ASSESSMENT OF RENAL FUNCTION IN MOTOR COMPLETE SPINAL CORD INJURY PATIENTS – CYSTATIN C AS AN ACCURATE SINGLE MARKER

Dissertation submitted to The Tamil Nadu Dr M.G.R Medical University Chennai, Tamil Nadu in partial fulfilment of the requirements for the M.D Degree Branch XIX (Physical Medicine and Rehabilitation) examination to be held in May 2020

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CERTIFICATE

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DECLARATION

I hereby declare that this dissertation titled **ASSESSMENT OF RENAL FUNCTION IN MOTOR COMPLETE SPINAL CORD INJURY PATIENTS – CYSTATIN C AS AN ACCURATE SINGLE MARKER** is a bonafide work done by me under the guidance of Dr. Henry Prakash, Professor, Department of Physical Medicine and Rehabilitation, Christian Medical College, Vellore. This work has not been submitted to any university in part or full.

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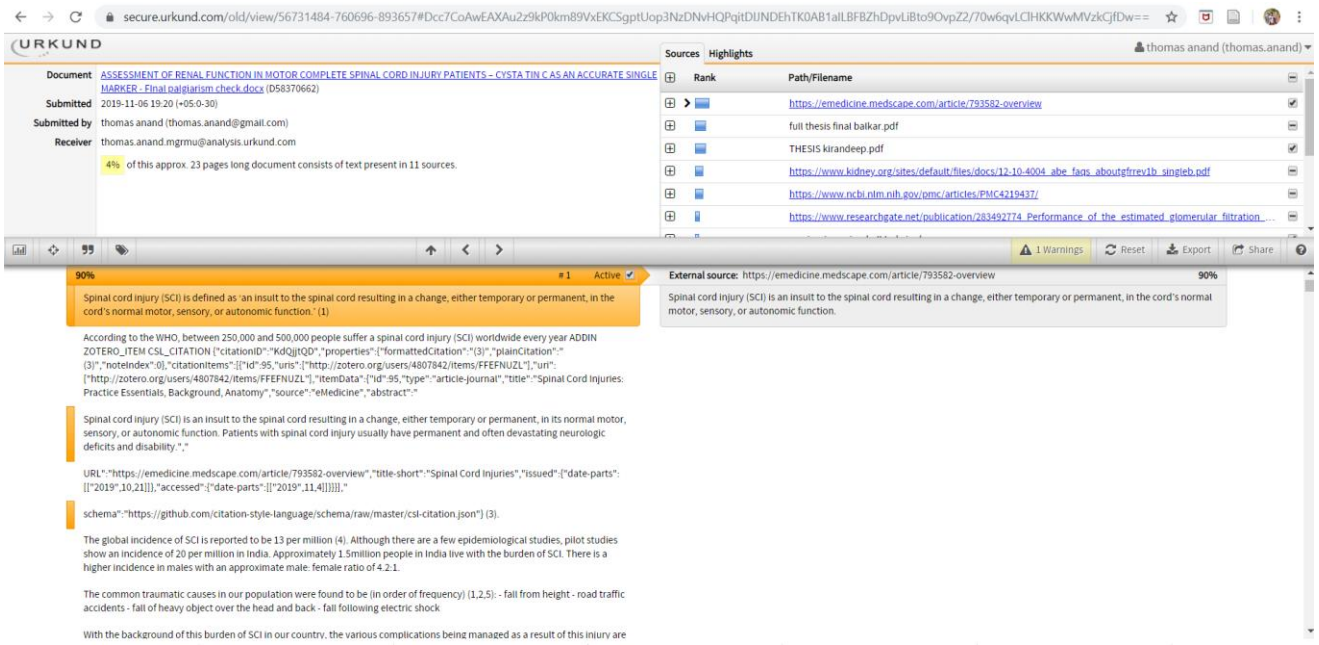
PLAGIARISM CHECK CERTIFICATE:



CERTIFICATE – II

This is to certify that this dissertation work titled **ASSESSMENT OF RENAL FUNCTION IN MOTOR COMPLETE SPINAL CORD INJURY PATIENTS – CYSTATIN C AS AN ACCURATE SINGLE MARKER** of the candidate Dr. Thomas Anand Augustine with registration number 201729054 for the award of MD in the branch of Physical Medicine and Rehabilitation. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows (4%) percentage of plagiarism in the dissertation.

Dr. Henry Prakash

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ABSTRACT

TITLE:

ASSESSMENT OF RENAL FUNCTION IN MOTOR COMPLETE SPINAL CORD INJURY PATIENTS – CYSTATIN C AS AN ACCURATE SINGLE MARKER

Investigators:

Thomas Anand, Henry Prakash, Santosh Varughese, Suceena Alexander, Julie Hephzibah, Mahasampath Gowri. S

Departments- Physical Medicine and Rehabilitation, Nephrology, Nuclear Medicine, Biostatistics

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Background:

Spinal cord injury (SCI) patients with neurogenic lower urinary tract dysfunction develop renal complications as a result of altered urodynamics and recurrent infections. There is a need for monitoring GFR in this population of patients.

At present, Serum Creatinine is used for estimating GFR. Among other factors that alter Serum Creatinine, loss of muscle mass is of interest in the current study.

Aim:

To show that Cystatin C is an accurate single marker to estimate GFR in motor complete SCI patients

Objectives:

- To assess if Cystatin C is an accurate for estimating GFR in patients with SCI with no preserved motor power

- To study if use of Serum creatinine for estimation of GFR SCI significantly overestimates GFR, thereby inaccurate and should be avoided in clinical practice.

Methodology:

Observational Cohort study

30 persons with SCI (ASIA A and B) fulfilling the inclusion criteria were recruited.

Previous studies with spinal cord injury were inclusive of patients with preserved motor function (ASIA C and D). By sampling only motor complete (ASIA A and B) patients, significant overestimate of GFR by Serum Creatinine due to muscle atrophy would be more evident.

Serum Creatinine and Serum Cystatin C values were obtained, GFR was calculated based on currently used formulae. 24 hour urine for urine creatinine clearance was collected and GFR obtained was used as a reference value.

A renal DTPA Tech99m scan was also done for estimating GFR using Gate's method in 10 subjects.

Results:

Analysis with Bland - Altman plot method showed that GFR estimated with CKD EPI formulae from Serum Cystatin C was more accurate than Serum Creatinine, using 24 hour urine creatinine as a reference value. Estimated GFR using Serum Creatinine significantly ($p < 0.0001$) overestimated GFR when compared to 24 hour urine creatinine clearance by a mean difference of 50.6%.

Estimated GFR using Serum Cystatin C showed a meagre mean difference of 0.5% when compared to 24 hour urine creatinine clearance with a p value of 0.91 (closer to 1, thus satisfying the null hypothesis that there isn’t much difference between the 2 methods)

The renal scan GFR by Gate's method showed marked variability with 24 hour urine creatinine clearance in the 10 subjects that were tested. The mean difference in comparison to 24 hour urine creatinine clearance was 29.3 with a p value of 0.0187.

Conclusion:

Serum Cystatin C is an accurate marker for estimating GFR in SCI. Using Serum Creatinine overestimates GFR in SCI, hence inaccurate to use.

Index:

Introduction

Spinal cord injury (SCI) is defined as ‘an insult to the spinal cord resulting in a change, either temporary or permanent, in the cord’s normal motor, sensory, or autonomic function.’ (1)

According to the WHO, between 250,000 and 500,000 people suffer a spinal cord injury (SCI) worldwide every year(3). The global incidence of SCI is reported to be 13 per million (4). Although there are a few epidemiological studies, pilot studies show an incidence of 20 per million in India. Approximately 1.5million people in India live with the burden of SCI. There is a higher incidence in males with an approximate male: female ratio of 4.2:1.

The common traumatic causes in our population were found to be (in order of frequency) (1,2,5):

- fall from height
- road traffic accidents
- fall of heavy object over the head and back
- fall following electric shock

With the background of this burden of SCI in our country, the various complications being managed as a result of this injury are many. There is a need for specific studies and evaluation of various complications that may differ in this population of patients from normal/non-SCI patients. The reason for this is alteration of their normal physiology in relation to sensory, motor, and autonomic functions.

Urinary bladder and renal related complications in this population of patients are significant and require evaluation with periodic monitoring.

Neurogenic lower urinary tract dysfunction in these patients put them at a risk of various complications, either as a direct result of the same or indirectly as a result of their method of bladder management.

SCI patients are at a risk of developing renal failure or chronic kidney disease (CKD) due to overactive/hyperreflexic detrusor which can cause contracted bladder with the risk of hydrouretronephrosis. Recurrent Urinary Tract Infections (UTI)- either due to external drainage devices used or significant residual urine, frequent use of antibiotics (nephrotoxic) without adequate renal adjusted dosing, use of NSAIDs and the risk of bladder/ureteric/renal calculi causing obstructive uropathy are also causes (6,7).

Estimating Glomerular Filtration Rate (GFR), is a widely used method to assess and monitor renal function. The current practice of using Serum Creatinine is questionable in the population of patients with SCI.

Another complication of SCI is significantly decreased muscle mass due to muscle atrophy as a result of longstanding disuse in motor complete SCI patients.

Serum Creatinine is a muscle mass dependant substance and hence will be an inaccurate measure to estimate GFR.

Hence there is a need to use an alternate method for estimation of GFR in this population.

Cystatin C is a 13kDa protein which is secreted in all nucleated cells in the body and has been proven to be a more accurate marker for estimating GFR in normal individuals/non-SCI patients.

There is a need to evaluate its use as an alternative in assessment and regular follow up of renal function.

There have been very few studies in the past concerning renal health in SCI patients and fewer done on its method of evaluation. There have not been any similar done in India to our knowledge.

AIMS and OBJECTIVES:

Aim of the study:

To show that Cystatin C is an accurate single marker to estimate GFR in motor complete spinal cord injury patients.

Objectives of the study:

1)To assess if Cystatin C is an accurate muscle mass independent maker for estimating GFR in patients with Spinal cord injury with no preserved motor power.

2)To study if the current use of Serum creatinine for estimation of GFR in patients with SCI significantly overestimates GFR, hence would be inaccurate and should be avoided in clinical practice.

Hypothesis:

Serum creatinine is muscle mass dependant and hence would significantly overestimate GFR in SCI. Serum Cystatin C is muscle mass independent and hence would be a more accurate measure of GFR in SCI.

LITERATURE REVIEW

SCI and skeletal muscle loss

Spinal cord injury has been known to cause significant skeletal muscle atrophy. (8) (9)

There isn't much data to show the time frame of the influence of spinal cord injury on skeletal muscle. The knowledge on activity of metabolic enzymes and their changes in skeletal muscle of spinal cord injured individuals is little. It was a hypothesis that increased activity augments mitochondrial content and enzyme level might be expected to be reduced after SCI because of disuse. In contrast, spinal transection studies of lower mammals suggested a relative independence of metabolic enzyme content of skeletal muscle and activation.

Michael J Castro et al study the influence of Spinal Cord Injury on skeletal muscle.(9) 12 patients with Spinal Cord Injury were studied and their Vastus Latralis muscle was biopsied at serial intervals. Biopsy was taken once they were clinically stable, which was around 6 weeks, once at 11 weeks and at 24 weeks. A control arm of 9 abled bodied men were studied with biopsies from the same site 18 weeks apart.

Electrical stimulation of the quadriceps femoris muscle was given and signs of fatigue were assessed in these individuals at the same time intervals.

Biopsies were analysed for the following:

- Cross sectional area (CSA)
- Fibre type specific succinic dehydrogenase (SDH) activity
- A-glycerophosphate dehydrogenase (GPDH)
- Myosin heavy chain percent.

CSA declined between 6-24 weeks (*P* 5 0.001), but did not change in able bodied controls.

Average fiber SDH and GPDH activity increased (*P* 0.001) in 6-24 weeks for spinal cord injury patients. There was no change in corresponding values for controls.

This showed that Spinal cord injury was the cause for the change in characteristics of skeletal muscle.

It was found that type I, IIa, and IIax1IIx showed significant atrophy (20-56%) within 6-24 weeks post injury.

There was a 22% decrease in CSA within 6-11 weeks and a 10% decline between 11 and 24 weeks.

The fibre size had reduced by around 60% in SCI patients in comparison to able bodied individuals by 6 weeks post injury.

SDH and GPDH activities increased within 6 months post injury. There was greater force reduction during electrical stimulation of the vastus lateralis.

The study showed that there is a significant change in skeletal muscle within 6 months of spinal cord injury.

There is also evidence to suggest that once muscle atrophy has set in, there isn't much difference between the atrophy at around 2 years and 17 years. Muscle atrophy reached a steady state after a period of time. (8)

H Kern et al showed that the force and size of thigh muscles only minimally differed between long term group of SCI patients (17 +/- 2.6 years) and mid-term group (2.2+/-0.5 years) (8). They studied 10 subjects who had suffered spinal cord injury with UMN type of lower limb features, levels between T4 and T12. Knee extension torque was measured as a marker for force, CT scan was used to measure atrophy and histopathology with electron microscopy for fibre morphology.

Muscle atrophy remains fairly stable once established after a period of time.

Complications of bladder management

UTI remains the most common cause of infection is spinal cord injury patients (10). Other causes include pneumonia, skin and soft tissue infections and blood stream infections. The incidence of UTI is highest among patients with indwelling catheters.

Kyle J. Weld et al studied the effect of bladder management on urological complications in spinal cord injured patients.

They retrospectively studied the different methods of bladder management amongst spinal cord injury individuals and categorised the kind of complications that arose as a result of the method of bladder management. (6)

313 male patients and 3 female patients were studied. Their methods of bladder management were characterised into:

- chronic urethral catheterization (114 cases)

- clean intermittent catheterization (92 cases)

-spontaneous voiding (74 cases)

- suprapubic catheterization (36 cases)

Complications noted were:

- infection – epididymitis, pyelonephritis

- stone disease (upper tract stones and bladder stones)
- urethral complications (periurethral abscess, urethral stricture disease).

Assessment of complications:

Fever, costovertebral angle tenderness and urine cultures positive were used to define the presence of pyelonephritis.

Fluoroscopic urodynamic studies were used to determine vesicoureteral reflux.

Renal ultrasound was used to determine moderate or severe hydronephrosis, renal scarring or renal size less than 8 cm.

Mercaptoacetyltriglycine renal scans were used to assess renal upper tracts

398 complications were recorded.

