CORRELATION OF SALIVARY AND SERUM CREATININE ESTIMATION IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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CHENNAI

DECLARATION BY THE CANDIDATE

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	EXPANSION
1	**CKD**	Chronic kidney disease
2	**Pmp**	Per million population
3	**GFR**	Glomerular filtration rate
4	**eGFR**	Estimated glomerular filtration rate
5	**KDIGO**	Kidney Disease Improving Global Outcomes
6	**CHC**	Community health center
7	**ROC**	Receiver Operator Characteristics
8	**EDD**	Endothelium-dependent dilation
9	**CKD-EPI**	Chronic Kidney Disease Epidemiology Collaboration
10	**MDRD**	Modification of Diet in Renal Disease
11	**PPV**	Positive predictive value
12	**NPV**	Negative predictive value

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INTRODUCTION

Saliva, being the mirror of body is a perfect medium to be explored for health and disease surveillance. The ability to utilize saliva to monitor the health and disease state of an individual is a highly desirable goal for health promotion and health care research.[26, 65] In the upcoming era of genomic medicine, sialochemistry will replace the biochemical analysis of blood in everyday medical practice. The ability to monitor health status, disease onset, progression and treatment outcome through non-invasive means is a most desirable goal in the health care promotion and delivery.[26,27]

Saliva, a multi-constituent biologic fluid secreted by the salivary glands, plays an important role in oral and systemic health. Its collection for biochemical analysis is preferable to blood because it is non-invasive, simple, and inexpensive, and can be performed more frequently. It also provides a cost-effective method for screening large populations.[60]

Saliva is considered as a filtrate of the blood where various molecules pass through transcellular (passive intracellular diffusion and active transport) or paracellular routes (extracellular ultrafiltration) into saliva.[67]As a result, saliva is equivalent to serum, thereby reflecting the physiological state of the body.

Chronic kidney disease (CKD) is characterized by high morbidity and mortality rates. The serum level of creatinine (primarily secreted by the kidneys) is used to determine renal function. This condition requires frequent serum analysis to diagnose and monitor therapeutic outcomes and to ascertain prognosis. Creatinine, a waste product of muscle metabolism, is primarily excreted by kidneys and its level in serum is used as an index to renal function. Collection of blood for serum analysis is an invasive procedure associated with fear and anxiety. Frequent blood sampling results in severe anaemia and an increase in the risk of infection.[62, 63]Also patients undergoing dialysis are at greater risk of developing Hepatitis B and C [64,] potentially increasing the risk of health care professional to blood borne diseases.

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. [58] 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. The increase in salivary creatinine due to concentration gradient diffusion makes saliva a potential tool for measuring renal function, and also it plays an important role when required a repeat sample for all age groups.[65]. It also provides a cost-effective approach for the screening of large

populations. Saliva as a diagnostic medium will also be a boon to patients suffering from clotting disorders like haemophilia and in patients with compromised venous access. [64]

There are several preliminary studies with promising results which show that saliva can be used to detect lung cancer, pancreatic cancer, breast cancer, and type II diabetes. With this background we planned a study to determine the diagnostic ability of saliva as an alternative to blood to estimate creatinine in chronic kidney disease patients and to evaluate the correlation between salivary and serum creatinine levels in these patients.

AIMS AND OBJECTIVES

AIM OF THE STUDY:

- To compare and correlate the salivary and serum creatinine levels in patients with chronic kidney disease.

OBJECTIVE OF THE STUDY

- The objective of the study is to correlate salivary and serum creatinine levels in patients with chronic kidney disease.
- To compare salivary creatinine and serum creatinine levels in male and female patients with chronic kidney disease.
- To compare salivary creatinine and serum creatinine levels in stage 4 and stage 5 chronic kidney disease patients.
- To evaluate the role of saliva as a non-invasive alternative to serum for creatinine estimation in chronic kidney disease patients.

REVIEW OF LITERATURE

The present study is about correlation of salivary and serum creatinine estimation in patients with chronic kidney disease. A detailed literature review will highlight the importance of the study and also briefs about various aspects of chronic kidney disease and obtains a correlation of salivary and serum creatinine in patients with chronic kidney disease .

CHRONIC KIDNEY DISEASE

Andrew S. Levey et al (2013) [1]defined kidney disease is a heterogeneous group of disorders, affecting kidney structure and function. It is recognized now that even mild abnormalities in kidney structure and function are associated with increased risk for developing complications in other organ systems as well as increased mortality. He enumerated rationale for classification of kidney disease for early diagnosis and intervention.

Arun et al (2012)[5] enumerated various stages of chronic kidney disease. Chronic renal failure or chronic kidney disease is defined as

- Kidney damage
- Glomerular Filtration Rate (GFR) < 60 ml/min/1.73 sq.m

For a period of ≥ 3 months (As per the National Kidney Foundation, Kidney Disease Outcomes Quality Initiative)

Stage 1: Kidney damage with normal or raised GFR,

GFR ≥ 90ml / min /1.73 sq.m.

Stage 2: Kidney damage with mild decrease in GFR,

GFR 60-89ml / min /1.73 sq.m.

Stage3: Moderately decreased GFR,

GFR 30-59ml/min/1.73 sq.m.

Stage4: Severely decreased GFR,

GFR 15-29ml/min/1.73 sq.m.

Stage 5 or ESRD (End stage renal disease): Kidney failure,

GFR 15-29ml/min/1.73 sq.m.

Stages of Chronic Kidney Disease

Nathan et al (2016)[38] noted that Chronic kidney disease (CKD) is a global health burden with a high economic cost to health systems and is an independent risk factor for cardiovascular disease (CVD). All stages of CKD are associated with increased risks of cardiovascular morbidity, premature mortality, and/or decreased quality of life. CKD is usually asymptomatic until later stages. He mentioned about the stage wise prevalence of chronic kidney disease.

STAGE WISE PREVALENCE OF CHRONIC KIDNEY DISEASE [38]

Stage-1	3·5%
Stage-2	3·9%
Stage-3	7·6%
Stage-4	0·4%
Stage-5	0·1%

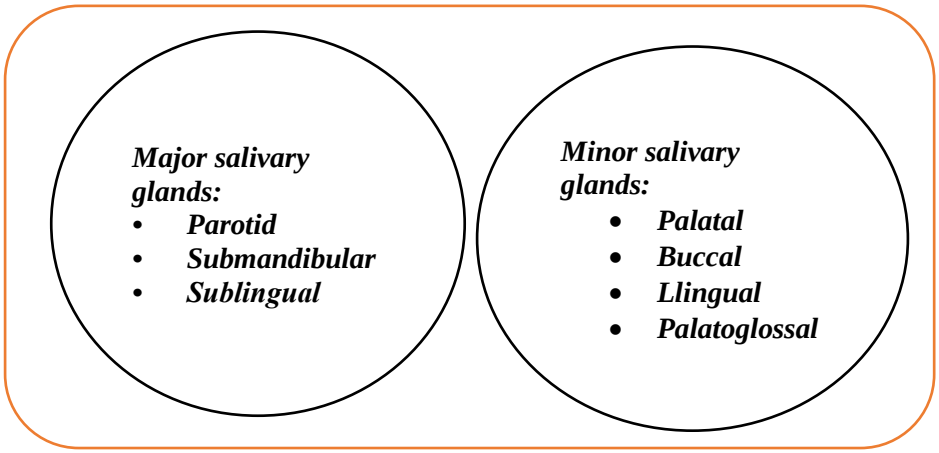
Gian Chand et al (2018) [16] stated that chronic kidney disease (CKD) encompasses a spectrum of different pathophysiological processes associated with abnormal kidney function and progressive decline in glomerular filtration rate, leading to abnormal blood urea, creatinine levels and electrolyte disturbances. Patients with CKD are subjected to repeated blood sampling to measure blood urea and serum creatinine, resulting in more pronounced anaemia. Frequent drawing of blood also adds to the psychological trauma to the patients. So to assess renal function in patients with CKD, an alternative sample source, other than blood is being investigated. In his study he concluded that there is significant positive correlation between salivary urea and salivary creatinine with blood urea and serum creatinine in patients with CKD.

Aravind P.S et al (2018) [2] noted the prevalence and incidence of chronic kidney disease is increasing worldwide. The condition requires frequent serum analysis to diagnose and monitor therapeutic outcomes and to ascertain prognosis. Creatinine, a waste product of muscle metabolism, is primarily excreted by kidney and its level in serum is used as an index to renal function.

In his study he concluded that salivary creatinine can be used as an alternative to serum creatinine in calculating eGFR and staging of CKD5. Also saliva could be an alternative to blood for diagnosis and monitoring patients with chronic kidney disease.

SALIVA: SECRETION, COMPONENTS AND COMPOSITION

The whole fluid present in the oral cavity originates mainly from major and minor salivary gland.



WHOLE SALIVA

Nunes S et al (2015) [44] mentioned the importance of whole saliva and explained about the contributions of different salivary glands. Secretions from major and minor salivary glands along with gingival crevicular fluid with bacteria, epithelial cells, erythrocytes, leukocytes and food debris are designated as "oral fluid" or "whole saliva". He concluded that whole saliva can be used as a diagnostic medium.

The average daily flow of whole saliva varies in health between 1 and 1.5 L / day

CONTRIBUTIONS OF DIFFERENT SALIVARY GLANDS

Edgar.WM (1992) [14] stated about the contributions of different salivary glands.

