normal range. This result is in line with the knowledge about CKD patients and is reflected in the classification of CKD, in which stage 1 patients usually have a normal estimated GFR (eGFR) and their creatinine values fall within normal range of serum and salivary creatinine.

In this study, we observed an increasing level of serum and salivary creatinine from CKD stages 2 to 5. CKD stage 5 has the highest values for both serum and salivary creatinine. This finding is in accordance with previous studies in which elevated levels of serum and salivary creatinine was found in end stage renal disease (Venkatapathy et al., 2014; Lasisi et al., 2016). The increase in serum creatinine, which progressively increased from stage 2 to 5, is due to the fall in GFR leading to reduced creatinine excretion via the kidneys. The fall in GFR has been attributed to a decrease in either glomerular capillary surface area or hydraulic permeability (Perrone et al., 1992). An increased serum creatinine creates a concentration gradient, which increases the diffusion of creatinine from serum to saliva, thereby increasing the salivary creatinine concentration.

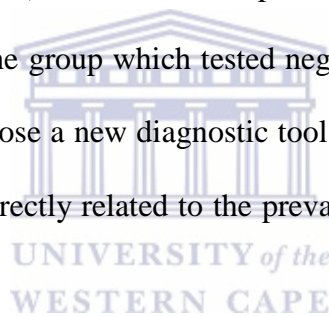
A Spearman's correlation analysis was done to determine the association between salivary and serum creatinine in CKD stages 1-5 and to determine their proportionality. A strong positive correlation ($r = 0.82$) between serum and salivary creatinine was found in the combined analysis of all CKD stages (Figure 1). Lloyd et al. (1996) reported a similar pattern in patients with CKD stages 4 and 5. The positive correlation observed in the present study may be due to the progressive alteration in salivary gland cell permeability as well as impaired endothelium-dependent dilation (EDD) associated with the vascular system of patients with chronic kidney diseases (Lakatta and Levy, 2003). This leads to an increased diffusion of creatinine from serum to saliva, (Nakahari et al., 1996).

Correlation studies between serum and salivary creatinine in healthy patients have reported conflicting results. Bader et al. (2015) found a positive correlation between the serum and salivary creatinine in patients without chronic kidney disease, while other studies reported a negative correlation (Venkatapathy et al., 2014; Lasisi et al., 2016). The different reports from the studies may be due to other local and systemic factors such as diabetes mellitus and hypertension and salivary gland diseases, which can alter the diffusion of creatinine from serum into the salivary glands (Ladgotraet al., 2016; Briet et al., 2011). Other factors that could be responsible for the difference in correlation are the medications, which the patients were using at the time the samples were collected, which were not considered in the present nor previous studies.

A linear regression analysis was used to determine the functional relationship between serum and salivary creatinine and to predict cut off values (Table 7). A significant relationship was found for serum and salivary creatinine in CKD stages 2 to 5 with stage 5 having the highest coefficient of determination of 27.5%. A linear regression established that salivary creatinine in stage 5 could statistically significantly predict serum creatinine concentration, $F(1, 38) = 39$, $p < .001$ and salivary creatinine accounted for 27.5 % of the explained variability in serum creatinine concentration. It should be noted that the generally low coefficient of determination (R^2) does not mean that salivary creatinine is not a good predictor of serum creatinine. Other confounding factors such as patients comorbidities and medications, which were not considered in this study can affect the level of serum creatinine and the rate at which it can be predicted by saliva. The significant relationship in stages 2-5 is in line with previous studies in which a significant predictive relationship was found between serum and salivary creatinine in CKD stages 4 and 5. For CKD stage 1, the relationship between serum and salivary creatinine was not significant. This was expected and consistent with results of previous studies (Venkatapathy et al., 2014; Lasisi et al., 2016), whose serum and salivary

creatinine values were within normal range. The non-significant relationship in stage 1 can be due to normal serum creatinine level found in stage 1 patients and which there is only minimal movement of creatinine from serum to saliva at this level.

