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**Assessment of Gamma Camera Based
^{99m}Tc-DTPA Glomerular Filtration Rate
Using Inoue Method**

تقييم معدل الرشح الكبيبي باستخدام القاما كاميرا علي طريقة اينوي
لمادة التكنيشيم ثنائي الايثالين ثلاثي الامينات خماسي الاستات

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا
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مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَارْحَمْنَا أَنْتَ مَوْلَانَا
فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ﴾

الله العظيم

Dedication

I dedicate this thesis and every effort and any benefit that may come from this thesis to the soul of my grandmother and my grandfathers, God be merciful on them.

I would like also to dedicate this thesis to my wonderful parents, whom they have led me through the valley of darkness with light of hope, support and endless love. Their words of encouragement and push for tenacity will always ring in my ears.

A very special dedication to my lovely fiancé for being there for me. “Without you darling I wouldn’t have made it”. I will always appreciate what you have done and I’m grateful for that.

I also dedicate this thesis to my lovely family, my brother and my sisters for their constant support and unconditional love.

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Abstract

Glomerular filtration rate (GFR) is generally accepted as the most representative parameter of renal function. It is relatively constant under standard conditions. GFR being reduced prior to the onset of symptoms of renal failure, its assessment enables earlier diagnosis and therapeutic interventions in patients at risk. Various methods are available for the measurement of GFR, including the inulin continuous perfusion method, methods related to creatinine clearance, methods using prediction equation, plasma radioactivity clearance method, and gamma camera methods. The aim of this study was to assess the gamma camera based of ^{99m}Tc -DTPA glomerular filtration rate using Inoue method. In this study, seventy-five subjects were studied, (50.7% men; 49.3% women), included patients with renal diseases, as well as potential donor for renal transplantation. All subjects were undergone dynamic renal scintigraphy after injection of ^{99m}Tc -DTPA and two blood samples were collected from each patient. And then using the dynamic images and with the aid of the software, GFR was measured applying Inoue method, and from the two blood samples GFR was again calculated using the two plasma sample method which was the reference method. In the result the correlation between the GFR estimated by the Inoue camera method and the Reference was high in overall ($r = 0.93$), however this high correlation was worth in severely ill patient with GFR of less than 15ml/min which had a correlation of $r = 0.53$. It was concluded that the method underassessment seems to enable estimation of GFR with acceptable accuracy in high and moderate GFR values, but its performance in low GFR values is of concern, which limit its applicability in routine clinical practice to estimate renal function of any severity. A modification to the Inoue method was recommended which may result in a marked improvement in its result that could lead to an expanding in the usefulness of the method.

يعتبر معدل الترشيح الكبيبي مقياساً جيداً لتحديد وظائف الكلى فى الانسان، وهو ثابت نسبياً تحت الظروف الطبيعية، يقل هذا المعدل دائما قبل ظهور أعراض الفشل الكلوي، ويمكن لقياسه ان يقود للتشخيص المبكر ومن ثم التدخلات العلاجية المناسبة فى المرضى او الافراد الذين لم تظهر عليهم الاعراض المرضية بعد. توجد طرق مختلفة لقياس معدل الترشيح الكبيبي تتضمن طريقة ضخ الانبولىين المستمر، طرق متعلقة بترشيح الكرياتينين باستخدام التنبؤ، طريقة ترشيح النشاط الإشعاعي للبلازما، و طرق باستخدام القاما كاميرا. تهدف هذه الدراسة لتقييم قياس معدل الترشيح الكبيبي عن طريق القاما كاميرا باستخدام طريقة انواي. شملت الدراسة خمسة و (منهم 50.7 % 49.3 % اناث) من مرضى ومتبرعى الكلى. تم عمل المسح الكلوى لكل المستهدفين بعد حقنهم باستخدام مادة ($^{99m}\text{Tc-DTPA}$)، و تم سحب عيني دم من كل شخص لقياس معدل الترشيح الكبيبي. باستخدام الصور الديناميكية وبمساعدة البرمجيات تم حساب معدل ترشيح الكبيبي باستخدام طريقة ، عيني الدم تم قياس معدل الترشيح الكبيبي باستخدام الترشيح الاشعاعي للبلازما والتي تم اخازها كطريقه مرجعيه لتقييم الطريقه تحت الدراسة. النتائج وضحت ان الارتبط بين طريقة القاما كاميرا و طريقه عيني اليلازما كان عاليا (0.93) علي نحون عام، لكن في الحالات التي يكون فيها معدل الترشيح الكبيبي اقل من 15 /دقيقه الارتباط ضعيفه جداً (0.53). هذا يوضح ان طريقة القاما كاميرا تسمح بقياس معدل الترشيح الكبيبي بدقه عالية في الحالات التي يكون فيها معدل الترشيح الكبيبي عالي او متوسط، اما في حالات التي يكون فيها معدل الترشيح الكبيبي منخفض فانها لا تعطي نتائج جيده. لهذا فأن اجراء بعض التعديلات علي طريقة القاما كاميرا قد يؤدي الي تحسن ملحوظ في نتائج قياس معدل الترشيح الكبيبي يعني زيادة الاستفادة و انتشار هذه الطريقه.