The distribution of the complications based on the type of method used were as follows:

- Indwelling urethral catheter - 236 complications developed in 61 patients (53.5%)

- Clean intermittent catheterization - 57 complications in 25 (27.2%)

- Spontaneous voiding - 57 complications in 24 (32.4%)

- Suprapubic catherization - 48 in 16 (44.4%)

The complications observed were as follows:

-Infectious complications: Epididymitis – 51 (16.1%)

Pyelonephritis – 11 (3.5%)

UTI – 94% patients were treated atleast once for UTI

- Upper tract stones – 111 (35.1%)
- Bladder stones – 46 (14.6%)
- Urethral strictures - 37 (11.7%)
- Periurethral abscesses - 9 (2.8%)
- vesicoureteral reflux in 50 patients (15.8%)
- upper tract abnormalities - 83 (26.3%)

The choice of bladder management for patients with spinal cord injury maybe based on convenience, comfort or maximum independence.

The safest method for bladder management remains Clean intermittent catheterisation with the least urological complications. Nevertheless, all methods are associated with the aforementioned complications.

All the complications mentioned are of relevance to this study and the need for regular monitoring of renal function in spinal cord injured individuals.

Of the complications observed, Urinary Tract Infections (UTI) seems to cause the largest burden of complications in SCI. (11) (6)

In this context, Ruz et al studied the epidemiology and risk factors for urinary tract infection in patients with spinal cord injury. (11)

128 Spinal cord injury patients were prospectively followed for 38 months (between October 1993 and December 1996 during the acute period of spinal cord injury) and data was collected:

- demographic characteristics - sex, age, days of hospitalization, time of evolution after spinal cord injury, co-morbidity

- injury type - neurological level, etiology, lesion degree and quantification on the American Spinal Injury Association scale (12)

- ADL – Functional Independence Measure (FIM) scale

- associated factors

- methods of urinary drainage

- bladder type

- urological complications

- predisposing factors of each infection episode

Inclusion criteria were acute spinal cord injury 60 days or less in duration before study enrolment, neurogenic bladder dysfunction, age older than 18 years and injury below C4 level.

Exclusion criteria were failure to comply with the protocol and overall hospitalization less than 120 days.

Urological complications noted were lithiasis and vesicoureteral reflux and UTI related factor was duration of injury.

Predisposing factors considered were:

- surgery
- invasive procedures - inserting, removing and replacing an indwelling catheter without prophylactic antibiotics
- immunosuppression
- previous antimicrobial treatment

Antimicrobial treatment and voiding method were documented.

Type of bladder dysfunction was noted:

- hyporeflexia or normoreflexia
- detrusor hyperreflexia
- external sphincter synergy or dyssynergia
- good or poor open bladder neck.

Total WBC counts from blood and Urine cultures were performed once in 10 days

Urinary tract infection was defined as:

- colony count of 10^5 colony-forming units per ml. or greater without fever
- + 2 symptoms: lower abdominal pain, bladder over distention, autonomic hyperreflexia, increased spasticity, increased urinary incontinence and/or increased sweating and malaise.

  Bacteriuria was defined as - colony count of 10^5 colony-forming units per ml. or greater without fever or any of the previously mentioned symptoms.

The following observations were made:

A total of 128 SCI patients were studied: 100 men and 28 women.

Mean age group was between 29.86 to 34.89 years old (median age plus or minus standard deviation 32.41 ± 14.52). They were hospitalized an average of 19 days post injury.

*100 person-days = 100 persons followed for 1 day who were free of urinary tract infection or bacteriuria throughout the day

Overall incidence for *bacteriuria*: 2.72/100 person days, accumulated incidence was 98%.

Based on method of drainage:

- male indwelling - 5/100 person-days (odds ratio 2.7)
- clean intermittent - 2.95 (odds ratio 1.16)
- condom - 2.41 (odds ratio 0.46)
- female suprapubic catheterization - 0.96 (odds ratio 0.06)
- normal voiding with an incomplete lesion - 0.33/100 person-days

For *Urinary Tract Infection:* 0.68 episode per 100 person-days - accumulated incidence was 78%

- male indwelling - 2.72/100 person-days (odds ratio 7.77)
- Clean intermittent - 0.41 (odds ratio 0.42)
- Condom - 0.36 (odds ratio 0.24)
- female suprapubic catheterization - 0.34 (odds ratio 0.04)
- normal voiding - 0.06/100 person-days (odds ratio 0.04)

Thus, indwelling catheter was again proved to be associated with the highest risk of both bacteriuria and UTI.

The most common organism that caused UTI was Escherichia coli (45%). Other organisms that grew on culture were Enterobacteriaceae (36%), Pseudomonas aeruginosa (15%), Acinetobacter species (15%), Enterococcus (12%), 6% others and 26% multiple strains.

The most commonly used antibiotic therapy used in the cases with UTI was

Among urodynamic abnormalities, a *hyperreflexic detrusor with a poor open neck* and *detrusor-sphincter dyssynergia* had a higher incidence of urinary tract infection. (11)

Chronic Kidney disease in Spinal Cord injury

Chronic kidney Disease or End stage Renal Disease (ESRD) is a chronic complication in Spinal Cord injury due to altered urodynamics, nephrolithiasis, risk of recurrent urinary tract infection and frequent use of antibiotics. It is a complication that is often overlooked and not adequately diagnosed.

There is evidence for kidney disease being a predictor of morbidity in this population of patients (13). Mark M Greenwell et al showed in a study that proteinuria and a creatinine clearance of less than 60ml.min independently increased morbidity in SCI patients.

The prevalence of CKD is alarmingly significant in SCI and hence there is a need to monitor renal function more closely in such patients.

It is a complication that results in high cost of treatment and follow up.

CKD was found to be a strong predictor of mortality in this population of patients and the prevalence of the same has lacked extensive research and study.

The understanding of CKD in SCI is also limited by the fact that the currently used equations for estimating glomerular filtration rate are inaccurate and significantly overestimate kidney function.

As a result, it is likely that CKD may not be accurately diagnosed and may be overlooked in this population of patients. (14)

Fischer et al, studied the prevalence of CKD in patients with spinal cord injuries. Veterans in the US with SCI were the subjects of the study. An MDRD equation which was modified for spinal cord injury was used to estimate GFR.

Spinal Cord injury veterans who were being treated and followed up by The Veterans Health Administration were recruited for this study. It is one of the largest

providers of care to adults with SCI and CKD in the United States.

Veterans who received treatment during the period from January 1st

through December 31st, 2006 were recruited. A total of 9,333 Veterans fit into the inclusion criteria.

An eGFR of less than 60 ml/min/1.73 m2 was defined as CKD. Methods used for calculating eGFR two:

1) MDRD equation
2) MDRD equation that incorporates an empirically derived correction factor SCI which was earlier developed and validated in a cohort of Veterans with SCI.

sociodemographic characteristics like sex, age, marital status, race/ethnicity, smoking status and medical conditions like diabetes, hypertension,

coronary artery disease, dyslipidemia, peripheral vascular disease, depression, CAD, congestive heart failure and CKD were retrieved from administrative data files.

It was found that without the SCI adjustment factor only 10.2% of Veterans with SCI/D fell into the category of CKD (less than 60 ml/min/1.73 m 2).

On the other hand, using the modified MDRD-SCI/D equation, 35.2% had CKD.

Following were the associations or added risk factors found with multivariate analysis in patients with CKD:

- older age
- female sex
- hypertension
- dyslipidemia
- depression
- congestive heart failure
- Nontraumatic SCI had a higher risk than traumatic SCI
- Black race had a decreased risk
- A duration of less than 10 years had a decreased risk when compared to more than 10 years.

This study highlighted 2 things that is of interest to us in this current study:

1) Use of the regular equation for estimation of GFR with Serum creatinine was highly underestimating the burden of CKD
2) The prevalence of CKD in the SCI cohort was a staggering 35.2%

Change in renal pathology in SCI patients with CKD

(15) C H Barton et al studied the changes in renal pathology in paraplegics with end stage renal disease. Renal pathological findings were noted from autopsy material along with relevant clinical data of 21 spinal cord injury patients who had end-stage renal disease (SCI-ESRD) and were being treated with maintenance haemodialysis.

These were compared to clinical and post mortem findings of 43 ambulatory patients who also expired in the same time period and were receiving haemodialysis.

The SCI-ESRD patients showed notably different renal histo-pathological and clinical data when compared to the ambulatory ESRD group.

The major causes of renal insufficiency in SCI ESRD patients were (15):

- Chronic pyelonephritis
- Amyloidosis

Dominant finding

- Acute pyelonephritis
- Papillary necrosis
- Calculous disease
- Pyonephrosis
- Perinephric abscess formation

The findings in *ambulatory* ESRD patients which were not so commonly seen in SCI:

- Hypertension
- Nephrosclerosis
- Acquired cystic disease (ACD)

The predominant cause of death in the SCI-ESRD patients was infection with gram negative sepsis. Urinary tract and infected decubitus ulcers were determined to be the most common source for sepsis in SCI -ESRD.

Death secondary to cardiovascular disease predominated in the ambulatory-ESRD group. It is evident from this study that prevention and control of these infections would lower incidence of renal failure and substantially reduced morbidity and mortality in chronic SCI.

Measurement of renal function – Glomerular Filtration Rate (GFR)

Glomerular filtration rate (GFR) is defined as the volume of plasma that is filtered by the glomeruli per unit of time, and is usually measured by estimating the rate of clearance of a substance from the plasma. (16,17)

Glomerular filtration rate (GFR) denotes the flow of plasma over a specified period from the glomerulus into Bowman's space. It is the chief measure of kidney function. The blood enters individual glomerular tufts through the afferent arteriole and exits through the efferent arteriole. (17)

Within this renal blood flow (RBF), only the plasma can cross the the glomerulus. Hence renal plasma flow (RPF) is a more accurate expression.

Organic and inorganic solutes are freely filtered from within the plasma. They are present in the ultrafiltrate (the fluid in Bowman's space) and plasma at the same concentrations.

- Blood passing through Kidneys - 20% to 25% of the cardiac output (1.0 to 1.1 L/min)
- RPF is roughly 600 to 720 ml per minute
- GFR is around 120 ml per min (180 litres per day)

- urine output - about 1.5 L day
- Reabsorption of 178.5 L occurs in the tubular network.

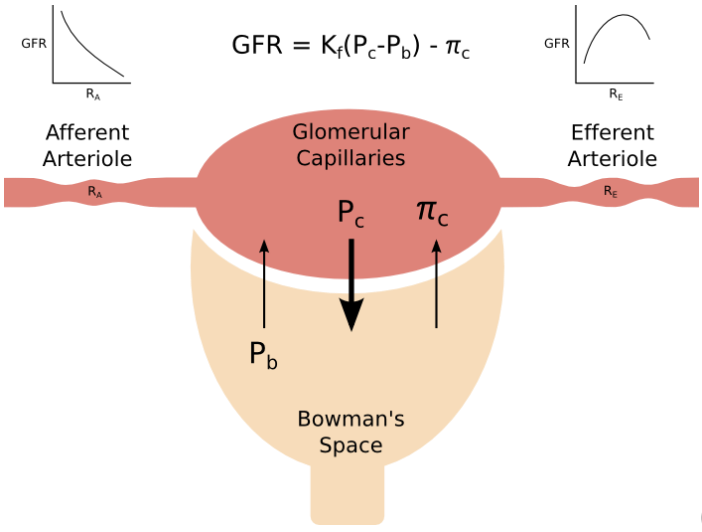
The endothelium of fenestrated capillaries allows only molecules of less than 70 nM to pass through it. The base membrane has a negative charge and hence favours cations and repels other proteins. The podocyte allows for only 14nM to pass through.

Factors that favour filtration in the tubule:

- Capillary hydrostatic pressure (Pc)
- Bowman's space oncotic pressure (πi)

Factors preventing filtration:

- Bowman's space hydrostatic pressure (Pi)
- capillary-oncotic pressure (πc)



(18)

Clearance of a particular solute is the plasma volume from which that particular solute is removed per unit time and is expressed in ml per minute.

It can be calculated for any substance by the formula- [urine concentration] x (urine flow rate) / [plasma concentration], or simply, $C = UV/P$.

For solutes that are freely filtered (not charge/size restricted) and not significantly reabsorbed, secreted, synthesized or metabolized in the kidney, the above method can be used an indicator of GFR (also ml per minute).

Ideally, inulin, a plant polysaccharide that is indigestible and administered exogenously can be used. This requires time to reach a steady state and is an expensive method hence not feasible in the clinical setting.

Alternately, creatinine has been used with this method. It is a breakdown product of creatine phosphate in skeletal muscle. In a healthy adult individual's metabolism (existing in catabolic and anabolic equilibrium), a constant amount of creatinine is released. In this setting, any change in creatinine is due to changes in clearance and hence GFR.

Nonrenal factors influencing plasma creatinine are:

- rapid muscle growth
- strenuous exercise
- endogenous consumption (muscle-building supplements)
- injury to a skeletal muscle (SCI, Rhabdomyolysis, burns)

Hence use of Creatinine as an estimation for GFR in the population of Spinal Cord Injury will be an inaccurate method to use.

Measurement of GFR is important to define and diagnose several pathologies.

Acute kidney injury (AKI/ acute renal failure) is associated with an increase in serum creatinine and is largely reversible. Chronic kidney disease is an irreversible damage and persists for over at least 3 months.

Staging of CKD based on GFR is as follows:

- Stage 1 normal- > 90 ml per minute
- Stage 2 mild - 60 to 89 ml per minute
- Stage 3a mild to moderate- 45 to 59 ml per minute
- Stage 3b moderate to severe - 30 to 44 ml per minute
- Stage 4 severe - 15 to 29 ml per minute
- Stage 5 failure - less than 15 ml per minute

There are many medications that affect GFR.

ACE inhibitors (or angiotensin receptor blockers) and NSAIDs both decrease GFR by different mechanisms.

NSAIDs inhibit synthesis of prostaglandin. PGE2 and PGI2 dilate the afferent arteriole, so NSAIDs constriction of afferent arterioles. Angiotensin II causes constriction of the efferent arteriole, so ACE inhibitors (or angiotensin receptor blockers) cause dilatation of the afferent arteriole. Endogenously produced compounds (Angiotensin II andPGE2) increase the GFR, and exogenous medications decrease the GFR (ACEi or ARBs and NSAIDS).

NSAIDS are medication that are widely used among Spinal Cord Injury patients and is yet another point of interest in relation to this study.

Methods for GFR estimation

Poul Brandt Rehberg published an article in 1926 titled – 'Studies on kidney function.