In order to replace the serum creatinine test with a salivary diagnostic test, the diagnostic value of salivary creatinine test must be compared with that of the presently accepted method for diagnosing of CKD. The accuracy of the new test is determined by the strength with which it separates the group being tested into those with the disease and without the disease (Brown Connolly, 2014). Specificity and sensitivity of the diagnostic test is the basic method used to measure accuracy of the diagnostic test. In addition, positive predictive value (PPV) and negative predictive value (NPV) which show the percentage of group who tested positive who truly have the disease and the group which tested negative who do not have the disease give a better indication of how close a new diagnostic tool is close the gold standard. This is because the PPV and NPV are directly related to the prevalence of the disease in the sample population.



ROC analysis (Figure 7) of salivary creatinine revealed a good sensitivity and specificity range. The area under the curve (0.839) obtained in the present study for salivary creatinine showed salivary creatinine to be a good alternative diagnostic test to differentiate healthy and CKD patients. Comparable large areas under the curve were obtained from previous studies. Xia et al. (2012) obtained an area under curve value of 0.897, while Ventakapathy et al. 2014 obtained an area under curve of 0.967.

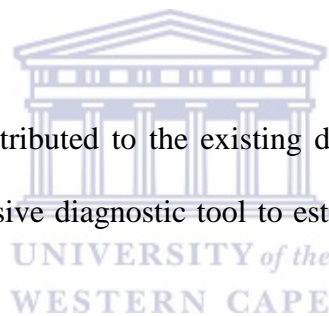
In the present study, significant ROC analysis cut-off points were measured at $8.50\mu\text{mol/L}$, which gave a sensitivity of 78.3% (false negative rate = 21.7%) and specificity of 74.0% (false positive rate = 26%) (Table 9). The PPV in this study is 91.6% while the NPV is 48.7%. The high PPV shows that the saliva as a diagnostic tool can be as good as serum

creatinine in diagnosis of CKD. However, the low NPV in this study is a reflection of the relatively high prevalence of CKD (17.3%) in the South African population. This suggests that people with salivary creatinine values above 8.50 μ mol/L be referred for further diagnostic evaluation and appropriate management.

Thus the results of the present study, supports the potential use of saliva as an alternative diagnostic medium to estimate serum creatinine in patients' with chronic kidney disease, but needs to be evaluated in longitudinal studies. The present study is the first to report a positive correlation as well as a predictive relationship in CKD stages 1 to 3.

Conclusion

The results of the study has contributed to the existing data on the diagnostic potential of salivary creatinine as a non-invasive diagnostic tool to estimate serum creatinine in patients with chronic kidney disease.



Limitations of the study

The limitation of this study design is that only a single salivary sample was taken from patients. Other limitation is non-inclusion of healthy control group. A prospective, cohort study is needed to test and verify our conclusions.

Recommendations

Patient stratification by sociodemographic factors such as gender, age and race, used in the calculation of glomerular filtration rate are needed to develop a specific GFR formula for calculating salivary creatinine. Further longitudinal studies are recommended to evaluate the potential use of saliva for monitoring CKD patients, as creatinine levels may be influenced by

different factors such as medications, diet, liver function and intense exercise. The effect of other systemic conditions such as diabetes mellitus, hypertension and obesity on salivary creatinine levels, as well as evaluation of other CKD markers (such as urea) in saliva at all stages of CKD, needs further investigation. Further investigations to determine if exogenous radioactive labelled tracers, which are more accurate but also costly, are detectable in saliva.



References

Adeniyi, A.B., Laurence, C.E., Volmink, J.A. and Davids, M.R., 2017. Prevalence of chronic kidney disease and association with cardiovascular risk factors among teachers in Cape Town, South Africa. *Clinical Kidney Journal*, p.sfw138.

Ahmadi Motamayel, F., Davoodi, P., Dalband, M. and Hendi, S.S., 2010. Saliva as a mirror of the body health. *DJH*, 2, pp.1-15.

