Should Glomerular Filtration Rate (GFR) be affected by the amount of viable, functioning tubular cells which in turn reflected by absolute renal uptake of Tc-99m DMSA

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For the memory of my father

Content

Acknowledgments

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Legend for Figures

Legend for Tables

Abstract

Aims:

This study was intended to quantify the cortical function of the kidneys by measuring the absolute Tc-99m DMSA renal uptake using the digital gamma camera. The second objective was to investigate whether there was any relationship between the absolute Tc-99m DMSA uptake with the corresponding Glomerular Filtration Rate (GFR).

Method:

Data was obtained from 47 patients whom were referred for the Tc-99m DMSA renal scintigraphy for differential function analysis. Absolute renal uptake of Tc-99m DMSA at 6 and 24 hours post injection were measured using a gamma camera based method (Picker dual headed 2000 XP) in the Prince of Wales Hospital, Shatin, Hong Kong. Their corresponding Glomerular Filtration Rate (GFR) were also estimated by single and double blood samples using Protein Free Plasma Clearance (PFPC) method as described by Rowell et al.,(1986). Body surface area, gender, age and serum creatinine level of each individual were also recorded.

Result:

The mean Tc-99m DMSA renal uptake measured at 6 and 24 hours post injection were 44 ± 13.3% and 44 ± 10% respectively in this population. The mean GFR calculated by PFPC method were 87.2 ± 35 ml/min when using single plasma sample and 87.5 ± 31 ml/min with double plasma samples. There was no statistical significant difference in the Tc-99m DMSA renal uptake measured at 6 and 24 hours post injection. There was also no statistical significant difference in the GFR estimated by single or double plasma samples. No gender difference was found in Tc-99m DMSA renal uptake measurement and in the GFR estimation.

For simplicity, the Tc-99m DMSA renal uptake measured at 6 hour post injection and the GFR estimated by single plasma sample (3 hr) were used for further analysis.

Both Tc-99m DMSA renal uptake measured at 6 hour post injection and the GFR estimated by single plasma sample responded exponentially with serum creatinine level.

There was a weak relationship between the Tc-99m DMSA renal uptake at 6 hours post injection with surface area and age.

A weak relationship was also noted between the GFR estimated by single plasma sample with surface area and age.

However a strong positive correlation was found between GFR estimated by single plasma sample and the Tc-99m DMSA renal uptake at 6 hours post injection with $r^2 = 0.685$, $p < 0.05$.

Discussion:

Based on the data, it was found that there was a strong positive correlation between the Tc-99m DMSA renal uptake measured at 6 hour post injection and GFR estimated using single plasma sample. In addition, serum creatinine level, surface area and age were also contributing factors to both the Tc-99m DMSA renal uptake measured at 6 hour post injection and the estimated GFR by single plasma sample.

Multiple regression analysis was performed using GFR(3 hour) as the dependent variable and Tc-99m DMSA renal uptake measured at 6 hour post injection, serum creatinine level, surface area and age as the independent variables. The result showed that GFR was significantly correlated with absolute Tc-99m DMSA uptake measured at 6 hour post injection, creatinine level and surface area ($r = 0.885$, $p<0.05$). The corresponding equation was expressed by GFR = {(absolute Tc-99m DMSA uptake measured at 6 hour post injection) + 54.9 (surface area) - 0.379 (creatinine level)} ml/min.

Conclusion:

In this study, I have demonstrated the validity and feasibility of determining global renal function in patients by using intravenously injected Tc-99m DMSA with planar imaging.

The technique for quantification of cortical function of the kidneys was simple and fast. The result was compatible with SPECT study.

Moreover Tc-99m DMSA renal uptake measured at 6 hour post injection was strongly correlated with the corresponding GFR.

Divided renal GFR was then calculated by multiplying the total GFR with their respective differential functions. In this way, it serves as an important and useful index for assessing cortical and renal mass function and offered a guideline to the referring clinician for their patient management.

Absolute quantification for all patients receiving renal scintigraphy with Tc-99m DMSA scan was suggested to be part of standard DMSA protocol.

論文題目

透過伽瑪射線數碼掃描機測定之腎皮質毛小管的活細胞量來鑑定是否直接影響腎小球的濾過功能。

摘要

目標

一) 利用伽瑪射線數碼掃描測定腎對靜態示蹤劑 (Tc-99m DMSA) 攝取量作爲腎皮質功能之量化。
二) 審查所得之數值與腎小球的濾過功能是否相關。

方法

研究對象爲 47 位腎結石病患者, 經轉介到本部作腎功能分析驗查，經靜脈注射 Tc-99m DMSA 約 3 mCi , 在 6 與 24 小時後作掃描及量化分析，所用影像儀器包括 Picker 公司雙頭 2000XP 相機 , 及 Odyssey VP 計算機。
腎小球的濾過功能的估值法是根據 Rowell 的脫蛋白質血漿肅清法(PFPC method)。
人體表面積、性別、年齡及血清內縮水肌氨酸的讀數也一併歸納分析。

結果及討論

於注射後 6 小時及 24 小時測定之腎皮質功能絕對平均值分別爲 44±13.3%及 44±10%。
從單一及雙血樣本所測定之腎小球的濾過功能的平均估值分別爲 87.2±35ml/min 及 87.5±31ml/min。
據統計學分析，在 6 小時及 24 小時測定之腎皮質功能定量並無顯著差異 (p=0.5610)。
由單一及雙血樣本而作出估值的腎小球的濾過功能同樣地沒有顯著分別 (p=0.2315)。
性別沒有對腎皮質功能及腎小球的濾過功能作出影響(p=0.5583; p=0.5729)。
因此以定量於 6 小時之腎皮質功質及由單一血樣本測定之腎小球的濾過功能數值作推算、分析，發現：

1. 兩者的相互關連性甚高，r=0.828，p<0.05。
2. 兩者對血清內縮水肌氨酸的讀數是按指數遞減 r^2 分別爲 0.655 及 0.462, p<0.05。
3. 腎皮質功能的數值與年齡及人體表面積作出弱的關連性，r^2 分別爲 0.063 與 0.072。
4. 腎小球的濾過功能與年齡及人體表面積均作出弱的關連性，r^2 分別爲 0.119 及 0.072。

綜合來說，從這次研究所得，腎小球的濾過功能可從以下的方程式中推斷：
（ 1(定量於 6 小時之腎皮質功能)+54.9(人體表面積)-0.379(血清內縮小肌氨酸的讀數)）ml/min ，r=0.885，p <0.05。

<u>總結</u>

腎皮質功能的定量可從平面影像中取得，這方法既方便及簡單。所得之數值可比美從單光子發射掃描儀所得之數據。
個別腎小球的濾過功能能從中運算出來，作爲主診醫生臨床參考的指標。

Chapter I Introduction

Renal disorders affect a wide spectrum of our population of different age groups. Numerous imaging modalities, including nuclear medicine, ultrasonography, x-ray florescence, computerized tomography and, more recently, magnetic resonance imaging, have been used in an attempt to provide a pathophysiologically related diagnosis with disease of the renal system. Apart from the imaging modalities that give anatomical as well as functional information, biochemical tests also serve to provide information on the functional status of renal system.

Among the various imaging modalities, ultrasonography has been used extensively in the diagnosis of renal disorders. It is said to be the most non-invasive and sensitive methods to visualize the anatomical detail and structural relations for the diagnosis of diseases. However, the function of the organ cannot be assessed accurately by ultrasound. Radionuclide imaging plays an important role in the diagnosis of renal disorder as it can integrate both morphological and functional information simultaneously.

Oeser and Bullion (1952) emphasized the advantage of using radioactive tracers over contrast agents for evaluating renal function, the measurements can be both qualitative and quantitative, and the tracers themselves do not disrupt function. Radionuclide measurements have remained the procedure of choice for measuring the function of each kidney (split function) and in the

detection of obstruction to urine flow. They also have the advantages in monitoring the progress of overall renal function and to decide whether nephrectomy will result in too great a diminution of overall renal function.

Various radiopharmaceuticals have been adopted for the evaluation of individual renal function (Taylor,1982; Chervu et al., 1982). The most commonly used are being Tc-99m DMSA , Tc- 99m DTPA or Tc- 99m MAG3.

Technetium-99m dimercaptosuccinic acid (DMSA) has been recognized as the radiopharmaceutical of choice for static renal imaging, because of its high affinity for the renal cortex and the fixation of the radioactive agent to the proximal tubular cells (Hosokawa et al., 1978). This makes it suitable for the assessment of the functioning mass of the cortex. Moreover its uptake has been shown to correlate well with the effective renal plasma flow (ERPF), glomerular filtration rate (GFR) and creatinine clearance (Taylor, 1982; Kawamura et al., 1978; Baillet et al., 1985).

Tc-99m DMSA renal scintigraphy not only can provide qualitative information on the morphology of the kidney but its functional status can also be computed, either as differential or absolute uptake. For the differential function, it is the percentage of right to left cortical uptake or retention as a percentage of total renal uptake. As it is the measurement of function of one kidney relative to the other, it may impose a diagnostic dilemma to the clinicians in deciding the actual change in functional status of one or both diseased kidney during follow up. In addition, there is no information on the actual amount of viable

functioning cortical tubular cells. Whereas for the absolute function, the renal uptake of Tc-99m DMSA is expressed as the percentage of injected dose and seems to be more informative in evaluation of cortical function.

However, the measurement of absolute renal uptake of Tc-99m DMSA is not simple and straight forward. It involves the calculations of net injected dose and the correction for various attenuation medium.

Despite all these potential problems, its clinical usefulness and benefit outweighs the difficulties encountered.

One of the objectives of my project is to develop a reliable method for quantifying Tc-99m DMSA renal uptake using a digital gamma camera image.

The second objective of this project is trying to establish a relationship between the absolute Tc-99m DMSA uptake with the corresponding Glomerular Filtration Rate (GFR).

The measurement of GFR is regarded as one of the most valuable assessment of renal function. Traditionally renal function has been measured by creatinine clearance. Obviously there are known limitation in the methods concerned such as technical problems with creatinine measurement and with urine collection. Moreover creatinine is excreted by the tubules as well as the glomeruli. Clinically creatinine clearance does not measure individual renal function unless ureteral catherization is performed to collect urine from each kidney. From the clinical point of view, its use in situations of rapidly changing renal function is limited because of the requirement for timed urine collections as well

as the dynamics of establishing a new steady state in terms of total body creatinine and its effect on measured serum creatinine concentration.

In my study, I adopt the two samples Protein Free Plasma Clearance (PFPC) as described by Rowell et al (1986) for the measurement of Glomerular Filtration Rate (GFR). It was recognized to be as equivalent to the gold standard of I125-iotholamate clearance with r = 0.99 , $p<0.05$ as reported by Goates et al., (1990).

If a positive relationship exists, then the absolute Tc-99m DMSA renal uptake value can be tabulated to give rapid and easy estimation of the corresponding GFR. Likewise individual kidney GFR can be calculated by multiplying the global GFR with the differential uptake without requiring ureteral catherization. In this way, the assessment of the function of each kidney separately is possible during the initial diagnosis and in the follow-up of patients with renal diseases. This is especially true in unilateral kidney diseases, where the blood biochemistry may be normal even when more than half of the parenchyma is nonfunctioning (Blaufox et al., 1984; George, 1978).

Moreover, knowledge of individual absolute renal function is important in rational patient management. When a restorative operation on a kidney or nephrectomy is about to be performed electively it is essential to know the contribution of that kidney to total renal function. In an adult with total GFR of over 50 ml/min in the presence of obstructive nephropathy or renovascular disorder a kidney contributing less than 7% of total function is usually not worth

preserving, but a kidney contribution over 16% is worth preserving by a restorative operation.

Objective

So in summary , there are two objectives in my study:

- To quantify the cortical function of the kidneys by measuring the absolute Tc-99m DMSA uptake using digital gamma camera
- Try to establish a relationship between the absolute Tc-99m DMSA uptake with the corresponding GFR

Chapter II Literature Review

II.1. Anatomy of the urinary system

Kidney is an organ which excretes many endogenous and exogenous substances; maintains water and electrolytes balances, and acid-base equilibrium; produces hormones, catabolism on low molecules weights proteins; and conserves a large number of constituents of extracellular fluid. These kidney functions regulate the volume and composition of extracellular fluid of the body. Under normal conditions, they also play an important role in circulatory dynamic because they receive one-fourth of the cardiac output (Langley, 1974).

The kidneys are located adjacent to the spine bilaterally at the level of upper lumbar vertebra with the right kidney slightly lower than the left. The majority of the kidney function takes within the cortex, the thin outer layer. The remainder of the kidney encapsulated by the cortex serves as a pathway for the drainage of urine by means of collecting tubules into the calyces, renal pelvis and finally the ureters.

Ureters are tubular structures, 24-30 cm in length and approximately 0.9 cm in diameter. They have walls linked by mucous membrane and are covered by a two layer muscular coat, a circular outer layer, and longitudinal inner layer. The function of the ureters is for the drianage of urine into the urinary bladder. The

muscular layers contract in peristaltic waves (1-5/min), forcing the urine into the bladder.

Located directly behind the pubic symphsis, the bladder resembles a collapsible bag. Its wall is composed of three smooth muscle layers linked by mucous membrane arranged in prominent folds, or rugae. Because of its elastic structure capacity that varies greatly with individuals (100-500 ml). Its function is to retain urine and expel from the body through the urethra. Distention of the bladder as it fills with urine triggers reflex contractions of the bladder wall. Simultaneous relaxation of the internal and external sphincters of the urethra cause the bladder to empty. Contraction of the bladder and relaxation of the internal sphincter (the bladder neck) are involuntary, whereas contraction of the external sphincter at the urogential diaphragm to prevent or terminate urination is learned.

The urethra is the tube leading from the floor of the urinary bladder to the exterior of the body. It is 2.5-3 cm in length in the female and 10-12 cm in male. Its function is to eliminate urine from the body. In male , it also serves a passage way for the seminal fluid.

Renal Circulation

Blood is supplied to the kidneys by the renal artery, which enters the renal hilum and divides into four or five branches, in turn giving rise to the interlobar arteries in the renal columns of the medulla. At the cortico-medullary junction

the interlobar arteries branch out into the arcuate arteries, which give rise at intervals to interlobular arteries that penetrate upward into renal cortex.

On entering the cortex the interlobular arteries branch out into the afferent arteries terminating as tufts of capillaries --- the glomeruli --- which number more than one million per kidney. These capillary beds do not reform into venues but into efferent arteries, which leaves the glomeruli and branch into a second network of capillaries (peritubular capillaries and vasa recta) enmeshing the entire tubular system of the nephron. From this second capillary bed the venules unite with the interlobular veins to initiate venous return and complete its transit through the arcuate, interlobar, and renal veins, which leave the renal hilum and empty into the inferior vena cava.

The Nephron

The functional unit of the kidney --- the nephron --- consists of a glomerulus and a renal tubule. It is located, for the most part, in the renal cortex. The glomerulus is enclosed in the Bowman capsule, which is actually an expanded portion of renal tubule. The tubule follows a tortuous course as it rises from Bowman capsule, hence its name proximal convoluted tubule. From this point, it proceeds on a straight course until it dips down into the medulla, where it makes a hairpin turn (Henle loop) upward and returns to the vicinity of the glomerulus. At this point the tubule again assumes a winding course (distal convoluted tubule) and terminates in a collecting tubule. The collecting tubule

passes downward through the medulla as a component of a renal pyramid, joining larger tubules that converge to form one tube that connects to one of the minor calyces. These minor calyces converge in the major calyces, which composes the renal pelvis and funnel into the ureter (Antony, 1977).

II.2. Physiology of the urinary system

The homeostatic functions of the kidneys are achieved through two simultaneous processes: (a) glomerular filtration, and (b) tubular resorption/ secretion. Glomerular ultrafiltration occurs as a result of the net Starling forces across the glomerular capillary wall. The net filtration pressure (NPF) is equal to the sum of the glomerular hydrostatic pressure and the colloid osmotic pressure in the capillary space into Bowman's capsule (which favors fluid movement from the capillary space into the Bowman's space) minus the sum of the mean glomerular capillary oncotic pressure and the hydrostatic pressure in Bowman's space (the principle forces opposing ultrafiltration). Because protein is not filtrated by the glomerulus, the fluid in the Bowman's space is protein-free and thus the colloid osmotic pressure in Bowman's space is negligible. The glomerular filtration rate (GFR) is determined by the NFP and the surface area available for ultrafiltration as well as the permeability of the glomerular capillary bed. Thus, GFR can be expressed as $GFR = K_f \times NFP$ where K_f is a constant expressing the product of the capillary permeability and the surface area of the capillary bed and NPF is the net filtration pressure.

Three processes are involved in urine formation: glomerular filtration, tubular reabsorption, and tubular secretion. Water and solutes filter out of the glomeruli into the Bowman capsule because a pressure gradient exists between the two areas. This gradient is usually determined by the glomerular

hydrostatic pressure of 75 mmHg, which tends to move fluid out of the glomeruli. The blood colloidal osmotic pressure of 30 mm Hg combined with the capsular hydrostatic pressure of 20 mmHg exerts force in the opposite direction. The net or effective filtration pressure is then 75- (30+20), or 25 mmHg.

The second process is the reabsorption of most of the water and part of the solutes from the glomerular filtrate back into the blood. This is carried out by the cells in the walls of the convoluted tubules, loop of Henle, and collecting tubules that absorb substances of vital need to the body, such as water, glucose, sodium, chloride, bicarbonate, amino acids, and other nutrients.

In addition to reabsorption, tubular cells extract and secrete certain substances from the blood into the filtrate. In reabsorption and secretion some substances are removed by active transport and some by passive mechanisms such as diffusion and osmosis. Ions and glucose are reabsorbed, potassium and hydrogen ions are secreted by active transport mechanisms, water is reabsorbed by osmosis, and ammonia is secreted by diffusion. The latter two mechanisms are passive. Of clinical importance is the secretion of certain substances and drugs.

II.3. Methods for investigating the urinary system

Introduction

Uroradiology is a discipline involving the use of different imaging techniques in the evaluation of renal disorders. The commonly used techniques include plain film radiography, excretory urogram, ultrasound, computed tomography(CT), magnetic resonance imaging (MRI), renal angiography and radionuclide imaging. The choice of individual technique is based on the clinical information given and the results of previous investigations.

II.3.1. Plain film radiography

This is mainly used for the detection of calcifications related to the urinary systems and sometimes can reveal the anatomical relationships of different organs. Although it is not specific in reaching a definitive diagnosis for urological disorders, it often serves as a baseline investigation for other urological examinations.

II.3.2. Excretory Urogram

The excretory urogram, sometimes referred as the intravenous pyelogram (IVP), is the imaging study for visualizing the entire urinary tract. It consists of a preliminary x-ray film of the abdomen (the KUB) prior to intravenous injection

of contrast medium. Following the injection, series of x-ray films are taken sequentially so that the entire urinary tract can be visualized as it is opacified by the contrast medium.

It enables us to have a rough idea about the anatomical and functional status of each kidney including its contour, alignment, size and the status of contrast excretion. The serial films also can provide information on the inner details of the drainage system.

II.3.3. Ultrasound

Ultrasound has now become the widely accepted and non-invasive examination of the urinary tract in the adult and it is probably the investigation of first choice in pediatrics. The advantages of this technique being non-invasive, without the hazard of contrast reaction and does not involve irradiation of either patient or operator.

The ultrasound beam behaves as light beam which can be focused, reflected and refracted. It is generated by vibration of the crystal within an ultrasound transducer. The crystal vibrates in response to an electrical stimulus and the frequency of vibrations is also a function of the shape and the thickness of the crystal itself. The ultrasound frequency used for the medical purpose ranged from 3.5-10 MHz and the same crystal acts as both transmitter and receiver. The returning echoes produce vibrations are being converted to a gray-scale

image. The strengths of returning echoes depend on the difference in the acoustic impedance. That is the higher the difference in acoustic impedance, the stronger the returning echo.

Clinically ultrasound can differentiate a renal mass from a cyst to tumour. In case of the renal failure, the cause can sometimes be found by judging the renal size, cortical thickness and the cortico-medullary details of the renal parenchyma. More recently, the introduction of doppler principle in ultrasound make it possible to assess the presence, direction, and velocity of blood flow and is of great value particularly in the transplanted kidney.

In the aspect of interventional radiology, it provides a guide for needle aspiration cytology, cyst aspiration, transrectal prostatic biopsy and for the percutaneous nephrostomy.

Although the advantages of ultrasound are significant, there are still some drawback. Ultrasound can not pass through the bone and gas that makes imaging impossible. Fat tends to attenuate the sound waves so studies of obese people are usually poor in quality. Moreover the examination is highly operator dependent.