The rate of filtration and reabsorption in the human kidney', which first introduced creatinine as a marker of GFR (7).

Serum creatinine and its limitations:

As discussed previously Serum Creatinine varies significantly with muscle mass. In the setting of spinal cord injury where there is significant muscle atrophy, this becomes a problem.

In the past, a single measure of plasma creatinine has been used to estimate GFR and this value is used for diagnosis and staging of chronic kidney disease (CKD).

The problem with this is that a person may show a significant increase in plasma creatinine with deterioration in renal function but their serum creatinine value may fall within the reference range.

Factors affecting Serum Creatinine concentrations include:

- Diet
- muscle mass
- age
- gender
- ethnicity

For example, a very lean elderly woman with renal impairment, may have normal plasma creatinine levels despite a low GFR.

A muscular subject may have an abnormally high creatinine despite having a normal GFR.

The characteristics of a good filtration marker should be the following(19) :

- freely filtered by the glomerulus
- not protein bound
- not metabolised by the kidneys
- without any tubular secretion
- physiologically inert

There are only a few substances that fulfil these criteria:

- Inulin - a plant polysaccharide – requires injection of exogenous substance with a complex protocol for collection
- radionuclides - ^{51}Cr-EDTA, ^{125}I-iothalamate, ^{99m}Tc-DTPA, (labour-intensive and too costly for routine use)

These techniques are not suitable as a screening procedure for the detection of CKD.

24 h urine creatinine clearance is regarded as a more sensitive tool for the detection of CKD than a single plasma creatinine measurement.

Yet, it is considered as an inconvenient method due to the following reasons: failure to collect the entire specimen, a timed urine collection and a wide (11%) within-subject variability.

These restrictions can be circumvented in the population of Spinal Cord injury as most of the subjects will be using external devices for bladder management either as indwelling or via clean intermittent catheterisation. Thereby, collection will be complete and can be exactly collected over 24 hours.

Cockcroft and Gault, in 1976, published an equation to predict creatinine clearance based on:

- Weight
- Age
- Height
- plasma creatinine

Although this was helpful, it had many limitations.

It used only a select cohort of mostly hospitalised men (with only nine women), all of whom had CKD. Also, it required weight and height to be provided and hence could not to be directly reported by the laboratory. Despite all the above limitations, it has achieved considerable importance, more through accumulated experience than a solid evidence base. The FDA (US Food and Drug Administration) and other bodies still stipulate that creatinine clearance should be estimated by the Cockcroft-Gault equation for guiding the administration of many drugs.

Cockroft Gault Formula (20):

C_{Cr}= {((l 40–age) x weight)/(72xS_{Cr})}x 0.85 (if female)

C_{Cr} (creatinine clearance) = mL/minute

Age = years

Weight = kg

SCr (serum creatinine) = mg/dL

The **MDRD study** was a multicentre trial studied 1628 patients with CKD to evaluate the effect of dietary protein restriction and blood pressure control on progression of renal disease. It also had an objective of developing an equation that would use plasma creatinine with a better prediction of GFR**.**

A 6-variable equation was initially derived, and subsequently reduced to a 4-variable version with gender, age, plasma creatinine value and race differentiation as white or black was published.

MDRD equation (20)**:**

eGFR = 175 x $(S_{Cr})^{-1.154}$ x $(age)^{-0.203}$

x 0.742 [if female]

x 1.212 [if Black]

The advantage of the MDRD equation over Cockcroft and Gault was that it did not require height or body weight to be supplied and it became the preferred equation.

It was subsequently validated in patients with renal transplant recipients, diabetic and non-diabetic kidney disease and African-Americans with non-diabetic kidney disease.

Given that the MDRD equation was originally derived from a group of CKD patients, its utility for healthy individuals remains unclear, and strictly it has not been validated in children under 18 years of age, in pregnant women, in patients above 70 years of age, and in ethnic groups other than African-American.

It hasn't been validated in pregnant women, in ethnic groups other than African-American in patients above 70 years of age, and in children under 18 years of age.

It also hasn't yet been validated at extremes of body weight, limiting its usefulness in these individuals who are at higher risks of developing CKD.

At low plasma creatinine concentrations, poorer performance of the MDRD formula has been reported.

CKD-Epidemiology Collaboration group (CKD-EPI) Equation:

They developed a new equation in 2009 and validated it. It was designed to match the accuracy of the MDRD equation at GFR <60 mL/min/1.73m^2. It also gave greater accuracy at higher GFR levels, thereby reducing the over-diagnosis of CKD that was reported with the MDRD equation (19).

It was developed from 8254 data points from four clinical populations and six studies. Original serum creatinine values were recalibrated to the Roche enzymatic method.

The CKD-EPI equation was as accurate as MDRD in the subgroup with eGFR less than 60 ml/min/1.73m^2 and substantially more accurate in the subgroup with eGFR more than 60 ml/min/1.73m^2. The initial goal of formulating the equation was to minimise the overdiagnosis of CKD with the MDRD equations which had a tendency to underestimate GFR especial across different racial profiles of people(19). By correcting this problem, health care could be targeted to people who actually had CKD.

CKD – EPI equation (20):

$eGFR = 141 \times \min(S_{Cr}/\kappa, 1)^{\alpha}$

$\times \max(S_{Cr}/\kappa, 1)^{-1.209}$

$\times 0.993^{Age}$

$\times 1.018$ [if female]

$\times 1.159$ [if Black]

Abbreviations:

eGFR (estimated glomerular filtration rate) = mL/min/1.73 m^2

S_{Cr} (standardized serum creatinine) = mg/dL

$\kappa = 0.7$ (females) or 0.9 (males)

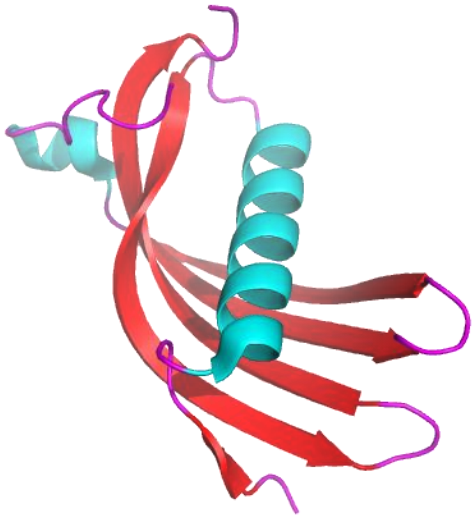
$\alpha = -0.329$ (females) or -0.411 (males)

min = indicates the minimum of S_{Cr}/κ or 1

max = indicates the maximum of S_{Cr}/κ or 1

age = years

CYSTATIN C



(21)

With the above limitations mentioned, there was a need to find an alternate method to accurately. It would need to be one which is not influenced by muscle mass which is of concern in the cohort of subjects being addressed.

With this came the use of a low molecular mass protein – *Cystatin C*

It was initially known as post-g-globulin, inter alia g-trace,

and gamma-CSF. The polypeptide chain amino acid sequence of the single

of human cystatin C was determined in 1981.

Cystatin C was the first sequence of the cystatin super family discovered.

There are a total of 12 Cystatin proteins of which Cystatin C is one of (22).

Family 1

Cystatin A

Cystatin B

Family 2

Intracellular cystatins Extracellular and/or transcellular cystatins

Cystatin C, Cystatin D, Cystatin E

Cystatin F, Cystatin G, Cystatin S, Cystatin SA, Cystatin SN

Family 3

Intravascular cystatins

LMW-kininogen

HMW-kininogen

Cys C is produced in all human nucleated cells and this makes it unique from all the other Cystatins (22). The Cys C gene is of the housekeeping type and hence indicates a stable production.

Studies in large patient cohorts have failed to correlate the serum level of Cys C to any pathophysiological state besides those affecting the glomerular filtration rate (GFR).

Its molecular mass is 13 kiloDalton (kDa)

Normal values of Cystatin C in the human body (22)*:*

Serum Cystatin C: 0.53 - 0.95 mg/dL

	(Mean; range) mg/dL
Blood plasma:	0.96; 0.57–1.79
Urine:	0.095; 0.033–0.29
Saliva:	1.8; 0.36–4.8
Cerebrospinal fluid:	5.8; 3.2–12.5
Amniotic fluid:	1.0; 0.8–1.4
Seminal plasma:	51.0; 41.2–61.8
Milk:	3.4; 2.2–3.9
Tears:	2.4; 1.3–7.4

As a marker for GFR:

It is almost freely filtered in the Glomeruli (94% in comparison to a gold standard - 51Cr-EDTA. 99% is degraded in the proximal tubular cells after passing through the Glomerulus. With the above 2 characteristics and the fact that there is a constant production, it makes Cystatin C an ideal marker for estimating GFR.

It has also been found to be a better GFR marker than the serum levels of the other low molecular mass proteins. These include:

- retinol binding protein
- beta2-microglobulin
- complement factor D

Laboratory methods:

Initially, its concentration was determined by *enzyme-amplified single radial immunodiffusion*. It was slow and required at least 10–20 h, and had a relatively high coefficient of variation (around10%). Later developed the method of *automated and rapid particle enhanced immunoturbidimetric and immunonephelometric*

methods, which are rapid and precise.

CKD – EPI equation (2012) (20):

$eGFR = 133 \times \min(S_{cys}/0.8, 1)^{-0.499}$

$\times \max(S_{cys}/0.8, 1)^{-1.328}$

$\times 0.996^{Age}$

$\times 0.932$ [if female]

Abbreviations / Units

eGFR (estimated glomerular filtration rate) = mL/min/1.73 m^2

S_{cys} (standardized serum cystatin C) = mg/l

min = indicates the minimum of $S_{cys}/0.8$ or 1

max = indicates the maximum of $S_{cys}/0.8$ or 1

age = years

Factors affecting Cystatin C:

There have been reported only 2 conditions that alter physiology causing change in serum levels of Cystatin.

1) *High dose Glucocorticoids* (22,23):

   Very large doses of glucocorticoids increase the production of cystatin C. Medium and low doses of glucocorticoids do not alter its production.

2) *Thyroid dysfunction* (22,24):

   Even mild thyroid dysfunction affects Cystatin C values.

   Hypothyroid states have lower values and Hyperthyroid states have higher Cystatin C values when compared to Euthyroid states. This is contrary to the change seen with Serum Creatinine values.

Other populations in question are the following (22):

- Elderly and Children: it is found to be a superior marker of renal function than most other methods in comparison to plasma clearance with a Gold standard.
- Pregnancy: requires more studies to establish its accuracy
- Post renal transplant: is still under evaluation but shows good results thus far.

RADIOPHARMACEUTICALS FOR ASSESSING GFR: (25)

The radiopharmaceuticals used for assessing renal function and anatomy is grouped into 3 broad groups: 1) filtration via the glomerulus, 2) those retained in the renal tubules via proximal tubule receptor-mediated endocytosis from the glomerular filtrate, 3) secreted via renal tubules by the organic anion transporter.

99mTc-Diethylenetriaminepentaacetic Acid

(DTPA) (Glomerular Filtration):

99mTc-DTPA is the only radiopharmaceutical that can be used to measure GFR as its the only radiopharmaceutical in its category which is available for routine imaging that is purely filtered by the glomerulus. The extraction fraction is relatively low as compared to extraction fraction of tubular tracers (41%–86%).

51Cr-Ethylenediaminetetraacetic Acid (EDTA)

(Glomerular Filtration):

51Cr-EDTA is a nonimaging radiopharmaceutical used to measure GFR through plasma sampling technique.

125I-Iothalamate (Glomerular Filtration):

125I-iothalamate is used to measure GFR through plasma sampling techniques. Not useful for renal imaging as it does not emit a photon of sufficient energy.

123I- and 131I-Orthoiodohippurate (OIH) (Tubular Secretion):

123I- and 131I-OIH are cleared via the proximal tubules and a small part is by the glomeruli. The clearance of OIH is approximately 500–600 mL/min in subjects with normal kidneys. 131I- and 123I-OIH are now replaced by 99mTc-mercaptoacetyltriglycine (MAG3).

99mTc-MAG3 (Tubular Secretion):

99mTc-MAG3 is highly protein-bound. It accumulates in the proximal tubular cells and is then transported into the tubular lumen through organic anion transporters located on the apical membrane or on the basolateral transporter. 99mTc-MAG3 has extraction fraction of 40%–50%, more than twice that of 99mTc-DTPA. Hence it is preferred over 99mTc-DTPA in patients with suspected obstruction and impaired renal function and is used in approximately 70% cases.

99mTc-Dimercaptosuccinic Acid (DMSA) (Cortical Retention)

99mTc-DMSA has cortical binding and imaging agent. It is used mainly in pediatrics to evaluate relative function, pyelonephritis, and renal scars.

99mTc-L,L- and D,D-Ethylenedicysteine (EC) (Tubular secreation):

These renal radiopharmaceuticals have clearances slightly higher than 99mTc-MAG3. Though 99mTc-D,D-EC is cleared faster than 99mTc-L,L-EC, 99mTc-L,L-EC was first described and is available as a kit formulation.

99mTc-(CO3)Tricarbonylnitriloacetic Acid (NTA) (Tubular Secretion):

Tc-(CO3)NTA is a newer renal radiopharmaceutical and has renogram curves and clearance equivalent to those of 131I-OIH. It is transported by the organic anion transporter 1. Preliminary studies have shown that unlike 99mTcMAG3, in patients with chronic kidney disease there is no evidence of gallbladder or gut activity.

99mTc-Glucoheptonate (GH) (Cortical Retention and GFR):

99mTc-GH is cleared primarily by glomerular filtration, but approximately10%– 15% of the injected dose is retained in the renal tubules, allowing delayed, high-resolution static images to be obtained (28). 99mTc-GH tends to be used for static imaging if 99mTc-DMSA is unavailable; because of the parenchymal retention, it should not be used for diuretic renography.

Radiopharmaceuticals for Renal PET PET provides high spatial resolution, sensitivity, and quantitative accuracy. Several PET radiopharmaceuticals are available for functional renal imaging; others are under development, but they are currently limited to research applications and were discussed in a previous review.