Unstimulated salivary flow

- 20% from parotid gland
- 65% from submandibular gland
- 7% to 8% from sublingual gland
- 10% minor glands.

Stimulated salivary flow

- 50% from parotid gland
- 35% from submandibular gland
- 7% to 8% from sublingual gland

Unstimulated salivary flow: 0.1 to 0.3 mL/min

Stimulated salivary flow: 7 mL/min

EFFECTIVENESS OF SALIVA [25, 56]

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 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.

Denny P et al (2006)[10] showed that a high proportion of proteins that are found in plasma and/or tears are also present in saliva along with unique components. The proteins identified are involved in numerous molecular processes ranging from structural functions to enzymatic/catalytic activities used to translate blood-based clinical laboratory tests into a format that utilizes saliva. He concluded that saliva can be used as a diagnostic medium.

Mittal et al (2011)[34] has enumerated the advantages of saliva being used as a diagnostic medium.

Advantages of salivary testing for diagnosis

- Non-invasive, easy to use, inexpensive
- Safer to administer than serum sampling (no needles)
- Real-time diagnostic values
- No need for trained medical staff
- Multiple samples can be obtained easily
- Collection and screening can be done at home
- Minimal risks of cross-contamination
- More economical sampling, shipping and storage compared to serum.

Advantages of salivary testing for diagnosis

Pfaffe, J et al (2011)[47] has enumerated the limitations of salivary diagnosis.

- Levels of certain markers in saliva are not always a reliable reflection of the levels of these markers in serum.
- Salivary composition can be influenced by the method of collection and degree of stimulation of salivary flow.
- Changes in salivary flow rate may affect the concentration of salivary markers and also their availability due to changes in salivary pH.
- Variability in salivary flow rate is expected between individuals and in the same individual under different conditions.
- In addition, many serum markers can reach whole saliva in an unpredictable way (*i.e.* gingival crevicular fluid flow and through oral wounds). These parameters will affect the diagnostic usefulness of many salivary constituents.
- Furthermore, certain systemic disorders, numerous medications and radiation may affect salivary gland function and consequently the quantity and composition of saliva.
- Whole saliva also contains proteolytic enzymes derived from the host and from oral microorganisms. These enzymes can affect the stability of certain diagnostic markers. Some molecules are also degraded during intracellular diffusion into saliva.

ANALYSIS OF SALIVA FOR OTHER CONDITIONS:[60]

- Hereditary disease
- Autoimmune disease
- Malignancy
- Infection
- Monitoring hormone levels
- Monitoring drug levels
- Bone turnover marker in saliva
- Forensic Evidence
- Dental caries and periodontal disease
- Diagnosis of Oral Disease with Relevance for Systemic Diseases.

PROPERTIES OF SALIVA AS A DIAGNOSTIC FLUID

Although the utility and advantages of saliva as a screening tool for cystic fibrosis has been identified in the early 1960s, its full diagnostic potential was discovered three decades later when studies revealed distinct advantages of saliva over serum.

Mohammed et al (2016) [36] said that saliva also contains hormones, antibodies, growth factors and enzymes like serum. Many of these constituents enter saliva through blood via passive diffusion, active transport or extracellular ultrafiltration. He concluded saliva as a reflection of the physiological function of the body.

Miller et al (1994) [33] have raised concerns about the use of saliva for diagnostic purposes due to its low concentration of analysts in comparison to blood. He concluded that with the advent of highly sensitive molecular methods and nanotechnology, this is no longer a limitation.

Nagler.M et al (2008) [40] mentioned the analysis of salivary composition as a diagnostic tool for the localization and assessment of various systemic diseases (such as end-stage renal disease) .He concluded that markers for monitoring patients with end-stage renal disease must fulfil 3 requirements:

- The markers should properly reflect serum concentrations of toxins to be dialyzed.
- The correlation between the serum and saliva concentrations of the markers should be as high as possible.
- The concentrations of the markers in saliva should not be altered by intraoral conditions or by processes associated with marker transport from serum into saliva.

Venkatapathy et al (2014) [63] listed the importance of salivary diagnostics for renal disease .Saliva has numerous analysts to 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. 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. In his study he concluded that saliva can be used as a non-invasive diagnostic tool for estimating serum creatinine in chronic kidney disease patients.

Malamud et al (2011) [37]enumerated Saliva as a diagnostic medium could be a valuable tool to patients with clotting disorders and those with compromised venous access. He stated that saliva can be used to detect disease conditions such as cardiovascular diseases, renal diseases, pancreatic, lung and breast cancer, and type II diabetes.

Motamayel A et al (2010)[35] stated saliva as an important body fluid for detecting the physiological and pathological situations of the human body. Saliva is a complex and dynamic biological fluid containing wide range of physical and chemical properties .He concluded that physical and chemical properties of saliva is influenced by systemic diseases.

Lazaro Alessandro Soares Nuns (2015)[24] had mentioned the importance of saliva as a diagnostic medium .Saliva being the first, in line to come in contact with any ingested substance its composition may be influenced by medication, oral lesions, intracellular diffusion, and proteolytic

enzymes. He concluded that standardized collection methods for saliva are essential.

Lawrence HP et al (2002)[25] had said that components of saliva act as a "mirror of the body's health," and the widespread use and growing acceptability of saliva as a diagnostic tool is helping individuals, researchers, health care professionals and community health programs to detect and monitor disease and to improve the general health of the public. He concluded that saliva is increasingly being used as an investigational aid in the diagnosis of systemic diseases.

Lassi et al (2016) [22] stated, that saliva as an important biomarker. Saliva, a multi constituent biological fluid secreted by salivary gland, is the major contributor to oral health .It has got a cutting edge over serum because saliva collection is a non-invasive, simple and economic procedure that can be performed by the patient with minimum involvement from the medical personnel. When required, a repeat sample can be easily obtained and is suitable for all age groups. They concluded that salivary creatinine and urea in patients with chronic kidney disease reflects their levels in blood. Hence, salivary creatinine and urea could be used as diagnostic biomarkers of chronic kidney disease.

Lee JM et al (2009)[26] stated saliva, as a bio fluid that is totally non-invasive and readily available. National Institute of Dental & Craniofacial Research (NIDCR) has created a roadmap to achieve these goals through the use of oral fluids as the diagnostic medium to scrutinize the health and/or disease status of individuals. Progress has shown this an ideal opportunity to bridge state of the art saliva-based biosensors, optimized to disease discriminatory salivary biomarkers, for diagnostic applications. Oral fluid being the 'mirror of body' is a perfect medium to be explored for health and disease surveillance. They have concluded saliva as an ideal translational research tool and diagnostic medium.

MECHANISM OF EXPRESSION OF SERUM CREATININE IN SALIVA

SOURCES AND SITES OF CREATININE GENERATION

Andrew et al (1988) [1] explained about the source of creatinine .Creatinine is a metabolic waste product, primarily excreted by the kidneys. Creatinine is formed as a result of the non-enzymatic dehydration of muscle creatine. Creatine is synthesized primarily in the liver and actively transported into muscle, which contains about 98% of the total body creatine pool. He concluded that approximately 1.6-1.7% of the total creatine pool is converted to creatinine every day.

Eliaz Kaufman et al (2002) [15] explained the diffusion of molecules into saliva. 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 mechanism. He concluded that creatinine enters via either transcellular or paracellular mechanism.

Lee Y.T et al (2009)[27] explained about transfer of biomolecules from serum into saliva. Mechanism of molecular transport from serum into salivary gland ducts. The image shows the proximity of a major salivary gland to the vascular system. Salivary glands are highly vascularized, allowing for the exchange of blood-based constituents. Acinus cells within the salivary glands absorb molecules from the blood and secrete salivary juices into the oral cavity. Alterations in the molecular composition of the blood may subsequently modify the composition of salivary secretions. He concluded that disease-specific blood-based biomarkers could sufficiently alter the output of salivary glands, yielding saliva-based biomarkers of systemic disorders.

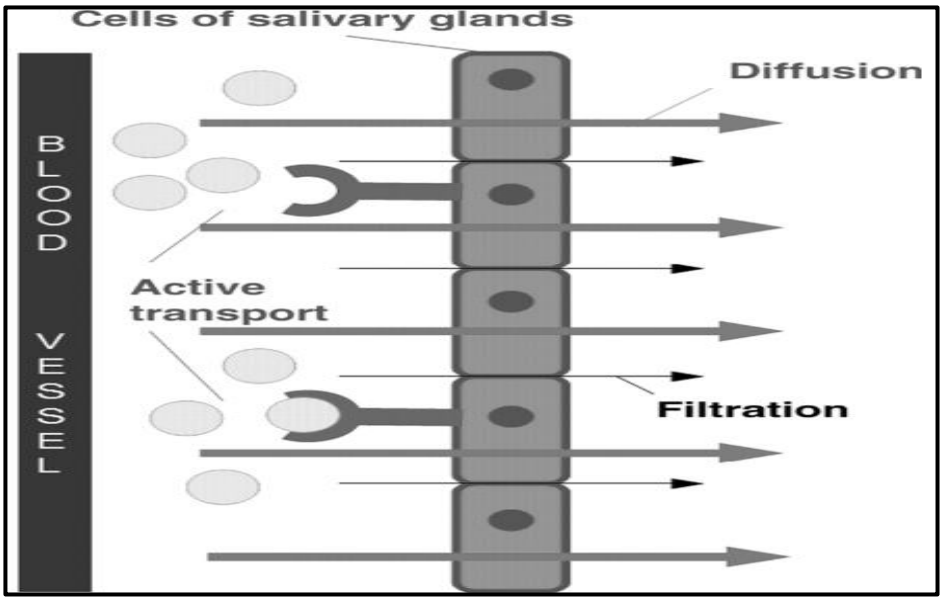


Fig:1 Mechanism of molecular transport from serum into salivary gland .