II.3.4. Computed Tomography

Computed Tomography (CT) has been in clinical use only since the late 1970s. It provides a cross-sectional imaging modality that represents a combination of x-ray and computer technology. A rotating fan-shaped x-ray beam emanates from a large doughnut - shaped gantry surrounding the patients and penetrates the body in a thin (1cm) transverse cross section. An array of detectors also located in the gantry records the distribution of attenuated X-ray beam by the body tissue and stores the digitized data in the system's computer. By solving multiple, complex mathematical equations, the computer reconstructs a two-dimensional image of anatomic cross-section from the x-ray data. The resulting images are far more superior quality than conventional tomographic radiography. Tissue characterization is by assigning different CT numbers to different tissues.

The indication for CT are many and include the evaluation of renal space occupying lesion that is not unequivocally characterized by ultrasound; the evaluation of suspected or known multiple mass lesions; the evaluation of suspected or known angiomyolipomas or other vascular malformation; the staging and follow up management of malignancies that related to the urinary tract.

Moreover, CT is very helpful in guiding biopsy needles used for percutaneous biopsy of the adrenal gland as well as other retroperitoneal

masses, and in the placement of drainage tubes for the treatment of perinephric and other retroperitoneal or pelvic fluid collections.

II.3.5. Renal Angiography

Renal angiography provides mapping for the kidney blood supply prior to partial nephrectomy, renal transplantation and the most rewarding if interventional procedures are being contemplated. It is carried under local anesthesia through a femoral artery puncture using Seldinger technique with proper catheter introducing via the puncture site into the aorta and then selectively into the appropriate renal artery.

II.3.6. Magnetic Resonance Imaging (MRI)

MRI is another technique which can provide sectional, anatomical information of the urinary system. The images obtained are the result of complex interactions that occur whenever hydrogen atoms, abundant in the human body, are exposed to an applied radiofrequency waves under a strong magnetic field. Excellent anatomic details can be obtained without exposing the patient to any ionizing radiation.

II.3.7. Radionuclide Imaging

All the imaging techniques mentioned previously seem to provide structural details. They are, in no way, accurate nor informative in evaluating renal function. Radionuclide Imaging technique is far more superior in providing both anatomical and functional of the kidneys. The most common indications of the radionuclide renal examination are:

- the assessment of renal perfusion and tracer drainage
- the evaluation of renal parenchymal scarring
- the measurement of renal function (GFR, ERPF)
- the assessment of vesico-ureteric reflux

II.4. Radiopharmaceuticals for renal parenchyma imaging

For optimal imaging of renal parenchyma, the tracer should be bound irreversibly to renal parenchyma and not be excreted into the urine. The first agent, radiolabelled mercurial diuretics, provides a useful agent. However, this agent is no longer in clinical use (Reba et al., 1963).

Nowadays the most popular renal parenchyma imaging agents are Tc-99m DMSA and Tc-99m Glucoheptonate (GHA).

II.4.1. Tc-99m GHA

II.4.1.1. Chemistry of Tc-99m GHA

Two molecules of glucoheptonate react with one atom of reduced technetium (+5 oxidation state) in alkaline medium to form a complex with a net negative charge. The Tc-99m(+5) bind to two oxygen atoms of the carboxylic and the adjacent hydroxyl groups with 99m Tc=0 as the core of the complex.

II.4.1.2. Preparation

3-6ml of Tc-99m pertechentate containing up to 3.7 GBq (100mCi) was added to freeze-dried calcium glucoheptonate and stannous chloride and then shaked the vial for 20 second to ensure complete dissolution of the powder. The preparation was stable for up to 6 hours.

II.4.1.3. Doses

The usual dose used for renal imaging is 370-555 MBq (10-15mCi)

II.4.1.4. Biological behavior

After intravenous injection of Tc-99m GHA, the rate at which the activity disappears from the blood and appears in the urine is comparable to that for filtrated agents, at least for the first few hours after administration (Arnold et al.,1975). Tc-99m GHA is excreted mainly by the kidneys through glomerular filtration and tubular secretion. Protein-bound Tc-99m GHA is excreted by tubular secretion, while the unbound component is excreted by glomerular filtration. The retention in renal cortex is 10% of the injection dosed at 1 hr (McAfee et al., 1979). By 3 hours, 12% of the dose has been accumulated in the kidneys and becomes static for 6 hours (Arnold et al., 1975). The urinary excretion is 70% within 24 hours (McAfee et al., 1979).

Imaging during the first hour after administration permits the evaluation of the collecting system, whereas delay imaging, preferably at 3 to 4 hours, can yield high quality images of the renal parenchyma.

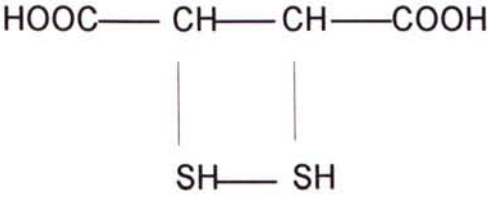
In my study, I need to measure the renal parenchyma uptake at 24 hours post injection. The fraction of administrated Tc-99m GHA dose retained by the kidney at that time is relatively small and affecting the counting statistics. Whereas the retained fraction of Tc-99m DMSA in the kidneys is about 50% and can provide a better counting statistics. Therefore the choice of radiopharmaceutical used for parenchyma imaging in this study is Tc-99m DMSA.

II.4.2. Tc-99m DMSA

II.4.2.1. Chemistry of Technetium-99m Dimercaptosuccinic Acid (Tc- 99m DMSA)

Unlabeled DMSA was originally used to treat heavy metal poisoning. The technetium chelate was found to localize in the kidneys and is used for imaging of the renal parenchyma (Lin et al., 1974).

Chemical structure of Unlabeled DMSA



The reasons for choosing the Tc-99m pertechentate as the radionuclide are as follows:

Desirable chemical characteristics

1. Its variable oxidation states and coordination numbers have led to the preparation of wide variety of Tc-99m radiopharmaceuticals.

2. Its ease of formation of complexes and chelate makes it suitable for the 'in-house' preparation of Tc-99m radiopharmaceuticals.
3. Its separation from its parent radionuclide (99 Mo) is easily accomplished in an enclosed and sterilized generator system.
4. Its generator eluate (Tc-99m pertechentate) does not need further processing or purification and can either be directly administered or used for the preparation of other Tc-99m radiopharmaceuticals.

Desirable Physical Characteristics

1. Its monoenergetic gamma ray of 140 KeV is easily collimated and highly suitable for gamma camera crystal to produce a sufficient number of photons.
2. Its physical half-life of 6.0 hours is long enough to obtain desirable diagnostic information in many studies and is short enough to cause any radiational hazard to the personnel and patients.

II.4.2.2. Chemical property of Tc-99m DMSA

Tc-99m pertechnetate can present in different oxidation states which can react with Dimercaptosuccinic Acid to form different complexes resulting in different biodistribution. The oxidation state of the technetium in the renal imaging

complex has been reported to be +3 or +4 and is formed at pH 2.5 (Ikeda et al., 1977). While the oxidation state for technetium for imaging the medullary carcinoma of thyroid is +5 and is formed at pH 7.5 - 8(Ohta et al., 1984).

II.4.2.3. Preparation

1-6 ml of Tc-99m pertechentate, containing up to 40 mCi was added into a vial containing sterile, pyrogen-free freeze-dried mixture of DMSA, stannous chloride dihydrate, ascorbic acid, sodium chloride and inositol and then carefully inverted the vial a few times until the powder was completely dissolved. The incubation time was at least 10 min at room temperature and should be used within 4 hours.

II.4.2.4. Radiochemical purity measurement

An assessment of the radiochemical purity of the preparation can be made using thin paper chromatography on Whartman No 1 paper with methyl ethyl ketone as solvent. Place a drop of Tc-99m DMSA preparation one cm from the lower end and develop in acetone up to 5 cm and dry air. Cut at the center, assay each piece in a dose calibrator and calculate the percentage labeling efficiency as follows:

Labeling efficiency (%) = 100 -% Tc-99m pertechenate

II.4.2.5. Doses

The usual dose for parenchymal image is 1-5 mCi

Table 1 Estimated radiation absorbed dose of 99m-Tc DMSA

Organ	rad/mCi
Kidneys	0.63
Bladder wall	0.07
Adrenals	0.05
Spleen	0.05
Ovaries	0.014
Testes	0.007
Uterus	0.017

(From Azuwuike 1995. The handbook of radiopharmaceuticals)

II.4.2.6. Pharmacokinetic of Tc-99m DMSA

After intravenous injection of Tc-99m DMSA, most of the circulating activity (75-90%) is bound to plasma proteins (Arnold et al., 1975). Its renal clearance is lower than for the filtered agents : a value of 34 ml per minute has been reported in normal adults (de Lange et al., 1989). Moreover, glomerular filtration is insignificant as compared with tubular secretion (Yee et al., 1981).

Most of the activity removed from blood is retained in the renal cortex, about a third passing unchanged into the urine (de Lange et al., 1989).

Tc-99m DMSA reached the tubules via the glomerular filtrate as well as tubular extraction from the peritubular capillaries.

Renal uptake of Tc-99m DMSA is 24% of the injected dose at 1 hour (Lin et al.,1974). Using an autoradiographic technique in rats, it has been shown that at 1 hour, Tc-99m DMSA accumulates mostly in the proximal and distal tubular sites of the cortex and to a lesser extent in the renal medulla, glomeruli, collecting tubules and blood vessels (Wills et al., 1977). The ratio of radioactivity in the cortex to medulla is 22:1, while that of tubules to glomeruli is approximately 27:1(Hosokava et al., 1978).

The accumulation of 99m-Tc DMSA in the kidney is probably due to its binding to metallothionein, a heavy metal binding protein that has approximately 50 mercapto groups per mole (Kagi et al., 1974).

Renal accumulation is unaffected by probenecid and PAH (Lee and Blaufox,1985). Accumulation is influenced by acidification of the urine in the rat (Yee et al., 1981). Uptake is decreased by angiotensin-converting enzyme (ACE) inhibitor, raising the possibility of tubular reabsorption (Hovinga et al., 1989: Kopecky et al., 1990). Urinary excretion is increased in the presence of tubular reabsorption (de Lange et al., 1989).

II.4.2.7. Renal handling of injected Tc-99m DMSA

After intravenous injection of Tc-99m DMSA, the extraction rate is 4-5% per renal passage. 25% of the injected dose reaching the kidneys within 1 hour. 50% of injected dose present in cortical tubules at 24 hour. Enlander and coworker investigated the biokinetics of Tc-99m DMSA in 35 patients and found that in most normal individuals the blood disappearance of Tc-99m DMSA followed a single exponential with a mean half-time of 56 minutes with only 6-9% of the dose present in the blood 14 hours after injection.

The renal uptake of Tc-99m DMSA is by means of active transport mechanism. In case of renal failure, Tc-99m DMSA is also uptake by the liver, gallbladder and the gut. Moreover oxidation will degrade the Tc-99m DMSA complex causing decrease uptake in the kidney and increase liver back ground activity.

The Tc-99m DMSA molecules split upon reaching kidney. One moiety binds to renal tubules, (that is **DMSA localize to the renal cortex by binding to sulfhydryl groups in proximal renal tubules**), other excreted. The elimination process is by means of complex interaction of glomerular filtration, peritubular uptake and eventually tubular secretion.

Enlander et al., reported that the cumulative urinary excretion of Tc-99m DMSA was 6% at 1 hour and 25 % at 14 hours.

II.5. General consideration for quantitative uptake measurement in organs

The quantitative assessment of organ radioactivity determined on the basis of external count rate measurement has many applications in the field of medical diagnosis (Clark et al., 1972; Ferrant et al.,1979; Fleming et al., 1979).

Most of the work accomplished has been performed with the rectilinear scanners or non-imaging fixed detectors. Nowadays, digital gamma cameras are widely available, and quantification can be performed on the digital image in a much easier way with the aid of advanced computer technology. However, the quantification assessment of the absolute uptake of an organ has never been straightforward and easy to achieve because the planar gamma camera is a two dimensional representation of a three dimensional radioactivity distribution in vivo. The image count obtained by the region of interest (ROI) defined around an organ is actually invalidated by the following factors:

- The count density recorded in a defined ROI is contributed by the counts from the region of interest, the overlying and underlying tissue, and the scattered radiation outside the ROI.
- There is never a sharp distinction between organ and background activity, due to the physical shape of the source and the smoothing effect of the modulation transfer function of the gamma camera.
- The distribution of the organ and background activity is not uniform.

- The response of the gamma camera is a function of patient-detector distance (sensitivity) and the image position with respect to the field of view (uniformity).

We should be borne in mind that the counts recorded over the ROI did not represent the true quantity of radioactivity within the organ and will be complicated by the above mentioned factors.

II.5.1. Clinical significance of renal Tc-99m DMSA uptake

Both the uptake and the binding of Tc-99m DMSA require the presence of functioning renal cellular element (Lin et al., 974).

Taylor et al., (1980) and Kawamura et al.,(1980) have shown that absolute quantitation of Tc-99m DMSA uptake can be used as an index of individual renal function.

Goldraich (1982) has shown that the absolute quantitation in the kidneys of children is possible 6 hours following intravenous injection when the uptake reaches a plateau. Others workers have shown that this is true in animals (Moretti et al.,1982, Godley et al., 1985).

Tc-99m DMSA can therefore act as an indicator of functioning renal mass (Taylor 1989) which expresses as tubular uptake of Tc-99m DMSA.

Numerous comparative studies also show good correlation between Tc-99m DMSA uptake and relative clearances of creatinine (Daly et al., 1979, Price et al., 1979), iothalamate (Powers et al., 1979), PAH (Kigashihara et al., 1988) and relative renal uptake (Choi et al., 1985, Bingham et al., 1978).

Pathophysiological conditions of kidneys characterized by an alteration in the ability to the renal uptake of Tc-99m DMSA. For this reason, the tubular uptake of Tc-99m DMSA can be utilized to monitor the renal function.

II.5.2. Special consideration and problems for quantitative renal Tc-99m DMSA uptake measurement

The major difficulties in measuring absolute individual renal function with radionuclides include the following:

- The rates of change of the renal input
- The blood and tissue background
- Renal uptake
- The effects of different renal depths on the attenuation of the renal count rate
- The problem of relating the detected activity to the injected dose
- The sensitivity of the gamma camera and the dead time losses in count rate if a syringe containing high activity is counted on its face

The detail of the above mentioned difficulties will be discussed in the following sections.

II.5.3. Suggestions and solutions for quantitative renal Tc-99m uptake measurement

II.5.3.1. Planar images Vs SPECT images for quantification

The renal uptake of Tc-99m DMSA provides a practical index for the evaluation of cortical function. Quantitation of Tc-99m DMSA uptake has been

attempted using planar scintigraphy (Kawamura et al., 1978; Baillet et al., 1985; Van et al., 1985) or Single Photon Emission Computed Tomography (SPECT) (Groshar et al., 1989).

In using SPECT, Groshar et al.,(1989) found that the absolute individual renal uptake of 14.7% of the injected dose of 99m-Tc DMSA could be act as index in separating normal kidney from diseased kidney with a sensitivity of 95%, a specificity of 92%, and an accuracy of 94%. However there are several factors affecting the quantitative reliability of SPECT:

1. The reconstruction algorithm with the chosen filter
2. The angular and linear sampling
3. The limited number of events
4. The attenuation and the scattering of gamma photons
5. The algorithm used to define the target
6. The performance of the gamma camera

Although, SPECT images are able to detect more defects, they are more technically demanding. Moreover, the effect of image blurring due to respiratory motion cannot be minimized and offer no statistically significant advantage (Semin Nucl Med, July 93, p.212.). A recent report (JNM,1996, p1346) showing SPECT defects in normal volunteers suggest specificity may decrease significantly with SPECT imaging.

In this project, the planar scintigraphy is adopted rather than SPECT for the absolute quantitation of the Tc-99m DMSA renal uptake, because there are

extremely limited amount of the scintigraphic events occurring in the delayed 24 hours imaging and the error obtained in quantitation may be substantial.

II.5.3.2. Background Subtraction

Background correction is designed to remove counts due to activity in those structures overlying and underlying the organ of interest. This is a very important step in uptake quantification. Estimation of background activity is usually performed by defining a background region of interest (ROI) close to or surrounding the organ of interest, but not overlapping. The average counts per pixel recorded in the background ROI are assumed to be the same as the background activity overlying and underlying the organ of interest.

The background corrected count in the organ ROI is then given by :

$$C_{net} = C_{orig} - C_{bkg}\frac{Npix_{organ}}{Npix_{bkg}}$$

where C_{orig} and C_{net} are the original and background corrected counts in the organ ROI respectively, C_{bkg} is the number of counts in the background ROI, the $Npix_{organ}$ and $Npix_{bkg}$ are the numbers of pixels in the organ and background ROIs respectively.

This technique assumes a uniform background distribution. However, background may be markedly non-uniform in certain cases. Overestimation of

background may also occur, because the full depth of the patient contributes to the background ROI, while, over the organ itself, background only comes from that part of the patient's depth not occupied by the organ.

In order to offset this error, a more complicated method known as ***interpolative background subtraction*** was introduced in estimating the background activity by Goris et al., (1976). It estimated the background activity at each pixel over the organ of interest based upon the background at points on a boundary circumscribing the organ.

Although the ***interpolative background substraction*** is more accurate in assessing the background, it is quite tedious and time consuming. So in this study, I choose a much simpler, but close to the reality, method of background substraction.

II.5.3.3. Choice of location for background ROI

The proper location of background ROI was very important in calculating the background corrected renal uptake values. It represented the amount of extrarenal activity that had to be subtracted from the renal image.

In the presence of poor renal function, the analogue images may show varying degrees of uptake by the reticulo-endothelial system mainly in the liver but also to a lesser extent in the bone marrow.

Inclusion of an area between the kidneys in the background has two problems; firstly Tc-99m DMSA uptake in the spine itself and secondly the scatter from the opposite kidney into the background region. Alternatively a background region simply above the kidneys would also be inappropriate. They obviously may overestimate the background activity.

On the contrary, inclusion only the lateral aspect of the kidney may underestimate the background activity.

Gordon et al. (1987) used different regions for background subtraction in assessing the mean percentage Tc-99m DMSA kidney uptake in 34 children with the following result:

1. With no background subtraction, the mean absolute left kidney uptake is 26.6 ± 3% and the mean absolute right kidney uptake is 27.1 ± 2.3%.

2. With inclusive regions from the above, below, in the lateral and medial aspect of the kidneys for the background subtraction the mean absolute left kidney uptake is 21.6 ± 2.8%, the mean absolute right kidney uptake is 21.6 ± 2.3% which acts as the standard for comparison.

3. With the regions chosen only from the medial aspect of the kidneys for the background subtraction, the mean absolute left kidney uptake is 19.2 ±

3.1%, the mean absolute right kidney uptake is 21.6 ± 2.4%. This seems to underestimate the kidney uptake.

4. With the regions chosen only from the lateral aspect of the kidneys for the background subtraction, the mean absolute left kidney uptake is 23.2 ± 2.7%, the mean absolute right kidney uptake is 23.4 ± 2.4%. This seems to overestimate the kidney uptake.

5. With the regions chosen from infero-medial and lateral aspect of the kidney for the background subtraction, the mean absolute left kidney uptake is 22.7 ± 2.8%, the mean absolute right kidney uptake is 23.1 ± 2.3%

6. With the regions chosen only from the superior aspect of the kidneys for the background subtraction, the mean absolute left kidney uptake is 21.8 ± 2.82% , the mean absolute right kidney uptake is 21.0 ± 2.2%. This seems to be quite close to the standard one for estimating the kidney uptake. Unfortunately it sounds good only for the patients with good renal function.

In my study, there is a wide spectrum of patients with different degree of renal function, I would rather adopt the regions chosen from infero-medial and lateral aspect of the kidney for the background subtraction in order to avoid the

varying degree of uptake by the liver. It seems to be acceptable as judging from the published work of Gordon et al., 1987.