Plasma Sample Clearances

Plasma concentration of the tracer is maintained at a constant level by a constant infusion technique and timely urine collection is done for measuring renal clearances. Subcutaneous injection is a variant of this technique uses; however, both are expensive, cumbersome, time-consuming, and limited to research applications. Alternative to this approach is substituting the plasma clearance for the renal clearance which avoids the necessity for urine collections. The single-injection, 2-compartment model of the plasma disappearance curve is used to obtain plasma clearance. The plasma disappearance curve is characterized by multiple blood samples obtained over 90 min for tubular tracers but requires a longer time for GFR tracers because they have slower clearance. Simpler techniques based on 1 or 2 plasma samples have been developed to estimate the multisample clearance. These techniques generally give reliable results and can be performed at the time of a standard renal scan using 99mTc-MAG3 or 99mTc-DTPA, and optimization of chemotherapy to an individual patient's underlying renal function. Plasma sampling techniques in cases of ascites, marked edema, or a large effusion can be erroneous as the tracer can diffuse into these extra fluid spaces and plasma clearance will not provide an accurate measure of renal clearance.

Camera-Based Clearances

Camera clearance methods do not require blood or urine samples and have been developed for 13II-OIH, 99mTc-DTPA, and 99mTc-MAG3 (63–67). The principle behind this is that the initial tracer accumulation by the kidneys is proportional to the renal clearance. At a defined period shortly after injection, camera-based techniques determine the tracer accumulation (counts) in the kidneys and divide by the counts injected to obtain a percentage injected dose in the kidneys. Using a validated nomogram, this is converted to a clearance measurement. For monitoring changes in renal function, camera-based clearances are reproducible and *superior to creatinine clearance*.

They however, have their own sources of error; to obtain the percentage injected dose in each kidney, kidney counts have to be corrected for background, infiltration, attenuation, and renal depth. Two common sources of error are estimation of renal depth and background subtraction.

For this study, camera-based clearance of 99mTc-DTPA was used in 10 subjects. Due to the limitations already mentioned above and the fact that it has not been validated in our population, it was not done on all the patients. During mid-term analysis it was also noted that there was a marked variability in its results.

GFR estimation in spinal cord injury

With the aforemention background of a valid need for monitoring GFR in SCI and with the limitations described with its evaluation, it becomes essential to explore and describe a more precise and validated tool.

Few studies have been done to establish the impact of SCI on Serum Creatinine levels and its use for estimation of GFR (7,26–30). Only 3 sudies have evaluated the usefulness of Cystatin C in this population (26,27,30) and only 2 have used an accepted gold standard for GFR measurement used for comparison(26,30)

Although Cystatin C has been established as a more accurate marker in other populations, its relevance and importance in SCI patients has not been adequately studied.

There have been reports of an initial increase in Serum Cystatin C levels in acute spinal cord injury(31). Its importance in this study is the effect of muscle atrophy in chronic SCI and its accuracy in this setting.

The studies done in the past have limitations. The use of a gold standard for comparison, definition of the population of SCI patients used and numbers that were studied are a few to state.

As described earlier, the use of 24-hour urine creatinine clearance has been accepted as a mode of estimating GFR in SCI patients. Unfortunately, it is a time-consuming procedure and hence requires a faster alternative. The advantage in this population is a better accuracy in 24-hour urine collection that can be obtained in comparison to normal individuals.

In a study published in 2002, titled *Cystatin C for estimation of glomerular filtration rate in patients with spinal cord injury***,** Margaret A Jenkins et al studied 64 spinal cord injury patients in Australia(27).

They compared estimated GFR from serum Creatinine and serum Cystatin C to 24-hour urine creatinine clearance. They compared these values also to non-SCI patients and studied if there was a difference in the correlation seen.

28 of these patients were paraplegic and 38 were tetraplegic.

Results showed a better correlation of GFR estimated from Serum Cystatin C with 24-hour urine creatinine clearance than Serum Creatinine. There were fairly similar values in the non-SCI group suggesting that the issue in estimation was with the SCI group.

Their study does not describe the level of injury of the patients studied nor the classification based on the ASIA grading (as to whether the injury was a complete or incomplete injury).

The other observation is the lack of an accepted gold standard for comparison of GFR.

In Denmark, SA Thomassen, studied 24 men and 7 women ASIA A and ASIA B according to ASIA impairment scale (30). They found a significantly better correlation of Cystatin C to the gold standard than Creatinine estimated GFR.

The gold standard used was 51Cr-EDTA-clearance (51chromium-ethylene-diamine-tetra-acetate com-plex) by a multiple plasma sample method.

In 2012, a similar study was done with a similar cohort of patients in the same region by EJ Erlandsen et al (a similar group of investigators as the previous study) (26). 145 patients were studied inclusive of ASIA A, B, C and D.

The results observed were similar and they further went on to develop an equation to strengthen the correlation of Cystatin C estimated GFR with the Gold standard.

The gold standard used for this study was plasma clearance of 51Cr-EDTA.

The equation suggested was:

eGFRCysC = 212 exp(0.914 . CysC).

Such an equation was not possible with the variability that was seen with Serum Creatinine.

Methodology

Setting

The study was conducted in Christian Medical College and hospital, Vellore, situated in the state of Tamil Nadu, India. It is a tertiary care hospital with an average out patient census of about 5000 patients per day and 2500 inpatient beds. Patients recruited were either inpatients or visited the outpatient services conducted by the Department of Physical Medicine and Rehabilitation in CMC. The department has 123 inpatient beds and an average of 150 outpatients per day. Patients were also recruited from the yearly Spinal Cord Injury mela conducted for the local population.

Study design:

Prospective observational cohort study.

34 patients fulfilling the inclusion and exclusion were recruited of which 4 patients did not complete the study and hence were excluded for analysis.

30 patients went through the studied and their data was analysed.

Demographic data like age, sex, marital status, mode of injury, duration of injury etc were collected from patient records.

Height and weight required for calculation were measured.

Height was measured as represented by arm span or length of a bed ridden patient (32).

Algorithm

Inpatient and outpatient SCI patients screened with inclusion and exclusion criteria

⇩

Patients fulfilling inclusion criteria were recruited

Obtained consent after procedure was explained

⇩

- blood sample: Serum Creatinine, Serum Cystatin C and Serum Albumin

⇩

- 24 hour urine collection: to calculate 24 hour urine creatinine clearance (***reference GFR***)

⇩

- Renogram – Technetium Tc^{99m} scan GFR (in 10 patients)

⇩

- Calculated: estimated GFR
  - serum Creatinine eGFR
  - serum Cystatin C
  - 24 hour urine creatinine clearance

⇩

- Analysed: 24 urine creatinine clearance GFR

Vs

-Creatinine eGFR

-Cystatin C eGFR

-Combined Cystatin C + Creatinine eGFR

-Renogram – Technetium 99m scan(done in 10 patients) by Gate's method

Participants

Inclusion criteria:

1) ASIA A and ASIA B according to ASIA Impairment Scale (12)
2) Age 18 years to 60 years
3) More than 6 months post injury

Exclusion criteria:

1) ASIA C and ASIA D
2) Less than 6 months post injury as it may take 6 months for muscle atrophy to set in
3) Known thyroid disease (hypothyroid or hyperthyroid)
4) Age less than 18 years or more than 60 years as that may be an additional factor leading to age related muscle atrophy
5) Pregnancy and breastfeeding mothers (as it may be a relative contraindication for renal scan)

Only ASIA A and ASIA B SCI patients were recruited (12). This was to ascertain that motor complete injury patients were recruited thereby maintaining that the overestimation of Serum Creatinine estimated GFR would be because of significant muscle atrophy which may not be elicited if motor incomplete SCI patients are included in the study.

ASIA A was defined as – complete - no sensory or motor function is preserved in the sacral segments S4-5.

ASIA B was defined as – sensory incomplete - Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-5 (light touch or pin prick at S4-5 or deep anal pressure) AND no motor function is preserved more than three levels below the motor level on either side of the body.

These were definition according to INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY (ISCOS) during the time the study was being performed.

From the recruited patients, blood was collected and tested for the following:

1) Serum Creatinine (mg/dL)
2) Cystatin C (mg/L)
3) Albumin (g/dL)

From the above values estimated GFR was calculated using the following formulae:

Creatinine eGFR:

1) Cockcroft Gault GFR (with Body wt.) – (ml/min/1.73m2) – without using the patient's body surface area. (Creat GFR1)
2) Cockcroft Gault GFR (with Body wt.) – (ml/min) – using the height and weight of the patient to derive body surface area. (Creat GFR2)
3) CKD-EPI creatinine – (ml/min/1.73m2) (Creat GFR3)

Cystatin C eGFR:

1) CKD – EPI - (ml/min/1.73m2) – without using the patient's body surface area. (Cys GFR1)
2) CKD – EPI - (ml/min) – using the height and weight of the patient to derive body surface area. (Cys GFR2)
3) Combined Cystatin C and Creatinine eGFR – ml/min/1.73m2 (Comb GFR)

Considering that the measurement of height and weight of a patient with SCI, we wished to evaluate eGFR using the standard Body Surface Area (BSA).

It also had implications in defining a person with CKD based on GFR.

24 – hour urine creatinine clearance

The patients were given urine collection cans to sample urine over a period of 24 hours. The measure of the 24 - hour urine volume would be accurate as most of them used an external device for bladder management.

GFR was calculated from 24-hour urine collection by the formula

[urine concentration] x (urine flow rate) / [plasma concentration], or simply, C = UV/P. This value was used as a reference value for our study.

Urie sample for UP/UC ratio was collected.

The serum creatinine samples were collected at the end of their urine collection.

The ten patients who had renograms had urine collection done prior to their renograms to prevent exposure to radionuclide.

This method was used as a reference for comparison of GFR.

Athough levels of Serum creatinine in SCI patients would be lower than normal due to muscle atrophy, its clearance in the kidneys measured as urine creatinine levels would remain a marker of GFR when compared their serum levels. (UV/P)

Renogram:

Technitium 99 -Tc ^{99m} DTPA scan using gamma camera imaging was used in 10 subjects. There wasn't a provision to use serial plasma clearance of a radionucleotide for this study.

It does not have much risk of exposure when subjected for testing.

Being exposed to 37- to 370-MBq (1–10 mCi) injection of 99mTc-DTPA ranges from 5% to 70% of the yearly background radiation from cosmic rays and naturally occurring radioactive sources in the environment.

This exposure is less than 5% of the yearly radiation dose considered safe in occupational exposure for personel working in areas with radiation.

There is no risk of allergic or anaphylactic reaction with its administration.

We employed the Gates formula for calculating GFR with the clearance of DTPA based on the Gamma camera images.

3-5mCi of DTPA was injected IV and 40mg (0.5mg-1mg/Kg) was given IV at 20 mins**.**

The patients were required to be careful with discarding their urine for 24 hours after the test following which there would be no risk of exposure to the environment.

This test was done for only 10 patients as it was found that there was marked variability in its results. It was also a time-consuming test which was expensive. It had not been validated in our population of study and hence the remainder of the subjects did not undergo the test.

An ideal gold standard (serial plasma clearance of 51Cr -EDTA or 99Tc – DTP) was not available during the time of the study for comparison but 24-hour urine creatinine clearance is accepted as a good reference value.

RESULTS:

Sample size calculation:

Using the results from a previous study, the estimated GFR (eGFR) calculated from Cystatin C mean (SD) reported was 83.7(19.8). 90% measured eGFR was conferred by eGFR (Cystatin C±30%). So, considering a 30% difference from Cystatin C eGFR, the difference was around 25 units (ml/min).

A sample of *15 subjects* will be sufficient to detect a mean difference of 20 units considering a standard deviation of 25 units, with a power of 80% and a significance level of 5%.

$$N_{pairs} = \frac{\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2}{\Delta^2} + \frac{z_{1-\alpha/2}^2}{2}$$

$$\Delta = \frac{(\mu_2 - \mu_1)}{\sigma} \qquad \sigma = \frac{\sigma_1 + \sigma_2}{2}$$

Where,

μ_1 = mean of Cystatin C

μ_2 = Mean of measured eGFR

σ_1 = Standard deviation of Cystatin C

σ_2 = Standard deviation of measured eGFR

$Z_{1-\alpha/2}$ = Desired confidence level

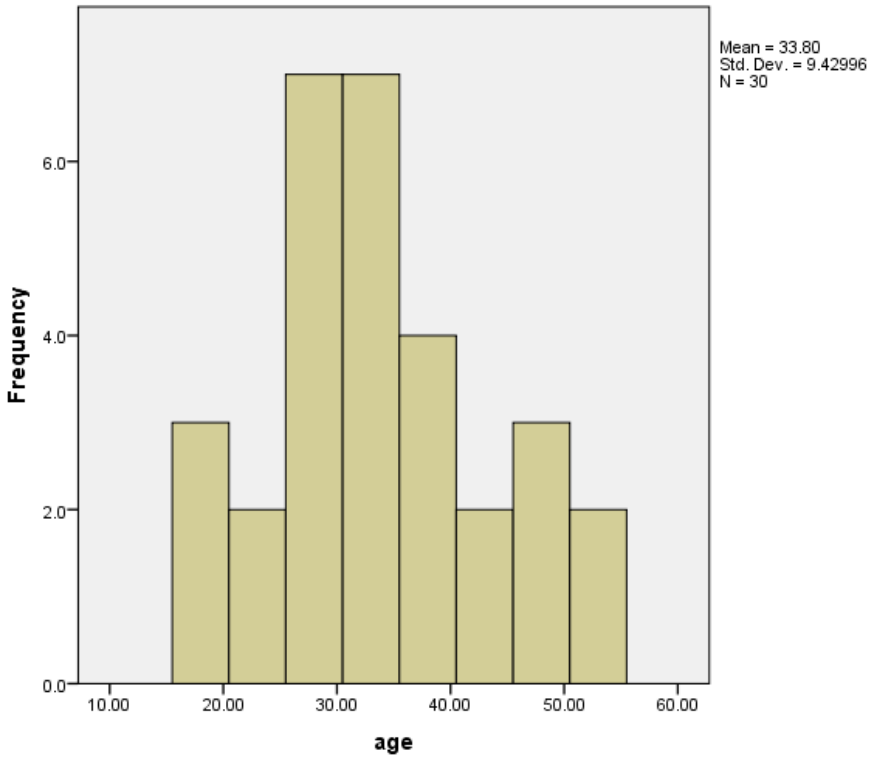
$Z_{1-\beta}$ = Power

A total of 34 patients were recruited and 30 patients completed the evaluation.

Hence analysis was done for 30 patients over a period of 1 year.