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List of Abbreviations

^{51}Cr	Chromium 51
$^{99\text{m}}\text{Tc}$	Technetium 99m
ARF	Acute renal failure
BSA	Body Surface Area
CCR	Creatinine Clearance Rate
cpm	Count per minute
CRF	Chronic renal failure
DTPA	Diethylene Triamine Penta acetic Acid
EDTA	Ethylene Diamine Tetra acetic Acid
FADS	factor analysis of dynamic structures
GCM	Gamma Camera Method
GFR	Glomerular Filtration Rate
LEHR	Low Energy High Resolution
LR	linear Regression
MAG ₃	Mercapto Acetyl Triglycine
MDRD	Modification of Diet in Renal Disease
MSM	Multiple Samples Method
ROI	Region Of Interest
SKGFR	Single-Kidney Glomerular Filtration Rate
SPECT	Single Photon Emission Computed Tomography
TPS	Two Plasma Sample

Chapter One

Introduction

1.1 Overview of Kidney Anatomy

The urinary system consists of two kidneys; two ureters, which carry urine from the kidneys to the urinary bladder; a single, midline urinary bladder; and a single urethra, which carries urine from the bladder to the outside of the body. The kidneys are the major excretory organs of the body, the skin, liver, lungs, and intestines eliminate some waste products as well; however, if the kidneys fail to function, these other excretory organs cannot adequately compensate.

The kidneys are bean-shaped, and each is about the size of a tightly clenched fist. They lie behind the peritoneum on the posterior abdominal wall on each side of the vertebral column near the lateral borders of the psoas major muscles (figure 1). The kidneys extend from the level of the last thoracic (T12) to the third lumbar (L3) vertebrae, and the rib cage partially protects them. The liver is superior to the right kidney, causing the right kidney to be slightly lower than the left. The kidneys are organized into two major regions: an outer cortex and an inner medulla surrounding the renal sinus (figure 2). The medulla is composed of coneshaped structures called renal pyramids. Medullary rays extend from the renal pyramids into the cortex. Renal columns, consisting of cortical tissue, project between the renal pyramids. The bases of the pyramids form the boundary between the cortex and the medulla. The tips of the pyramids, the renal papillae, point toward the renal sinus. Minor calyces are funnel-shaped chambers into which the renal papillae extend. The minor calyces of several pyramids merge to form larger funnels, the major calyces. Each kidney contains 8–20 minor calyces and 2 or 3 major calyces. The major calyces converge to form an enlarged chamber called the renal pelvis, which is surrounded by the renal sinus. The renal pelvis narrows into a small-diameter tube, the ureter, which exits the kidney at the hilum and connects to the urinary bladder. (Cinnamon, Jennifer & Andrew 2014; Gerard & Bryan 2009)