II.5.3.4. Attenuation

Attenuation of gamma-rays by the body itself and the imaging table will lead to an underestimate of the radioactivity in the organ. If the depth of the organ and the attenuation coefficients of the tissues transversed are known, an attenuation correction factor can be calculated. The attenuation-corrected count C_{corr} can be obtained from the following equation

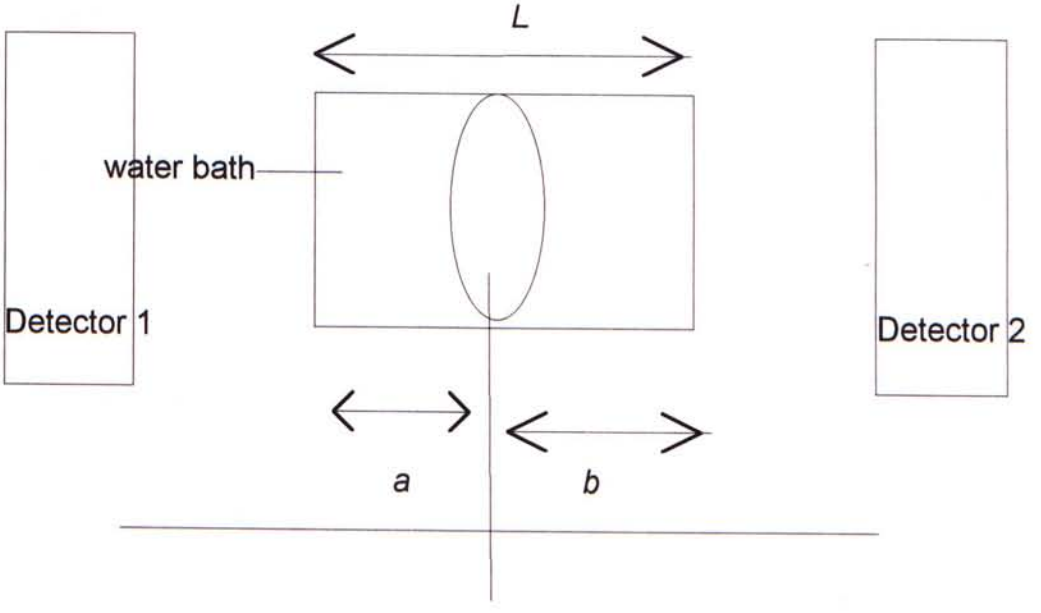
$C_{corr} = C_o e^{\mu d}$

where C_o is the measured count and $e^{\mu d}$ represent the effective attenuation factor for a point source at depth d.

Unfortunately the distribution of attenuation and the depth of the organ are unknown, we frequently use the conjugate view method. A series of experiments were also conducted in my study to determine the table attenuation and approximate the tissue attenuation.

II.5.3.5. Principle of the conjugate view method

It uses the geometric mean on the counts obtained from opposing views of an organ. The geometric mean of two quantities is defined as the square root of their product.



The conjugate view method of correcting for attenuation for oval source of activity

From the above illustrated diagram

I_1 = *measured counts from detector 1*

= $I_o e^{-\mu a}$

where Io is the unattenuated counts measured from oval source and a is the distance measured from the centre of oval source to surface of detector 1.

I_2 = measured counts from detector 2

$= Io\, e^{-\mu b}$

where Io is the unattenuated counts measured from oval source and b is the distance measured from the centre of oval source to surface of detector 2 and b=*L-a*

The geometric mean counts = $(I_1 I_2)^{1/2}$

$$= Io\ e^{1/2\,-\mu L}$$

Therefore the geometric mean count is independent of *a* and *b*, that is independent of the depth of the source in the attenuating medium.

It has a direct relationship with the object's thickness and the effective attenuation factor, μ.

The methods for estimating the attenuation due to body and imaging table will be discussed in the section **III**.

II.5.3.6. Body thickness and kidney depth measurement

In evaluating the absolute renal function, the correction for attenuation due to kidney depth is a necessity. Using sodium Tc-99m pertechenate, 1cm difference may be responsible for radiation attenuation of about 14%

(Grunewald 1985), so an accurate and reliable renal depth measurement method is important.

Hambye et al (1992) compared 50 measurements of renal depth using Tc-99m DMSA to that measured by computed tomography, a gold standard. They found a mean underestimation of 0.78 cm for the right kidney and 0.77 cm for the left kidney when using Tc-99m DMSA, with a correlation coefficient of 0.85 for the right kidney and 0.79 for the left kidney ($r = 0.82$ for both kidneys). Moreover they found, in comparison, using the Tonnesen's formula with regard to the CT method, the mean underestimation's for both kidneys are 1.7cm with a correlation coefficient of 0.77.

By using the radioisotope method or using an empirical formula in calculating renal depth, there exists a large discrepancy from the 'gold standard' and will affect the absolute quantitation of renal uptake.

A method, such as conjugate view method, which can quantify the organ uptake without knowing the organ depth seems more reliable. Furthermore, the body thickness is a parameter which can be easily and reliably to measure.

II.6. Glomerular Filtration

II.6.1. Introduction

The measurement of glomerular filtration rate, which is expressed as milliliters of filtrate formed in the glomeruli per minute, is an important milestone in renal physiology and the consequence of the combined efforts of various workers in the 1920s and 1930s. GFR is expressed as a clearance value, that is, a parameter of renal function determined from the clearance of a compound from the blood by the kidney.

Mathematically the volume of blood cleared of a substance in 1 minute's excretion of urine is determined by the following formula:

$$V_b = \frac{C_u x V_u}{C_b}$$

Where

- V_b = volume of blood cleared of a substance per unit time (ml/min)
- C_b = blood concentration of substance being cleared (in milligrams per deciliter)
- V_c = timed volume of urine containing the cleared substance (24 --hour urine collection expressed in ml/min)
- C_u = urine concentration of the substance whose clearance is being measured (mg/dl)

Clinically, any agent used in measuring glomerular filtration should satisfy the following criteria:

1. Completely filtrated at the glomerulus
2. Not synthesized, destroyed, reabsorbed, or secreted by the renal tubules
3. Physiologically inert
4. Not bound to plasma protein

II.6.2. Gold standard for GFR measurement

Inulin meets all the above mentioned criteria as the agent used in measuring the GFR and was proposed as a reference standard for GFR measurement by Richards et al (1934) as well as by Shannon and Smith (1935). Inulin is an inert fructose polysaccharide originally derived from dahlia roots and Jerusalem articholes and was found to be an ideal substance, from a theoretical standpoint, for measuring GFR because it is removed from the blood stream exclusively by glomerular filtration. Practically, the requirement for constant intravenous infusion, frequent blood sampling, timed catherizes urine collections, and a somewhat cumbersome analytical technique has limited inulin clearance determination in daily clinical practice.

II.6.3. Laboratory Studies for the measurement of glomerular filtration: Serum Creatinine and Blood Urea Nitrogen (BUN) levels

Serum Creatinine and Blood Urea Nitrogen (BUN) levels are two of the more common chemical determinations used to measure glomerular filtration. The creatinine level is much more reliable as a parameter of glomerular filtration because creatinine is only minimally secreted by the renal tubules and offers the clinician a far better measurement of actual glomerular function than does the BUN level.

Urea is significantly reabsorbed by the renal tubules, the rate of reabsorption is inversely related to the rate of urine flow through the renal tubules; therefore, the slower the flow rate, the more urea is reabsorbed by the tubules and returned to the general circulation, the higher the resulting BUN levels become.

Moreover there are numerous non-renal factors that can affect the plasma concentration of BUN while leaving the level of serum creatinine relatively unaffected. First, bleeding into the gastrointestinal tract will lead to an increased breakdown in red blood cells, with increased reabsorption of protein producing the same net effect as though the individual patient was on a "high-protein diet". This high-protein diet would cause an increase in the BUN level whereas a low-protein diet would cause a decrease in the BUN level.

Dehydration of the patient would bring about a hypovolemia and a slow flow rate through the kidneys, thereby leading to increased reabsorption of the urea from the renal tubules and an increase in concentration of BUN. Although severe dehydration can also result in a rise of the serum creatinine without any intrinsic renal disease being present, the creatinine levels are not so seriously affected by state of hydration as are the BUN levels.

Unfortunately the creatinine level is a function of and proportional to the muscle mass of the individual. The normal serum creatinine level for a large, muscular man would be expected to be higher than that for a small, unmuscular woman.

Renal function is not constant throughout life, and with advancing age glomerular filtration tends to decreases, leading to elevation in the normal serum creatinine levels for different age groups. Under the age of 60 years the upper limit of normal for creatinine in man and woman is probably around 1.4 mg/dl. Above age 70 years, the normal creatinine levels can be around 1.8 mg/dl

Technically, the laboratory determination of serum creatinine levels is a colorimetric one, any other non creatinine chromatogens, in particular, acetone and glucose will falsely raise the creatinine concentration. Therefore the 24-hour creatinine clearance test is more appropriate in assessing the renal function.

II.6.3.1. Calculation of Creatinine Clearance Rate

Creatinine Clearance Rate (an estimate of glomerular filtration rate) is best determined on a 24-hours urine collection. The formula for creatinine clearance, first introduced by Van Slyke, is

$$C = \frac{UxV}{P}$$

where

- *C* is the creatinine clearance in milliliters per minute
- *U* is the concentration of the creatinine in the urine expressed as milligrams per milliliter or per deciliter
- *V* is the urine flow rate in milliliters per minute (24-hour volume divided by 1440 min/day)
- *P* is the concentration of creatinine in the plasma in milligrams per milliliter or per deciliter

Therefore the creatinine clearance

$$C(ml/\min) = \frac{U(mg/dl)xV(ml/\min)}{P(mg/dl)}$$

Creatinine clearance should be corrected to 1.73 square meter of surface area for comparison.

Glomerular filtration rate can be estimated by determining the clearance of creatinine. The GFR determined in women is approximate 10 % less than in men owing to the difference in the amounts of muscle mass between the sexes even after correcting for the body surface area as stated by Ganong (1977). However, gender difference can be eliminated by correcting for lean body weight (Doolan et al.,1962)

II.6.3.2. Critique for using creatinine clearance as a measurement of renal function

Creatinine is cleared almost entirely by glomerular filtration, although some tubular secretion occurs (Ganong,1977; McNeely,1980) that may progressively increase as GFR decrease to approximate 20 ml/min, below which the tubular secretion of creatinine is progressively impaired (Bauer et al., 1982).

In view of the limitation and inaccuracy of the creatinine clearance , that is the urinary creatinine concentration is raised both by tubular secretion (Bauer et al., 1982) and by dietary meat (Pasternack et al.,1971), and the timed urine collection, the measured creatinine clearance is often inaccurate.

Payne (1986) argued that creatinine clearance should be scrapped and serum creatinine concentration used alone as a measure of renal function.

Several published formulae (Cockcroft et al., 1976; Gates, 1986; Jelliffe, 1973) were used to estimate the creatinine clearance from a serum creatinine value.

These methods are good for approximately total GFR in steady states and are useful as rapid "bedside" estimates of renal function, particularly in patients in whom drug dose requirements are being calculated (Jelliffe, 1973; Lesar et al., 1982).

Here below is one of those such formulae as suggested by Gates (1985)

Men

Creatinine clearance = $(89.4)(X^{-1.2}) + (55 - A)\ [0.005\ (89.4)\ (X^{-1.2})]$

Women

Creatinine clearance = $(60)\ (X^{-1.1}) + (56 - A)\ [0.005\ (60)\ (X^{-1.1})\ ]$

where

- X is the serum creatinine concentration (mg/dl)
- A is the patient age (in years)
- Calculated creatinine values are normalized to standard surface area of 1.73 m^2

As reported by Gates (1985), the accuracy of estimation was acceptable, especially below 100ml/min.

II.6.3.3. Limitation of the serum creatinine concentration used alone as a measurement of renal function

Serum creatinine does not have a linear relationship to the glomerular filtration rate measured using chromium-51 edetic acid as reported by Gabreil et al.,1975 (unpublished data). Moreover their use is limited to steady-state conditions.

With regard to dietary intake, in one study, serum creatinine concentration risen by 70-80% after an unphysiological meal containing 300g protein (Jacoben et al., 1980). The source of protein is probably important since 90g of milk protein did not change the creatinine clearance, while in the same group of eight healthy volunteers 90g of meat protein increased the clearance by 18% (Jones et al., 1985)

In women, clearance increases up to 20% premenstrual, slight reducing the serum creatinine concentration (Davison et al., 1981).

Moreover if serum creatinine is to replace creatinine clearance, doctors will require age related range of serum creatinine concentration (and 95 % confidence intervals) for men and women, in pregnancy, in childhood, and in renal impairment. Although the figures from one large study of 50,000 subjects provide the data for serum creatinine concentrations in healthy men and women (De Lauture et al., 1973), and the preliminary figures for pregnancy are available (Davison et al., 1981; Moniz et al., 1985), data for infancy and

childhood are sparse (Moniz et al., 1985; Rudd et al., 1983). No reference ranges are available for patients with renal impairment.

Judging from the above argument, Gabriel (1986) stated creatinine clearance with urine collected under controlled conditions remained the simplest, cheapest, and most useful measure of renal function. And it may be replaced by the concentrations of serum creatinine once tables correcting for age, sex, weight, pregnancy, and renal failure are available.

II.6.4. Radionuclide technique for the assessment of the glomerular function

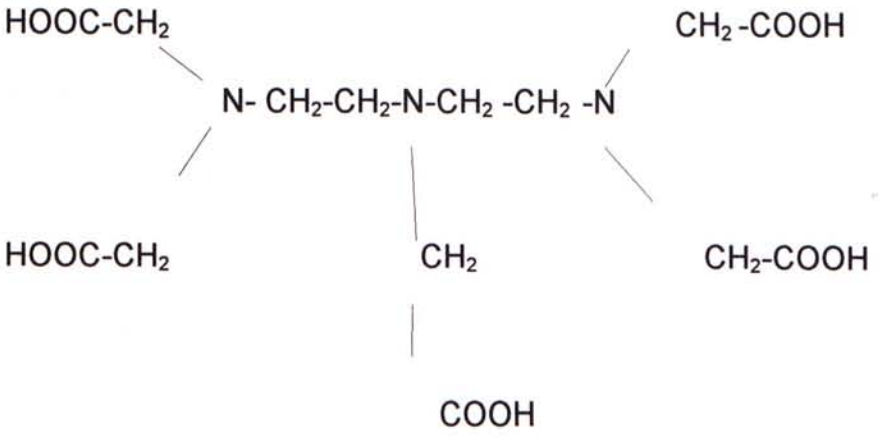
Owing to the disadvantages and limitations of the above-mentioned methods, an alternative technique using radionuclides was introduced in measuring GFR. This technique was modified from the ERPF-based renography. The main principle was to label the GFR agent with radioactive isotopes. For instance, radiolabeled (cobalt-57) vitamin B_{12} has been used in attempts to measure GFR (Nelp et al., 1964). Similarly radiographic contrast agents labeled with either ^{125}I or ^{131}I have been used to measure GFR. And Ethylenediaminetetraacetic acid (EDTA) labeled with ^{51}Cr has a plasma clearance that correlates very well with inulin clearance as reported by Brochner-Mortenson (1969) and Chantler (1969). However the use of these agents offered no renal imaging and the individual renal GFR cannot be obtained.

This problem was finally solved with the development of gamma camera and subsequent use of Tc-99m pertechnetate plus Tc-99m labeled renal scanning agents. Diethylene Triamine Penta Acetic acid (DTPA) labeled with Tc-99m is the radiopharmaceutical of choice when performing a radionuclide renogram during which GFR can be calculated.

II.6.4.1. Diethylene Triamine Penta Acetic acid (DTPA)

Structurally DTPA ,is a soluble organic molecule, which is stable at physiological pH.

Chemical structure of DTPA:



When stannous ions (Sn^{++}) are mixed with pertechnetate, the chelating properties of DTPA result in technetium stannous DTPA with a net negative charge in neutral or weakly acidic solutions (Russel et al., 1980). The oxidation state of Tc-99m in the complex is not known, but it has been reported to be III, IV, V or a combination of these as reported by Russel (1982).

Biologic behavior

Following intravenous injection, Tc-99m DTPA is rapidly cleared from the blood by glomerular filtration (Kloppen et al., 1972). Renal retention is 7% of the

injection at 1 hour and 95% is excreted within 24 hours (Arnold et al., 1975). It is neither secreted nor reabsorbed by the renal tubules and has negligible biliary excretion and elimination in feces.

The images of the kidneys obtained in the first few minutes after injection represent the vascular pool, while subsequent images represent parenchymal handling of the radiopharmaceuticals with subsequent excretion into the collecting system.

In the plasma, 2-6% of the Tc-99m DTPA is bound to protein. The protein binding is more significant with constant infusion of DTPA compared with bolus injection (average protein binding at 1 hour of 9.7% versus 3.7%, respectively) as reported by Klopper et al.,(1972). Therefore clinically the clearance of Tc-99m DTPA is slightly lower than I-125 iothalamate clearance.

II.6.4.2. Methods

II.6.4.2.1. Measurement of Glomerular Filtration Rate using Tc-99m DTPA with single injection techniques

Several different techniques are available to measure the total GFR using Tc-99m DTPA. These include:

1. Calculation of a two-compartment clearance curve constructed from multiple venous or capillary blood samples
2. A single exponential curve fitted to several plasma samples
3. A modification of Tauxe's OIH method in which counts in a single plasma sample correlated with a GFR nomogram
4. A modification of Schlegel's OIH technique introduced by Gates

Each of these methods will be discussed in the following sections.

II.6.4.2. Compartment model

II.6.4.2.2a. Two-compartment model

The plasma disappearance curve appears to be a double exponential process as shown in Fig.1.

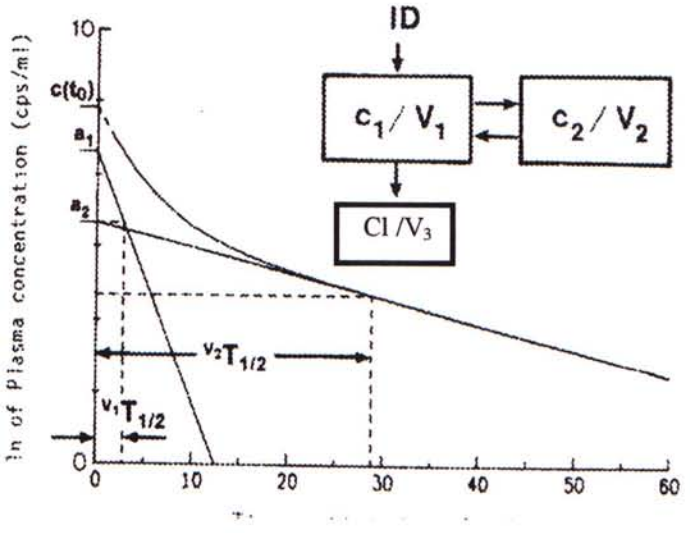


Fig.1 Schematic representation of the two-compartment model according to Sapirstein et al., (1955)

According to Sapirstein(1955), the curve can be described as follows:

$y = a_1 \times e^{-b1t} + a_2 \times e^{-b2t}$

a_1 and a_2 correspond to the intercepts of the partial components with y-axis , b_1 and b_2 corresponding to the decay constants of the respective compartments. V_1 is the theoretical volume including plasma volume. V_2 is the closed system in which the material equilibrates. V_3 is the excreted volume. Multiple plasma

samples are obtained and counts are plotted against time on a semilog paper. A biexponential curve is found with a fast and slow component. The fast component represents intravascular renal clearance; slow component reflects clearance from the interstitial space. The slow component (with decay constant of b_2) is subtracted from the curve deriving the fast component (with decay constant of b_1) , and the intercepts of a_1 and a_2 along the Y-axis (counts per minute) are determined.

$$GFR(ml/\min) = \frac{Ixb_1xb_2}{(a_1xb_2)+(a_2xb_1)}$$

Where *I = total radioactivity injected*

Clinically single-injection methods have been reported by some authors (Ash et al., 1980; Braren et al., 1979; Klopper et al.,1972) using Tc-99m DTPA for determining GFR using the plasma disappearance rate of tracer to determine glomerular function. Multiple blood samples are required for up to 4-hour interval after injection to perform the exponential analysis of the plasma disappearance curve of Tc-99m DTPA and from which GFR is ultimately calculated.

Russell (1993) reported the best accuracy for GFR estimations in adult using Tc-99m DTPA, and a multiple blood sampling technique is achieved by obtaining the initial sample 10 minutes after tracer injection and continuing at

intervals for 3, if not 4, hours to account for the slow component of the plasma -activity curve and its important contribution to calculated clearance. As the multiple blood sample technique is so tedious, a more simplified dual blood sample method has been studied by several authors. Duffy et al.,(1982) showed that two blood samples obtained at 2 and 4 hours after Tc-99m DTPA injection allowed for GFR estimations that closely resembled those obtained by use of ^{51}Cr-EDTA clearance. Waller et al., (1987) compared the clearance calculations obtained by 2 and 4 hour blood samples to a seven plasma sample technique conducted over 4 hours with blood drawing starting at 1 hour and continuing every 30 minutes thereafter to the end of the study. The coefficient of correlation, r, is 0.996 and standard error of estimate is 2.8. Similarly Russell et al., (1985) require samples between 1 and 3 hours after injection yet yields results virtually comparable to the multiple sampling technique with r =0.962.

II.6.4.2.2b. Single-compartment model

By using monoexponential single compartment model, a smaller number of blood samplings are necessary at a time when redistribution of the clearance agent from the extravascular space into the intravascular space is dependent primarily on the excretion by the kidneys. The clearance agent is distributed in a single compartment from which it is eliminated exclusively by the kidneys.