Demographic data:

Age: Mean = 33.8 ± SD 9.43, (18-54 years)



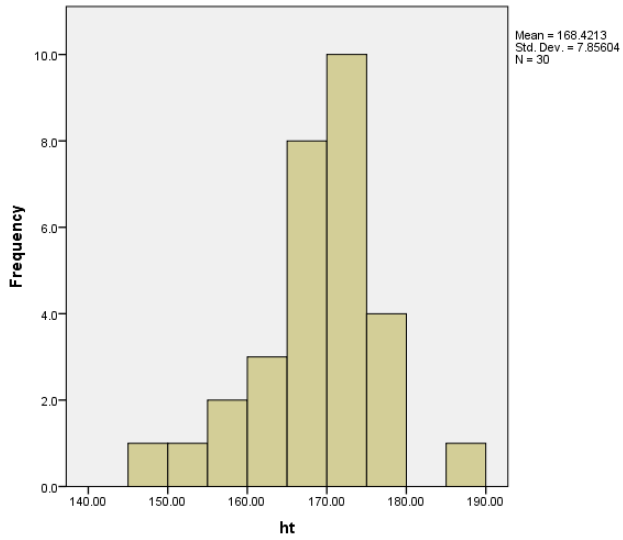
Sex distribution:

Male = 29

Female = 1

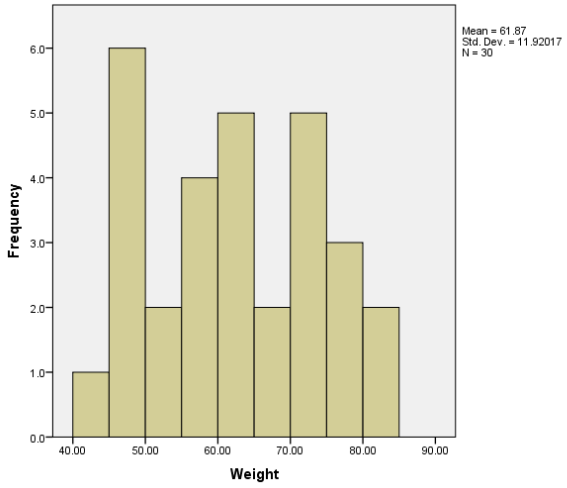
Height distribution:

Mean = 168.45cm ± SD 7.85



Weight distribution:

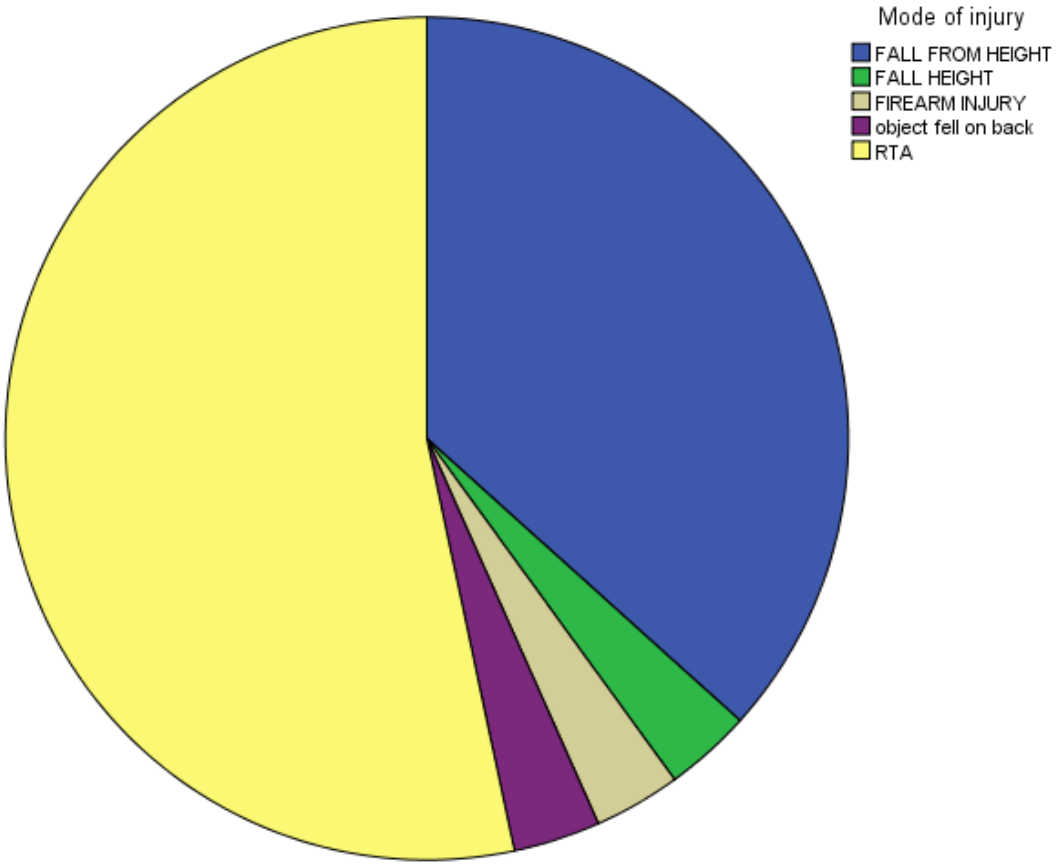
Mean = 61.87 Kgs ± SD 11.92 (41.80 - 82.00 Kgs)



Mode of injury:

Majority of cases were caused by RTA – 53% followed by fall from height – 40%

	Frequency	Percent
FALL FROM HEIGHT	12	40.0
FIREARM INJURY	1	3.3
object fell on back	1	3.3
RTA	16	53.3
Total	30	100.0



Level of injury:

No of tetraplegics: 6

No of paraplegics: 24

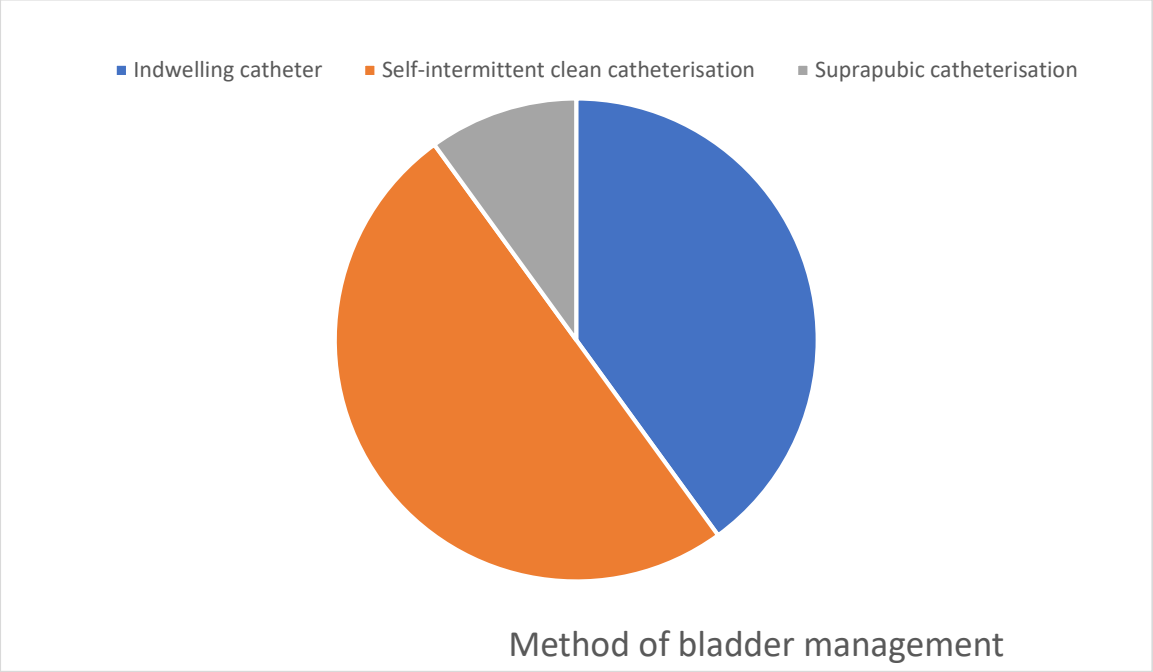
ASIA A: 25

ASIA B: 5

	Frequency	Percent
C 4 ASIA A	2	6.7
C 4 ASIA B	1	3.3
C 6 ASIA A	1	3.3
C 8 ASIA B	1	3.3
C7 ASIA B	1	3.3
L 2 ASIA A	1	3.3
T 4 ASIA A	3	10.0
T 4 ASIA B	1	3.3
T 5 ASIA A	3	10.0
T 6 ASIA A	2	6.7
T 7 ASIA A	1	3.3
T 8 ASIA A	4	13.3
T10 ASIA A	5	16.7
T11 ASIA A	1	3.3
T11 ASIA B	2	6.7
T12 ASIA A	1	3.3
Total	30	100.0

Method of bladder management:

	Frequency	Percent
Indwelling catheter	12	40.0
Self-intermittent clean catheterisation	15	50.0
Suprapubic catheterisation	3	10.0
Total	30	100.0

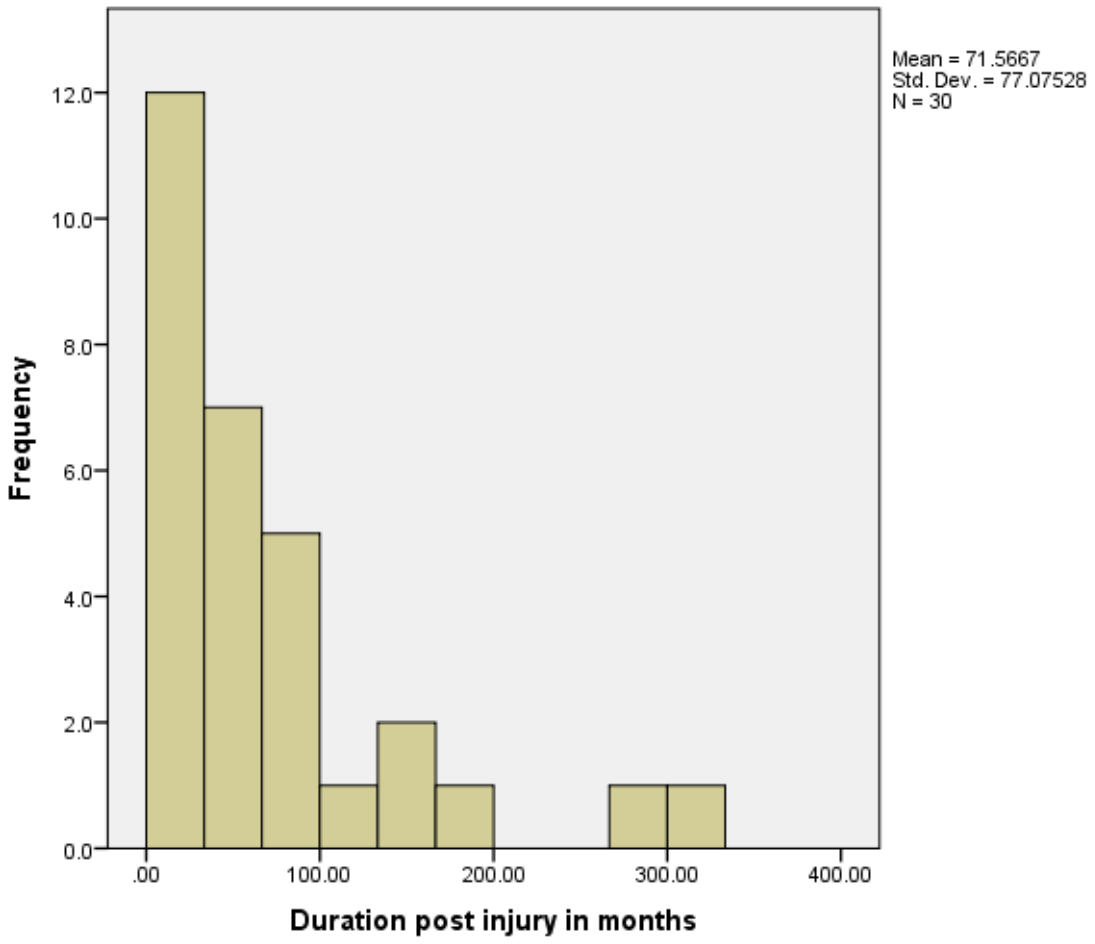


Duration post injury:

Minimum – 8 months

Maximum – 312 months (26 years)

Mean – 71.57 months (around 6 years)



Descriptive Statistics for serum and urine sample values:

	N	Minimum	Maximum	Mean	Std. Deviation
Serum creatinine (mg/dL)	30	.33	3.30	.8720	.56660
Serum Cystatin C (mg/L)	30	.83	2.48	1.1960	.40416
24-hour urine creatinine clearance (GFR) (ml/min)	30	15.79	113.00	79.1737	24.37936
Valid N (listwise)	30				

Descriptive statistics of *estimated GFR*:

	N	Minimum	Maximum	Mean	Std. Deviation
Cr GFR1	30	22.60	173.71	114.9013	33.04011
Cr GFR2	30	22.32	178.61	111.4517	32.35225
Cr GFR3	30	23.21	218.70	108.1120	45.71754
Cys GFR1	30	25.52	111.41	75.7710	22.04437
Cys GFR2	30	23.49	123.35	74.5730	23.87744
Comb GFR	30	22.98	126.21	91.6870	24.22783
24-hour urine creatinine clearance (GFR)	30	15.79	113.00	79.1737	24.37936
Renogram GFR	10	31.00	130.00	96.0000	30.12197

Mean GFR calculated from 24-hour urine creatinine clearance is 79.17 ml/min which is less than the normal level of 90 ml/min

- Cr GFR1 – CKD-EPI creatinine – (ml/min/1.73m2)
- Cr GFR2 – Creatinine- Cockcroft Gault GFR (with Body wt.) – (ml/min/1.73m2)
- Creat GFR3 – Creatinine Cockcroft Gault GFR (with Body wt.) – (ml/min) - using body surface area.
- Cys GFR1 - CKD – EPI Cystatin C - (ml/min/1.73m2)
- Cys GFR2 - CKD – EPI Cystatin C- (ml/min) – using body surface area.

- Comb GFR - Combined Cystatin C and Creatinine eGFR – ml/min/1.73m2

Bland Altman Method for analysis:

The Bland Altman method was first described in 1986 to compare 2 measurement techniques. It has both good reliability and good validity.

A modification of this method in 2008 by Krouwer was used for comparison.

Comparison between the percentage mean difference between GFR estimated by each separate method Vs the reference GFR (which is 24-hour urine creatinine clearance) was analysed.