Andrassy KM., 2013. National Kidney Foundation: comments on 'KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease'. *Kidney Int*, 84, p.622-3.

Bader, R.S., Kora, M.A.A.A., El-Shalakany, A.H. and Mashal, B.S.A.A.B., 2015. Clinical significance of saliva urea and creatinine levels in patients with chronic kidney disease. *Menoufia Medical Journal*, 28(2), p.406.

Baumgarten, M. and Gehr, T., 2011. Chronic kidney disease: detection and evaluation. *American family physician*, 84(10), p.1138.

Bayraktar G, Kurtulus I, Kazancioglu R, et al., 2009. "Oral health and inflammation in patients with end-stage renal failure," *Peritoneal Dialysis International*, vol.29, no.4, pp.472-479.

Briet, M., Collin, C., Karras, A., Laurent, S., Bozec, E., Jacquot, C., Stengel, B., Houillier, P., Froissart, M., Boutouyrie, P. and Nephrotest Study Group, 2011. Arterial remodeling associates with CKD progression. *Journal of the American Society of Nephrology*, 22(5), pp.967-974.

Brown Connolly, N.E., 2014. A better way to evaluate remote monitoring programs in chronic disease care: receiver operating characteristic analysis. *Telemedicine and e-Health*, 20(12), pp.1143-1149.

Burtis, C.A. and Bruns, D.E., 2014. *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics-E-Book*. Elsevier Health Sciences.

Carobene, A., Ferrero, C., Ceriotti, F., Modenese, A., Besozzi, M., De Giorgi, E., Franzin, M., Franzini, C., Kienle, M.G. and Magni, F., 1997. Creatinine measurement proficiency

testing: assignment of matrix-adjusted ID GC-MS target values. *Clinical chemistry*, 43(8), pp.1342-1347.

Chianeh, Y.R. and Prabhu, K., 2014. Biochemical markers in saliva of patients with oral squamous cell carcinoma. *Asian Pacific Journal of Tropical Disease*, 4, pp.S33-S40.

Coresh, J., Astor, B.C., Greene, T., Eknoyan, G. and Levey, A.S., 2003. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *American journal of kidney diseases*, 41(1), pp.1-12.

Coresh, J., Byrd-Holt, D., Astor, B.C., Briggs, J.P., Eggers, P.W., Lacher, D.A. and Hostetter, T.H., 2005. Chronic kidney disease awareness, prevalence, and trends among US adults, 1999 to 2000. *Journal of the American Society of Nephrology*, 16(1), pp.180-188.

Coresh, J., Selvin, E., Stevens, L.A., Manzi, J., Kusek, J.W., Eggers, P., Van Lente, F. and Levey, A.S., 2007. Prevalence of chronic kidney disease in the United States. *Jama*, 298(17), pp.2038-2047.

de Jong, E.P., van Riper, S.K., Koopmeiners, J.S., Carlis, J.V. and Griffin, T.J., 2011. Sample collection and handling considerations for peptidomic studies in whole saliva; implications for biomarker discovery. *Clinica Chimica Acta*, 412(23), pp.2284-2288.

Dodder, N.G., Tai, S.S.C., Sniegowski, L.T., Zhang, N.F. and Welch, M.J., 2007. Certification of creatinine in a human serum reference material by GC-MS and LC-MS. *Clinical chemistry*, 53(9), pp.1694-1699.

Gao, K., Zhou, H., Zhang, L., Lee, J.W., Zhou, Q., Hu, S., Wolinsky, L.E., Farrell, J., Eibl, G. and Wong, D.T., 2009. Systemic disease-induced salivary biomarker profiles in mouse models of melanoma and non-small cell lung cancer. *PLoS One*, 4(6), p.e5875.

Gonwa, T.A., Jennings, L., Mai, M.L., Stark, P.C., Levey, A.S. and Klintmalm, G.B., 2004. Estimation of glomerular filtration rates before and after orthotopic liver transplantation: evaluation of current equations. *Liver transplantation*, 10(2), pp.301-309.