The curve would then be identical with the second, slower exponential of the two-compartment model. For the GFR determination, blood sampling should be performed between 3 and 4 hours after injection as reported by Chantler et al., (1969) and, Tauxe et al., (1984).

II.6.4.2.3. Single blood sample technique: a modification of Tauxe's OIH method in which counts in a single plasma sample correlated with a GFR nomogram

Tauxe et al., (1975) presented single sample algorithms for ERPF determination in children. In the same year Fisher and Veall modified this single blood sampling technique to estimate GFR. The technique relied on determination of a semiempirical relationship between plasma clearance of tracer (due to GFR) and the volume distribution expressed as injected activity divided by the plasma sample activity.

Russell et al (1985) introduced a method for calculating total GFR by a plasma clearance technique using a single blood sample. Basically the technique measures the plasma concentration of Tc-99m DTPA at that time and compares it with the amount of tracer injected using an in vitro laboratory technique with carefully calibrated standards. They reported the optimal sampling time in a patient population to be 180 minutes after injection of Tc-99m DTPA, although the best time for an individual patient's blood sample depended on the underlying renal function (Tauxe,1985). And it is supported by Tauxe et al (1985) who reported the overall best accuracy for GFR estimation was achieved when a single sample was drawn 180 minutes after injection with the standard error of estimate of 8ml/min.

Russell et al (1985) compared GFR calculation using both Tc-99m DTPA and Yb-169 DTPA using eight blood samples (obtained at 10, 20, 30, 45, 60, 120, 180, and 240 minutes after injection) with one-point and two-point GFR estimates in 40 patients. The eight-point GFR was calculated by dividing the administrated dose by the integral of plasma time-activity curve, whereas the one-point and two-point GFR estimations were obtained by fitting the data into an open linear two-compartment model. They showed that GFR calculated from two points (1 and 4 hours) had an excellent correlation (R=0.998) with eight-point method. Moreover, a single blood sample drawn at 180 minutes was shown to give reasonable estimated of GFR, although the error was about the twice that of the two-samples technique (3.1 $\pm$ 0.7 ml/min versus 6.6 $\pm$ 1.4 ml/min for the two-point and one-point technique). The one-sample method was judged to be accurate for routine clinical work, whereas the two-sample technique was generally recommended for either investigation use or requiring special accuracy. Russell et al (1985) further stated that second blood sample be obtained at three hours in order to shorten the test without a significant loss of accuracy. Moreover it is supported by the work of Mulligan et al (1990) whom reported the Russell et al (1985) two-point plasma clearance technique (sampling at 60 and 180 minutes) correlated best with the six-point plasma test.

II.6.4.2.4. Gamma camera based method

II.6.4.2.4a. Gates -modification of Schlegel's OIH technique

Introduction

The desire to rapidly measure GFR in clinical setting without using blood and / or urine samplings has lead to the development of techniques using gamma camera imaging in combination with radiopharmaceuticals. Moreover it can be used to measure individual kidney's GFR in the form of split renal function by determining each kidney's percentage uptake of tracer rather than to use radionuclide methods merely to report total glomerular filtration.

The first of the gamma camera methods, the simplest, and the one in widest use today was introduced by Schlegel and Hanway in 1976. Background corrected renal uptake of I-131OIH are summed for the interval from 1 to 2 minutes after injection ($C_{1\text{-}2\ min}$) , corrected for renal depth, and compared with counts obtained by imaging the dose in the syringe prior to injection (C_{inj}) which then is converted into REPF value (Schlegel et al., 1976; Schlegel et al., 1979).

An inverse square law was used to correct uptake (U) for kidney depth (Y) rather than employing a standard attenuation correction technique, the Tonnesen's equation is being used for determining the kidney depth:

$$U = \frac{C_{1-2\,min} Y^2}{C_{inj}}$$

And empirically derived equation based on the 30 minute OIH urine excretion and reports the ERPF in units of ml/min.

ERPF = 5.029 {(0.3698707 x % corrected Uptake) - (2.31476 x 10^{-4} x % corrected uptake ^{2})}

In 1979, Schlegel et al., used a gamma camera to estimate GFR by an I 131 - OIH renogram performed in combination with either a Tc-99m DTPA or Tc-99m Fe-ascorbate study. The main principle use the renal uptake based on premise that corrected renal uptake of 131I -OIH in first 1 to 2 minutes following injection reflects total ERPF to each kidney as discussed in the previous section. Then the GFR can be calculated by multiplying the ERPF with the Filtration Fraction (FF).

But there are several drawbacks on this method.

1. The I131-OIH offering a low count rate that does not permit a perfusion study.
2. Contamination with 3 to 5% free I131 produces errors of up to 25 % on ERPF.
3. OIH calculated ERPF usually 10% below PAH levels owing to the localized protein or cellular binding, differences in handling of two agents by kidney.

Bratt et al. (1981) modified Schlegel's depth correction to include attenuation, scatter, and collimation factors and then applied the resulting method to Tc-99m DTPA and OIH.

Quantitative methods designed for children have been developed by Piepsz et al., (1978) and Shore et al., (1984).

Piepsz et al., (1978) developed the technique for estimating GFR in children by assuming the time-activity cardiac blood pool curve would be identical with the plasma curve. The renal clearance values were calculated for each kidney using the slope of a background corrected renal curve divided by the plasma concentration of tracer, the latter was obtained from the cardiac blood pool time-activity curve and one blood sample drawn at 20 minutes.

They reported a good correlation (r = 0.89) between camera-estimated GFR and creatinine clearance. The Piepsz method has been validated by independent investigators (Vivian and Gordon, 1983) but there is one main drawback of requiring a blood sample at 20 minutes.

Shore et al., (1984) analyzed the slope of the accumulation phase of Tc-99m DTPA renogram curves to estimate total as well as individual kidney GFR in children. They proposed that the initial portion of the renogram curve reflected the rate of renal uptake of tracer and could be related to clearance with the knowledge of plasma concentration of tracer, which in turn was estimated as a value proportional to dose divided by body weight.

Shore reported an excellent correlation (r =0.971) between camera estimated GFR and Tc-99m DTPA clearance as determined by a multiple blood sampling technique.

In 1982, based on Schlegel's OIH technique, Gates introduced a rapid method for estimating total as well as individual kidney GFR using a gamma camera without requiring blood nor urine samples, which was subsequently validated by other investigators (Chachati et al., 1987; Fawdry et al., 1985; Russell, 1985)
The technique converts the fractional renal uptake of Tc-99m DTPA that occurs in the time period from 2- 3 minutes post injection, after background and depth correction, and was expressed as a percentage of net injected counts. The net injected counts were determined by measuring a 1-minute pre and post-injection syringe counts using the gamma camera. The percentage of injected activity localizing within the kidneys was entered into an equation based on a linear regression analysis of the renal uptake of Tc-99m DTPA versus creatinine clearance. The kidney depth was measured by ultrasound in transplant patients or estimated by the Tonnesen's equation.

The Tonnesen's equation calculates the mid-plane kidney depth in centimeters based on patient's height and weight:

$$\textit{Right kidney's depth(cm)} = \frac{13.3 x Weight(Kg)}{Height(cm)} + 0.7$$

$$\textit{Left kidney's depth(cm)} = \frac{13.2 x Weight(Kg)}{Height(cm)} + 0.7$$

The linear attenuation coefficient of Tc-99m DTPA in soft tissue, μ, was 0.153/cm (Gates et al., 1983, Taylor et al., 1991)

The Gates formula was based on correlating the percentage uptake of injected Tc-99m DTPA (Diagnostic Isotopes brand) in 51 adult patients with 24-hour creatinine clearance (range = 1 to 116 ml/min). There was a linear relationship with good correlation (r = 0.97; Sy.x =4.7).

The empirically derived equation depicts the relationship between kidney uptake and GFR:

GFR = {(% kidney uptake x 9.81270) - 6.82519} ml/min

II.6.4.2.4b. Critique for the Gamma camera technique for measuring GFR

There are many debatable questions concerning about the gamma camera technique.

The Gates formula calculates GFR by determining percentage uptake of injected tracer without a requirement for determining plasma concentration. It makes an important underlying assumption that the volume of distribution are approximately equal in all patients but has been validated only in adults. Practically there are many techniques and intrisinic factors that may contribute to the generation of unreliable GFR or ERPF as shown in table 2.

Table 2 Detail of technique factors that affecting the accuracy of estimated GFR and ERPF by gamma camera method.

GFR and ERPF Impact	Factor	Description
Decrease	Inaccurate Start Time	The GFR and ERPF calculations are based on measuring the kidney uptake in the 1-2 minute or 2-3 minute time period after the bolus injection. If there is a delay between the acquisition start time and the bolus arrival time that is not accounted for by an accurate start time, the kidney uptake will most likely be underestimated. The GFR and ERPF will also be underestimated.
Increase or Decrease	Kidney ROI Definition	The kidney regions of interest should be drawn accurately and using a standardized technique (color stripe and window settings). This will minimize intra-observer and inter-observer variability. Smaller regions will result in lower GFR and ERPF values while larger regions will result in higher GFR and ERPF values.
Decrease	Infiltration at	If dose infiltration occurs at the Injection

	Injection Site	site, the kidney uptake will be effectively lowered because less tracer is available in the blood pool. The GFR or ERPF can be lowered significantly.
Increase or Decrease	Quantitative Accuracy	It is important that the syringe and the kidney images all be acquired with identical collimators, identical energy windows, and with minimal time delay to ensure quantitative accuracy. The recommended acquisition protocols should be strictly followed.

For the intrisinic factors, namely, the kidney depth measurement and background correction, are the main sources of error that affecting the accuracy of estimated GFR and ERPF by gamma camera method.

Kidney depth measurement :

Many camera based techniques for measuring GFR require an estimation of kidney depth within the body (i.e. distance from skin surface to kidney center) to compensate for the attenuating effects of soft tissues on emitted gamma rays (Gates ,1982; Gates ,1983; Schlegel et al., 1976). Piepsz et al (1978) determined depth correction in children from a lateral view scintigram. Shore

et al., (1984) measured renal depth by ultrasound but subsequently reported that depth correction was unnecessary in children. However, Maneval et al., (1990), using CT measurements to determine kidney depth in children, reported nearly 40% of kidneys varied by > 1 cm from the average renal depth with 8% > 2 cm. Moreover Gruenewald et al (1985) showed a poor correlation between estimated renal depth by the use of Tonnesen formula in individual patients compared with upright ultrasonographic measurements. Gruenewald et al., (1985) pointed out the importance of correct renal depth estimations on computed uptake of Tc-99m DTPA. Mathematically they showed that a 14% error in uptake calculation would result from a kidney being 1 cm deeper than predicted, while a 26% error results from a 2 cm variation between actual and predicted depth.

Talyor et al., (1993) measured renal depth in 126 patients by CT to compare these values to Tonnesen formula since the latter are based on data from the oblique ultrasonograhy in upright patients; most renograms today are performed supine, and the kidney position may be different when upright. They reported a significant underestimation of renal depth by use of Tonnesen's equation compared with CT measurements and developed a new formula to estimate supine renal depth based on patient's height, weight, and age. However, Maneval et al (1990) stated the importance of using low osmolality contrast agent in measuring the renal depth by CT methods as the high

osmolality contrast agent would raise the possibility of renal swelling which will result in inaccurate kidney depth measurement.

In summary, there is no direct, accurate method in assessing the true kidney depth for the GFR computation.

Background correction

In order to perform accurate measurements of renal activity for purposes of either directly measuring GFR or merely determining uptake ratios between the two kidneys, accurate background correction is of utmost importance. The low extraction ratio of Tc-99m DTPA results in high background activity. The interstitial activity in the perirenal area is presumed to represent interstitial within the kidney, but the vascular supply of the kidney is greater than the vascular supply of the surrounding region. Thus, it is not possible to assign a background area that will easily correct for both interstitial and vascular components. Gates (1982) tested both using a nearly circumferential background area around the kidney, avoiding the anticipated location of renal arteries and vein, and semilunar regions of interest (area adjacent to the inferior and lower lateral kidney margin, avoiding region of liver and spleen). Gates found the semilunar ROI appeared to be better.

Piepsz et al (1990) accounted for the background correction by applying double background correction method. They combined an area ratio method with a linear fit method.

Judging from the above agrument, gamma camera techniques are considered somewhat less accurate in estimating GFR than in vitro measurements despite its ease of performance, reproducibility of results and proven suitability for clinical renography. The most accurate methods for measuring renal function are based on plasma clearance. Methods are available that are based on timed blood samples following a bolus intravenous injection, and these are more accurate and though not simpler than any of the camera-based methods

II.7. The relationship between the Tc-99m DMSA uptake and GFR

The relative renal uptake of Tc-99m DTPA from 90-150 seconds after bolus injection was compared with the relative DMSA uptake at 3 hours post-injection by Bingham and Maisey, they showed excellent correction, r =0.96, between the two measurements (Bingham et al., 1978).

Taylor et al (1985) showed the relative DMSA uptake determined at 24 hours post-injection using computer-assisted regions of interest produce an excellent correlation with the relative GFR determined by either the integrating the counts from 1-3 minutes postinjection and correcting background or totalizing the individual renal counts in a single 15 second frame from 2.45 minutes to 3.00 minutes post-injection and correcting for background, r =0.98.(Daly et al., 1979).

Moreover determination of the relative renal uptake of DMSA from the simple quantitation of a delayed static image has been shown to correlate well with the relative effective renal plasma flow in patients with a wide variety of renal disease (Kawamura et al., 1978; Prince et al., 1978; Taylor, 1980; Taylor,1982).

However all these methods derived only the relative, differential or split renal function without addressing the global or total renal function.

In my study, I try to investigate whether any relationship exists between the absolute Tc-99m DMSA uptake with the corresponding GFR. If a significant relationship exists, then the absolute Tc-99m DMSA renal uptake value can be tabulated to give rapid and easy estimation of the corresponding GFR. Likewise individual kidney GFR can be calculated by multiplying the global GFR with the differential renal function without subjecting the patient to ureteral catherization.

Chapter III Material and Methods

III.1. Subjects and Sampling Methods

47 patients with renal calculus disease whom were referred for Tc-99m DMSA renal scintigraphy were studied. Their renal calculus disease were of different degree so that a wide range of GFR values could be included. All subjects were adults, with an average age of 50 (range 18-70). There were 15 male and 32 female. They all underwent a Tc-99m DMSA renal scintigraphy and the absolute renal uptake of Tc-99m DMSA were calculated at 6 and 24 hours post injection. Four days after the Tc-99m DMSA scan, they all underwent a Tc-99m DTPA renography and their GFR were estimated using the Two Sample Protein Free Plasma Clearance (PFPC) method with informed consent. The serum creatinine level was also obtained on the same day as the patient attended for the Tc-99m DTPA renography. Subjects with edema or ascites were excluded from the study because of possible alterations in the distribution of radionuclides into the extracellular fluid.

III.2. Quantitation of Absolute DMSA uptake

III.2.1. Parameters for Tc-99m DMSA uptake study

III.2.1.1 Materials and methods

III.2.1.1.1. Instrumentation

The gamma camera used was dual headed Picker 2000 XP equipped with a low energy parallel hole general purpose (LEGP) collimator and interfaced with an odyssey VP computer. The energy was set at 140 KeV with a 20% symmetrical window and the image data was stored in 128 x 128 word mode matrix.

III.2.1.1.2. Dosage

Unit dose of 3 mCi of Tc-99m DMSA was supplied by our local supplier, Syncor, Hong Kong. The activity in the syringe was measured by an ionization chamber (Radionuclide Manager Model 4045 by Radcal Corporation, USA.) before and after injection. The radiopharmaceutical was injected by direct venous injection.

III.2.1.1.3. Optimum acquisition start time

Taylor et al., (1980) and Kawamura et al., (1980) showed that Tc-99m DMSA uptake reached a plateau at 6 hours post injection. In line with our

departmental protocol, all the Tc-99m DMSA renal scan were acquired at 2 hours post injection. Additional images were acquired at 6 and 24 hours post injection.

III.2.1.1.4. Length of acquisition time

The imaging time should be sufficiently long so as to render the statistical fluctuation in the counts negligible. In this project, the preset time for the Tc-99m DMSA renal image was five minutes that were sufficiently long to bring the mean counting error down to less than 0.5% for all cases.

III.2.1.1.5. Acquisition parameter

Simultaneous planar acquisition in both anterior and posterior views with both kidneys included in the field of view were obtained. The 128x128 matrix size images were stored in the computer for later analysis.

III.3. Calculation of absolute Tc-99m DMSA renal uptake

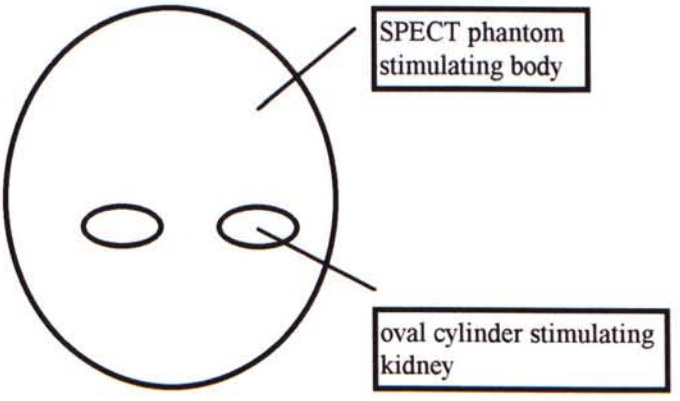
In evaluating the absolute Tc-99m DMSA renal uptake, the correction for attenuation due to kidney depth and table were needed.

As we were using the geometrical mean count based on the conjugate view method to assess the Tc-99m DMSA uptake. The measurement can be done without knowing the renal depth. However, as described in sections **II.5.3.5.** and **II.5.3.6.**, we need to estimate the effective attenuation, μ, by the tissue, the body thickness and the table attenuation.

Therefore, several experiments were conducted to assess the table attenuation and tissue attenuation. Further experiment was also use to validate the method for measuring body thickness.

III.3.1. Attenuation Coefficient factor (μ)

A phantom model was designed to estimate the attenuation coefficient for soft tissue. A circular SPECT phantom of 22 cm in diameter and 21 cm in height was used to stimulate the patient's body. The total volume was 7982 cm^3. It was then filled with water. Two oval symmetrical cylinders with dimension 5cm x 6cm x 10.5 cm simulating the two kidneys were filled with 1 mCi sodium Tc-99m pertechnetate individually and placed obliquely within the SPECT phantom and at variable depths as shown in the following diagram.



The depth was measured from the center of the cylinder to the outside edge of the phantom and ranged from 6.5 to 19 cm. Simultaneous anterior and posterior image of the phantom was taken, with the cylinders placed at

different depths within the phantom. After correction for background activity, physical decay, the resultant count rate at different depth were shown in the following table 3.

Table 3 Count rate Vs Depth

Depth/cm	count rate/ Count/min	ln count rate
6.5	194702	12.17
9	141601	11.86
11.5	97928.8	11.49
14	69723.5	11.15
16.5	46010	10.73
19	31592.4	10.36

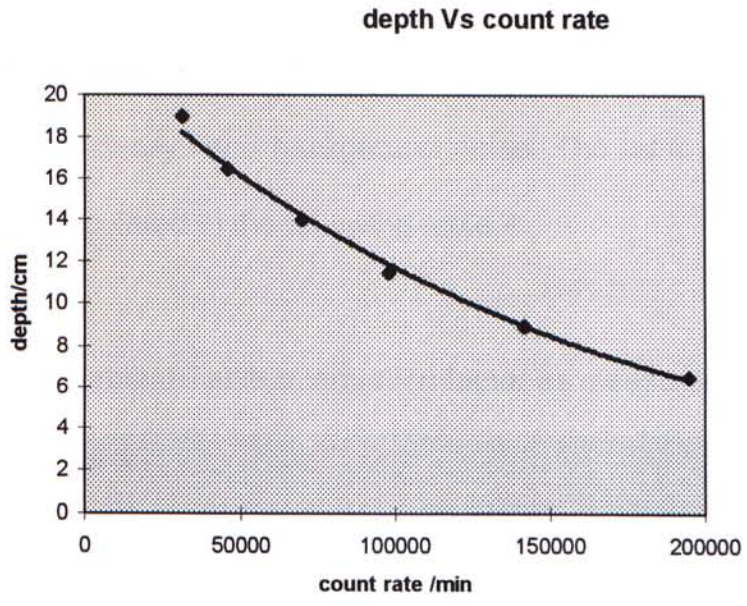


Fig.2 Count rate Vs depth

The count rate was plotted Vs depth as shown in Fig.2. The linear attenuation coefficient for the sodium Tc-99m pertechnetate in water was estimated to be 0.1446/cm.