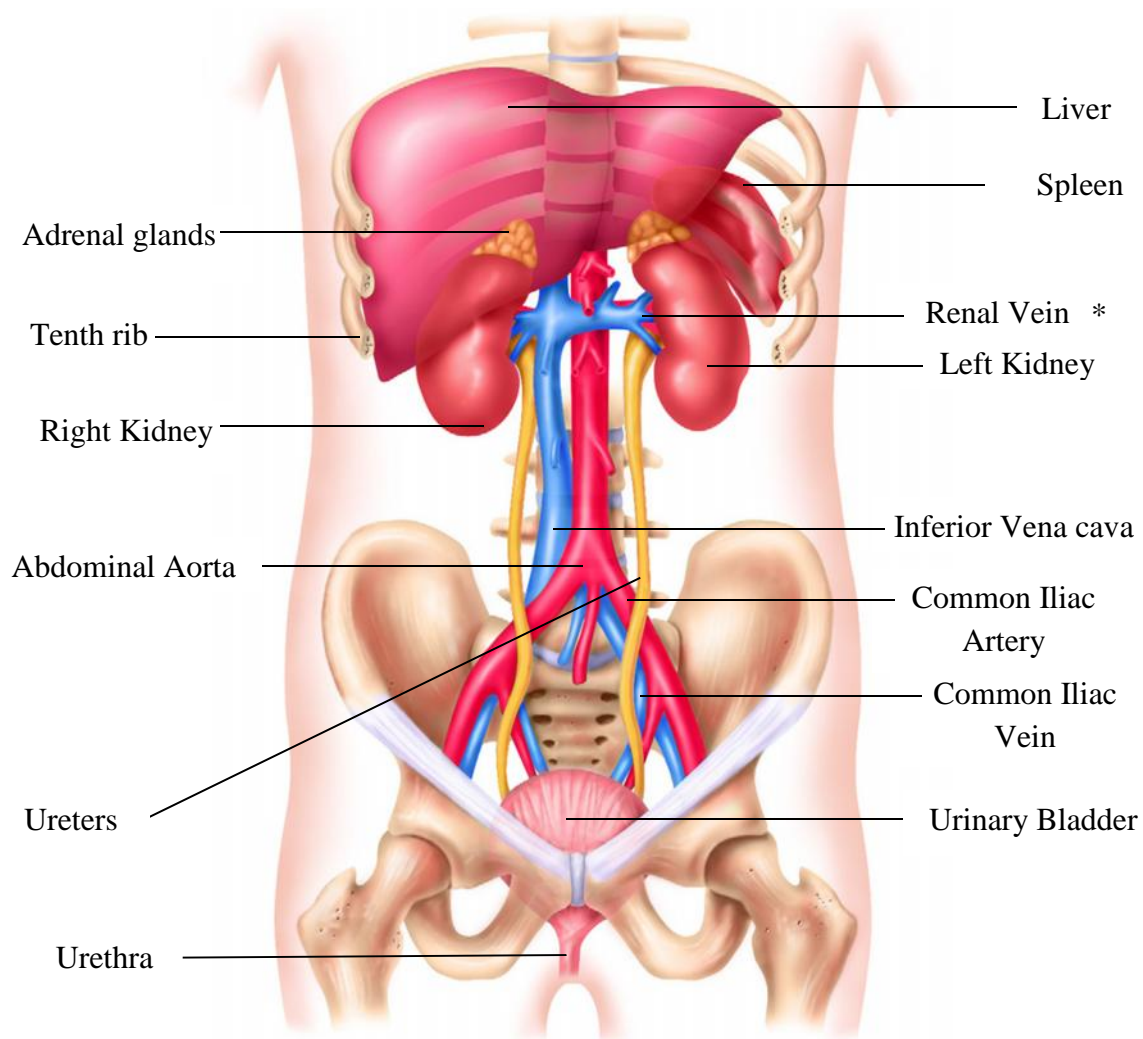


Figure 1.1: An anterior view shows the anatomy of the urinary system. Note: * The renal vein is superimposed on the renal artery. (Cinnamon, Jennifer & Andrew 2014)