DIFFUSION

The most common route for substances to migrate from blood to saliva is via unaided or passive diffusion. The capillaries surrounding the salivary glands are quite porous for many small molecules. A serum molecule reaching saliva by diffusion must cross 5 barriers: the capillary wall; the interstitial space; the basal cell membrane of the acinus cell or duct cell; the cytoplasm of the acinus or duct cell; and the luminal cell membrane.

ACTIVE TRANSPORT

A second pathway for the entry of molecules into saliva is active transport through the secretory cells of the glands against concentration gradient.

ULTRAFILTRATION

Ultrafiltration (an extracellular mechanism), a third means of transportation of molecules from blood stream into saliva, is filtration through the spaces between acinus and ductal cells.

Thus the movement of creatinine molecules from blood stream into saliva occurs most commonly through these processes.

Lee et al (2009) [26] stated that 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. He concluded that creatinine enters saliva through process of diffusion.

Reference values for serum creatinine:

- ***Adult males***: 0.8 - 1.4 mg/dL: values are slightly higher in males due to larger muscle mass.
- ***Adult females***: 0.6 - 1.1 mg/dL: creatinine clearance is increased in pregnancy, resulting in lower serum levels
- ***Children***: 0.2 - 1.0 mg/dL: slight increases with age because values are proportional to body mass.

Reference values for salivary creatinine:

- **Salivary creatinine**: 0.05- 0.2 mg/dL.

Vijayalaxmi et al (2013)[64] specified the ultra-filtration of blood substance 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. She concluded that creatinine is very small sized molecules transported through ultrafiltration and filtration may also occur through the gap junctions between cells of secretory units.

Herenia et al (2002)[18] said that 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. He concluded that increase in salivary creatinine due to concentration gradient diffusion makes saliva a potential tool for measuring renal function.

Eliaz Kaufman et al (2002)[15] explained the diffusion of molecules into saliva. 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 trans-cellular (passive and active transport) or para-cellular (extra cellular ultrafiltration) diffusion mechanism. He concluded that creatinine enters via either trans-cellular or para-cellular mechanism.

PRINCIPLE OF CREATININE ESTIMATION:

Kirtimaan sayal et al (2013)[52] enumerated about creatinine estimation. Creatinine is one of the most common analysts used as the indicator of glomerular filtration rate (GFR) and kidney function. In 1886, Jaffe reported a reaction of creatinine and picric acid in an alkaline medium forming complex having absorbance maxima at 520 nm. The reaction has been brought to use for the measurement of creatinine by Folin and Wu in 1919. He concluded that Jaffe's reaction has been used for estimation of creatinine in serum and urine sample.

Jonathan E Lloyd (1996)[21] stated that creatinine is produced as the by-product of creatinine metabolism and is transported through bloodstream to the kidneys for excretion. Malfunctioning of kidney is reflected by lowering of the amount of creatinine in urine and rise of its level in blood. He concluded that salivary creatinine concentrations are 10-15% of serum creatinine concentrations in healthy populations.

Laisi et al (2016)[23] mentioned the importance of creatinine for measuring glomerular filtration rate. Creatinine levels in blood and urine also indicate the creatinine clearance, accounting for GFR. Creatinine readings indicate the total kidney GFR which is the sum of filtration rates of all

functional nephrons. Early structural damage to the renal cells involving reduction in functional nephron number may not affect an individual's total GFR as remaining renal units may perform compensatory function, enabling the kidneys to maintain kidney function temporarily even after the loss of functional tissue. Thus, creatinine/GFR may not indicate the early damage to kidney tissue. Jaffe also elaborated the interference in alkaline picrate reaction by number of organic compounds (e.g. acetone, glucose) which has been described as pseudochromogens. He concluded that creatinine remains the gold standard for measuring the glomerular filtration rate and kidney functioning.

Levey et al (2011)[29] highlighted the jaffe's kinetic reaction for estimation of serum creatinine. The assay is based on the reaction of creatinine with sodium picrate as described by Jaffe. Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents. The intensity of the colour formed is proportional to the creatinine concentration in the sample. He concluded that Jaffe's kinetic reaction and its accuracy for play a valuable tool for estimation of creatinine.

Nisha et al (2017) stated estimation of creatinine levels using auto analyser. The creatinine levels in the samples were assessed by Jaffe Kinetic assay. Creatinine in the serum sample reacted with picric acid in an alkaline solution (i.e., alkaline picrate) of the reagent and developed an orange

coloured complex. The quantity of creatinine in the test samples was calculated against the intensity of the colour developed during the fixed time. The intensity of the colour was measured using a fully automated Cobas C311 analyzer for detection of serum creatinine. He concludes the importance of auto analyser for estimation of serum creatinine.

Aravind PS et al (2018)[2] has enumerated the estimation of salivary creatinine using auto analyser. All collected saliva samples were centrifuged at 3000RPM for 10 minutes. Salivary supernatant and serum were separated. He concluded that samples were assayed immediately in automatic analyser (EM360 chemistry analyser with ISE module) using creatinine estimation kit (Swemed diagnostics) by Jaffe kinetic reaction.

Divya Panday et al (2016) [11] had mentioned that serum urea and creatinine are most widely accepted parameters to assess Chronic Kidney Disease (CKD) status as well as to assess renal status in susceptible diabetic and hypertensive subjects. In her study she concluded that there is a significant positive relationship between salivary and serum urea and creatinine. Thus, salivary urea and creatinine levels can be used non-invasively to detect serum urea and creatinine levels respectively in renal disease and diabetic, hypertensive and nephropathic cases.

Barder RS et al (2015)[6] enumerated, monitoring of markers in saliva instead of serum is advantageous because saliva collection is a non-invasive, simple, and inexpensive approach. Measurement of biomarkers in saliva may be an effective alternative method for monitoring the effectiveness of hemodialysis. The levels of urea and Cr in saliva and serum are closely related. He concluded that concentration of salivary urea and Cr can reflect renal damage, monitor the kidney function of CKD patients, and help in the diagnosis of middle-stage and late-stage CKD.

Rahime Renda et al (2017)[54] stated that collection of blood for serum analysis is an invasive procedure causing anxiety and discomfort for the patient. Certain amount of blood loss is associated with each dialysis procedure in CKD patients which amounts to about 4 to 20ml, with additional loss which results from frequent blood sampling ,also the patient undergoing dialysis are at greater risk of developing Hepatitis B and C potentially increasing the risk of health care personal to blood borne diseases . Thus, a simple diagnostic test that provides reliable evaluation of disease status and stages would be of value of both the clinicians and patient. Based on the positive correlation between the serum and saliva creatinine levels observed in the study, they concluded that saliva analysis could be used as a non-invasive alternative to blood analysis for diagnosing CKD in children.

Naresh Yajamanam t al (2016)[38] mentioned the diagnosis of renal diseases by assessing renal parameters in saliva. Biochemical investigations using serum form important component of monitoring patients with renal disease. Utility of saliva, in diagnosis and monitoring of patients with renal disease and for calculation of estimated glomerular filtration rate (eGFR), was studied. Positive correlation was observed between serum and salivary urea and creatinine ($P < 0.0001$). eGFR values calculated from salivary creatinine showed good agreement with those calculated form serum creatinine. He concluded that salivary urea (>6 mmol/L) and creatinine (>14.6 μmol/L) and eGFR calculated from salivary creatinine can be used to identify patients with renal disease.

MATERIALS AND METHODS

This is a hospital based study designed to compare and correlate the serum creatinine and salivary creatinine in chronic kidney disease patients. Patients were selected from Department Of Urology, VHS Multispeciality Hospitals, Madhya Kailash, Chennai.

TYPE OF STUDY

Prospective study

STUDY PERIOD

The study was done from February 2018 to August 2018.

PLACE OF THE STUDY

- This study was carried out in VHS multispecialty hospitals , Madhya Kailash, Chennai.
- Laboratory investigations were carried out in Gift Laboratory Services, Kanathur, Chennai.

STUDY POPULATION

- The study population comprised of 50 subjects who were diagnosed with chronic kidney disease stage 4 and Stage 5 (with increased levels of urea and creatinine) reported to VHS Multispecialty Hospital, Madhya Kailash Chennai.
- The patients were either under medical management alone or were also undergoing haemodialysis or peritoneal dialysis.

ETHICAL APPROVAL

Ethical clearance was obtained from the Institutional Review Board of Ragas Dental College and Hospital.

SELECTION CRITERIA

INCLUSION CRITERIA

- The study subject should be above the age of 18 years.
- Patient diagnosed with stage 4 and stage 5 kidney diseases.

EXCLUSION CRITERIA

- Subject diagnosed with stage 1 and stage 2 kidney disease.
- Urinary tract infections
- Rhabdomyolysis
- Patients under medication (other than insulin and antihypertensive) that could affect saliva production.
- Smokers
- Alcoholics
- Pregnant women.
- Patients with recent history of hospitalization and infusions

METHODOLOGY

The participants were clearly informed and explained about the study in local language and consent was obtained.