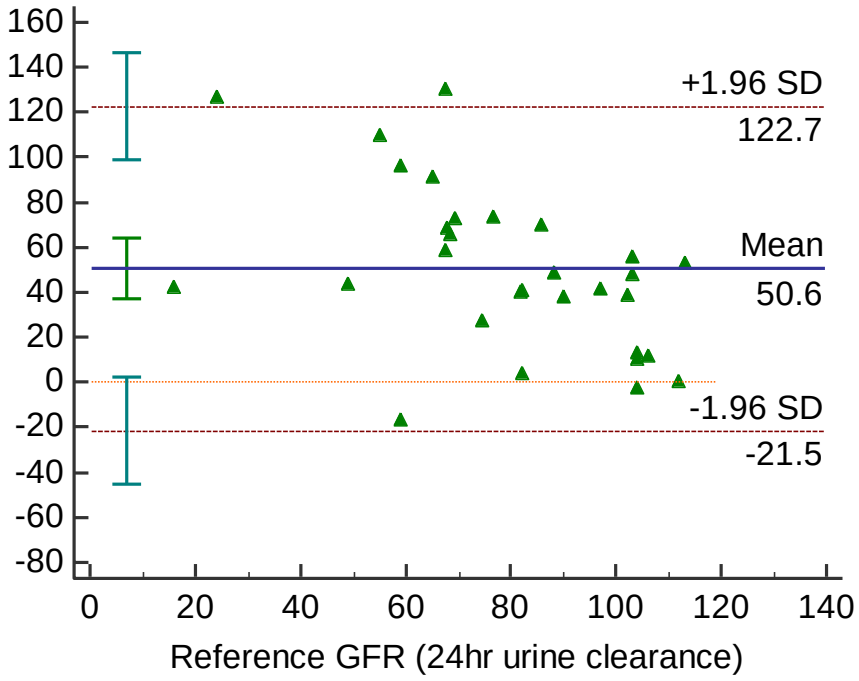
Percentage mean difference was calculated by:

$$\frac{\text{Estimated GFR (by the various methods)} - \text{reference GFR}}{\text{reference GFR}} \times 100$$

This percentage mean difference was plotted against the Y – Axis and the Reference GFR value was plotted on the X – axis. Thus, the overestimate or underestimate of the estimated GFR values from the reference values could be observed at difference GFR levels.

The arithmetic mean and the 95% confidence intervals for the upper and lower limits were calculated.

Comparison between Creatinine CKD-EPI formula *VS* reference GFR:



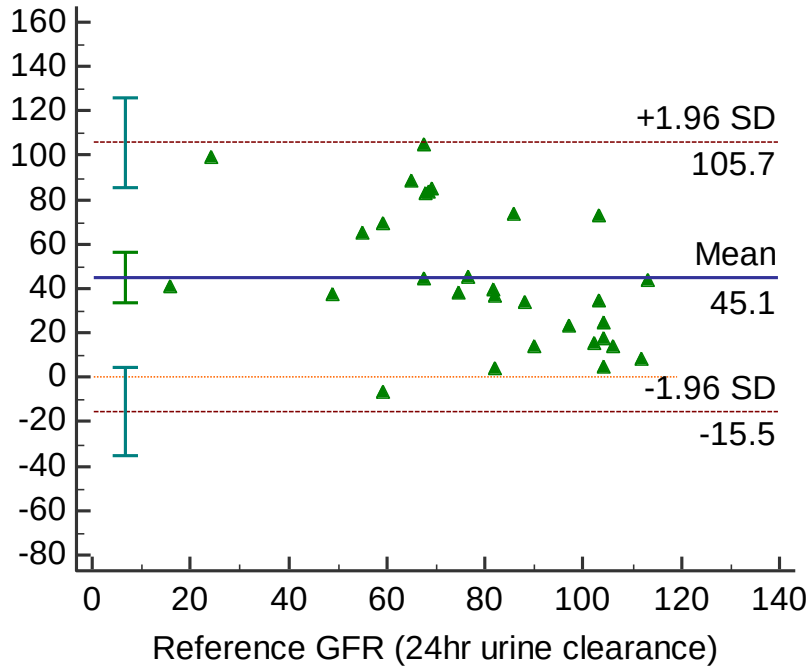
Cr GFR1: CKD-EPI creatinine – (ml/min/1.73m2)

Reference GFR: 24-hour urine creatinine clearance (ml/min)

mean	50.6141
95% CI	36.8810 to 64.3473
P (H_0: Mean=0)	<0.0001
Lower limit	-21.4709
95% CI	-45.2060 to 2.2642
Upper limit	122.6992
95% CI	98.9641 to 146.4342

The CKD – EPI folmula overestimates GFR by a mean of 50.6%.

Comparison between Creatinine based Cockcroft Gault formula *VS* reference GFR:



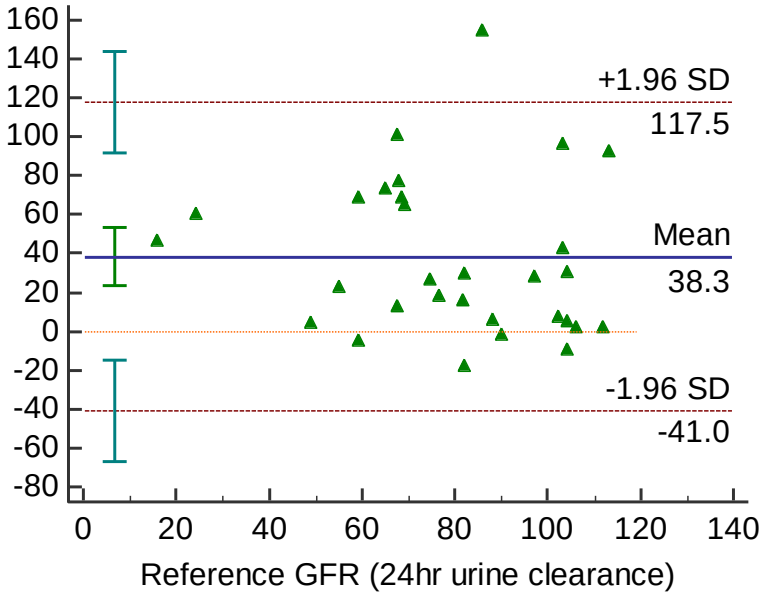
CrGFR 2: Creatinine- Cockcroft Gault GFR (with Body wt.) – (ml/min/1.73m2)

Reference GFR: 24-hour urine creatinine clearance (ml/min)

mean	45.0881
95% CI	33.5376 to 56.6386
P (H_0: Mean=0)	<0.0001
Lower limit	-15.5400
95% CI	-35.5028 to 4.4227
Upper limit	105.7162
95% CI	85.7535 to 125.6789

The Creatinine based Cockcroft Gault formula overestimates GFR by a mean of 45.09%.

Comparison between Creatinine based Cockcroft Gault formula, using Body Surface Area calculated from height and weight, *VS* reference GFR:



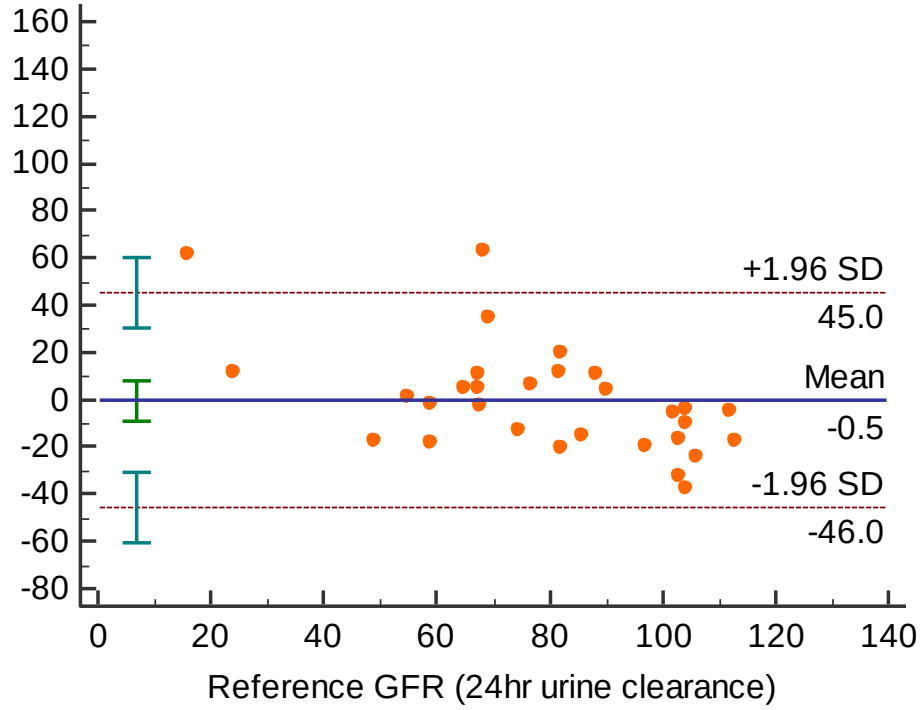
CrGFR 3: Creatinine Cockcroft Gault GFR (with Body wt.) – (ml/min) -using body surface area

Reference GFR: 24-hour urine creatinine clearance (ml/min)

mean	38.2687
95% CI	23.1656 to 53.3718
P (H_0: Mean=0)	<0.0001
Lower limit	-41.0072
95% CI	-67.1100 to -14.9044
Upper limit	117.5446
95% CI	91.4418 to 143.6474

The Creatinine based Cockcroft Gault formula, using body surface area overestimates GFR by a mean of 38.27%.

Comparison between Cystatin C based CKD - EPI formula *VS* reference GFR:



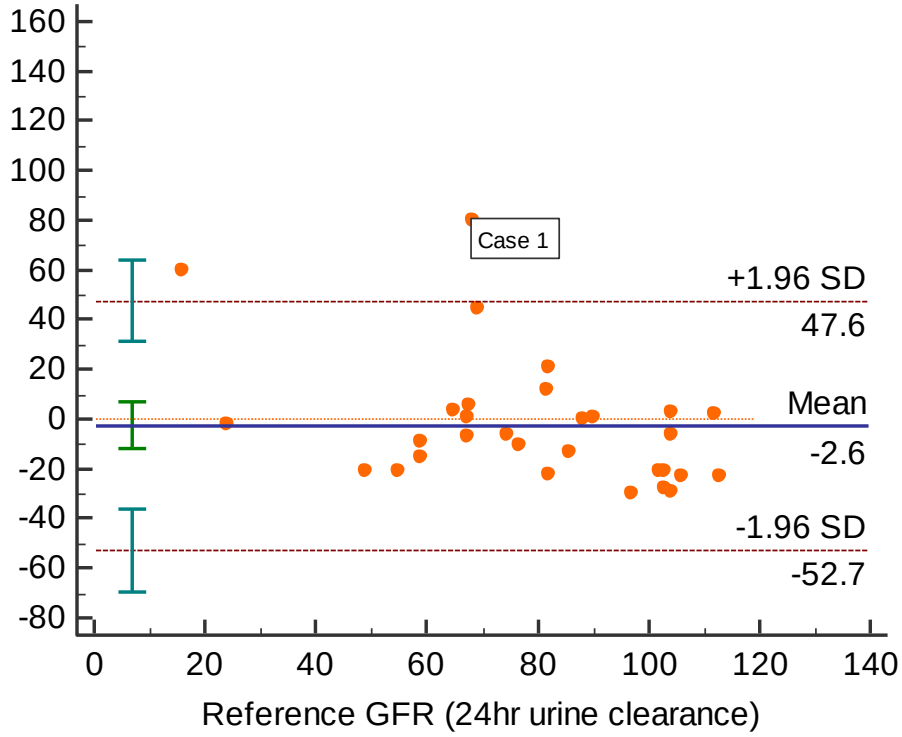
CysGFR 1: CKD – EPI Cystatin C - (ml/min/1.73m2)

Reference GFR: 24-hour urine creatinine clearance (ml/min)

mean	-0.4727
95% CI	-9.1373 to 8.1918
P (H_0: Mean=0)	0.9119
Lower limit	-45.9529
95% CI	-60.9279 to -30.9778
Upper limit	45.0074
95% CI	30.0324 to 59.9824

The Cystatin C based CKD - EPI formula remains close to the reference GFR by a mean difference of -0.47%. The p value is closer to one, indicating the null hypothesis that there isn't much significant difference between the 2 methods.

Comparison between Cystatin C based CKD - EPI formula, using Body Surface Area calculated from height and weight, *VS* reference GFR:



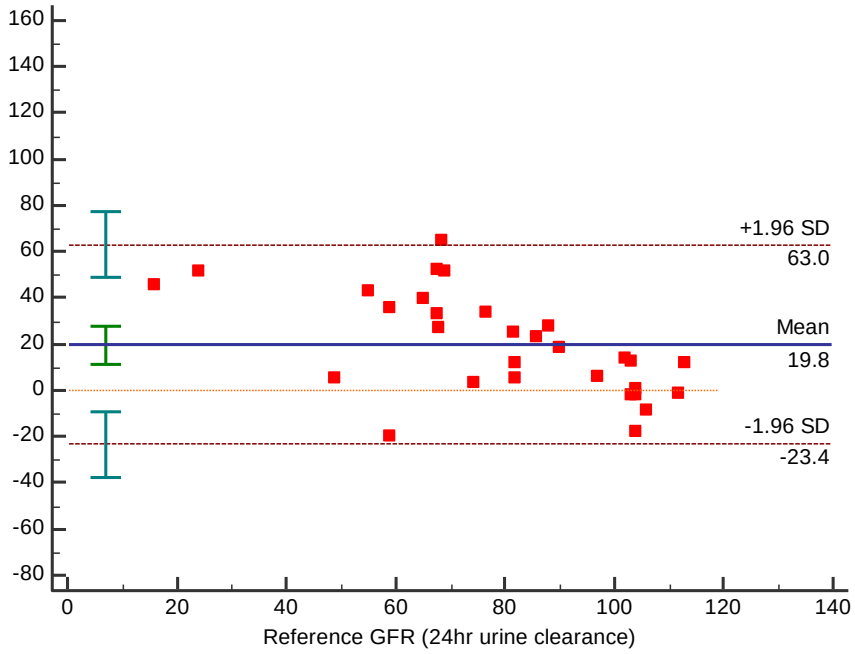
CysGFR 2: CKD – EPI Cystatin C- (ml/min) – using body surface area

Reference GFR: 24-hour urine creatinine clearance (ml/min)

mean	-2.5586
95% CI	-12.1169 to 6.9998
P (H_0: Mean=0)	0.5883
Lower limit	-52.7303
95% CI	-69.2501 to -36.2105
Upper limit	47.6132
95% CI	31.0934 to 64.1330

The Cystatin C based CKD - EPI formula, using Body Surface Area calculated from height and weight, remains close to the reference GFR by a mean difference of -2.56%.