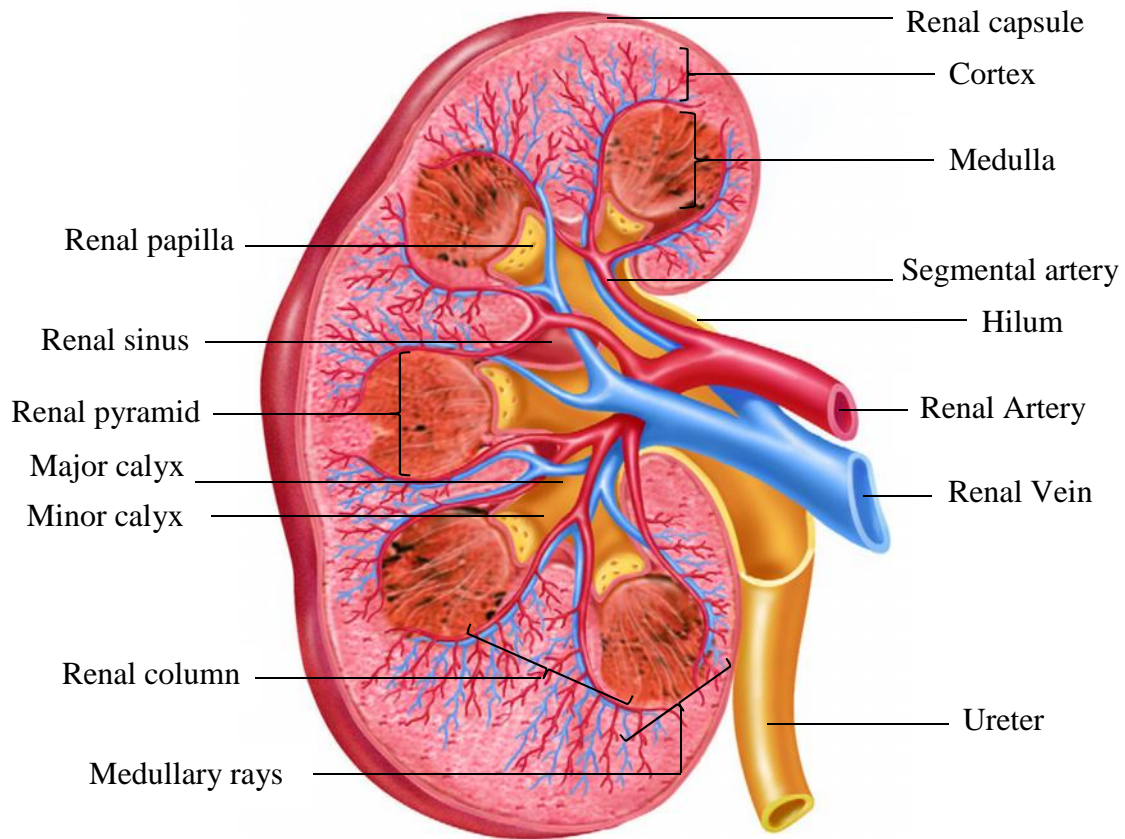


Figure 1.2: A frontal kidney section showing the cortex forming the upper part of the kidney, and the medulla forms the inner part. A central cavity called the renal sinus contains the renal pelvis. The renal columns of the kidney project from the cortex into the medulla and separate the pyramids. (Cinnamon, Jennifer & Andrew 2014)

1.1.1 The Nephron

Nephrons are the functional units of the kidneys. Each nephron consists of two parts: a renal corpuscle, where blood plasma is filtered, and a renal tubule into which the filtered fluid passes (Figure 3). The two components of a renal corpuscle are the glomerulus and the glomerular (Bowman's) capsule, a double walled epithelial cup that surrounds the glomerular capillaries. Blood plasma is filtered in the glomerular capsule, and then the filtered fluid passes into the renal tubule, which has three main sections. In the order that fluid passes through them, the renal tubule consists of; (1) a proximal convoluted tubule, (2) loop of Henle, and (3) distal convoluted tubule. The renal corpuscle and both convoluted tubules lie within the renal cortex; the loop of Henle extends into the renal medulla, makes a hairpin turn, and then returns to the renal cortex.