Gröschl, M., 2008. Current status of salivary hormone analysis. *Clinical chemistry*, 54(11), pp.1759-1769.

Gupta, R., 2004. Trends in hypertension epidemiology in India. *Journal of human hypertension*, 18(2), p.73.

Guyton, A.C. and Hall, J.E., 2006. *The body fluids and kidneys. textbook of medical physiology*, 6, p.339.

Hansen, Å.M., Garde, A.H., Christensen, J.M., Eller, N.H. and Netterstrøm, B., 2003. Evaluation of a radioimmunoassay and establishment of a reference interval for salivary cortisol in healthy subjects in Denmark. *Scandinavian journal of clinical and laboratory investigation*, 63(4), pp.303-310.

Hilawe, E.H., Yatsuya, H., Kawaguchi, L. and Aoyama, A., 2013. Differences by sex in the prevalence of diabetes mellitus, impaired fasting glycaemia and impaired glucose tolerance in sub-Saharan Africa: a systematic review and meta-analysis. *Bulletin of the World Health Organization*, 91(9), pp.671-682D.

Hill, N.R., Fatoba, S.T., Oke, J.L., Hirst, J.A., O'Callaghan, C.A., Lasserson, D.S. and Hobbs, F.R., 2016. Global prevalence of chronic kidney disease—a systematic review and meta-analysis. *PLoS One*, 11(7), p.e0158765.

Kaufman, E. and Lamster, I.B., 2002. The diagnostic applications of saliva—a review. *Critical Reviews in Oral Biology & Medicine*, 13(2), pp.197-212.

Krishnegowda, A., Padmarajaiah, N., Anantharaman, S. and Honnur, K., 2013. Spectrophotometric assay of creatinine in human serum sample. *Arabian Journal of Chemistry*.

LADgotrA, A., Verma, P. and Raj, S.S., 2016. Estimation of Salivary and Serum Biomarkers in Diabetic and Non Diabetic Patients-A Comparative Study. *Journal of clinical and diagnostic research: JCDR*, 10(6), p.ZC56.

Lakatta, E.G., 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. *Circulation*, 107(3), pp.490-497.

Lamb, E., Newman, D.J. and Price, C.P., 2006. *Kidney function tests. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Burtis, CA, Ashwood, ER & Bruns, DE (Eds). Elsevier Inc., 24, pp.797-835.

- Lasisi, T.J., Raji, Y.R. and Salako, B.L., 2016. Salivary creatinine and urea analysis in patients with chronic kidney disease: a case control study. *BMC nephrology*, 17(1), p.10.
- Lee, Y.H. and Wong, D.T., 2009. Saliva: an emerging biofluid for early detection of diseases. *American journal of dentistry*, 22(4), p.241.
- Lee, Y.H., Kim, J.H., Zhou, H., Kim, B.W. and Wong, D.T., 2012. Salivary transcriptomic biomarkers for detection of ovarian cancer: for serous papillary adenocarcinoma. *Journal of Molecular Medicine*, 90(4), pp.427-434.
- Levey, A.S., Coresh, J., Balk, E., Kausz, A.T., Levin, A., Steffes, M.W., Hogg, R.J., Perrone, R.D., Lau, J. and Eknoyan, G., 2003. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Annals of internal medicine*, 139(2), pp.137-147.
- Levey, A.S., Eckardt, K.U., Tsukamoto, Y., Levin, A., Coresh, J., Rossert, J., Zeeuw, D.D., Hostetter, T.H., Lameire, N. and Eknoyan, G., 2005. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney international*, 67(6), pp.2089-2100.
- Levin, A., Stevens, P.E., Bilous, R.W., Coresh, J., De Francisco, A.L., De Jong, P.E., Griffith, K.E., Hemmelgarn, B.R., Iseki, K., Lamb, E.J. and Levey, A.S., 2013. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney International Supplements*, 3(1), pp.1-150.
- Lloyd, J.E., Broughton, A. and Selby, C., 1996. Salivary creatinine assays as a potential screen for renal disease. *Annals of clinical biochemistry*, 33(5), pp.428-431.
- Malamud, D., 2011. Saliva as a diagnostic fluid. *Dental Clinics of North America*, 55(1), pp.159-178.
- Matsha, T.E., Yako, Y.Y., Rensburg, M.A., Hassan, M.S., Kengne, A.P. and Erasmus, R.T., 2013. Chronic kidney diseases in mixed ancestry south African populations: prevalence, determinants and concordance between kidney function estimators. *BMC nephrology*, 14(1), p.75.