ARMAMENTARIUM REQUIRED

For collection of venous blood

- Syringes
- Blood collection tubes
- Tourniquets
- Antiseptic
- 2×2 gauze or cotton balls
- Sharpe disposal container
- Bandages or tape

For collection of unstimulated saliva

- Antiseptic
- Saliva collection disposal container
- Gauze rolls
- Saliva collection tubes

COLLECTION OF VENOUS BLOOD

After obtaining a written informed consent, a clinical examination of the oral cavity was performed and the case details were recorded on a special clinical proforma. Blood and whole unstimulated saliva samples were obtained. All the samples were collected between 9:00 and 11:00 a.m. to minimize the effect of diurnal variation. In patients undergoing haemodialysis, both the samples were collected prior to dialysis.

SAFETY PRECAUTIONS

- Hands were washed in warm, running water with an antiseptic.
- Gloves were worn during the procedure
- A lab coat or gown was worn during the blood collection procedures.
- Needles and hubs were single use and were disposed in an appropriate sharps container.
- Gloves were discarded in the appropriate container immediately after the phlebotomy procedure.
- All other items used for the procedure were disposed according to proper Bio-hazardous waste disposal policy.
- Contaminated surfaces were cleaned with freshly prepared 10% bleach solution. All surfaces were cleaned daily with bleach.

Phlebotomy procedure

- The necessary equipment appropriate to the patient's physical characteristics was assembled.
- Patients were informed that minimum amount of blood required for testing will be drawn.
- Hands were washed and gloves were worn.
- The patient was positioned with the arm extended to form a straight line from shoulder to wrist.
- Appropriate vein for venepuncture was selected. The large median cubital vein was used.
- The tourniquet was applied 3-4 inches above the collection site.
- The puncture site was cleaned by making a smooth circular pass over the site with the 70% alcohol pad.
- The syringe was taken from the cover, the cap was removed and the bevel was turned up.
- The skin was pulled tightly with thumb or index finger just below the puncture site.
- The needle was held in line with the vein, using a quick, small thrust, the needle was penetrated into the skin and into the vein in one motion. The desired amount of blood was drawn by pulling back slowly on the syringe stopper.

- The tourniquet was released. Gauze pad was placed over the puncture site and the needle was removed quickly.
- Pressure was applied immediately. When bleeding stopped, a fresh bandage, gauze or tape was applied. The drawn blood was transferred into the appropriate tubes.

COLLECTION OF UNSTIMULATED WHOLE SALIVA

The subjects received detailed information about the collection protocol. In this study, unstimulated saliva was used, as stimulation affects quantity, concentration and pH of saliva. Unstimulated whole saliva can be collected with several oral fluid collector devices and commercial devices are also available. For our study spitting method was used.

SPITTING METHOD

- The participants were instructed to refrain from eating and drinking at least 90min before collection and thoroughly rinse mouth with deionised/distilled water prior to the collection.
- They were asked to sit in a comfortable position with eyes open and head tilted slightly forward and to avoid swallowing and oral movements during collection and to pool the saliva in the floor of the mouth and spit every 60 seconds or when they experience an urge to swallow the fluid accumulated. This was done until 2mL of whole saliva was obtained.

- Two mL of whole saliva was obtained under restful conditions, in a sterile graduated container by spitting method.

BIOCHEMICAL ANALYSIS

Preparation of serum

The collected sample was collected in a plain tube and allowed to stay for 1 hour at 37 degree C, to enable clotting of the blood.

The sample was then maintained at 4 degree C overnight to allow the clot to contract.

- The glass tube was then centrifuged at 4000 rpm for 20 minutes at 4 degree C.
- Remove the serum from the clot by gently pipetting off into a clean tube using a glass Pasteur.
- The test tube was then labelled.

Preparation of salivary supernatant

- The collected saliva sample was then transferred into a test tube.
- It was then allowed to settle down for 15- 20 minutes.
- The test tube with saliva was then centrifuged for at 4000 rpm for 15 minutes at 4 degree C.

- The supernatant is then carefully pipetted out into a new clean test tube and was refrigerated at 4 degree C.

Estimation of serum creatinine

Principle: Jaffe's method

Calorimetric estimation of creatinine using the alkaline picrate method.

Creatinine +Picric acid $\longrightarrow$ Creatinine Picrate (orange)

Materials:

A. Chemicals:
    - Serum standard (3mg/dl)
    - Serum sample
    - Picric acid
    - 2.5m NaoH
    - Distilled water

B. Instruments
    - Pipette with different volume capacity
    - Cuvette
    - Water bath
    - Test tubes
    - Aluminium foil

The samples were assayed immediately in automatic analyser (EM360 chemistry analyser with ISE module) using creatinine estimation kit (Swemed diagnostics) by Jaffe kinetic reaction.

STATISTICAL ANALYSIS

SPSS for windows 13.0 (Statistical Package for the Social Sciences, Chicago, IL) were used. Evaluation of results and statistical analysis was carried out using descriptive, correlation and regression analysis. In all the above mentioned tests, $P < 0.05$ was taken to be statistically significant. When the correlation values were between -1 and 0, then it is interpreted as negative correlation and when the correlation values were between 0 and 1, then it is interpreted as positive correlation.

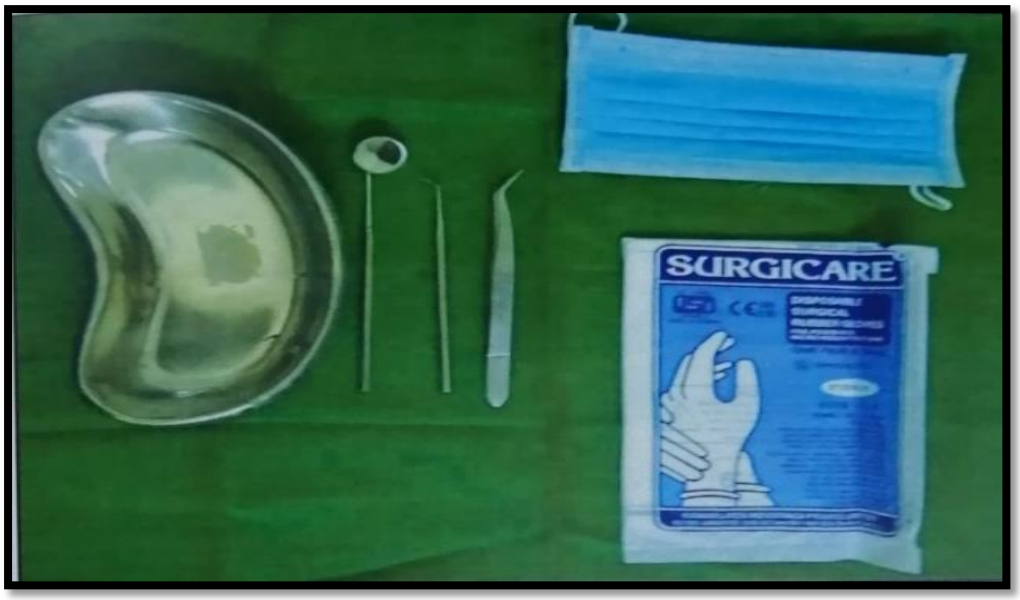


Fig 2: Armamentarium for clinical examination

Fig 3: Phlebotomy procedure for blood collection

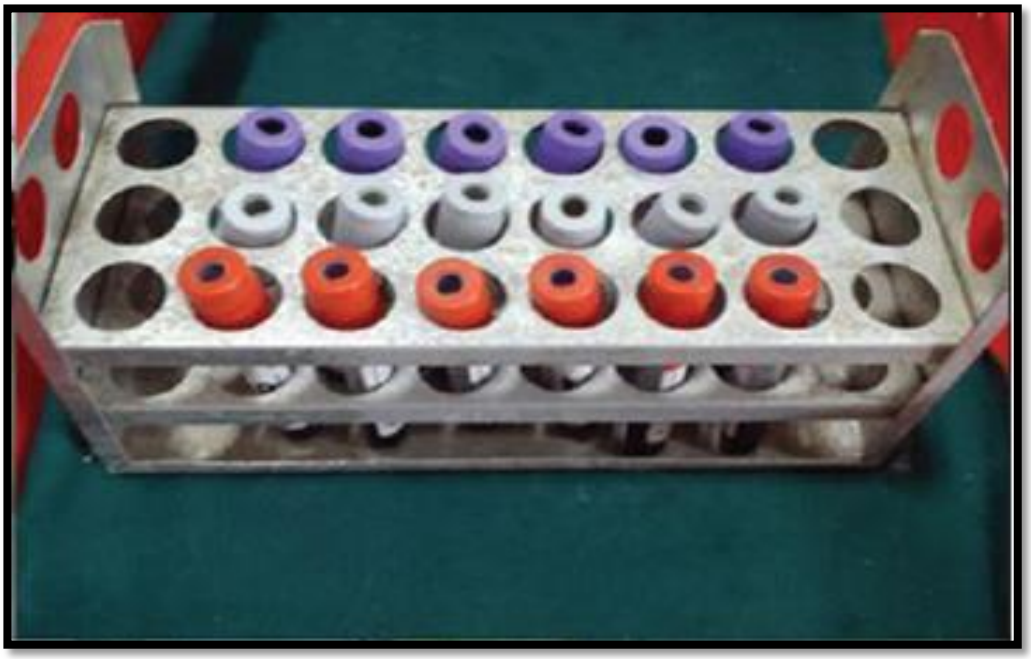


Fig 4: Collection of blood sample for centrifugation

Fig5: Preparation of Blood Sample for Centrifugation

Fig 6: Centrifugation of blood sample

Fig7 : Creatinine Estimation Kit

Fig 8: Centrifugation tube for saliva collection

Fig 9: Centrifugation of the salivary sample

Fig 10: Auto analyser for serum and salivary creatinine estimation

Fig 11: Salivary and serum sample in auto analyser

RESULTS

The present study was conducted in the Department Of Urology, VHS Multi-speciality Hospital, Madhya Kailash, Chennai. The study was conducted from February 2018 to August 2018.