The distal convoluted tubules of several nephrons empty into a single collecting duct, the collecting ducts then unite and converge into several hundred large papillary ducts, which drain into the minor calyces. The collecting ducts and papillary ducts extend from the renal cortex through the renal medulla to the renal pelvis. So one kidney has about 1 million nephrons; but it has a much smaller number of collecting ducts and even fewer papillary ducts. (Gerard & Bryan 2009)

1.2 Overview of the Kidney Physiology

The kidneys do the major work of the urinary system. The other parts of the system are mainly passageways and storage areas. Functions of the kidneys include the following:

- a) Regulation of blood ionic composition; The kidneys help regulate the blood levels of several ions, Na^+ , Cl^- , K^+ , Ca^{2+} , HCO_3^- , and HPO_4^{2-} they also regulate other solute concentration, such as urea.
- b) Regulation of blood pH; it excretes a variable amount of hydrogen ions (H^+) into the urine and conserve bicarbonate ions (HCO_3^-), which is an

important buffer of H^+ in the blood. Both of these activities help regulate blood pH.

c) Regulation of blood volume; they adjust blood volume by conserving or eliminating water in the urine. An increase in blood volume increases blood pressure; a decrease in blood volume decreases blood pressure.

d) Regulation of blood pressure; it also helps regulate blood pressure by secreting the enzyme renin, which activates the renin angiotensin aldosterone pathway. Increased renin causes an increase in blood pressure.

e) Maintenance of blood osmolarity; by separately regulating loss of water and loss of solutes in the urine, the kidneys maintain a relatively constant blood osmolarity close to 300 milliosmoles per liter.

f) Production of hormones; they produce two hormones. Calcitriol, the active form of vitamin D, helps regulate calcium homeostasis and erythropoietin stimulates the production of red blood cells.

g) Regulation of blood glucose level; like the liver, the kidneys can use the amino acid glutamine in gluconeogenesis, the synthesis of new glucose molecules. They can then release glucose into the blood to help maintain a normal blood glucose level.

h) Excretion of wastes and foreign substances. By forming urine, the kidneys help excrete wastes—substances that have no useful function in the body. Some wastes excreted in urine result from metabolic reactions in the body. These include ammonia and urea from the deamination of amino acids; bilirubin from the catabolism of hemoglobin; creatinine from the breakdown of creatine phosphate in muscle fibers; and uric acid from the catabolism of nucleic acids. Other wastes excreted in urine are foreign substances from the diet, such as drugs and environmental toxins. (Cinnamon, Jennifer & Andrew 2014; Gerard & Bryan 2009)