Mazzachi, B.C., Peake, M.J. and Ehrhardt, V., 2000. Reference range and method comparison studies for enzymatic and Jaffé creatinine assays in plasma and serum and early morning urine. *Clinical laboratory*, 46(1-2), pp.53-55.

Mbanya, J.C.N., Ngogang, J., Salah, J.N., Minkoulou, E. and Balkau, B., 1997. Prevalence of NIDDM and impaired glucose tolerance in a rural and an urban population in Cameroon. *Diabetologia*, 40(7), pp.824-829.

Moorman, J.E., Rudd, R.A., Johnson, C.A., King, M., Minor, P., Bailey, C., Scalia, M.R. and Akinbami, L.J., 2007. Centers for Disease Control and Prevention (CDC). *MMWR Morb Mortal Wkly Rep*, 56, p.154.

Myers, G.L., Miller, W.G., Coresh, J., Fleming, J., Greenberg, N., Greene, T., Hostetter, T., Levey, A.S., Panteghini, M., Welch, M. and Eckfeldt, J.H., 2006. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. *Clinical chemistry*, 52(1), pp.5-18.

Nagarathinam AE, Kumar TD, Kumar AR, Vasanthira K, Lakshmi RS, Jayant VS, 2017. Salivary Urea and Creatinine as a Diagnostic Marker of Chronic Kidney Disease – Review. (*IOSR-JDMS*, 16:95-100)

Naicker, S., 2009. End-stage renal disease in sub-Saharan Africa. *Ethnicity & disease*, 19(1), p.13.

Nakahari, T., Yoshida, H. and Imai, Y., 1996. Transepithelial fluid shift generated by osmolarity gradients in unstimulated perfused rat submandibular glands. *Experimental physiology*, 81(5), pp.767-779.

Nunes, L.A.S., Brenzikofer, R. and Macedo, D.V., 2011. Reference intervals for saliva analytes collected by a standardized method in a physically active population. *Clinical biochemistry*, 44(17), pp.1440-1444.

Peake, M. and Whiting, M., 2006. Measurement of serum creatinine—current status and future goals. *Clinical biochemist reviews*, 27(4), p.173.

Perrone, R.D., Madias, N.E. and Levey, A.S., 1992. Serum creatinine as an index of renal function: new insights into old concepts. *Clinical chemistry*, 38(10), pp.1933-1953.

Pfaffe, T., Cooper-White, J., Beyerlein, P., Kostner, K. and Punyadeera, C., 2011. Diagnostic potential of saliva: current state and future applications. *Clinical chemistry*, 57(5), pp.675-687.

Piéroni, L., Bargnoux, A.S., Cristol, J.P., Cavalier, E. and Delanaye, P., 2017. Did creatinine standardization give benefits to the evaluation of glomerular filtration rate?. *EJIFCC*, 28(4), p.251.

Prakash, S. and O'Hare, A.M., 2009, September. Interaction of aging and chronic kidney disease. In *Seminars in nephrology* (Vol. 29, No. 5, pp. 497-503). WB Saunders.

Proctor, R., Kumar, N., Stein, A., Moles, D. and Porter, S., 2005. Oral and dental aspects of chronic renal failure. *Journal of dental research*, 84(3), pp.199-208.