III.3. 2. Table attenuation

With the imaging table removed, a syringe containing approximately 30 μCi sodium Tc-99m pertechnetate was placed directly on the collimator and a one minute planar image was acquired with 128x128 matrix. Data was stored for subsequent analysis. The imaging table was then replaced over the gamma camera so that the bottom of the table touched the collimator surface. The

same procedure was repeated several times by placing the syringe in different locations on the imaging table. The counts measured were corrected for both physical decay and background noise. The table attenuation at these points were calculated in the following table 4.

Table 4 Table attenuation factor as measured in different location

position	count rate with table =G counts / min	count rate without table=H counts / min	table attenuation coefficient =G/H
A	11837.4	13270.8	0.891
B	12066.5	13496.2	0.894
C	13496.2	12066.5	0.894
D	14456.2	12909.3	0.893
E	15500.0	13841.5	0.893
F	10555.5	9415.5	0.892

Judging from the result, the table attenuates about 10% of the emitting photons. For the sake of convenience, the table attenuation factor was converted into the body thickness which was equivalent to 0.727cm.

III.3.3. Body thickness measurement

The body thickness was estimated from a true lateral scintigraphic image of the patient. At 2 hours after injection of the Tc-99m DMSA , a true lateral image of the abdomen was taken with the patient lying supine and under the same acquisition parameter. The body thickness was determined by measuring the distance, at the center of kidney level, between the anterior and posterior limit of the body contour as shown in the following Fig.3.

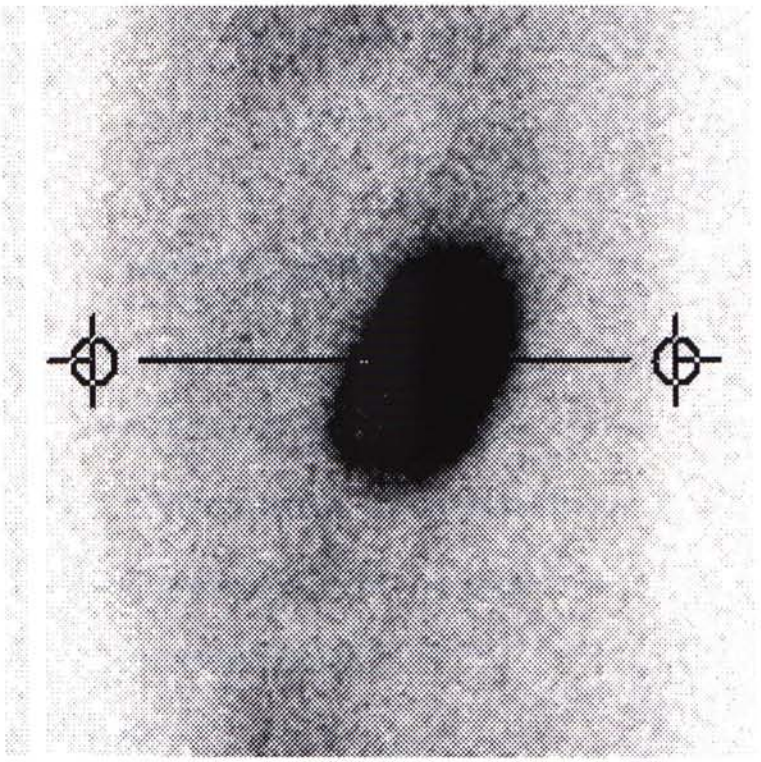


Fig.3 Body thickness measurement

III.3.4. Decay correction

All measurements of the Tc-99m DMSA were corrected for radioactive decay. The time of every measurement was recorded and the activity was converted to a common reference time with the use of the equation for radioactive decay.

$A_t\, e^{kt} = Ao$

where A_t = activity (or counts) at time t

Ao = activity (counts) at time zero (the reference time)

K = the decay constant (0.1155 hr^{-1} for Tc-99m)

III.3.5. Calculation of DMSA uptake

The calculation of Tc-99m DMSA uptake after correction for background activity, physical decay, attenuation due to body and table are as follows:

1. Count measured from the anterior image after correction for background activity and physical decay:

$$RUA = (C_{tA} - N_{tA} B_{tA})\, e^{kt2}$$

2. Count measured from the anterior image after correction for background activity, physical decay and attenuation corrected due to body.

$$RUAT = (C_{tA} - N_{tA} B_{tA})\, e^{kt2} e^{\mu D}$$

3. Similarly for count measured from the posterior image after correction for background activity, physical decay and attenuation due to body and imaging table:

$$RUPT = (C_{tP} - N_{tP} B_{tP}) e^{kt2} e^{\mu(L-D+T)}$$

4. Geometrical mean count equals to the square root of the product of *RUAT* and *RUPT* and was shown in the following equation:

$$RUGM = \sqrt{(C_{tA} - N_{tA} B_{tA})\ (C_{tP} - N_{tP} B_{tP})\ e^{kt2} e^{\mu(L+T)} e^{kt2}}$$

where:

$C_{t\,A}$ = total counts of renal ROI / minute in anterior image

N_{tA} = no. of pixels in renal ROI in anterior image

$B_{t\,A}$ = counts / pixel of renal background / minute in anterior image

C_{tP} = total counts of renal ROI / minute in posterior image

N_{tP} = no. of pixels in renal ROI in posterior image

$B_{t\,P}$ = counts / pixel of renal background / minute in posterior image

K = the decay constant (0.1155 hr^{-1} for Tc-99m)

L = patient's body thickness in cm

T = the table attenuation, in terms of the linear attenuation coefficient, it equals to 0.727cm

μ = Attenuation Coefficient factor, equals to 0.1446/cm

D = kidney's depth in cm

III.3.6. Counting the Dose Injected

The pre-injected counts were obtained by putting directly on the collimator's surface. Similarly the post-injected counts were also obtained in the same manner after the dose injected to the patient. It was corrected for time decay and subtracted from the pre-injection syringe counts to yield the net injected dose.

Dose injected = $(A_s - A_r e^{kt1})$

where :

A_s = activity in syringe before injection in terms of counts /minute

A_r = activity in syringe after injection in terms of counts /minute

t^1 = time elapsed from the reference (injection) time when the residual activity was measured

K = the decay constant (0.1155 hr^{-1} for Tc-99m)

III.3.7. Calculation of absolute Tc-99m DMSA uptake

The percentage of an administered dose of Tc-99m DMSA that were concentrated in the kidneys at any given specific time was defined as the absolute Tc-99m DMSA uptake at that time.

The time of every measurement was recorded and values were converted to a common reference time (the time at which patient dose was injected).

Where RU = Tc-99m DMSA renal uptake in %

Mathematically absolute DMSA uptake, RU, at any hour can be defined as follows:

$$RU = \frac{100\% x RUGM}{A_s - A_r e^{kt1}}$$

III.3.8. Dose infiltration

At the completion of the study, an image was obtained over the injection site. If any infiltration was noted, a tight ROI was drawn around the area of infiltration and the counts were time decay corrected and divided by the injected counts. There was no correction for depth or attenuation. If infiltration exceeded 0.5 % , the patient was excluded from the study.

III.4. GFR measurement

III.4.1. Instrumentation

The machine used for centrifuging the blood sample was known as Haraeus Christ Labofuge GL, made in Germany, and for the ultrafiltering of plasma was known as eppendorf centrifuge 5415 C, made in Germany. The 30,000 NMWL filter unit (Millipore Bedford) was used in this study. All the samples counting were done by ANSR gamma counter (Abbott Laboratories U.S.A.)

III.4.2. Methods

The estimation of GFR using the ultrafiltration of plasma after injection of Tc-99m DTPA was performed according to the method described by Rowell and associates (1985). Two equal aliquots containing 5 mCi of Tc-99m DTPA in 1 ml were supplied by our local contractor, Syncor, Hong Kong. One of them was used as a standard and other was used as the dose to be injected intravenously. Bloods samples were taken from the arm opposite to the injection site at 60 and 180 min after injection. The blood samples were then subjected to centrifugation at 2,000 rpm for 10 min and the plasma was removed. To obtain protein-free fluid, the plasma was transferred to Millipore filter unit and subjected to centrifugation for 30 min at 14,000 rpm. The

resultant ultrafiltration is 99.9 % free of plasma proteins. Duplicate aliquots of ultrafiltered samples and equal volumes of standard dilution were counted in a gamma well counter.

(1) Calculation of GFR with single (3 hr) blood sample by the following formula:

$$GFR = 82.42 \ln(\frac{D}{P}) - 800.5 \ ml/min$$

where:

D= Standard dose activity , counts/min

P = Ultrafiltrate activity x 0.94 , counts/min-ml

(2) Calculation of GFR with double blood samples (1 & 3 hours) by the following formula:

$$GFR = \{D\frac{\ln(P1/P2)}{(T1-T2)}\exp\frac{(T1\ln P2 - T2\ln P1)}{(T2-T1)}\}^{0.979} \ ml/min$$

where :

D= standard dose activity, count/minute

T1=time of collection of first blood sample in minutes (60)

T2=time of collection of second blood sample in minutes (180)

P1=ultrafiltrate activity (in cpm/ml) at T1 x0.94

P2=ultrafiltrate activity (in cpm/ml) at T2 x0.94

III.5. Statistical and analytical methods

In this study, the data for the independent variables which included surface area, absolute Tc-99m DMSA uptake at 6 hour, absolute Tc-99m DMSA uptake at 24 hr, plasma creatinine level and gender were collected. The GFR obtained from the single plasma sample and double plasma sample using PFPC method were treated as dependent variable.

All the Tc-99m DMSA uptake measurements were normalized to 2 mCi.

Descriptive statistic : mean, standard deviation (SD), maximum, minimum and number of case will be given for each variable.

The statistical tests used in data analysis were as follows:

1. Kolmogorov - Smirnov Goodness of Fit Test was used to test whether the variables were normally distributed.
2. Nonparametric analytic methods were used as the sample size was only 47.
3. Wilcoxon Signed-Rank Test was used to test whether there was any statistical difference exist between the GFR measured by the single (3 hr) blood sample and double (1 & 3 hr.) blood samples.

4. Wilcoxon Signed-Rank Test was used to test whether there was any statistical difference exist between the absolute Tc-99m DMSA uptake measured at 6 hour and absolute Tc-99m DMSA uptake measured at 24 hr post injection.
5. Wilcoxon rank sum (Mann-Whitney U) Test was used to test whether there was any statistical difference in sex in relation to the GFR and DMSA uptake measurements.
6. Univariate regression analysis was used to evaluate the relationships between the GFR measurement using single (3 hr) blood sample and those independent variables namely age, surface area, absolute Tc-99m DMSA uptake measured at 6 hour and the plasma creatinine levels.
7. Univariate regression analysis was used to evaluate the relationships between absolute Tc-99m DMSA uptake measured at 6 hour and those independent variables such as age, surface area and the plasma creatinine levels.
8. Multiple stepwise regression analysis was employed to analyze more than one independent variable simultaneously with the dependent variable GFR measurement by the single (3 hr) blood sample.
9. The null hypothesis was the absence of correlation or difference between the variables considered. The probability value taken to indicate statistical significance was $\rho = 0.05$.

10. In all the tests, statistical difference was considered as significant only when the probability value (ρ - value) was < 0.05.

All the statistical analyses were performed using Microsoft Excel (version 5) and Statistical Package for the Social Sciences (SPSS release 6).

Chapter IV Results

In terms of statistic glossary, the patient pool used in this project was a sample of the population. Therefore Kolmogorov - Smirnov Goodness of Fit Test (K-S) was used to test the normality of the study population with the result tabulated in table 5.

Table 5 Kolmogorov - Smirnov Goodness of Fit Test (K-S) for different variables

variables	K-S Z	2-tailed P
absolute DMSA (6 hr.) uptake	0.7038	0.7079
absolute DMSA (24 hr.) uptake	0.5413	0.9313
surface area	0.768	0.597
GFR (3hr)	0.5449	0.9278
GFR(1&3hrs)	0.4947	0.9672

From the table 5, the *p* value of the all variables were > 0.05 which validated the normality of the study population as there was no sufficient evidence to reject the null hypothesis (Ho).

IV.1. Characteristics of experimental subjects and their serum creatinine profile.

The anthropometric characteristics of 47 patients (28 males and 19 females) were summarized in table 6. The values (mean ± S.D.) for the serum creatinine were also included.

Table 6 The anthropometric characteristic of 47 patients

Parameter	mean ± S.D.	actual range
Age (yr.)	48 ± 14.2	18 ----->73
Surface Area ($/m^2$)	1.63 ± 0.158	1.27-----> 2.1
Serum creatinine (μ mol/l)	110 ± 46.7	63 ------>290

IV.2. Absolute Tc-99m DMSA uptake

IV.2.1. The change of absolute Tc-99m DMSA uptake with time

Table 7 showed the absolute Tc-99m DMSA uptakes (%) values for four patients out of the 47 at 2, 3, 6 and 24 hours post injection

Time	% uptake 1	% uptake 2	% uptake 3	% uptake 4
0	0	0	0	0
2	53.35	18.75	46.4	32.56
3	61.75	24.19	55.4	48.24
6	65.5	24.2	59.6	54.15
24	59.28	22.02	44.9	46.18

Table 7 Absolute Tc-99m DMSA uptake pattern in 4 patients

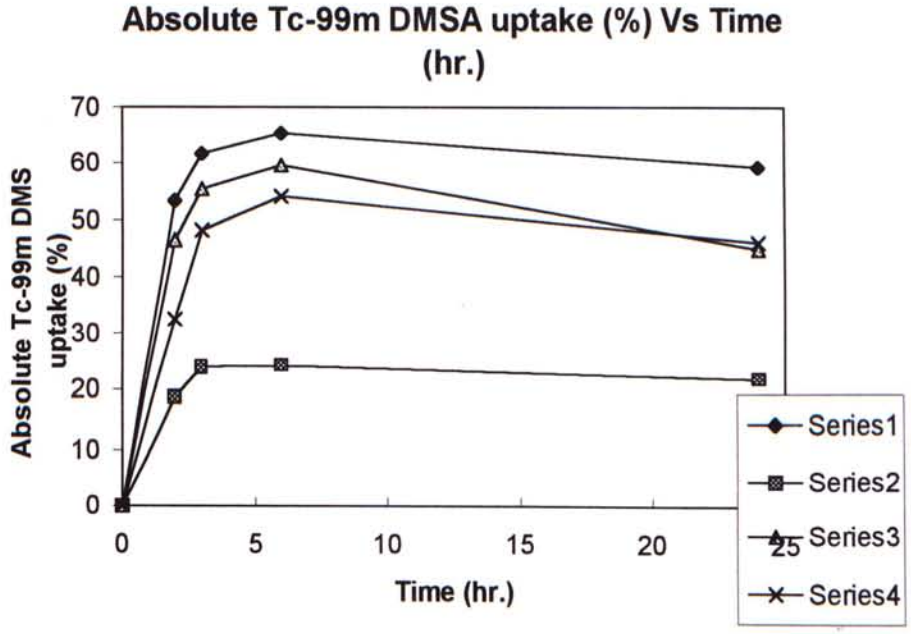


Fig.4 Absolute Tc-99m DMSA uptake (%) Vs Time (hr)

As shown from Fig.4, the absolute Tc-99m DMSA uptake reached a plateau from the 6 hr onward. Therefore the absolute Tc-99m DMSA uptake measured at 6 hr. and 24 hr. were used for further analysis.

IV.2.2. Absolute Tc-99m DMSA uptake measurement at 6 hour and 24 hours

The mean absolute DMSA uptake for the 47 patients at 6 hours and 24 hours were 44.03% and 44.07% respectively and the results were tabulated in table 8.

Table 8 The absolute DMSA (6 hour) uptake and absolute DMSA (24 hours) uptake for the 47 patients

Parameter	mean ± S.D.	actual range
absolute DMSA (6 hr.) uptake	44.03 ± 13.2	17.9 --------> 68.5
absolute DMSA (24 hr.) uptake	44.07 ± 10.1	19.8 ---------> 62

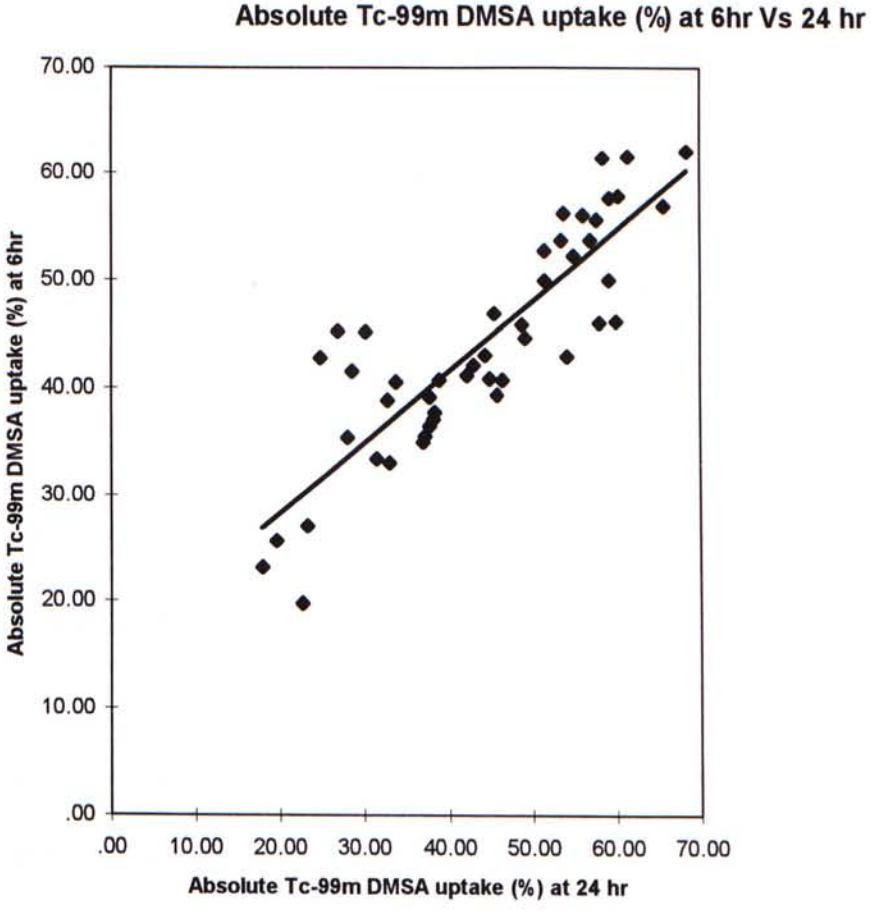


Fig 5. Relationship of absolute Tc-99m DMSA uptake(%) at 6 and 24 hr.

As shown in Fig.5, there was a strong positively correlation between the absolute Tc-99m DMSA uptake(%) at 6 and 24 hr with $r^2 = 0.748$, $p>0.05$.

Absolute Tc-99m DMSA uptake at 6 hr and 24 hr were then tested for any statistically significance difference by Wilcoxon Signed -Rank Test.

The null and alternative hypotheses were be:

H_o : $\mu(6) = \mu(24)$; there was no difference in the mean absolute DMSA uptake in 6 hours and 24 hours

H_A : $\mu(6) \neq \mu(24)$; the mean absolute DMSA uptake in 6 hours and 24 hours were different.

The calculated 2-tailed p value was 0.5610 which was greater than 0.05 , therefore statistically there is no difference between the absolute Tc-99m DMSA uptake at 6 hr and 24 hr. For simplicity, absolute Tc-99m DMSA uptake at 6 hr was used for further analysis.

IV.2.3. Gender difference in absolute Tc-99m DMSA uptake in measurement at 6 hour

As shown in Fig.6, there was no gender difference in the absolute Tc-99m DMSA uptake (%) measured at 6 hour as tested by Mann-Whitney U test with 2-Tailed $p = 0.5583$.