It was devised to compare salivary creatinine and serum creatinine on a total of 50 individuals with stage 4 and stage 5 chronic kidney diseases. In the study population out of 50 individuals, 34 were males and 16 were females. Based on their estimated GFR, 31 patients were classified into stage 4 CKD (GFR: 15–30 mL/min) and 19 patients into stage 5 CKD (GFR: <15 mL/min). Majority of patients being referred to nephrology department were in late stages of CKD and the consecutive patients selected in the present study happened to be in stage 4 and stage 5. Patients with stage 4 CKD were only under medical management without dialysis. Among 19 patients with stage 5 CKD patients, 12 were undergoing hemodialysis and 7 were undergoing peritoneal dialysis along with medical management.

The samples (saliva and serum) were centrifuged and assayed immediately in automatic analyser (EM360 chemistry analyser with ISE module) using creatinine estimation kit (Swemed diagnostics) by Jaffe kinetic reaction. The data obtained from the study were statistically analysed. The

results extracted are compared with various variables included in the study and are presented here.

The descriptive analysis of serum and salivary creatine of subjects with stage 4 and stage5 CKD were obtained and correlated. The ***serum creatinine level ranged between 2.8 and 14.8mg/dL with a mean of 6.8mg/dL (SD 2.3031***) and range of ***the salivary creatinine level was found to be 0.3-2.0mg/dL with a mean of 0.66mg/dL (SD 0.317).***The mean serum and the salivary creatinine concentration were found to be higher in stage 4 and stage5 CKD patients and serum and salivary methods have significant correlation.

Mean salivary creatinine was found to be 10% of the mean serum creatinine level in stage 4 and stage 5 chronic kidney disease patients.

GENDER– WISE DISTRIBUTION OF CASES:

Distribution of salivary creatinine and serum creatinine in the study population of 50 individuals with stage 4 and stage 5 CKD, 34 were males and 16 were females, is analysed by Mann Whitney U Test and the results shows that there is ***high salivary and serum creatinine in males with a mean of salivary creatinine 0.7mg/dl and serum creatinine 7.1mg/dl, whereas in females with a mean of salivary creatinine 0.5mg/dl and serum creatinine 6.2mg/dl and the p value is statistically significant p < 0.000.***

COMPARISON BETWEEN STAGE IV AND STAGE V:

On comparison of stage 4 and stage 5 CKD subjects, by Mann Whitney U Test the results showed with high salivary and serum creatinine in stage 5 CKD subjects with a ***mean of salivary creatinine 0.9mg/dl and serum creatinine 8.3mg/dl whereas in stage 4 CKD subjects with a mean of salivary creatinine 0.5mg/dl and serum creatinine 5.9mg/dl and the p value is statistically significant p < 0.000.***

Therefore, we conclude that there is significance correlation between salivary creatinine and serum creatinine in stage 4 and stage 5 CKD subjects.

Table 1 -Distribution of mean Serum and Salivary Creatinine Levels among Chronic renal failure patients

	N	*Minimum (mg/dl)*	*Maximum (mg/dl)*	*Mean (±S.D)(mg/dl)*
Serum Creatinine level	*50*	*2.8*	*14.3*	*6.892(±2.3031)*
Salivary Creatinine level	*50*	*0.3*	*2.0*	*0.672(±0.3175)*

Table 2: Tests of Normality showing statistically significant differences between salivary and serum creatinine levels

	Kolmogorov-Smirnov[a]			*Shapiro-Wilk*		
	Statistic	*Df*	*Sig.*	*Statistic*	*df*	*Sig.*
Serum	.141	50	.014	.918	50	.002
Saliva	.203	50	.000	.728	50	.000

Table 3: Nonparametric correlations showing statistically significant between salivary and serum creatinine levels

			Serum	*Saliva*
Spearman's rho	*Serum*	*Correlation Coefficient*	*1.000*	*.532***
		Sig. (2-tailed)	*.*	*.000*
		N	*50*	*50*
	Saliva	*Correlation Coefficient*	*.532***	*1.000*
		Sig. (2-tailed)	*.000*	*.*
		N	*50*	*50*

Table 4: ANOVA showing statistically significant differences between the mean salivary creatinine and serum creatinine.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	81.760	1	81.760	22.028	.000
Residual	178.156	48	3.712		
Total	259.917	49			

Table 5 -*Correlation between Serum and Salivary Creatinine levels*

	Salivary Creatinine	
	Spearman's rho (r)	*P –value*
Serum Creatinine	*0.532*	*0.000**

* Correlation is significant at the 0.01 level (2-tailed)

Regression Equation :Y =4.158+4.068X	$R^{2=}$0.315

Figure 1- Correlation between Serum and Salivary Creatinine levels

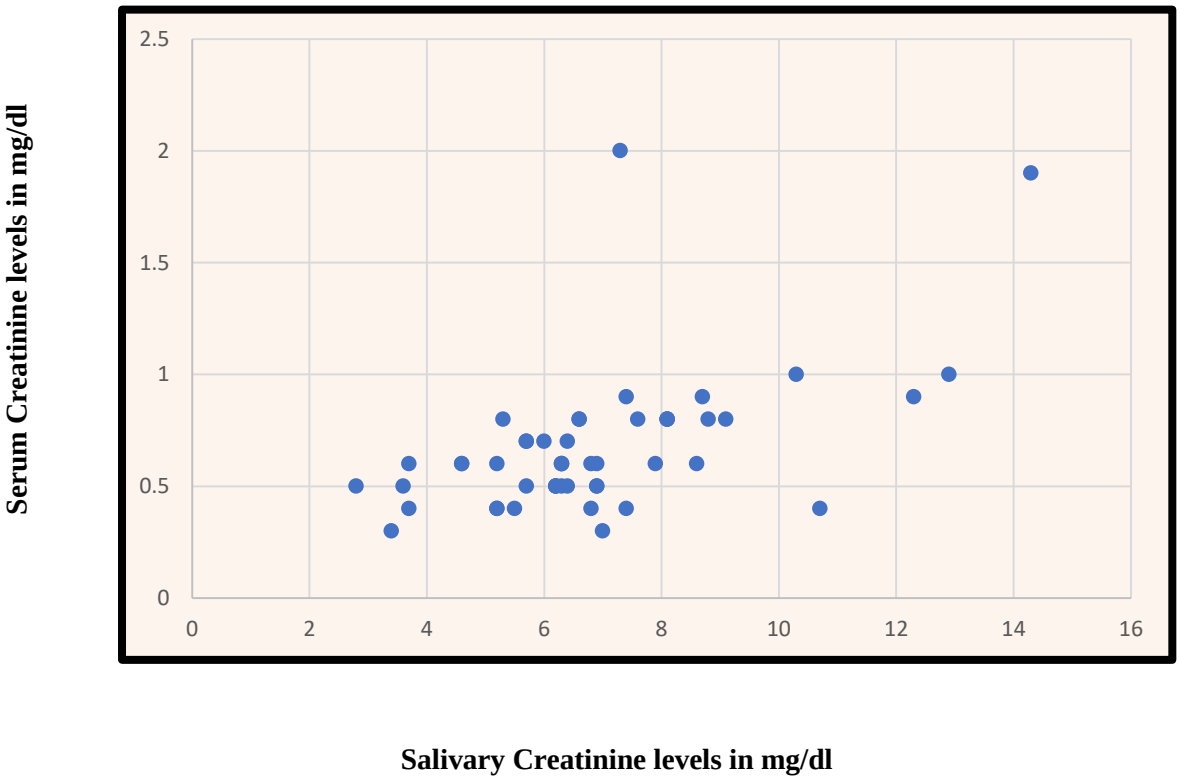


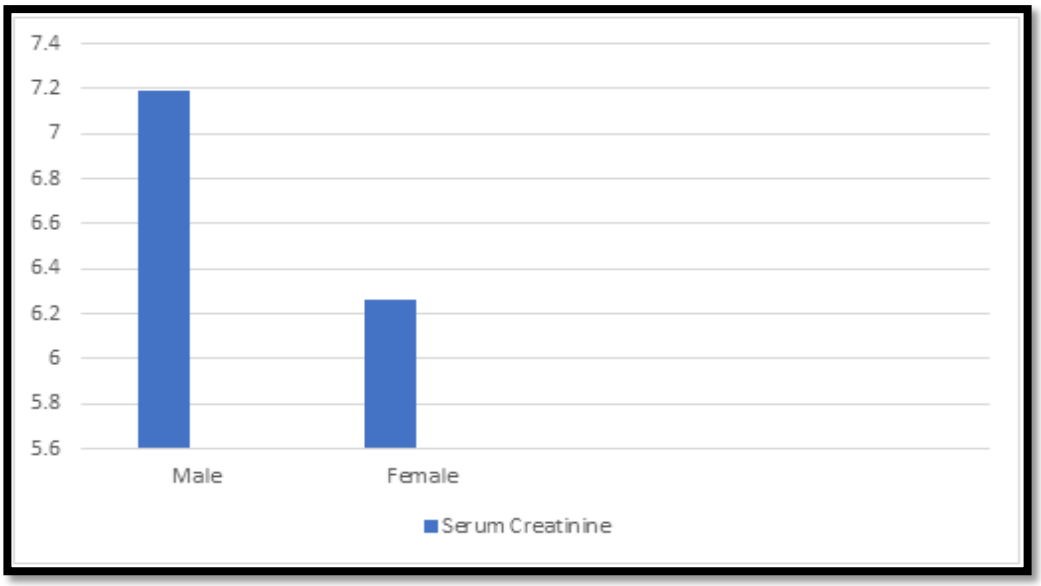
Table 6: Mann-Whitney Test showing statistically significant differences between the means in male and female subjects