Rao, P.V., Reddy, A.P., Lu, X., Dasari, S., Krishnaprasad, A., Biggs, E., Roberts Jr, C.T. and Nagalla, S.R., 2009. Proteomic identification of salivary biomarkers of type-2 diabetes. *Journal of proteome research*, 8(1), pp.239-245.

Rose, B.D., Post, T.W. and Rose, B., *Clinical Physiology of Acid-Base and Electrolyte Disorders*, 2001.

Ruggenti, P., Schiepati, A. and Remuzzi, G., 2001. Progression, remission, regression of chronic renal diseases. *The Lancet*, 357(9268), pp.1601-1608.

Seethalakshmi, C., Koteeswaran, D. and Chiranjeevi, V., 2014. Correlation of serum and salivary biochemical parameters in end stage renal disease patients undergoing hemodialysis in pre and post-dialysis state. *Journal of clinical and diagnostic research: JCDR*, 8(12), p.CC12.

Snyder, S. and Pendergraph, B.E.R.N.A.D.E.T.T.E., 2005. Detection and evaluation of chronic kidney disease. *Interventions*, 100(1), pp.24-25.

Stanifer, J.W., Jing, B., Tolan, S., Helmke, N., Mukerjee, R., Naicker, S. and Patel, U., 2014. The epidemiology of chronic kidney disease in sub-Saharan Africa: a systematic review and meta-analysis. *The Lancet Global Health*, 2(3), pp.e174-e181.

Tomás, I., Marinho, J.S., Limeres, J., Santos, M.J., Araújo, L. and Diz, P., 2008. Changes in salivary composition in patients with renal failure. *archives of oral biology*, 53(6), pp.528-532.

Turpeinen, U., Välimäki, M.J. and Hämäläinen, E.S.A., 2009. Determination of salivary cortisol by liquid chromatography-tandem mass spectrometry. *Scandinavian journal of clinical and laboratory investigation*, 69(5), pp.592-597.

van Deventer, H.E., George, J.A., Paiker, J.E., Becker, P.J. and Katz, I.J., 2008. Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. *Clinical Chemistry*, 54(7), pp.1197-1202.

Venkatapathy, R., Govindarajan, V., Oza, N., Parameswaran, S., Pennagaram Dhanasekaran, B. and Prashad, K.V., 2014. Salivary creatinine estimation as an alternative to serum creatinine in chronic kidney disease patients. *International journal of nephrology*, 2014.

Weber, J.A. and Van Zanten, A.P., 1991. Interferences in current methods for measurements of creatinine. *Clinical chemistry*, 37(5), pp.695-700.

Wild, S., Roglic, G., Green, A., Sicree, R. and King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030 *Diabetes Care* 27: 1047–1053. Find this article online.

Xia, Y., Peng, C., Zhou, Z., Cheng, P., Sun, L., Peng, Y. and Xiao, P., 2012. Clinical significance of saliva urea, creatinine, and uric acid levels in patients with chronic kidney disease. *Zhong nan da xue xue bao. Yi xue ban= Journal of Central South University. Medical sciences*, 37(11), pp.1171-1176.

Yoshizawa, J.M., Schafer, C.A., Schafer, J.J., Farrell, J.J., Paster, B.J. and Wong, D.T., 2013. Salivary biomarkers: toward future clinical and diagnostic utilities. *Clinical microbiology reviews*, 26(4), pp.781-791.

Zhang, L., Farrell, J.J., Zhou, H., Elashoff, D., Akin, D., Park, N.H., Chia, D. and Wong, D.T., 2010. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. *Gastroenterology*, 138(3), pp.949-957.



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WESTERN CAPE

Kidney Disease Questionnaire

Record No.

1 Age Sex M F

2 Marital status single never married married divorced

3 Highest education level Primary school Secondary school Technikon/University Post graduate

4 Do you smoke? Yes No

5 If YES, when did you start? years ago

6 If you no longer smoke, when did you stop? years ago

7 How many cigarettes do you smoke per day?

8 Do you drink alcohol? Yes No

9 If yes to above question, answer the following questions
When did you start taking alcohol? years ago

What is the average number of glasses of alcohol you take in a day?