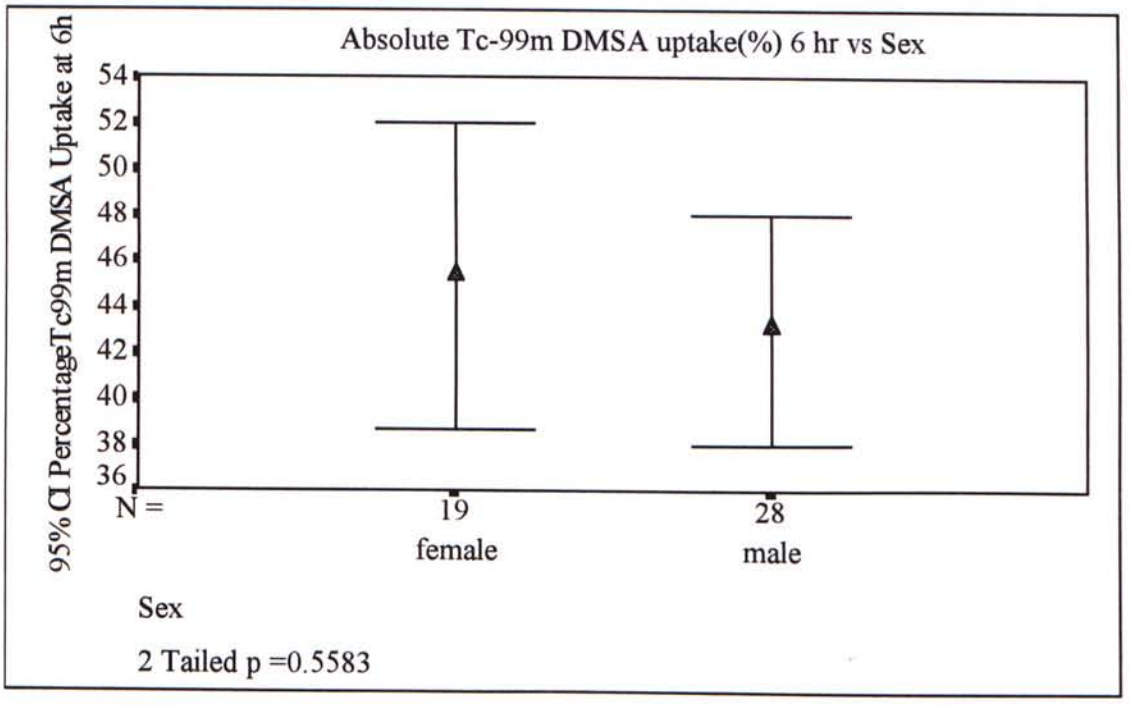


Fig. 6 Relationship of absolute Tc-99m DMSA uptake (%) at 6 hr. and Sex

IV.3. GFR measurement

IV.3.1. GFR measurement by single (3 hr) and double (1&3 hrs) plasma sampling

The mean GFR measured by single plasma sample and double plasma samples were 87.59 ml/min and 87.49 ml/min. The results were shown in Table 9.

Table 9 GFR measurement by single (3 hr) and double (1&3) plasma samples

Parameter	mean ± S.D.	actual range
GFR (3) (ml/min)	87.59 ± 34.73	28------>150
GFR(1&3) (ml/min)	87.45 ± 31.29	32.9 -----> 145

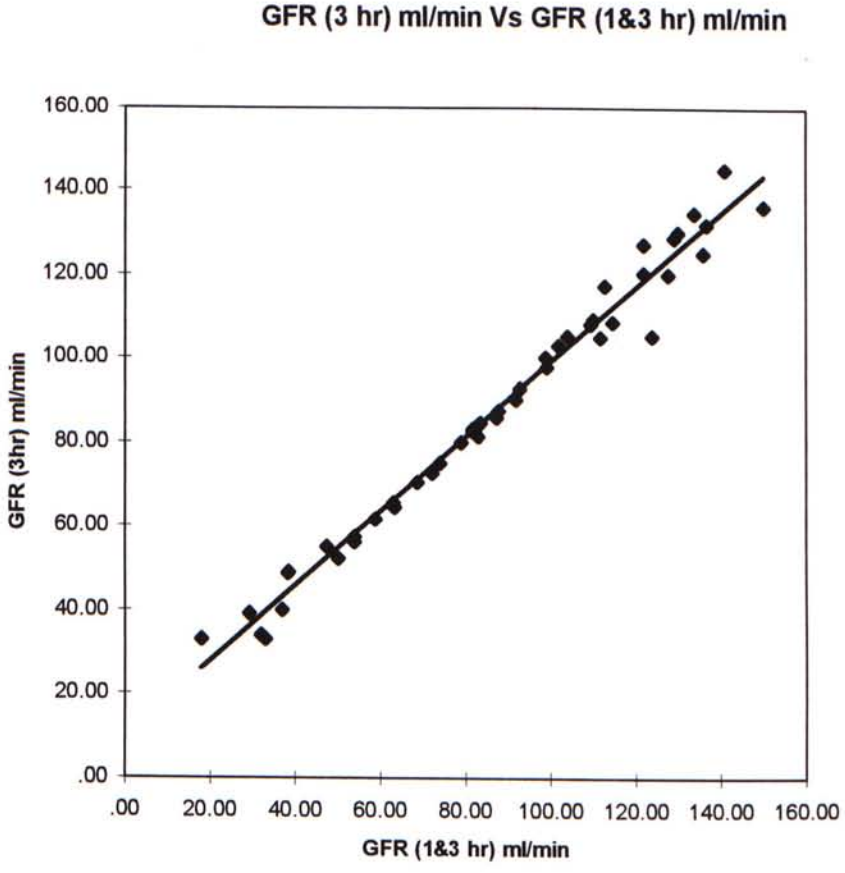


Fig. 7 Relationship of GFR (3hr) and GFR (1&3 hr)

As shown in Fig.7 , there was a strong positive correlation between the GFR measurement using single (3hr) and double (1&3 hr) plasma with $r^2 = 0.9808$, $p < 0.05$.

GFR(3hr) and GFR (1 & 3 hours) were then tested for any statistically significance difference by Wilcoxon Signed-Rank Test.

The null and alternative hypotheses would be

H_o : $\psi(3) = \psi$ (1&3); there was no difference in the GFR estimated by single plasma sample and double plasma samples.

H_A : $\psi(3) \neq \psi$ (1&3); the GFR estimated by single plasma and double plasma samples were different.

The calculated 2-tailed *p* value is 0.2315 which was greater than 0.05 , therefore statistically there was no difference between the GFR(3 hr) measured using single plasma sample and GFR(1&3 hr) measured using double plasma samples. For simplicity GFR (3 hr) is used for further analysis.

IV.3.2. Gender difference in GFR measurement using single plasma sampling

As shown in the Fig 8, there was no gender difference in the GFR (3 hr) measured using single plasma sample as tested by Mann-Whitney U test with 2-Tailed $p = 0.5729$.

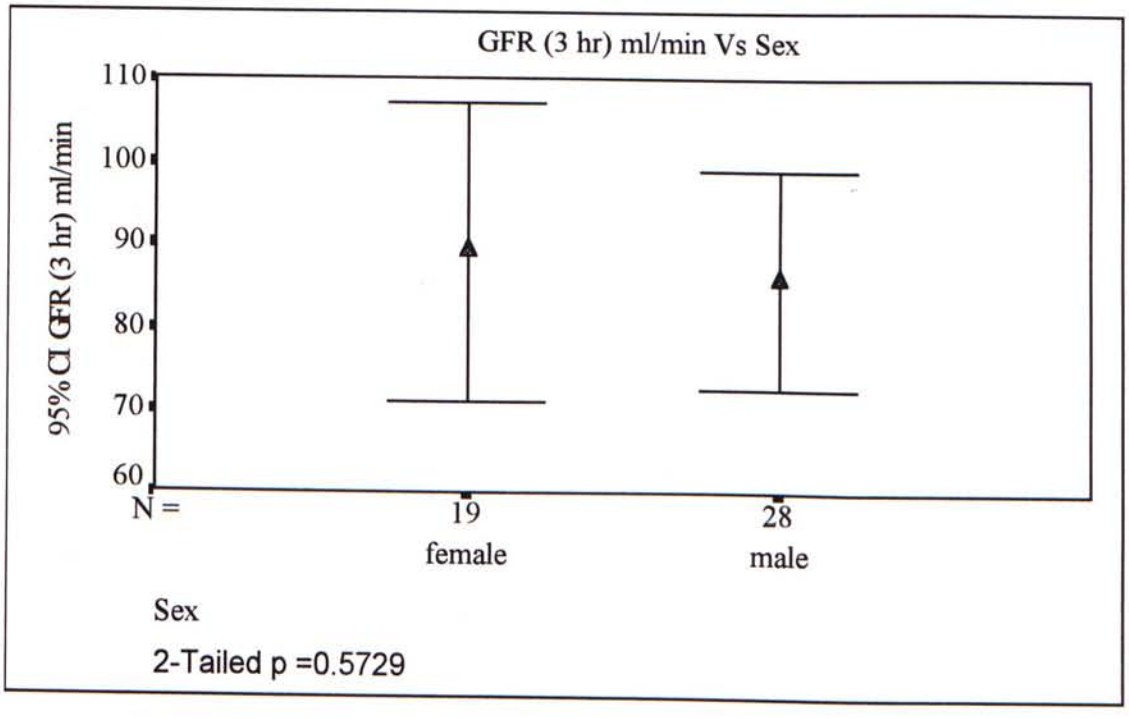


Fig.8 Relationship of GFR (3hr) and Sex

IV.4. Univariate Correlation

IV.4.1. Correlation between GFR using single plasma sampling and absolute Tc-99m DMSA uptake measurement at 6 hour

The absolute Tc-99m DMSA uptake measured at 6 hours for the 47 patients were plotted against their corresponding GFR measured using single plasma sample (Fig.9). The calculated correlation coefficient (Pearson's product moment correlation coefficient) was 0.828, $p < 0.05$.

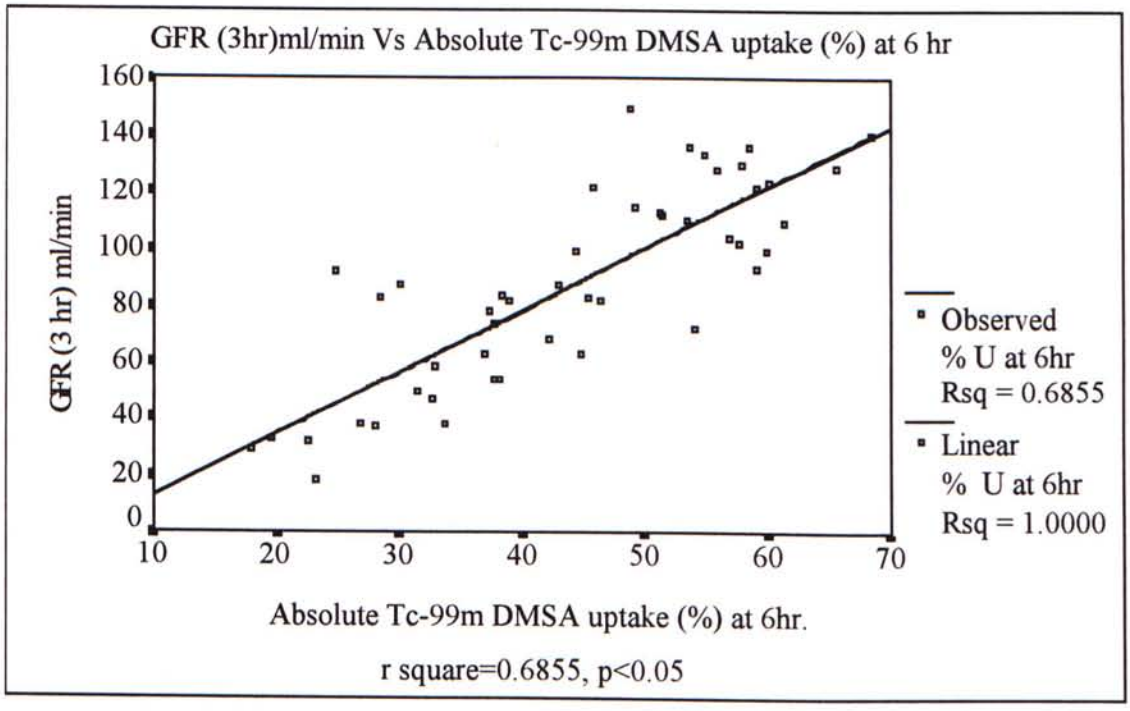


Fig.9 Relationship of GFR (3 hr) and absolute Tc-99m DMSA uptake measured at 6 hr.

IV.4.2. Correlation between GFR using single plasma sampling and plasma creatinine levels

The serum creatinine level for 47 patients were plotted against their corresponding GFR using single plasma sample (Fig.10). An exponential relationship between the 2 sets of data were found and with a $r^2 = 0.655$, $p < 0.05$.

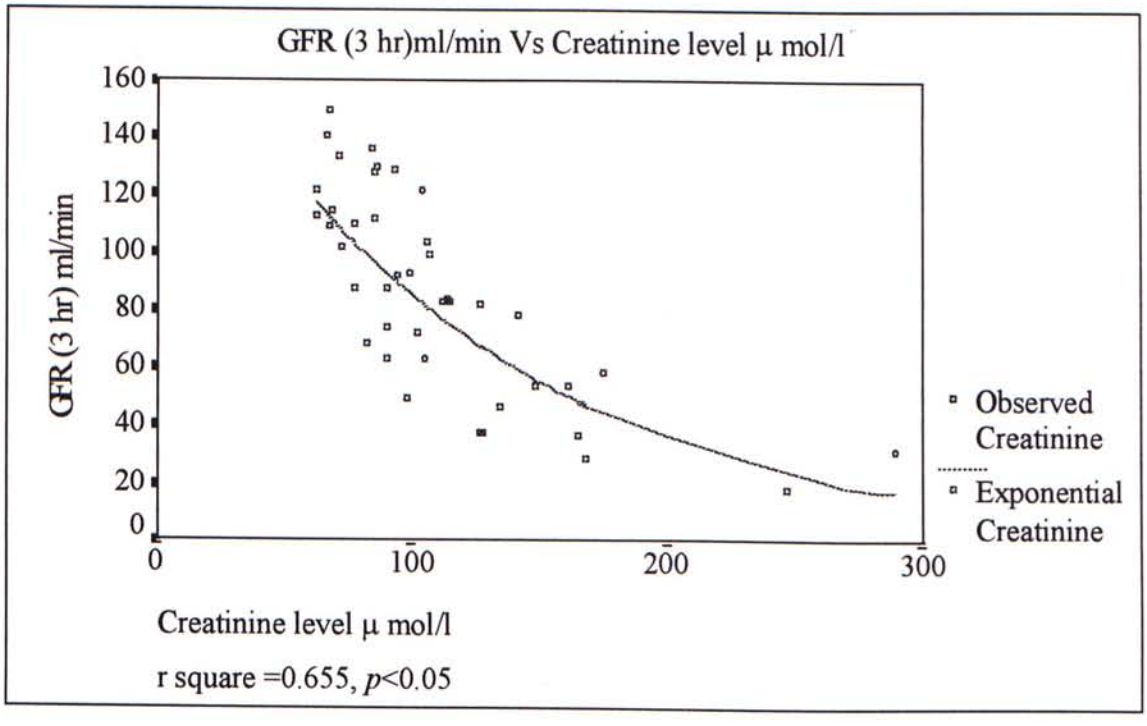


Fig.10 Relationship of GFR (3 hr) and creatinine level

IV.4.3. Correlation between anthropometric variables on GFR (3 hr)

The value of GFR of these 47 patients measured using single plasma sample were plotted against their corresponding surface area and age as shown in Fig. 11 and Fig.12. A weak, inverse correlation was found in both situations with $r^2 = 0.119$, $p = 0.018$ for GFR against age and with $r^2 = 0.072$, $p = 0.065$ for GFR against surface area.

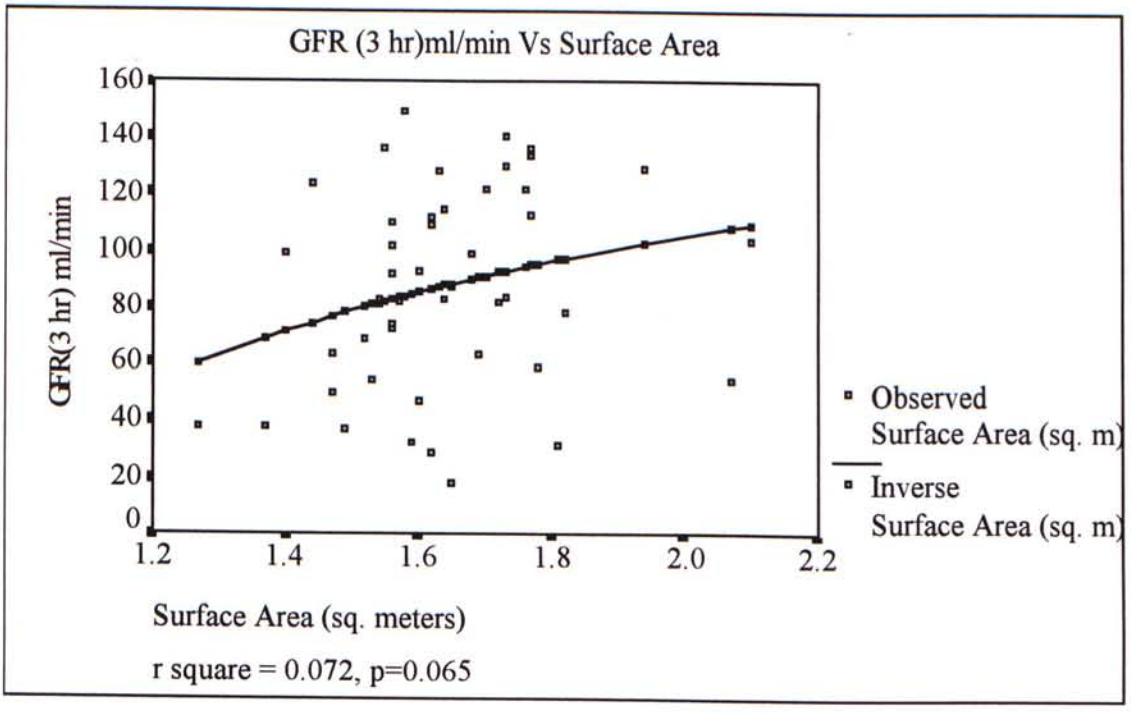


Fig.11 Relationship of GFR (3hr) and surface area

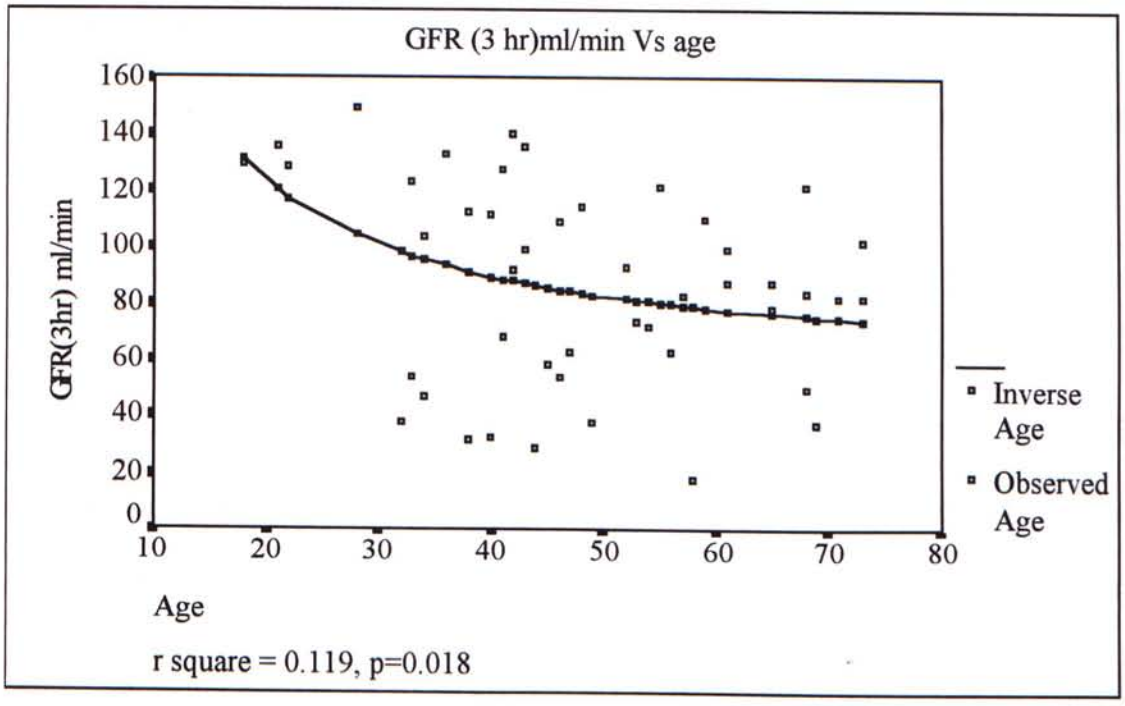


Fig.12 Relationship of GFR (3hr) and age

IV.4.4. Correlation between anthropometric variables and serum creatinine plasma level on absolute Tc-99m DMSA uptake measurement at 6 hour

Similar results were obtained when we plotted the TC-99m DMSA uptake at 6 hours against serum creatinine level, age and surface area (Fig.13, 14 and 15). The absolute Tc-99m DMSA uptake (%) at 6 hr responds exponentially with serum creatinine level (r^2 =0.462, $p < 0.05$) and weakly inversely with age (r^2 =0.063, $p = 0.074$) and surface area ($r^2 = 0.066$, $p = 0.082$).