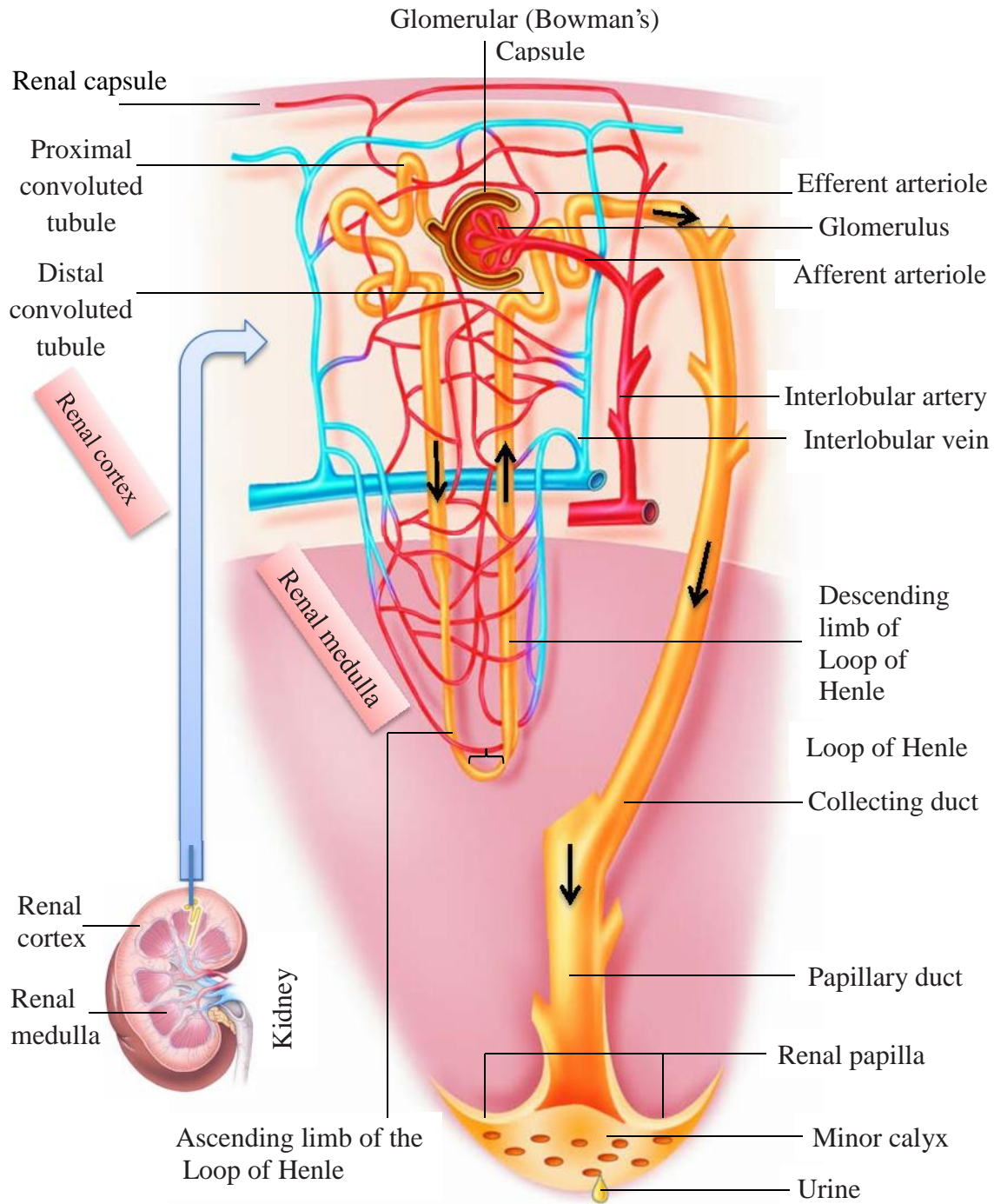


Figure 1.3: Presents the Nephron “the functional unit of the kidney” consists of a renal corpuscle, proximal convoluted tubule, loop of Henle, and distal convoluted tubule. (Gerard & Bryan 2009).

1.3 Overview of Kidney Pathology

Traditionally, diseases of the kidneys are divided into 4 major groups according to the predominant involvement of corresponding morphologic components:

1. Glomerular diseases: These are most often immunologically-mediated and may be acute or chronic.
2. Tubular diseases: These are more likely to be caused by toxic or infectious agents and are often acute.
3. Interstitial diseases: These are likewise commonly due to toxic or infectious agents and quite often involve interstitium as well as tubules (tubulo-interstitial diseases).
4. Vascular diseases: These include changes in the nephron as a consequence of increased intra-glomerular pressure such as in hypertension or impaired blood flow.

In addition to other diseases include; congenital anomalies, obstructive uropathy and tumors of the kidneys. (Harsh 2010)

The major morphologic involvements of the kidneys in the initial stage is confined to one component (glomeruli, tubules, interstitium or blood vessels), but eventually all components are affected leading to end-stage kidneys. Regardless of cause, renal disease usually results in the evolution of one of the two major pathological syndromes: acute renal failure and chronic renal failure. Acute renal failure (ARF) is a syndrome characterized by rapid onset of renal dysfunction, chiefly oliguria or anuria, and sudden increase in metabolic waste-products (urea and creatinine) in the blood with consequent development of uremia. Chronic renal failure (CRF) is a syndrome characterized by progressive and irreversible deterioration of renal function due to slow destruction of renal parenchyma, eventually terminating in death when sufficient numbers of nephrons have been damaged. (Harsh 2010)