10 Do you have any of the following medical conditions?

Diabetes	Hypertension	Nephrotic syndrome	Urinary tract infection	Other ...
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11 Which medications are you taking presently?

10 The presence of any of the following oral lesions

Oral candida	Lichen planus	Apthous ulcer	Gingival hyperplasia	Leukoplakia	Other ...
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11 Teeth present 1st quadrant 2nd quadrant 3rd quadrant 4th quadrant

Serum creatinine value

Salivary creatinine value

Nier Siekte Vraelys

Rekordgetal.

1 Ouderdom Geslag M F

2 Huwelikstatus Enkol Nooit getroud Getroud Geskei

3 Hoogste vlak van onderwys Laerskool Hoërskool universiteit Nagraadse

4 Rook jy? Ja Geen

5 Indien ja, wanneer het jy begin? Jare terug

6 As jy nie meer rook, wanneer het jy oophou? Jare terug

7 Hoeveel sigarette rook jy per dag?

8 Jy alkohol drink? Ja Geen

9 Indien wel om bogenoemde vraag, antwoord die volgende vrae
Wanneer het jy begin om alkohol? Jare terug

Hoeveel glase alkohol wat jy neem in 'n dag?

10 Het jy enige van die volgende mediese toestande?

<input type="checkbox"/> Suikersiekte	<input type="checkbox"/> Hoë bloeddruk	<input type="checkbox"/> Nefrotiese sindroom	<input type="checkbox"/> Urienweginfeksie	<input type="checkbox"/> Ander ...
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11 Wat medikasie neem jy tans?

10 Die teenwoordigheid van enige van die volgende mondelinge letsels

<input type="checkbox"/> Oral candida	<input type="checkbox"/> Lichen planus	<input type="checkbox"/> Aphthous ulcer	<input type="checkbox"/> Gingival hyperplasia	<input type="checkbox"/> Leukoplakia	<input type="checkbox"/> Other ...
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11 Tande teenwoordig

<input type="checkbox"/> 1 st kwadrant	<input type="checkbox"/> 2 nd kwadrant	<input type="checkbox"/> 3 rd kwadrant	<input type="checkbox"/> 4 th kwadrant
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Scrum creatinine value



Salivary creatinine value

Ikhweshine yasifo sezintso

Irekhodnamba

1 Ubudala Isini M F

2 Isimo somtshato

enye	zange atshate	tshatile	umtshato
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3 Kwincanaba el phezuu lemfundo

Amabanga aphantsi	kumabanga aphakathi	Iyur Ivesithi	Isidanga
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4 Uyatshaya? Ewe Hayi

5 Ukuba ewe, Waqala nini? Kwimiryaka ayadlulayo

6 Ukuba akukho kuphinda utshaye, xa ngaba uyeka? Kwimiryaka ayadlulayo

7 Zingaphi icuba ukuba utshaye ngosuku?

8 Ingabo uyabusela utywala? Ewe Hayi

9 Ukuba uthi "ewe" kulombuzo ungentla, phendula lemibuzo ilandelayo: Uqale nin ukuthatha utywala? Kwimiryaka ayadlulayo

Useta iiglas ezingaphi zitywala ngosuku?

10 Ngaba unayo na enye yezimeko zeconyarigo?

isifo seswekile	Iwegazi	Nephrotic syndrome	losu eleko wokuchama ipheca	Omnye ...
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11 Ngawaphi amayeza owathathayo?

10 Ubukho naphi eziqhekebeni zomlomo zilandelayo

Oral candida	Lichen planus	Aphthous ulcer	Gingival hyperplasia	Leukoplakia	Other ...
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11 Amazinyo akhoyo

1 st inxalenye	2 nd inxalenye	3 rd inxalenye	4 th inxalenye
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Serum creatinine value

Salivary creatinine value

|Salivary Creatinine as a Diagnostic Tool for evaluating Chronic Kidney Diseases study

Information Sheet

Date:

Good day Sir/Madam

My name is Dr. Temilola Dada from the Department of Oral Medicine and Periodontology, Faculty of Dentistry, University of the Western Cape, Tygerberg.