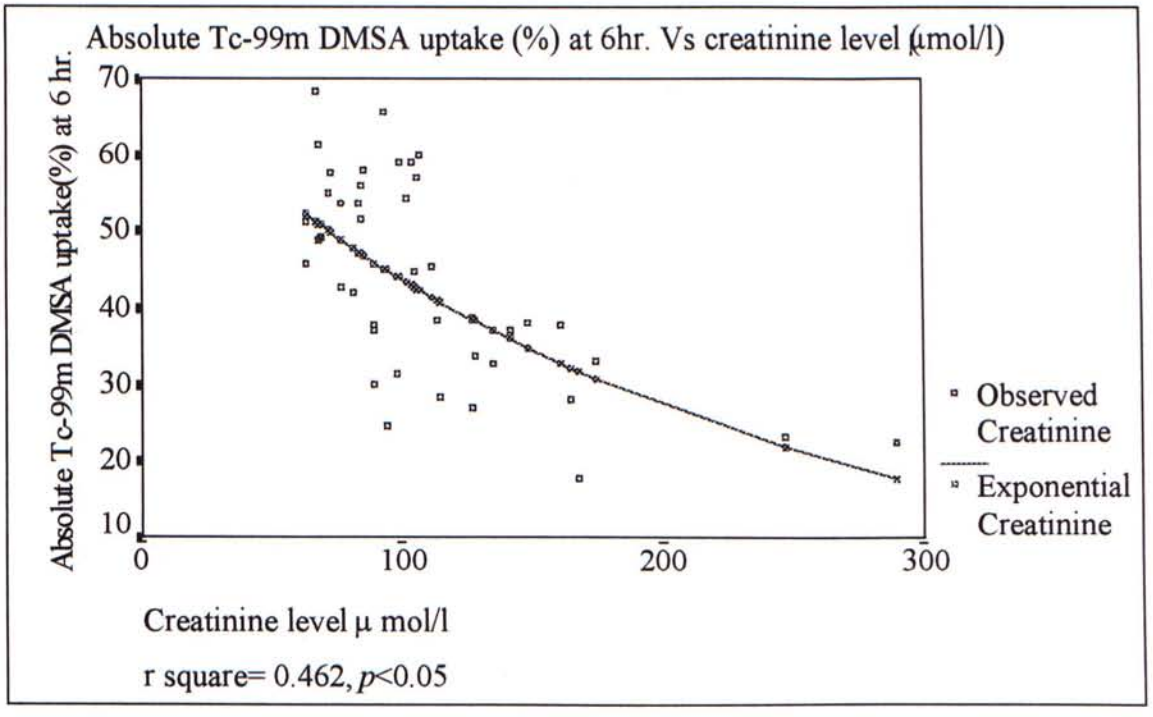


Fig.13 Relationship of absolute Tc-99m DMSA uptake (%) and creatinine level

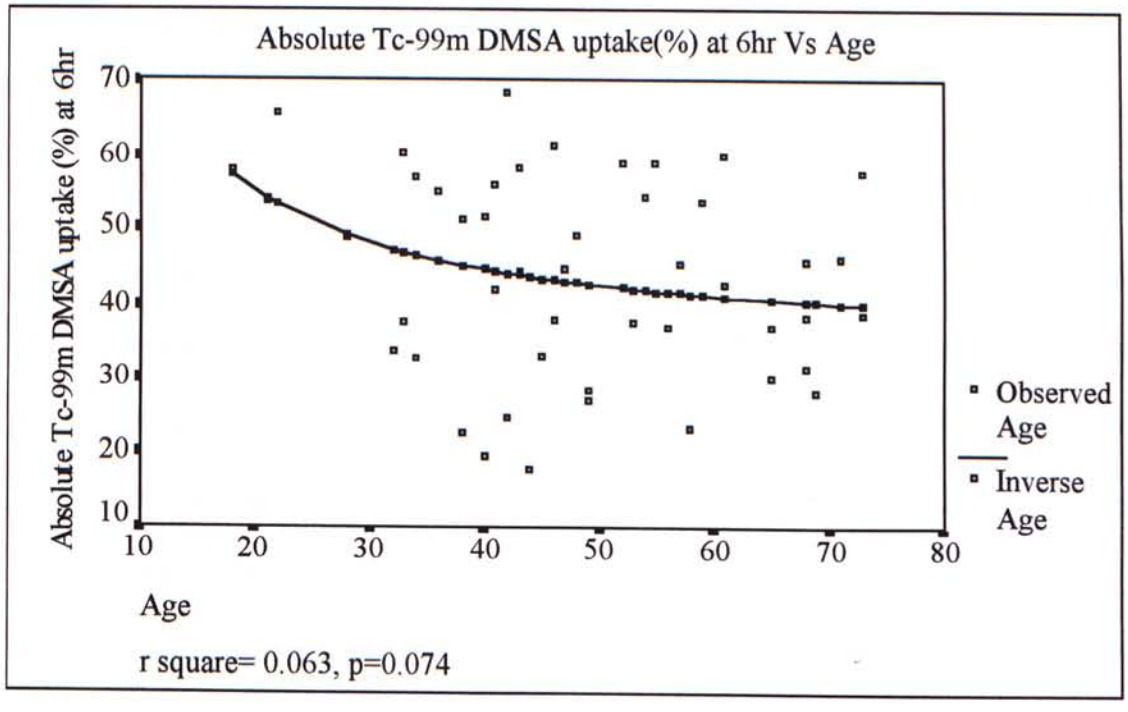


Fig.14 Relationship of absolute Tc-99m DMSA uptake (%) and age

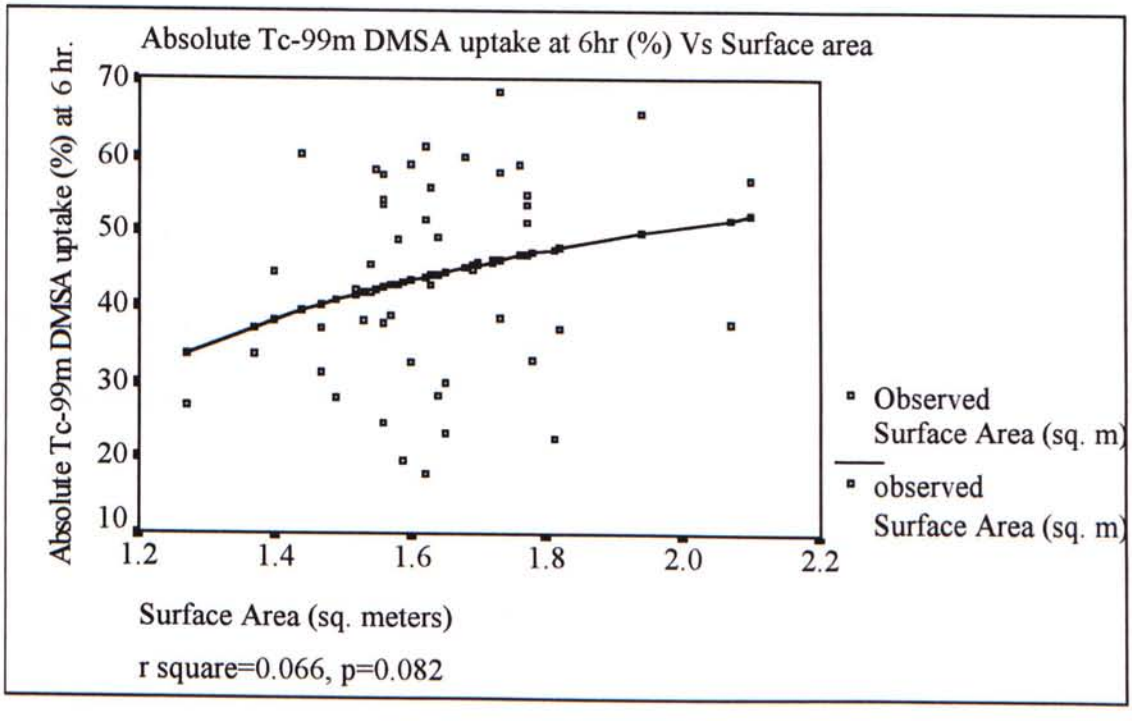


Fig.15 Relationship of absolute Tc-99m DMSA uptake (%) and surface area

IV.5. Multiple linear stepwise regression analysis

Based on the data, it was found that there was a strong positive correlation between the Tc-99m DMSA renal uptake measured at 6 hour post injection and the estimated GFR using single plasma sample. Serum creatinine level, surface area and age were also the contributing factors to both the Tc-99m DMSA renal uptake measured at 6 hour post injection and estimated GFR by single plasma sample.

Multiple regression analysis was performed using GFR(3 hours) as the dependent variable and Tc-99m DMSA renal uptake measured at 6 hour post injection, serum creatinine level , surface area and age as the independent variables. Result showed that GFR was significantly correlated with absolute Tc-99m DMSA uptake measured at 6 hour post injection, creatinine level and surface area ($r = 0.885$, $p < 0.05$). The corresponding equation was expressed by GFR ={1 (absolute Tc-99m DMSA uptake measured at 6 hour post injection) + 54.9 (surface area) - 0.379 (creatinine level)} ml/min. A validation comment for this equation was complied in the Appendix II.

Chapter V Discussion

V.1. Review of the study

Results : Summary of the major findings.

V.1.1. Experimental subjects and their absolute Tc-99m DMSA uptake (%) at 6 hour

1. As summarized in the table 8, the mean (± S.D.) values of absolute Tc-99m DMSA uptake (%) at 6 hr in 47 patients were all in the range of the typical finding as documented in the literature (Kawamura et al (1978) using planar technique with total kidney uptake 54 ± 8.8% 2 hr after injection; Groshar et al., (1989) using SPECT with total kidney uptake 41.2 ± 8.5% 6 hr after injection).
2. There was no gender difference in values for total Tc-99m DMSA renal uptake.
3. There was no statistical difference in the absolute Tc-99m DMSA uptake (%) at 6 and 24 hr.
4. A strong exponential relationship was found between absolute Tc-99m DMSA uptake (%) measured at 6 hour and their corresponding serum creatinine level and was similar to the finding of Groshar et al., (1989).
5. A weak relationship was found between absolute Tc-99m DMSA uptake (%) measured at 6 hour with age and surface area.

A strong positive linear relationship was found between absolute Tc-99m DMSA uptake (%) measured at 6 hour with their corresponding GFR (3hr).

V.1.2. Experimental subjects and their GFR (3hr)

1. There was no statistical difference in the GFR measurement by single (3 hr) plasma sample and double (1 &3 hr) plasma samples.
2. A strong exponential relationship was found between GFR (3hr) using single plasma sample with their corresponding serum creatinine level and was similar to the finding of Gabriel (1986).
3. A weak relationship was found between GFR using single plasma sample (3hr) with age and surface area.
4. No gender difference existed in values for GFR measurement.

V.2. Discussion on subject

47 patients whom were referred for the Tc-99m DMSA renal differential function analysis in the period from November 1995 to January 1997, were recruited with wide range of renal function status as reviewed by their serum creatinine level (see Appendix I).

V.2.1. Subject preparation

Adequate hydration was given to all subjects who underwent for both Tc-99m DMSA scan and Tc-99m DTPA examination in order to minimize the radiation dose to the bladder and to enhance the target to background ratio.

V.3. Discussion of method

V.3.1. Equipment

(a) Dose calibrator

The activity in the syringe was measured by an ionization chamber (Radionuclide Manger Model 4045 by Radcal Corporation , USA). The error associated with the measured activity was estimated to be 5 % including the inherent offset of the dose calibrator.

(b) The sensitivity of the head 1 and 2 of the gamma camera

The relationship between the activity being imaged and the count rate recorded by the individual camera head and was tested separately by placing different activity of sodium Tc-99m pertechenate in a phantom. The values were plotted in Fig.16 for head 1 and in Fig.17 for head 2 respectively.

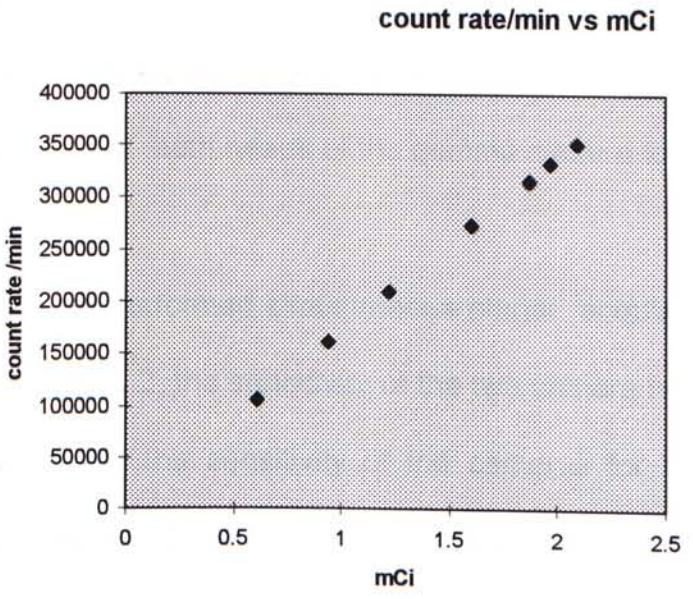


Fig. 16 The recorded count rate Vs activity for head 1

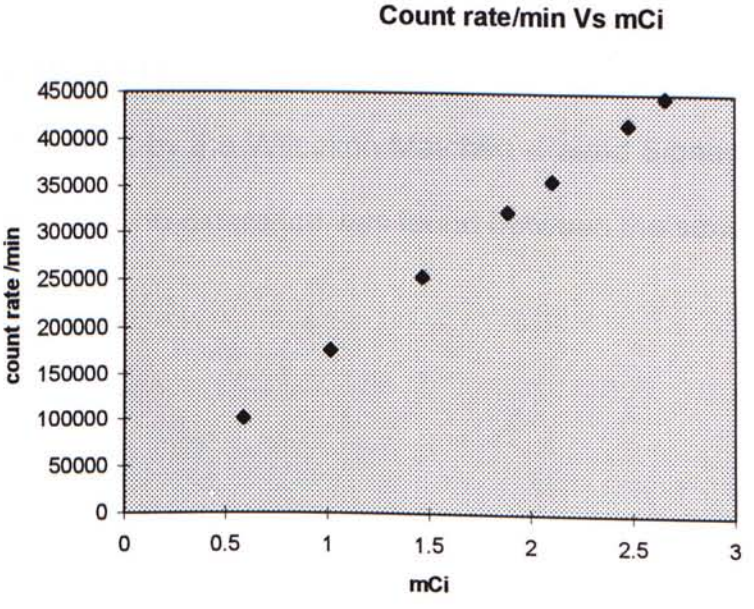


Fig. 17 The recorded count rate Vs activity for head 2

As shown in Fig.16 & 17, there was a positive linear relationship exist between the activity being imaged and the count rate recorded by the cameras. A linear response of both heads of the gamma camera to dose was validated.

Since we performed simultaneous planar acquisition of the images from head 1 and head 2, the sensitivity of the two camera heads should be the same. The variation in the sensitivity of the cameras for head 1 and 2 in counts per minute per mCi were being tested by placing different amount of sodium Tc-99m pertechenate in the phantom directly on the collimator surface of the head 1 and head 2 cameras alternatively at the dates as shown in the table 10 and 11. The mean value of 189491 counts per minute per mCi was found under the project settings for the head 1 and 189819.8 counts per minute per mCi for head 2.

Statistically, by the Wilcoxon Matched -Paired Signed-Ranks test with 2 tailed $p = 0.7353$, no difference was found between the sensitivity of head 1 & 2.

Date	Net syringe counts /1min	Source activity/mCi	Cts/ min / mCi	% difference compared with the mean
5-Sept.-96	191755.5	1	191755.5	1.19
12-Sept.-96	95362.4	0.5	190724.8	0.65
19-Sept.-96	239093.6	1.25.	191275	0.94
26-Sept.-96	129615.4	0.69	187848.5	0.866
3-Oct.-96	159770	0.85	187965	0.8
10-Oct.-96	138366	0.74	186980	1.32
17-Oct.-96	119630	0.63	189889	0.2

Table 10 Data for calculation of sensitivity of head 1

Date	Net syringe counts /1min	Source activity/mCi	Cts/min/mCi	% difference compared with the mean
5-Sept.-96	191669.4	1	191669.4	1.09
12-Sept.-96	96419.2	0.5	192838.4	1.71
19-Sept.-96	237274.7	1.25.	189819.8	0.124
26-Sept.-96	130308	0.69	188852.3	0.38
3-Oct.-96	159352.7	0.85	187473.8	1.11
10-Oct.-96	138095.5	0.74	186615.6	1.56
17-Oct.-96	119586.4	0.63	189819.7	0.124

Table11 Data for calculation of sensitivity of head 2

(c) Validation of quantification of injected activity by gamma camera method - constancy of performance for camera gamma

The net injected activity was being measured by placing the syringe containing the radiopharmaceutical directly on the surface of the head 1 collimator of gamma camera before and after injection. Therefore the constancy of performance of the gamma camera can directly affected the actual amount of injected dose. It was tested by placing a syringe containing different amount of sodium Tc-99m pertechenate directly on the surface of the head 1 collimator during a time interval was shown in the Fig.18. The mean value of 189491 counts per minute per mCi was found under the project settings.

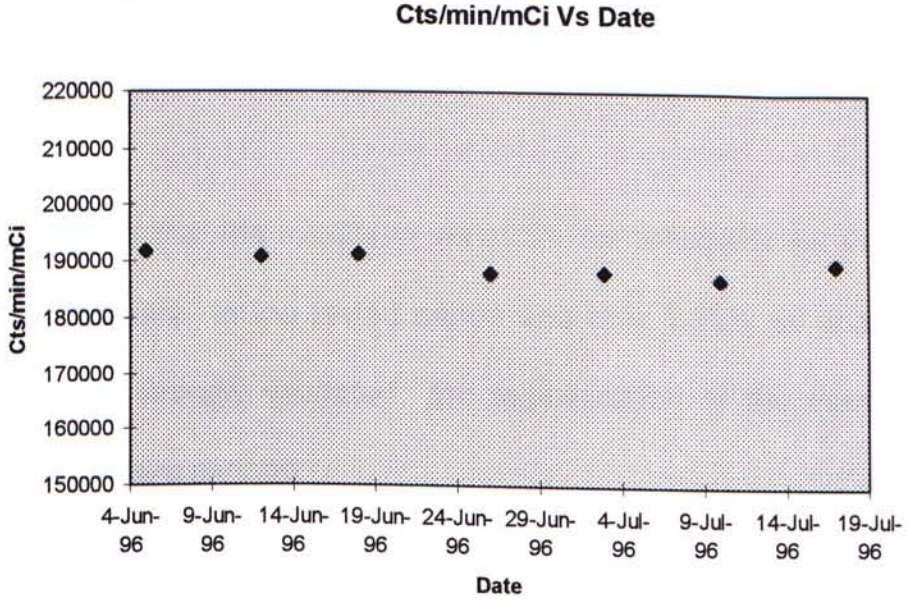


Fig.17 The variation of sensitivity of head 1 during the tested period

Date	Net syringe counts /1min	Source activity/mCi	Cts/min/mCi	% difference compared with the mean
5-Jun.-96	191755.5	1	191755.5	1.19
12-Jun.-96	95362.4	0.5	190724.8	0.65
18-Jun.-96	239093.6	1.25.	191275	0.94
26-Jun.-96	129615.4	0.69	187848.5	0.866
3-Jul.-96	159770	0.85	187965	0.8
10-Jul.-96	138366	0.74	186980	1.32
17-Jul.-96	119630	0.63	189889	0.2

Table 12 Data for testing the constancy of performance of head 1

It was found that the maximum variation for sensitivity for the head 1 of the gamma camera in the tested period was only 1.32% as shown in Table 12. This result strongly validated the quantification of the injected activity by gamma camera method.

(d) LEHR Collimator

A low energy parallel hole high resolution general purpose (LEHR) collimator was used in this project which was in line with our imaging protocol. The additional advantage was that the response of the gamma camera would not depend on the source detector distance. Therefore the geometric mean of anterior and posterior count rate was independent of the kidney depth, the only additional measurement needed being the body thickness. Moreover a parallel hole collimator introduced no image distortion and could provide a constant field of view.

(f) Dead time loss

Approximately a count loss of **2 %** for the Picker Prism 2000XP detectors with an LEHR collimator for 3 mCi of inject dose as stated by the manufacturer. No correction for the dead time loss was necessary to the calculation in this study.