Comparison between Combined Cystatin C and Creatinine CKD - EPI formula *VS* reference GFR:



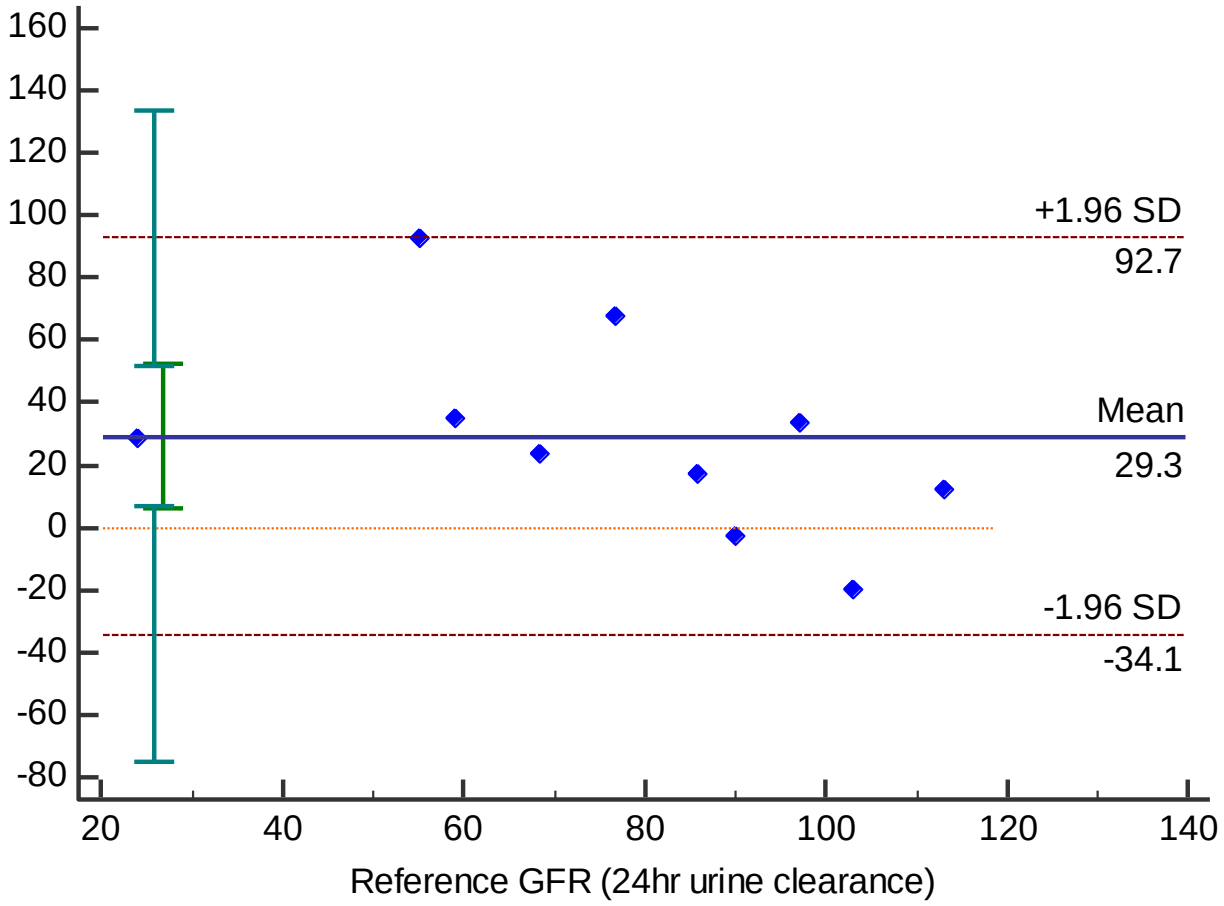
Combined GFR - Combined Cystatin C and Creatinine eGFR(CKD-EPI) – ml/min/1.73m2

Reference GFR: 24-hour urine creatinine clearance (ml/min)

mean	19.7676
95% CI	11.5359 to 27.9993
P (H_0: Mean=0)	<0.0001
Lower limit	-23.4405
95% CI	-37.6675 to -9.2136
Upper limit	62.9757
95% CI	48.7488 to 77.2027

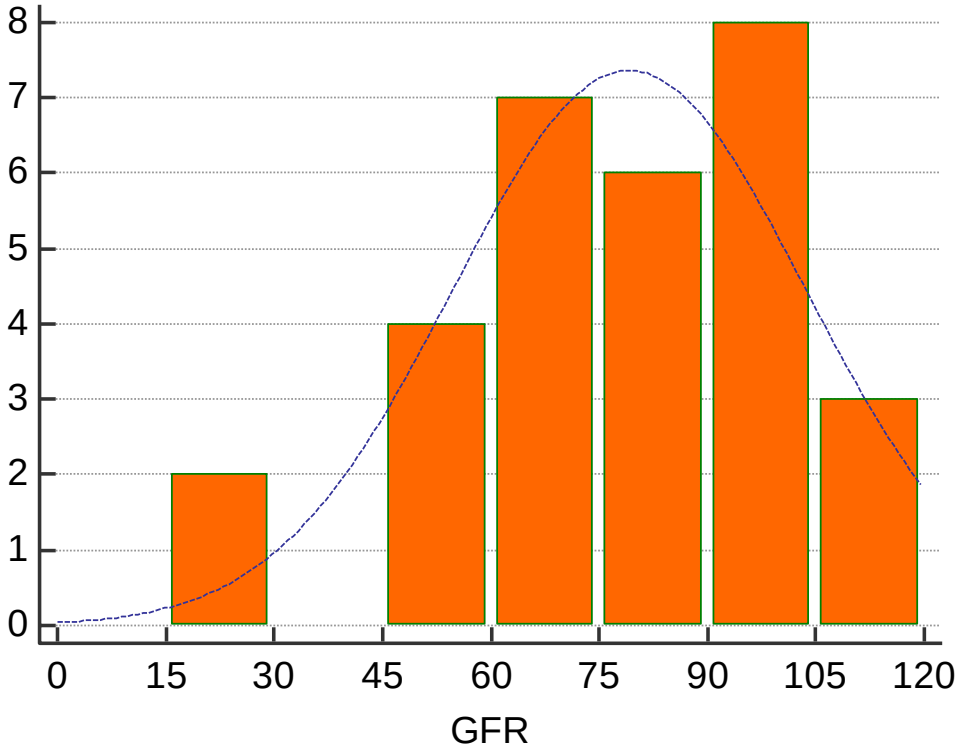
The combined GFR (Using cystatin C and creatinine, CKD-EPI) overestimated GFR by 19.76%.

Comparison between Renogram GFR using Gates method (99Tc – DTPA) *VS* reference GFR (in 10 patients):



mean	29.2922
95% CI	6.1398 to 52.4445
P (H_0: Mean=0)	0.0187
Lower limit	-34.1426
95% CI	-75.1309 to 6.8456
Upper limit	92.7269
95% CI	51.7387 to 133.7152

Abnormal GFR values amongst patients studied:



Based on CKD stages:

Normal GFR – 11 patients (>90ml/min) 36.67%

Stage 2 – 13 patients (60 to 89 ml/min) 43.33%

Stage 3 – 4 patients (30 to 59 ml/min) 13.33%

Stage 4 – 2 patients (15 to 29 ml/min) 6.67%

63.33% of the patients had GFR below 90ml/min (normal).

Discussion:

Among the cohort of patients studied, road traffic accidents seem to be the leading cause for SCI (50%) after fall from height (40%). This pertains to the trend of increasing incidence of road traffic accidents contrary to older studies that stated fall from a height being the most common.

Most patients studied were male, which could be explained by the lower incidence of SCI in women.

26 patients were paraplegic and 6 tetraplegic with 25 falling into the ASIA A category and 5 in the ASIA B category according to the ASIA impairment scale.

Half the patients used Self Intermittent Catheter as their mode of bladder management with 40% using indwelling catheter and the remaining suprapubic catheterisation.

This made collection of 24-hour urine more accurate, although this can only be a subjective observation made.

The average duration post injury was around 6 years and the longest duration post injury was 23 years.

Creatinine based equations significantly overestimate GFR:

The Bland Altman plot method clearly shows the overestimation of Serum Creatinine derived GFR equations.

The magnitude of overestimation can only be seen by the large percentages (50.6%, 45.1% and 38.3% - 3 different Serum Creatinine based equations) by which they differed from the reference GFR Value. All them showed significant P values (<0.0001).

The percentages seen are overestimations that are *not acceptable in clinical practice*.

This brings us to the first conclusion that Serum Creatinine may have no role for estimation of GFR with currently available formula in Spinal Cord Injury patients with motor complete injuries.

It was interesting to note that the newest developed Creatinine based CKD-EPI formula showed the largest mean difference. Nevertheless, all the methods tested showed a large overestimation.

The Combined Cystatin C and Creatine based CKD – EPI equation also showed 19.6% mean overestimation. While this is lower than the Creatinine based equations, it was still significantly higher.

Renogram GFR calculated with Gate's method using 99Technitium – DTPA

(in 10 patients):

This method was the only available radionuclide-based method for GFR estimation in our institution during the time of the study. The Gamma camera-based method was used, depth correction was also made while performing the study. Calculation of GFR was done using Gate's formula.

10 patients under went Renal scans and at interim analysis there was marked variability noted in the results. It showed a mean difference of around 30% with a 95% confidence interval between 6.1398 and 52.4445 %. Hence further patients in the study did not undergo the test as it was expensive and time consuming, also being invasive for delivering the substance via intravenous route. They also found it difficult maintain the advised safety precautions with taking care while discarding their urine for 24 hours post the study, considering their method of bladder management.

This method is yet to be validated in our population of patients and hence may not be of much use until then. Serial sampling/plasma clearance of 99Technitium – DTPA may be a good Gold standard to use if feasible.

Cystatin C is a good single marker for estimating GFR in SCI:

The difference noted between the mean differences of the Cystatin C based CKD-EPI equation GFRs and the reference GFR was very minimal (-0.5% and -2.6%).

Their P values were also closer to one suggesting that there isn't much difference between the 2 methods studied.

Serum Cystatin C can be used as an alternate accurate single marker for estimation of GFR in Spinal Cord Injury patients who are ASIA A and B.

The Serum Cystatin C levels can be estimated fast and will not require 24 hours to wait for urine collection.

The equations used for estimation of GFR where the Cystatin C based CKD – EPI equations. The first equation did not require height and weight of the patient and the other used the height and weight of the patients to calculate body surface area which was used in the formula. Both showed similar results and were accurate in measuring GFR. Measure of height and weight in spinal cord injury patients is a cumbersome task and hence using the equation without its requirement may be more feasible in routine clinical practice.

Cost accounting:

Serum Cystatin C is not significantly more expensive than 24 hour urine creatinine clearance or Serum Creatinine.

During the time of the study the cost of 24 hour urine creatinine clearance test was Rs. 250 /-.

The cost of Serum Cystatin C was Rs. 400 /-.

The cost of Serum Creatinine was Rs. 130 /- but this is not of much significant as we have seen that it is a test that currently has little value in motor complete SCI patient.

In situations where Cystatin C is not available, 24-hour urine creatinine clearance can be used as a substitute. As mentioned before in conditions where Cystatin C levels may be affected (as in the case of Thyroid dysfunction and patients receiving high dose glucocorticoids as stated previously), this method may be the cheapest and a reliable method in SCI. The drawback of this test remains the long duration of 24 hours for collection and analysis of GFR.

Incidental finding of renal failure in patients with spinal cord injury:

It was a significant majority of patients (63.33%) who had below normal (90ml/min) GFR values. The burden of renal failure in spinal cord injury patients has not been looked into careful. This requires further evaluation and screening.

20% fell into the Stage 3 and 4 categories of CKD which require management and periodic follow up. 2 of these patients had a GFR of less than 30ml/min.

These patients were referred to the department of Nephrology for further management and were also advised precautions to be followed – avoiding nephrotoxic drugs, renal adjusted dosing of antibiotics, periodic follow up etc…

A lot of factors like low socio-economic status, inadequate diet, recurrent infections, hygiene and limited access to healthcare, to list a few, which may be more pertinent to developing nations like ours, put them at a higher risk of developing CKD. It is likely that this problem has been overlooked due to inadequate methods for screening which has been observed in this study.

Methods of long-term bladder management in SCI patients and their effect on renal health need to be established to be able to advice the right method in the future.

The question of which the safest method for bladder management, considering long term ill effects on renal health, still remains unanswered.

Although there was no method used to assess muscle mass in this study, it seems to indirectly correlate well with the fact that there is a significant overestimation when Serum Creatinine is used for GFR estimation.

Using Dual Xray Energy Absoptiometry (DEXA) was considered to quantify skeletal muscle loss, but as it has not yet become a validated method and is till under scrutiny, it was not done.

Conclusion:

- Cystatin C proves to be a single accurate marker for estimation of GFR in motor complete spinal cord injury patients

- Using Serum Creatinine for estimation of GFR in motor complete Spinal Cord Injury patients significantly overestimates GFR and should be avoided in clinical practice

- Compromised renal function is a significant complication in spinal cord injury and needs to be evaluated and screened for more vigorously.

- There is a need for further evaluation of renal health in spinal cord injury patients.

Limitations of the study:

- An accepted Gold standard like plasma clearance of a radionuclide –

51Cr – EDTA or 99Tc – DTPA would have been a better method for comparison.

- Female population was not adequately represented.

- Further evaluation, follow up and collection of additional data from SCI patients who had affected renal function could have given more information on risk factors, although it may not have been within the scope of this study

- Correlation of patients with low GFR with Sonological findings of Kidneys could have been done.

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ANNEXURES

Participant information sheet

The study requires your blood sample to be drawn and 24 hour urine sample to be collected by the method you are currently practising for bladder drainage. You will be required to undergo a renal scan wherein you will be given an Intravenous injection and a scan will be taken to assess the function of your kidneys.

What harm can I expect from the study?