Gender	*N*	*Serum Creatinine*	*Salivary Creatinine*
Male	*34*	*7.188(±2.4942)*	*0.709(±0.3630)*
Female	*16*	*6.262(±1.7378)*	*0.594(±0.1731)*
P value		*0.479*	*0.316*

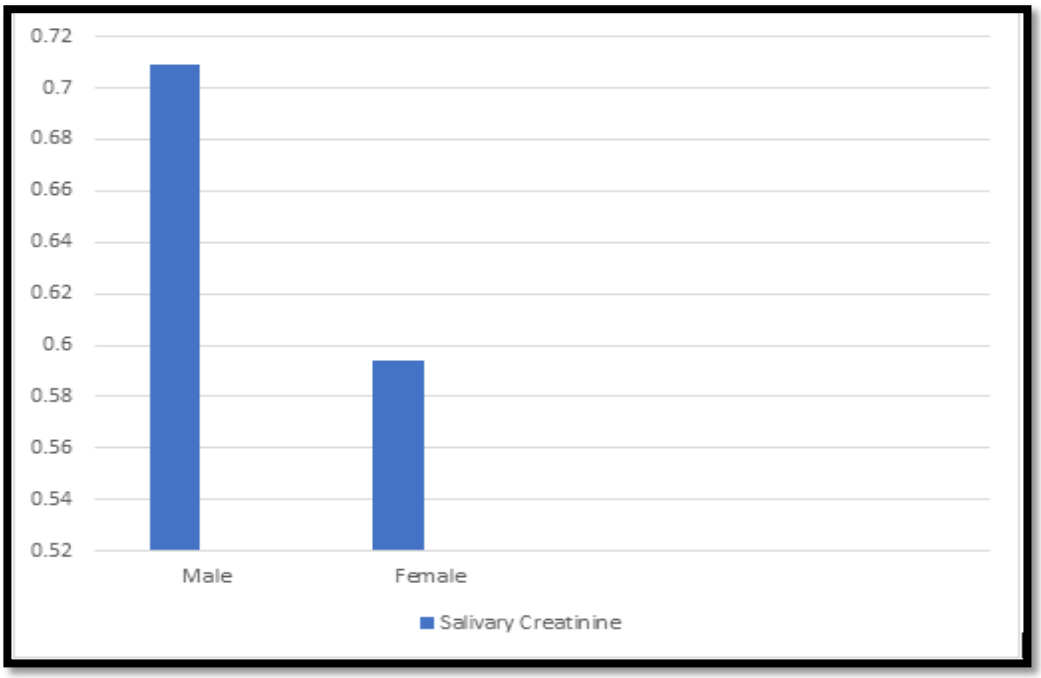
Table 7: Mann-Whitney Test showing statistically significant differences between the means in stage 4 and stage 5 CKD subjects

Stage	***N***	***Serum Creatinine***	***Salivary Creatinine***
Stage IV	*31*	*5.977(±1.6256)*	*0.506(±0.0998)*
Stage V	*19*	*8.384(±2.4989)*	*0.942(±0.3656)*
P value		*0.000*	*0.000*

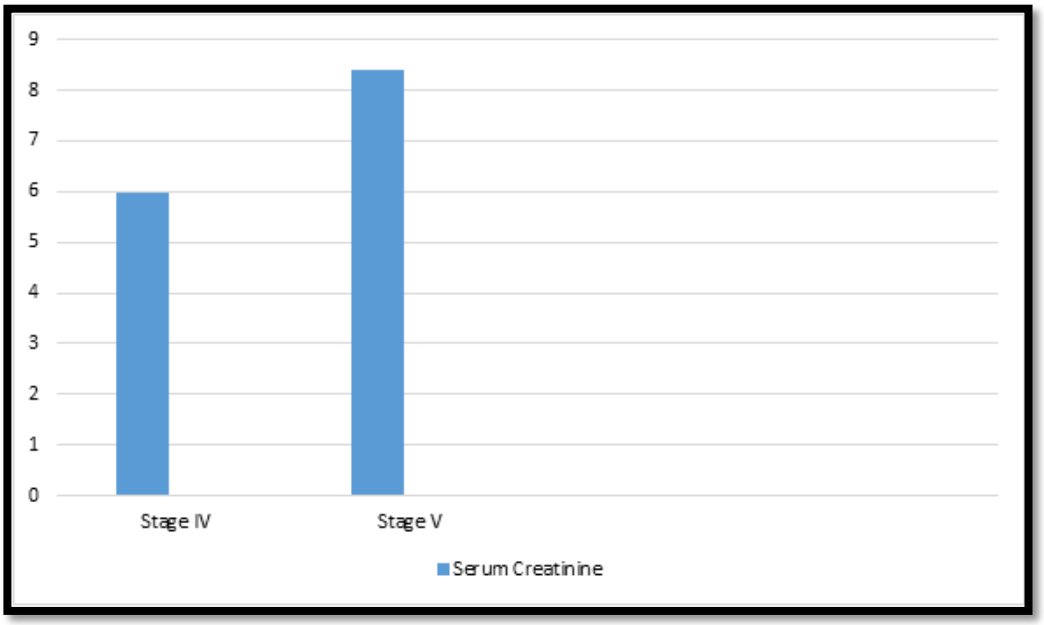
Graph 1: Distribution of serum creatinine in male and female CKD patients



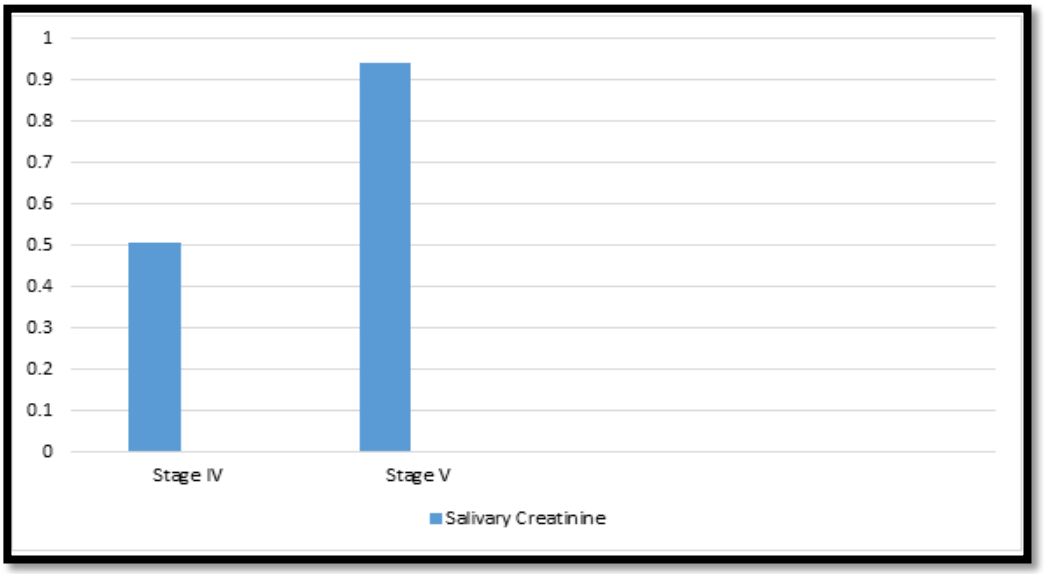
Graph 2: Distribution of salivary creatinine in male and female CKD patients



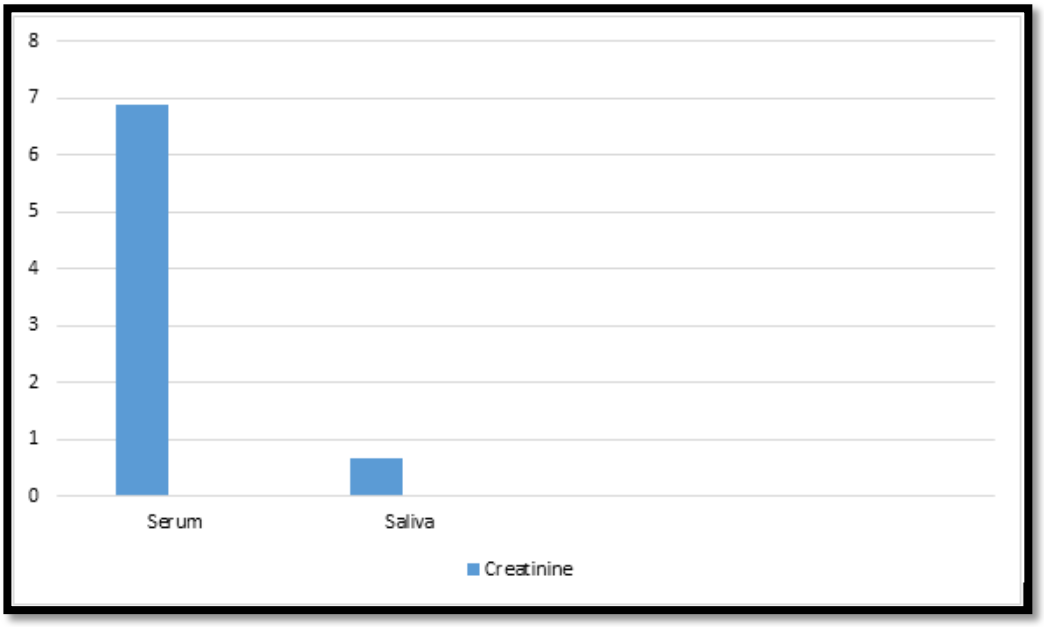
Graph 3: Distribution of serum creatinine in stage 4 and stage 5 CKD patients