I would appreciate your consideration to participate in a study to determine if saliva could be used to diagnose kidney disease. The diagnosis and monitoring of kidney disease is usually done by taking a blood sample and sending it to a laboratory for evaluation. Enrollment in this study would require that spit into a container in which about 2ml of your saliva and will be collected. Blood will be drawn in the usual manner at your clinic. Both samples will be sent to the laboratory for evaluation. Study enrollment and sample collection will only take approximately 30 minutes of your time.

Your participation would be voluntary and refusal/withdrawal would not incur any penalty nor influence your usual treatment. Enrollment in the study will not incur any additional costs to the participants.

The results of the study could be beneficial to all patients with kidney disease if the results of the study support that saliva is as reliable as blood samples in monitoring kidney disease. It could prove to be a less invasive diagnostic method and improve patient compliance. Patient anonymity will be ensured by assigning record numbers to your specimens and the samples will be disposed of in the usual manner at the laboratory.

This study has been ethically reviewed and approved by the UWC Biomedical Research Ethics Committee (approval number _____).

In the event of any problems or concerns/questions you may contact the researcher at Department of Oral Medicine and Periodontology, Faculty of Dentistry, University of the Western Cape, Tygerberg, or the UWC Biomedical Research Ethics Committee, whose contact details as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
University of the Western Cape
Research Office
New Arts Building
C-Block, Top Floor, Room 28.
Western Cape, SOUTH AFRICA
Tel: 021 959 2948



**Office of the Deputy Dean
Postgraduate Studies and Research**
Faculty of Dentistry & WHO Collaborating Centre for Oral Health



UNIVERSITY OF THE WESTERN CAPE
Private Bag XI, Tygerberg 7505
Cape Town
SOUTH AFRICA

Date: 07th December 2016

For Attention: Dr DO Temilola

Dear Dr Temilola

STUDY PROJECT: Salivary creatinine as a diagnostic tool for evaluating chronic kidney disease

PROJECT REGISTRATION NUMBER: BM/16/5/4

ETHICS: Approved

At a meeting of the Senate Research Committee held on Thursday 24th November 2016 the above project was approved. This project is therefore now registered and you can proceed with the study. Please quote the above-mentioned project title and registration number in all further correspondence. Please carefully read the Standards and Guidance for Researchers below before carrying out your study.

Patients participating in a research project at the Tygerberg and Mitchells Plain Oral Health Centres will not be treated free of charge as the Provincial Administration of the Western Cape does not support research financially.

Due to the heavy workload auxiliary staff of the Oral Health Centres cannot offer assistance with research projects.

Yours sincerely

Professor Lawrence Stephen



Western Cape
Government

Health

TYGERBERG HOSPITAL
REFERENCE: Research Projects
ENQUIRIES: Dr GG Marinus
TELEPHONE: 021 938 5752

Ethics Reference: BM/16/5/4


TITLE: Salivary creatinine as a diagnostic tool for evaluating chronic kidney disease.

Dear Dr Temilola

PERMISSION TO CONDUCT YOUR RESEARCH AT TYGERBERG HOSPITAL.

1. In accordance with the Provincial Research Policy and Tygerberg Hospital Notice No 40/2009, permission is hereby granted for you to conduct the above-mentioned research here at Tygerberg Hospital.
2. Researchers, in accessing Provincial health facilities, are expressing consent to provide the Department with an electronic copy of the final feedback within six months of completion of research. This can be submitted to the Provincial Research Co-Ordinator (Health.Research@westerncape.gov.za).


DR GG MARINUS
MANAGER: MEDICAL SERVICES [RESEARCH CO-ORDINATOR]


DR D ERASMUS
CHIEF EXECUTIVE OFFICER
Date: 19 January 2017