The amount of blood drawn for the study is around 2ml. This is not known to cause any significant clinical problems. If in case you do experience any discomfort (nausea, light-headedness, generalized weakness), you will receive immediate medical attention. You will be subjected to a renal scan where you will be injected with a dye and images will be capture for assessing kidney function. There has been no known allergic reaction to the substance with neglible exposure to gamma radiation which is less than the daily environmental exposure . In the event that you develop any discomfort, it will be managed accordingly in the ward.

Will I be charged extra for the test performed under the study?

You will NOT be charged any additional fee for the test performed in the study and no incentives will be provided for your participation.

Can I withdraw my consent even after starting the study? Yes. You are free to withdraw your consent at ANY point of time during the study.

What benefits can I expect from the study?

You may not experience any immediate benefits from the study but by the tests performed we will be able to achieve a more accurate estimation of the current status of your kidney function. It will also help in understanding how better we can, in general, assess kidney function in patients who have spinal cord injuries thereby changing management in the context of diagnostic evaluation of kidney function in such patients.

By participating in the study, you agree to share information about yourself, including your past medical and surgical history, and answer the questions that the investigators ask you about your symptoms and consent for drawing blood, 24 urine sample, renal scans during your admission/treatment under Department of Physical Medicine and Rehabilitation in CMC, Vellore. It also means that we are allowed to obtain your lab reports. The investigators may publish the information they get from the study, but your identity will be kept confidential

Clinical Research Form

ASSESSMENT OF RENAL FUNCTION IN MOTOR COMPLETE SPINAL CORD INJURY PATIENTS – CYSTATIN C AS AN ACCURATE SINGLE MARKER

Study number:

Name:

Age:

Sex:

Hospital number:

Address:

Diagnosis:

Mode of spinal cord injury:

Duration of injury:

Bladder management:

Indwelling urethtral catheter / SICC / Crede's

Serum levels:

- Creatinine:

- Cystatin C:

- Albumin:

24 hour urine creatinine clearance :

Renal scan based GFR :

Form completed by:

Name and signature

Date:

Informed Consent Form for Subjects

Informed Consent form to participate in a research study

Study Title: ASSESSMENT OF RENAL FUNCTION IN MOTOR COMPLETE SPINAL CORD INJURY PATIENTS – CYSTATIN C AS AN ACCURATE SINGLE MARKER

Study Number: _____

Subject's Initials: _____ Subject's Name: _____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

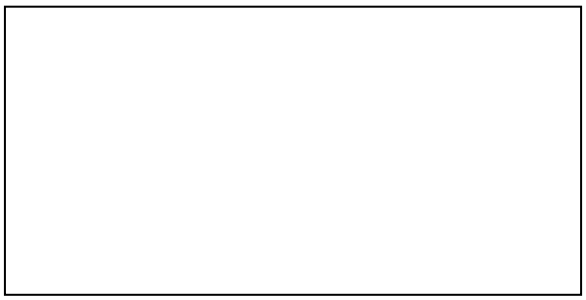
(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or



Representative: _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

_____

INSTITUTIONAL REVIEW BOARD APPROVAL (IRB) FORMS:

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

August 01, 2018

Dr. Thomas Anand Augustine,
PG Registrar,
Department of PMR,
Christian Medical College,
Vellore – 632 002.

Sub: **Fluid Research Grant: New Proposal:**
Assessment of renal function in motor complete spinal cord injury patients – cystatin C as an accurate single marker.

Dr. Thomas Anand Augustine, PG Registrar, PMR, Dr. Henry Prakash (emp. No.20322), PMR, Dr. Santosh Varughese (Emp. No. 28219), Nephrology, Dr. Julike Hephzibah, Nuclear Medicine, Dr. Suceena Alexander (emp. No. 51288), Nephrology.

Ref: IRB Min. No. 11341 [OBSERVE] dated 04.06.2018

Dear Dr. Thomas Anand Augustine,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Cc: Dr. Henry Prakash, Dept. of PMR, CMC, Vellore

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
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Ref: IRB Min. No. 11341 [OBSERVE] dated 04.06.2018

Dear Dr. Thomas Anand Augustine,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Assessment of renal function in motor complete spinal cord injury patients – cystatin C as an accurate single marker" on Junel 04th 2018.

The Committee reviewed the following documents:

1. IRB application format
2. Patient information Sheet and Consent form (Tamil, English, Hindi)
3. Clinical Research Form
4. International STDS diagram
5. Cvs of Drs. Henry Prakash, Julie, Santosh Varughese, Suceena Alex, Thomas Anand Augustine.
6. No. of documents 1- 5.

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on June 04th 2018 in the New IRB Room, Bagayam, Christian Medical College, Vellore 632 004.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Tunny Sebastian	P.hd., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Nirmala Margaret	MSc Nursing	Addl. Deputy Nursing Superintendent, College of Nursing, CMC,Vellore	Internal, Nurse
Dr. Sathish Kumar	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Barney Isaac	M.B.,B.S. D.N.B (Respiratory Diseases)	Associate Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Dr. John Antony Jude Prakash	MBBS, MD	Professor, Clinical Microbiology, CMC, Vellore.	Internal, Clinician.
Dr. Ajith Sivadasan	MD, DM	Professor, Neurological Sciences, CMC, Vellore	Internal, Clinician
Dr. Rekha Pai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal, Basic Medical Scientist
Dr Sneha Varkki	MBBS, DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of Withdrawals for the study entitled: "Assessment of renal function in motor complete spinal cord injury patients – cystatin C as an accurate single marker" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:
A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty thousand only) each will be released at the end of the first year as 2 nd Installment.

Yours sincerely,

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board
IRB Min. No. 11347 [OTHER] dated 04.06.2018

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Muscle Function Grading

0 = total paralysis

1 = palpable or visible contraction

2 = active movement, full range of motion (ROM) with gravity eliminated

3 = active movement, full ROM against gravity

4 = active movement, full ROM against gravity and moderate resistance in a muscle specific position

5 = (normal) active movement, full ROM against gravity and full resistance in a functional muscle position expected from an otherwise unimpaired person

5* = (normal) active movement, full ROM against gravity and sufficient resistance to be considered normal if identified inhibiting factors (i.e. pain, disuse) were not present

NT = not testable (i.e. due to immobilization, severe pain such that the patient cannot be graded, amputation of limb, or contracture of > 50% of the normal ROM)

Sensory Grading

0 = Absent

1 = Altered, either decreased/impaired sensation or hypersensitivity

2 = Normal

NT = Not testable

When to Test Non-Key Muscles:

In a patient with an apparent AIS B classification, non-key muscle functions more than 3 levels below the motor level on each side should be tested to most accurately classify the injury (differentiate between AIS B and C).

Movement	Root level
Shoulder: Flexion, extension, abduction, adduction, internal and external rotation **Elbow:** Supination	**C5**
Elbow: Pronation **Wrist:** Flexion	**C6**
Finger: Flexion at proximal joint, extension. **Thumb:** Flexion, extension and abduction in plane of thumb	**C7**
Finger: Flexion at MCP joint **Thumb:** Opposition, adduction and abduction perpendicular to palm	**C8**
Finger: Abduction of the index finger	**T1**
Hip: Adduction	**L2**
Hip: External rotation	**L3**
Hip: Extension, abduction, internal rotation **Knee:** Flexion **Ankle:** Inversion and eversion **Toe:** MP and IP extension	**L4**
Hallux and Toe: DIP and PIP flexion and abduction	**L5**
Hallux: Adduction	**S1**

ASIA Impairment Scale (AIS)

A = Complete. No sensory or motor function is preserved in the sacral segments S4-5.

B = Sensory Incomplete. Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-5 (light touch or pin prick at S4-5 or deep anal pressure) AND no motor function is preserved more than three levels below the motor level on either side of the body.

C = Motor Incomplete. Motor function is preserved at the most caudal sacral segments for voluntary anal contraction (VAC) OR the patient meets the criteria for sensory incomplete status (sensory function preserved at the most caudal sacral segments (S4-S5) by LT, PP or DAP), and has some sparing of motor function more than three levels below the ipsilateral motor level on either side of the body.
(This includes key or non-key muscle functions to determine motor incomplete status.) For AIS C – less than half of key muscle functions below the single NLI have a muscle grade ≥ 3.

D = Motor Incomplete. Motor incomplete status as defined above, with at least half (half or more) of key muscle functions below the single NLI having a muscle grade ≥ 3.

E = Normal. If sensation and motor function as tested with the ISNCSCI are graded as normal in all segments, and the patient had prior deficits, then the AIS grade is E. Someone without an initial SCI does not receive an AIS grade.

Using ND: To document the sensory, motor and NLI levels, the ASIA Impairment Scale grade, and/or the zone of partial preservation (ZPP) when they are unable to be determined based on the examination results.

ASIA
AMERICAN SPINAL INJURY ASSOCIATION

INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY

ISCOS
INTERNATIONAL SPINAL CORD SOCIETY

Steps in Classification

The following order is recommended for determining the classification of individuals with SCI.

1. Determine sensory levels for right and left sides.
The sensory level is the most caudal, intact dermatome for both pin prick and light touch sensation.

2. Determine motor levels for right and left sides.
Defined by the lowest key muscle function that has a grade of at least 3 (on supine testing), providing the key muscle functions represented by segments above that level are judged to be intact (graded as a 5).
Note: in regions where there is no myotome to test, the motor level is presumed to be the same as the sensory level, if testable motor function above that level is also normal.

3. Determine the neurological level of injury (NLI)
This refers to the most caudal segment of the cord with intact sensation and antigravity (3 or more) muscle function strength, provided that there is normal (intact) sensory and motor function rostrally respectively.
The NLI is the most cephalad of the sensory and motor levels determined in steps 1 and 2.

4. Determine whether the injury is Complete or Incomplete.
(i.e absence or presence of sacral sparing)
*If voluntary anal contraction = **No** AND all S4-5 sensory scores = **0** AND deep anal pressure = **No**, then injury is **Complete.***
*Otherwise, injury is **Incomplete.***

5. Determine ASIA Impairment Scale (AIS) Grade:

Is injury Complete? **If YES, AIS=A** and can record ZPP (lowest dermatome or myotome on each side with some preservation)

NO ↓

Is injury Motor Complete? **If YES, AIS=B**

NO ↓ (No=voluntary anal contraction OR motor function more than three levels below the motor level on a given side, if the patient has sensory incomplete classification)

Are at least half (half or more) of the key muscles below the neurological level of injury graded 3 or better?

NO ↓ **AIS=C** **YES** ↓ **AIS=D**

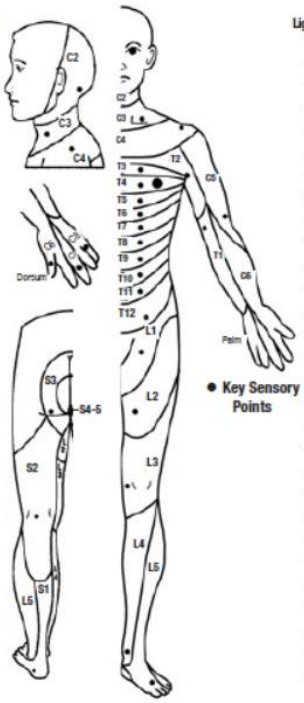
If sensation and motor function is normal in all segments, AIS=E
Note: AIS E is used in follow-up testing when an individual with a documented SCI has recovered normal function. If at initial testing no deficits are found, the individual is neurologically intact; the ASIA Impairment Scale does not apply.

INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY (ISNCSCI)

Patient Name _____ Date/Time of Exam _____

Examiner Name _____ Signature _____

RIGHT		MOTOR KEY MUSCLES	SENSORY KEY SENSORY POINTS Light Touch (LTR)	SENSORY KEY SENSORY POINTS Pin Prick (PPR)		SENSORY KEY SENSORY POINTS Light Touch (LTL)	SENSORY KEY SENSORY POINTS Pin Prick (PPL)	MOTOR KEY MUSCLES	LEFT
	C2								
	C3								
	C4								
UER (Upper Extremity Right)	Elbow flexors C5							C5 Elbow flexors	UEL (Upper Extremity Left)
	Wrist extensors C6							C6 Wrist extensors	
	Elbow extensors C7							C7 Elbow extensors	
	Finger flexors C8							C8 Finger flexors	
	Finger abductors (little finger) T1							T1 Finger abductors (little finger)	
	T2								
	T3								
	T4								
	T5								
	T6								
	T7								
	T8								
	T9								
	T10								
	T11								
	T12								
	L1								
LER (Lower Extremity Right)	Hip flexors L2							L2 Hip flexors	LEL (Lower Extremity Left)
	Knee extensors L3							L3 Knee extensors	
	Ankle dorsiflexors L4							L4 Ankle dorsiflexors	
	Long toe extensors L5							L5 Long toe extensors	
	Ankle plantar flexors S1							S1 Ankle plantar flexors	
	S2								
	S3								
(VAC) Voluntary Anal Contraction (Yes/No)	S4-5							(DAP) Deep Anal Pressure (Yes/No)	
RIGHT TOTALS (MAXIMUM)		(50)	(56)	(56)		(56)	(56)	(50)	LEFT TOTALS (MAXIMUM)



● Key Sensory Points

Comments (Non-key Muscle? Reason for NT? Pain?):

MOTOR (SCORING ON REVERSE SIDE)

0 = total paralysis
1 = palpable or visible contraction
2 = active movement, gravity eliminated
3 = active movement, against gravity
4 = active movement, against some resistance
5 = active movement, against full resistance
5* = normal corrected for pain/disuse
NT = not testable

SENSORY (SCORING ON REVERSE SIDE)

0 = absent 2 = normal
1 = altered NT = not testable

MOTOR SUBSCORES

UER [ ] (MAX 25) + UEL [ ] (25) = UEMS TOTAL [ ] (50)

LER [ ] (MAX 25) + LEL [ ] (25) = LEMS TOTAL [ ] (50)

SENSORY SUBSCORES

LTR [ ] (MAX 56) + LTL [ ] (56) = LT TOTAL [ ] (112)

PPR [ ] (MAX 56) + PPL [ ] (56) = PP TOTAL [ ] (112)

NEUROLOGICAL LEVELS (Steps 1-5 for classification as on reverse)	R	L				
1. SENSORY			3. NEUROLOGICAL LEVEL OF INJURY (NLI)		4. COMPLETE OR INCOMPLETE? (Incomplete = Any sensory or motor function in S4-5)	
2. MOTOR					5. ASIA IMPAIRMENT SCALE (AIS)	

(In complete injuries only) ZONE OF PARTIAL PRESERVATION (Most caudal level with any innervation)

	R	L
SENSORY		
MOTOR		

REV 11/15