Graph 4: Distribution of salivary creatinine in stage 4 and stage 5 CKD patients



Graph 5: Distribution of salivary creatinine and serum creatinine in stage 4 and stage 5 CKD patients



DISCUSSION

Chronic kidney disease is a progressive reduction in renal function. The prevalence and incidence of chronic kidney disease are increasing worldwide. The condition requires frequent serum analysis to diagnose and monitor therapeutic outcomes and to ascertain prognosis.[1]

Creatinine is a waste product of muscle metabolism and is primarily excreted by kidneys. Virtually all the creatinine that is filtered at the glomerulus is excreted without reabsorption in the proximal tubules of kidney. Creatinine levels in the blood are used as an index to renal function. The normal range of serum creatinine is 0.6–1.5 mg/dL and salivary creatinine is 0.05- 0.2 mg/dL.[16, 24]

Saliva, a multi constituent biological fluid secreted by salivary gland, is the major contributor to oral health .It has got a cutting edge over serum because saliva collection is a non-invasive, simple and economic procedure that can be performed by the patient with minimum involvement from the medical personnel. When required, a repeat sample can be easily obtained and is suitable for all age groups. Oral fluid being the 'mirror of body' is a perfect medium to be explored for health and disease surveillance.

This is hospital based study conducted between february 2018 to august 2018.The study was conducted among a total of 50 individuals with stage 4 and stage 5 chronic kidney disease. In the study population out of 50 individuals, 34 were males and 16 were females. Based on their estimated GFR, 31 patients were classified into stage 4 CKD (GFR: 15–30 mL/min) and 19 patients into stage 5 CKD (GFR: <15 mL/min). Majority of patients being referred to nephrology department were in late stages of CKD and the consecutive patients selected in the present study happened to be in stage 4 and stage 5. Patients with stage 4 CKD were only under medical management without dialysis. Among 19 patients with stage 5 CKD patients, 12 were undergoing haemodialysis and 7 were undergoing peritoneal dialysis along with medical management.

In the present study we found significantly high creatinine level in both serum and saliva of CKD patients. Similar observation was made by **Xia et al.[12] and Davidovich et al**.[66] This is because the kidneys are unable to excrete creatinine in renal failure and hence its concentration in blood increases. The increased concentration in saliva may be because of increased serum creatinine which creates an increased concentration gradient which in turn increases the diffusion of creatinine from serum to saliva in CKD patients.[34] It is also possible that saliva may be an attempted alternative route of excretion by the body in a compromised renal function state[40].

In the present study we found mean salivary creatinine was found to be 10% of the mean serum creatinine level in stage 4 and stage 5 chronic kidney disease patients. ***Jonathan E Lloyd***[21] in his study stated that salivary creatinine concentrations are 10-15% of serum creatinine concentrations in healthy populations, which was in accordance with our study.

In the present study we performed a correlation analysis and found a positive correlation between salivary and serum creatinine in CKD patients. Similar observation was made by **Naresh Yajamanam et al**[39].

In the present study we did a correlation analysis for Gender– wise distribution of cases. Distribution of salivary creatinine and serum creatinine in the study population of 50 individuals with stage 4 and stage 5 CKD, 34 were males and 16 were females, the results shows that, there is high salivary and serum creatinine in males with a mean of salivary creatinine 0.7mg/dl and serum creatinine 7.1mg/dl whereas in females with a mean of salivary creatinine 0.5mg/dl and serum creatinine 6.2mg/dl and the p value is statistically significant $p < 0.000$.

In the present study we also did a correlation analysis for stage – wise distribution of cases. On comparison of stage 4 and stage 5 CKD subjects, the results shows that there is high salivary and serum creatinine in stage 5 CKD subjects with a mean of salivary creatinine 0.9mg/dl and serum creatinine 8.3mg/dl whereas in stage 4 CKD subjects with a mean of salivary creatinine

0.5mg/dl and serum creatinine 5.9mg/dl and the p value is statistically significant $p < 0.000$.

Venkatapathy R et al (2014)[63] in his study concluded that serum and salivary creatinine levels were significantly higher in CKD patients than controls which is in accordance with the present study.

Divya Pandya et al (2016) [11] obtained a positive co-relation between salivary creatinine and serum creatinine. The correlation coefficient for serum creatinine and salivary creatinine was 0.976, with p-value <0.001 which is in accordance with the present study.

Lessi et al (2016) [23] in his study found a positive co-relation between serum creatinine and salivary creatinine. He obtained median salivary creatinine levels as 0.20 mg/dL in patients with chronic kidney disease. Salivary levels of creatinine were significantly elevated in chronic kidney disease patients ($p < 0.001$). This is also in accordance with the study.

Creatinine is a large molecule, with high molecular weight (MW 113Da and molecular radius of 3.2 ˚ A) maintained at constant plasma levels by kidneys. They also exhibit low lipid solubility. Thus in a healthy state under

normal conditions owing to its physical properties it is unable to diffuse easily across the cells and the tight intercellular junction of the salivary gland . Hence, a low negative correlation was obtained in controls. But in the diseased state possibly there is an alteration in the permeability of the salivary gland cells.[35] Also the increased serum creatinine levels in CKD patients create a concentration gradient that facilitates increased diffusion of creatinine from serum in to saliva . So, a good positive correlation was obtained in CKD patients.[36]

These findings provide salivary creatinine values above 0.2 mg/dL are more likely to suffer from CKD and must be subjected for further medical evaluation for appropriate management. The results show positive correlation between salivary creatinine and serum creatinine in chronic kidney disease patients.

Thus the results of the present study suggest that the saliva is used as an alternative diagnostic medium for estimating serum creatinine in chronic kidney disease patients.

SUMMARY AND CONCLUSION

The present study titled "Correlation of salivary and serum creatinine estimation in patients with chronic kidney disease " was conducted in the Department of Urology, VHS Multispeciality Hospital, Madhya Kailash, Chennai, to evaluate the role of saliva as a non-invasive alternative to serum creatinine estimation in patients with chronic kidney disease .

The study population comprised a total of 50 individuals among which 34 were males and 16 were females. Based on their estimated GFR, 31 patients were classified into stage 4 CKD (GFR: 15–30 mL/min) and 19 patients into stage 5 CKD (GFR: <15 mL/min). Among the 19 stage 5 CKD patients, 12 were undergoing hemodialysis and 7 were undergoing peritoneal dialysis along with medical management. The stage 4 CKD patients comprising a total of 31 were only under medical management without dialysis.

The study documents the following data:

- The study shows the mean salivary creatinine levels in 50 subjects with chronic kidney disease is 0.6 mg/dL and the mean serum creatinine level is 6.8 mg/dL.
- The p value obtained on comparison of salivary creatinine and serum creatinine of the study subjects was $p < 0.000$ which is statistically significant and less than 0.01%. i.e. 10% level of significance . Therefore we conclude that there is a significance correlation of salivary creatinine and serum creatinine in patients with chronic kidney disease.
- Mean salivary creatinine was found to be 10% of the mean serum creatinine level in stage 4 and stage 5 chronic kidney disease patients.

Gender– wise distribution of cases:

- Distribution of salivary creatinine and serum creatinine in the study population of 50 individuals with stage 4 and stage 5 CKD, 34 were males and 16 were females, the results shows that there is high salivary and serum creatinine in males with a mean of salivary creatinine 0.7mg/dl and serum creatinine 7.1mg/dl whereas in females with a mean of salivary creatinine 0.5mg/dl and serum creatinine 6.2mg/dl and the p value is statistically significant $p < 0.000$.

Stage – wise distribution of cases:

- On comparison of stage 4 and stage 5 CKD subjects, the results shows that there is high salivary and serum creatinine in stage 5 CKD subjects with a mean of salivary creatinine 0.9mg/dl and serum creatinine 8.3mg/dl whereas in stage 4 CKD subjects with a mean of salivary creatinine 0.5mg/dl and serum creatinine 5.9mg/dl and the p value is statistically significant $p < 0.000$.

This study was an effort to enumerate the advantage of saliva as a non-invasive diagnostic fluid in chronic kidney disease patients. Salivary creatinine can be used as an alternative to serum creatinine for diagnosis and monitoring patients with CKD. Saliva collection is a non-invasive method for obtaining diagnostic fluid in patients with CKD, and can reduce the anxiety and discomfort associated with blood collection procedures and also increases their willingness to undergo frequent health inspections that will greatly increase the opportunity to monitor their general health over time and to diagnose morbidities in the early stage. Sampling saliva instead of blood is suitable for all age groups and also reduces the occupational risks to laboratory personnel.