V.4. Discussion on Measurement

(a) Length of acquisition time

The imaging time should be sufficiently long so as to render the statistical fluctuation in counts negligible. In this project, the preset time for the Tc-99m DMSA renal image was five minutes that were sufficiently long to bring the mean **counting error down to less than 0.5%.**

(b) Attenuation Coefficient factor (μ)

The estimated value of μ in my study was 0.1446/cm and the detail described in section **III.3.1.** which was similar to those reported by Fleming et al (1987) (0.12/cm) , Cosgriff (1990) (0.11/cm) and Corrigan (1984) (0.14/cm).

(c) Body thickness, L, measurement

In my study, the body thickness was measured by means of a lateral body image that was validated by the water phantom study as shown below:
A hollow cylinder of dimension 210mm in height and 220mm in diameter was filled with 1 mCi of sodium Tc-99m pertechnetate. Different known volume of tracer were used so as to give a different water level that stimulated variation

in body thickness. The water height measured by the gamma camera was then compared with the true water height in the water phantom.

Table 13 True water phantom's height Vs height measured by gamma camera

height in the water phantom (cm)	height measured by gamma camera (cm)
21	20.8
17.9	18
17	17
16	15.9
15.8	15.6
15.2	15.2
14.7	14.7
13.9	14
13.2	13.3
18.4	18.4

A high correlation was found between the height measured by the water phantom and by the gamma camera with $r^2 = 0.998$, $p < 0.05$. The **estimated error is only 1 % .**

(d) Optimum acquisition time for data collection

Our study showed that Tc-99m DMSA uptake reached a plateau following i.v. injection from 6 hour onwards. It was compatible to the finding of Taylor et al., (1980) and Kawamura et al., (1980).

V.5. Discussion on overall error estimation

(a)Tc-99m DMSA uptake measurement at 6 hour

The error associated with Tc-99m DMSA uptake measurement at 6 hr was calculated in the following way. In order to simplify the calculation, a modified form of equation was used, provided that all activity measurements and count rate were corrected for decay beforehand.

$$RU\% = \frac{100xRUGM}{D}$$

where *RU %* = absolute Tc-99m DMSA uptake (%)

RUGM = net Tc-99m DMSA uptake (CPM)

D = net injected dose (CPM)

Hence, $\frac{\Delta RU}{RU} = \sqrt{(\frac{\Delta RUGM}{RUGM})^2 + (\frac{\Delta D}{D})^2}$

The percentage error estimated for the net injected dose, *D*, arising from dose calibrator=7.07%

The percentage error estimated for the net Tc-99m DMSA uptake, *RUGM*, arising from body thickness measurement and dead time loss = 2.23%

Hence, the overall percentage error estimated for the absolute Tc-99m DMSA uptake

$= \sqrt{(7.07\%)^2 + (2.23\%)^2}$

$= 7.4\%$

(b) GFR measurement by single (3 hr) sample

It was reported by Rowell et al., (1986) the error estimated by using single plasma (3 hr) sample method in calculating the GFR was only an 8ml/min. The mean GFR measured in my study was 80 ml/min in this project which made the average estimated percentage error to be 10 %.

Chapter VI Conclusion

The usefulness of the Tc-99m DMSA renal scintigraphy in providing qualitative information on the renal morphology was well established. The calculation for the differential function by Tc-99m DMSA renal scintigraphy was only partially quantitative and no information was given on the actual amount of viable functioning tubular cells.

In my study, I tried to develop a method which can measure the absolute Tc-99m DMSA renal uptake using gamma camera and tried to incorporate it as part of the standard renal parenchymal scintigraphic procedure. The aim was to provide more comprehensive renal evaluation, including not only the assessment of renal cortical integrity, but also the total renal function in a single routine Tc-99m DMSA renal scan.

The method of Tc-99m DMSA renal uptake quantification using planar imaging was simpler than that using SPECT quantitation and the results were found to be comparable.

47 patients with a wide range of renal function status, and their plasma creatinine level ranged from 63-290 μ mol/l. They were found to have Tc-99m DMSA renal uptake at 6 hour of 17.9 - 68.5% of the injected dose and with a mean of 44 $\pm$ 13.3%.

Estimation of their corresponding GFR for this group of patients were also performed in lieu with method of PFPC (Rowell et al.,1986).

The results showed that that there was a strong positive correlation between the Tc-99m DMSA renal uptake at 6 hour and their corresponding GFR (r =0.828, $p<0.05$).

Serum creatinine level, surface area and age were also contributing factors in affecting both the Tc-99m DMSA renal uptake at 6 hour and their corresponding GFR.

Multiple regression analysis was performed using GFR as the dependent variable and Tc-99m DMSA renal uptake at 6 hour, serum creatinine level, surface area and age as the independent variables. Results showed that, GFR(3 hour) was significantly correlated with absolute Tc-99m DMSA uptake (%) at 6 hour, creatinine level and surface area (r = 0.885, $p<0.05$). The corresponding equation was expressed by GFR = {1(absolute Tc-99m DMSA uptake (%) at 6 hour) + 54.9 (surface area) - 0.379 (creatinine level)} ml/min. The validation comment for this equation was complied in the appendix II.

In summary, the technique was simple to perform and was able to provide a global GFR determination. The divided renal GFR's could then be calculated by multiplying the total GFR by their respective differential function. It would serve as an important and useful index for assessing cortical and renal mass function and be able to provide guideline to the referring clinicians for patient management.

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Appendix I -- Patient Data

Patient no.	Age	Sex	S_Area/ m square	GFR_S /ml/min	GFR_D/ ml/min	%H6_ 2mCi	%H24_2 mCi	Creatinine µmol/litre
1	42	F	1.73	141.00	145.00	68.50	62.07	67.00
2	69	F	1.49	37.00	40.00	28.07	35.40	165.00
3	43	F	1.55	136.00	125.00	58.50	61.38	#NULL!
4	65	M	1.82	79.10	80.00	37.30	35.50	142.00
5	65	M	1.65	87.90	87.30	30.20	45.10	90.00
6	49	F	1.27	38.50	48.80	26.90	45.20	127.00
7	42	M	1.56	92.00	90.00	24.80	42.80	94.00
8	71	M	1.72	81.82	83.12	46.40	40.65	#NULL!
9	73	M	1.57	81.80	82.60	38.90	40.65	127.00
10	38	M	1.81	32.00	34.00	22.60	19.80	290.00
11	40	M	1.59	33.00	33.00	19.60	25.56	#NULL!
12	61	M	1.68	99.00	100.00	60.00	46.10	107.00
13	68	F	1.47	50.00	52.00	31.50	33.49	98.00
14	59	M	1.56	110.00	109.00	53.50	53.70	77.00
15	49	M	1.64	82.96	81.28	28.50	41.56	115.00
16	38	F	1.77	113.00	117.30	51.40	50.00	63.00
17	57	M	1.54	82.88	83.88	45.40	46.90	112.00
18	41	M	1.63	127.88	120.00	56.00	56.00	85.00
19	68	F	1.70	121.90	120.19	45.80	39.40	63.00
20	22	M	1.94	129.00	128.50	65.68	56.90	93.00
21	61	M	1.63	87.29	85.89	43.00	42.00	77.00
22	45	M	1.78	58.76	61.40	33.00	33.00	175.00
23	43	F	1.40	99.24	97.65	44.40	43.00	#NULL!
24	32	F	1.37	38.11	48.60	33.75	40.50	128.00
25	54	M	1.56	72.18	72.70	54.18	42.90	102.00
26	28	M	1.58	150.00	136.13	48.80	45.90	68.00
27	40	F	1.62	111.80	104.90	51.50	52.80	85.00
28	36	M	1.77	133.80	134.30	54.90	52.30	72.00
29	21	M	1.77	136.60	131.70	53.80	56.20	84.00
30	68	M	1.73	83.66	84.55	38.40	37.70	114.00
31	48	F	1.64	114.60	108.50	49.20	44.60	69.00
32	47	M	1.69	62.96	65.36	44.90	40.79	105.00
33	18	M	1.73	130.00	130.00	58.00	46.00	86.00
34	56	F	1.47	63.30	64.50	37.00	35.00	90.00

Patient no.	Age	Sex	S_Area/ m square	GFR_S /ml/min	GFR_D/ ml/min	%H6_ 2mCi	%H24_2 mCi	Creatinine umol/litre
35	33	M	2.07	54.03	55.90	37.75	36.50	161.00
36	41	F	1.52	68.69	70.43	42.20	41.11	82.00
37	73	F	1.56	102.00	103.00	57.70	55.60	73.00
38	34	M	1.60	47.36	55.00	32.70	38.80	135.00
39	52	M	1.60	93.00	92.80	59.20	57.60	99.00
40	33	F	1.44	124.00	105.00	60.28	57.80	#NULL!
41	53	F	1.56	73.98	75.11	37.79	39.10	90.00
42	46	M	1.53	54.00	57.20	38.24	37.10	149.00
43	58	M	1.65	18.00	32.90	23.28	27.00	247.00
44	44	F	1.62	29.00	39.00	17.90	23.10	168.00
45	34	M	2.10	104.00	105.00	56.90	53.70	106.00
46	46	F	1.62	109.60	108.00	61.40	61.50	68.00
47	55	F	1.76	122.00	127.00	59.10	50.00	104.00

Appendix II ------- Validation comment

Introduction

In 1985, Gates derived regression formula with age corrected resulting in mathematical models useful in estimating creatinine clearance from serum creatinine concentrations. His formula was tested by prospectively in another group of 100 patient studies in which creatinine clearance (24 hour) had been determined. Both values were normalised to standard surface areas of 1.73 m^2 . He stated that the accuracy of stimation was acceptable especially below 100 ml/min.

The regression formula for men in computing creatinine clearance (Cr Cl) with age correction :

creatinine creatinine = $(89.4)(X^{-1.2}) + (55 - A)\{0.005(89.4)(X^{-1.1})\}$ ml/min

Similarly, the regression formula for women in computing creatinine clearance (Cr Cl) with age correction :

creatinine creatinine = $(60)(X^{-1.1}) + (56 - A)\{0.005(60)(X^{-1.1})\}$ ml/min

where "X" in the above formulae was serum creatinine in mg/dl and "A" was patients age in years.

In this retrospective review and analysis, Gate's creatinine clearance method was used to estimate the creatinine clearance (24 hour).

Methods

GFR in the present group of patients were then estimated using the newly proposed formula as well as the Rowell's PFPC method. The computed values were then plotted against the creatinine clearance as estimated by Gate's creatinine clearance method. All the data were normalised to standard body surfaced area of 1.73 m^2 with the data shown in Appendix III.

Result

Univariate Correlation

1. Correlation between estimated GFR using single plasma sampling and the Gate's creatinine clearance (Cr Cl) method

The estimated normalised GFR using single plasma sampling for the 39 patients were plotted against those normalised data by Gate's creatinine clearance (Cr Cl) method (Fig.1). The calculated correlation coefficient (Pearson's product moment correlation coefficient) was 0.847, p <0.05.

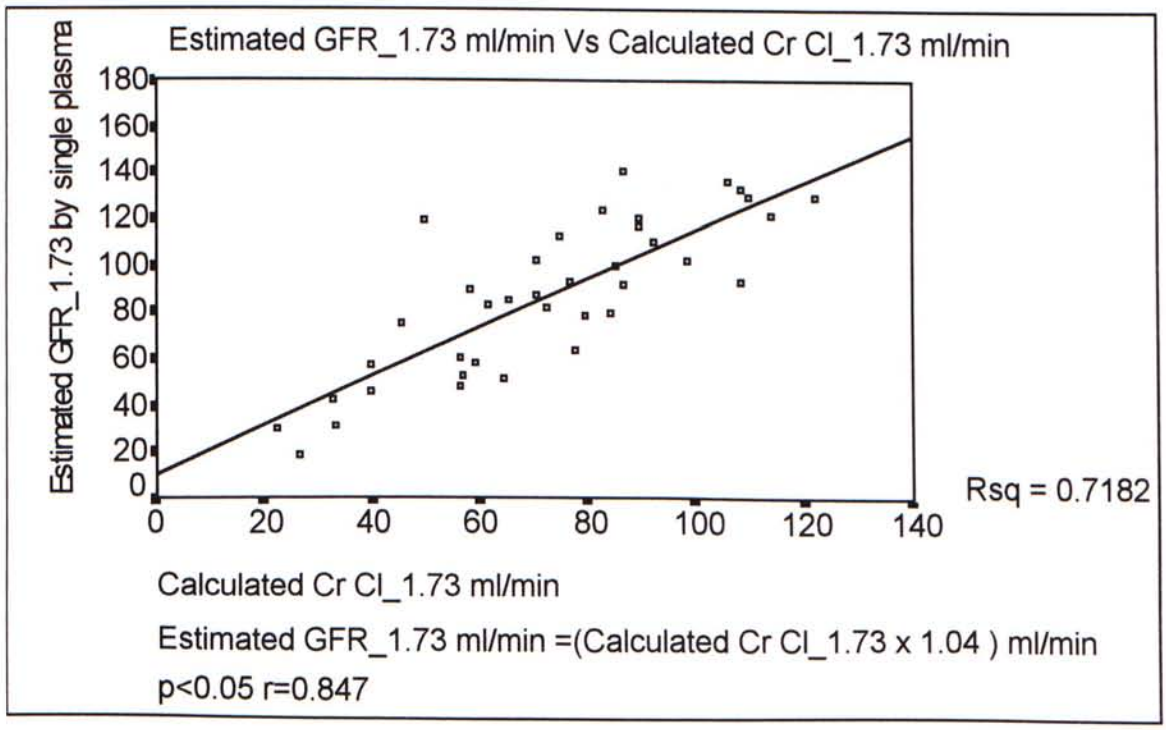

Fig.1 Relationship of normalised estimated GFR and Cr Cl

2. Correlation between predicted GFR using newly proposed formula and the Gate's creatinine clearance (Cr Cl) method

The normalised predicted GFR using newly proposed formula for the 39 patients were plotted against those normalised data by the Gate's creatinine clearance (Cr Cl) method (Fig.2). The calculated correlation coefficient (Pearson's product moment correlation coefficient) was 0.77, p <0.05.

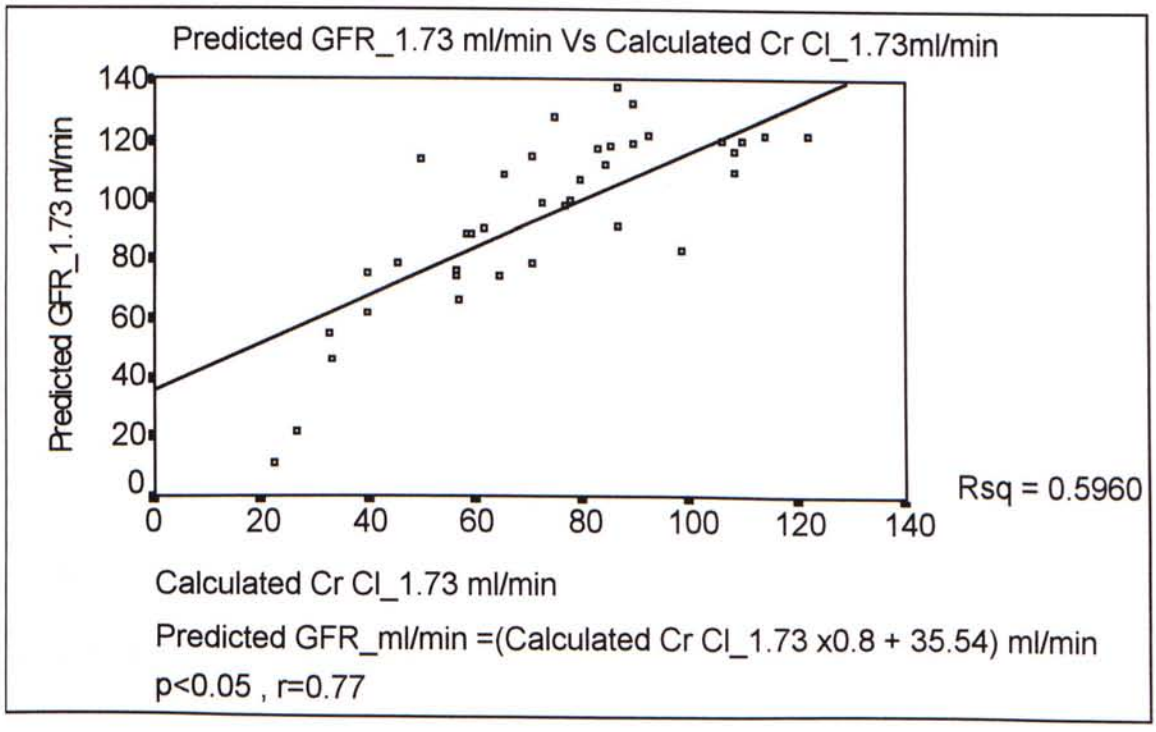

Fig.2 Relationship of normalised predicted GFR and Cr Cl

Conclusion

Clinically, the clinicians relied on the serum creatinine level and creatinine clearance (24 hours) to monitor the patient's renal function. In this study, it had been shown that there was a strong positive correlation between the normalised estimated GFR using single plasma sampling and the Gate's creatinine clearance (Cr Cl) method with $r=0.847$, $p<0.05$. Therefore it validated the method that I used to assess the GFR namely Rowell's PFPC method in my study.

Moreover as shown in the fig.2, there was a strong positive correlation between normalised predicted GFR using newly proposed formula and the Gate's creatinine clearance (Cr Cl) method with $r=0.77$, $p<0.05$.

The close relationship between the creatinine clearance, a measure of glomerular function, and tubular DMSA binding made the concept of glomerular-tubular balance strong.

Microscopically it may indicate that the entire nephron was affected in renal disease rather than separate effects on glomerular and tubular function.

Clinically, the divided renal GFR's could then be calculated by multiplying the total GFR by their respective differential function. It would provide a valuable guideline to the referring clinicians for patient management.

Appendix III-- Patient Data

Pt.No	Predict_ GFR ml/min	Predict GFR_ 1.73 ml/min	CrCl ml/min	CrCl_ 1.73 ml/min	Plasma GFR ml/min	S.A.	Plasma GFR_ 1.73 ml/min
1	138.00	138.00	87.00	87.00	141.00	1.73	141.00
2	47.30	54.90	28.20	32.70	37.00	1.49	42.90
4	83.40	79.20	47.90	45.60	79.10	1.82	75.18
5	86.60	90.70	83.03	87.00	87.90	1.65	92.10
6	48.49	66.05	41.89	57.06	38.50	1.27	52.40
7	74.80	82.90	88.77	98.40	92.00	1.56	102.00
9	79.96	88.10	52.96	58.30	81.80	1.57	90.10
10	12.00	11.40	23.57	22.52	32.00	1.81	30.50
12	111.60	114.90	68.86	70.90	99.00	1.68	101.90
13	75.00	88.20	50.32	59.20	50.00	1.47	58.80
14	109.90	121.87	103.10	114.30	110.00	1.56	121.90
15	74.90	79.00	67.20	70.80	82.96	1.64	87.50
16	124.60	121.60	94.80	92.60	113.00	1.77	110.00
17	87.40	98.10	68.60	77.00	82.80	1.54	93.00
18	113.20	120.10	100.30	106.40	127.80	1.63	136.00
19	115.20	117.20	81.78	83.20	121.90	1.70	124.00
21	103.13	109.40	102.40	108.60	87.29	1.63	93.30
22	64.30	62.40	41.20	40.00	58.76	1.78	57.10
24	60.40	76.27	44.60	56.30	38.11	1.37	48.10
25	101.00	112.00	75.96	84.23	72.18	1.56	80.00
26	109.70	120.10	138.80	151.90	150.00	1.58	164.00
28	124.70	121.80	125.12	122.20	133.80	1.77	130.00
29	119.00	116.30	111.15	108.60	136.60	1.77	133.50
30	90.10	90.10	61.50	61.50	83.66	1.73	83.66
31	113.00	119.20	85.10	89.70	114.60	1.64	120.80
32	97.80	100.10	76.22	78.00	62.96	1.69	64.45
33	120.30	120.30	109.84	109.84	130.00	1.73	130.00
34	69.00	74.60	59.76	64.60	47.36	1.60	51.20
35	90.00	75.20	47.90	40.00	54.30	2.07	45.38
36	94.00	106.90	70.00	79.60	68.69	1.52	78.18
37	115.60	128.10	67.74	75.12	102.00	1.56	113.00
39	109.50	118.30	79.20	85.60	93.00	1.60	100.00
41	89.30	99.00	65.52	72.66	73.98	1.56	82.00
42	65.70	74.20	50.11	56.60	54.00	1.53	61.00
43	20.25	21.23	25.69	26.90	18.00	1.65	18.87
44	43.00	45.90	31.34	33.40	29.00	1.62	30.96
45	132.00	108.70	79.37	65.30	104.00	2.10	85.60
46	124.00	132.40	83.97	89.67	109.60	1.62	117.00
47	116.00	114.00	50.62	49.75	122.00	1.76	119.90