In conclusion, we suggest that saliva can be used as a non-invasive diagnostic tool for estimating serum creatinine in chronic kidney disease patients.

The present involved a small sample size and needs to be confirmed in larger longitudinal population studies. Further research can be directed at all stages of CKD and healthy controls thus laying the foundation to enumerate the role of saliva analysis in the diagnosis and treatment.

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ANNEXURE I

CASE SHEET

RAGAS DENTAL COLLEGE & HOSPITAL

2/102, EAST COAST ROAD, Uthandi, Chennai – 600119

DEPARTMENT OF ORAL MEDICINE & RADIOLOGY

CORRELATION OF SALIVARY AND SERUM CREATININE ESTIMATION IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Date:

S.No :

OP.No :

Study Group : Stage 4 And Stage 5 Chronic Kidney Disease Patients

Name :

Age/Sex :

Address :

Phone number :

Occupation :

Monthly income :

Past medical /surgical/dental /history :

Personal history :

Provisional diagnosis :

ESTIMATION	**STAGE 4**	**STAGE 5**
Salivary creatinine		
Serum creatinine		

ANNEXURES II

STUDY SUBJECTS

Name	Age	sex	Serum Creatinine	Salivary Creatinine	Stage
Gopi	31	M	6.3 mg/dl	0.5 mg/dl	4
Kalyani	60	F	2.8 mg/dl	0.5 mg/dl	4
Rajasekar	52	M	6.9mg/dl	0.6mg/dl	4
Loganathan	62	M	7.0mg/dl	0.3mg/dl	4
Siva Kumar	52	M	5.7mg/dl	0.7mg/dl	5
Murugesan	50	M	7.3mg/dl	2.0mg/dl	5
Tamilarasi	48	F	8.8mg/dl	0.8mg/dl	5
Lakshmi	53	F	7.4mg/dl	0.9 mg/dl	5
Ravi	45	M	10.7mg/dl	0.4mg/dl	4
Dhilshad Begum	66	F	3.7mg/dl	0.6mg/dl	4
Jayapal	52	M	12.9mg/dl	1.0mg/dl	5
Sasi Kumar	38	M	12.3mg/dl	0.9mg/dl	5
Yuvaraj	35	M	14.3mg/dl	1.9mg/dl	5
Rajendran	57	M	8.6mg/dl	0.6mg/dl	4
Mohan	68	M	10.3mg/dl	1.0mg/dl	5
Shantha Kumari	41	F	3.7mg/dl	0.4mg/dl	4
Gnana Sugumar	60	M	9.1mg/dl	0.8mg/dl	5
Palkia Das	70	M	4.6mg/dl	0.6mg/dl	4
Balaji	41	M	4.6mg/dl	0.6mg/dl	4
Ramuammal	60	F	8.7mg/dl	0.9mg/dl	5
Chandra Sekar	42	M	5.3mg/dl	0.8mg/dl	5
Bala Murugan	44	M	3.4mg/dl	0.3mg/dl	4
Ebinesar	60	M	3.6mg/dl	0.5mg/dl	4
Sasikala	50	F	7.9mg/dl	0.6mg/dl	4
Sabapathy	70	M	6.4mg/dl	0.5mg/dl	4
Selvi	51	F	7.4mg/dl	0.4mg/dl	4

Shalmakhadri	69	F	6.6mg/dl	0.8mg/dl	5
Munirathinam	50	M	6.8mg/dl	0.4mg/dl	4
Murthy	60	M	5.7mg/dl	0.5mg/dl	4
Bhavani	26	F	5.2mg/dl	0.4mg/dl	4
Govindammal	37	F	6.2mg/dl	0.5mg/dl	4
Indira	40	F	6.3mg/dl	0.6mg/dl	4
Babu	51	M	8.1mg/dl	0.8mg/dl	5
Shantha Kumari	48	F	5.5mg/dl	0.4mg/dl	4
Sampath	73	M	6.0mg/dl	0.7mg/dl	4
Sekar	64	M	6.9mg/dl	0.5mg/dl	4
Pandiyan	74	M	7.6mg/dl	0.8mg/dl	5
Johnson	50	M	6.4mg/dl	0.7mg/dl	5
Kuppu	60	F	6.8mg/dl	0.6mg /dl	4
Ramesh	57	M	8.1mg/dl	0.8mg/dl	5
Parthiban	61	M	6.2mg/dl	0.5mg/dl	4
Kandaswamy	55	M	6.3mg/dl	0.6mg/dl	4
Arnold	48	M	5.7mg/dl	0.7mg/dl	5
Sakthivel	51	M	5.2mg/dl	0.6mg/dl	4
Jayaselvi	49	F	6.3mg/dl	0.6mg/dl	4
Nageshwari	53	F	6.9mg/dl	0.5mg/dl	4
Krishna	59	M	5.2mg/dl	0.4mg/dl	4
Prakash Raj	69	M	6.2mg/dl	0.5mg/dl	4
Mageshwaran	63	M	8.1mg/dl	0.8mg/dl	5
Mannikandan	65	M	6.6mg/dl	0.8mg/dl	5

ANNEXURE III

CONSENT LETTER

I ....., the undersigned hereby give my consent for the performance of the study " CORRELATION OF SALIVARY AND SERUM CREATININE ESTIMATION IN PATIENTS WITH CHRONIC KIDNEY DISEASE ", By Dr. R.GEETHA under the able guidance of Dr. S. Kailasam, B.Sc, M.D.S., Professor and Head,Department of Oral Medicine and Radiology, Ragas Dental College and Hospital,Chennai-600119. I have been informed and explained the procedure and the purpose of the study. I also understand and accept this as a part of the study protocol there by voluntarily, unconditionally and freely give my consent without any fear or pressure in a mentally sound and conscious state to participate in the study.

Witness/Representative: Patient's signature:

Date:

ANNEXURE IV

ஒப்புதல் படிவம்

---------- என்கின்ற நான், சென்னை ராகாஸ் பல் மருத்துவக் கல்லூரி மற்றும் மருத்துவமனையின் வாய் மருத்துவம் மற்றும் ஊடுகதிர் துறையில் பேராசிரியர் **மரு. S கைலாஸம்** (M.D.S) அவர்களின் மேற்பார்வையில், முதுநிலை (M.D.S) பட்டப்படிப்பு பயிலும் **மரு.ர.கீதா** அவர்கள் மேற்கொள்ளும் **"நீர் அழிவு நோய்யால் (நிலை 4 மற்றும் நிலை 5) பாதிக்கபட்டவர்களிடம், உமிழ் நீர் மற்றும் இரத்தத்தில் உள்ள கிரியக்ட்டின் அளவு கணிப்பு மற்றும் ஒப்பீடு கண்டறியும் ஆய்வு"** என்கின்ற ஆராய்ச்சிக்கான பரிசோதனைக்கு என்னை உட்படுத்துவதற்கு எனது மனமுவந்த பரிபூரண சம்மதத்தினை அளிக்கிறேன்.

மேலும் எனக்கு என்னுடைய நோயின் தன்மையைபற்றியும், அதனால் ஏற்படக்கூடிய விளைவுகளைப் பற்றியும் எடுத்துக் கூறப்பட்டுள்ளது எனவும், இந்த பரிசோதனைக்கு நான் எந்தவித அச்சமுமின்றி தன்னிச்சையாகவும், தெளிவான முழு மனதுடன் என்னுடைய பரிபூரண சம்மதத்தினை அளிக்கிறேன் என இதன் மூலம் தெரியப்படுத்துகிறேன்.

இப்படிக்கு

சாட்சியாளர்கள் :

ANNEXURE V

RAGAS DENTAL COLLEGE & HOSPITAL
(Unit of Ragas Educational Society)
Recognized by the Dental Council of India, New Delhi
Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai
2/102, East Coast Road, Uthandi, Chennai - 600 119. INDIA.
Tele : (044) 24530002, 24530003-06. Principal (Dir) 24530001 Fax : (044) 24530009

TO WHOMSOEVER IT MAY CONCERN

Date: 12.01.2019

Place: Chennai

From

The Institutional Review Board,

Ragas Dental College and Hospital,

Uthandi, Chennai - 600119.

The dissertation topic titled **"CORRELATION OF SALIVARY AND SERUM CREATININE ESTIMATION IN PATIENTS WITH CHRONIC KIDNEY DISEASE"**submitted by **Dr.R.GEETHA,** has been approved by the Institutional Review Board of Ragas Dental College and Hospital.

Dr. N. S. AZHAGARASAN, M.D.S.,
Member Secretary,
Institutional Ethics Board,
Ragas Dental College and Hospital,
Uthandi, Chennai – 600119.

ANNEXURE –VI

URKUND

Urkund Analysis Result

Analysed Document:	Correlation of salivary and serum creatinine estimation in patients with chronic kidney disease..docx (D47292140)
Submitted:	1/27/2019 4:07:00 PM
Submitted By:	r_geetha3@yahoo.co.in
Significance:	8 %

Sources included in the report:

https://www.researchgate.net/publication/314118371_Diagnostic_accuracy_of_salivary_creatinine_urea_and_potassium_levels_to_assess_dialysis_need_in_renal_failure_patients
https://www.researchgate.net/publication/233828939_Clinical_significance_of_saliva_urea_creatinine_and_uric_acid_levels_in_patients_with_chronic_kidney_disease

Instances where selected sources appear:

51