1.4 Glomerular Filtration Rate

The amount of filtrate formed in all the renal corpuscles of both kidneys each minute is the glomerular filtration rate (GFR). In adults, the GFR averages 125 mL/min in males and 105 mL/min in females. Homeostasis of body fluids requires that the kidneys maintain a relatively constant GFR. If the GFR is too high, needed substances may pass so quickly through the renal tubules that some are not reabsorbed and are lost in the urine. If the GFR is too low, nearly all the filtrate may be reabsorbed and certain waste products may not be adequately excreted. GFR is directly related to the pressures that determine net filtration pressure; any change in net filtration pressure will affect GFR. Severe blood loss, for example, reduces mean arterial blood pressure, decreases the glomerular blood hydrostatic pressure. Filtration ceases if glomerular blood hydrostatic pressure drops to 45 mmHg because the opposing pressures add up to 45 mmHg. Amazingly, when systemic blood pressure rises above normal; net filtration pressure and GFR increase very little. GFR is nearly constant when the mean arterial blood pressure is anywhere between 80 and 180 mmHg. (Gerard & Bryan 2009)

The mechanisms that regulate glomerular filtration rate operate in two main ways: (1) by adjusting blood flow into and out of the glomerulus and (2) by altering the glomerular capillary surface area available for filtration. GFR increases when blood flow into the glomerular capillaries increases. Coordinated control of the diameter of both afferent and efferent arterioles regulates glomerular blood flow. Constriction of the afferent arteriole decreases blood flow into the glomerulus; dilation of the afferent arteriole increases it. Three mechanisms control GFR: renal autoregulation, neural regulation, and hormonal regulation. (Gerard & Bryan 2009)

1.5. Common Indications for GFR Measurement

The glomerular filtration rate (GFR); the plasma volume filtering through the glomerulus per minute, is a significant value for the evaluation of renal function, and an objective index for the assessment of the state of illness and the effectiveness of treatment (Li et al. 2007). It is considered to be the best overall index of renal function in health and sickness (Aydin et al. 2008), It could be indicated for:

- i. Monitoring of drugs that might cause nephrotoxicity.
- ii. Calculation of dose in chemotherapy (Calvert et al, 1989).
- iii. Detection of renal failure in patients in whom; (a) serum creatinine results might be misleading (b) missing a decline in renal function might be disastrous e.g. single kidney, renovascular disease or renal transplant and (c) a 24 h clearance measurement is difficult e.g. elderly or those with learning difficulties.
- iv. Assessment of potential live donors for transplantation e.g. relative of patient on dialysis.
- v. Evaluation and follow up of renal function in chronic glomerulonephropathies such as hemolytic uremic syndrome and diabetes mellitus.
- vi. The evaluation of single kidney function in conjunction with relative renal function measurements from static or dynamic radionuclide imaging.

1.6 Measurement of Glomerular Filtration Rate

The ideal way of assessing GFR is to measure the clearance of a substance that is freely filtered by glomeruli and does not undergo reabsorption or tubular secretion (Assadi et al. 2008). Various methods are available for the measurement of GFR, including the inulin continuous perfusion method, methods related to creatinine clearance, methods using prediction equation, plasma radioactivity clearance method, and radionuclide uptake method.

1.6.1 Inulin Perfusion

The determination of GFR became possible through the introduction of the clearance concept, and was initially determined by the use of inulin, it is ideally measured with agents that are excreted solely by glomerular filtration, which is typically offered by inulin (Gates 1982). The generally accepted gold-standard technique for GFR assessment is the inulin continuous perfusion method which requires constant intravenous infusion of inulin and bladder catheterization (Cousins et al. 1999). The clearance of inulin is proved as the gold standard for GFR determination. Although it is an ideal method for determining of GFR as reported by Berliner, the technique has limited clinical application because the technique is difficult and time-consuming (Berliner 1971). Therefore it regarded as inappropriate for routine uses and is seldom applied in clinical practice due to the technical complexity and limited availability (Aydin et al. 2008).

1.6.2 Creatinine Clearance

The twenty-four-hour endogenous creatinine clearance, which requires 24-h urine collection, is one of the methods for the assessment of GFR. Determination of creatinine clearance is simpler and has been adopted as a clinical means of determining GFR (Gates 1982). It is a sensitive parameter of renal function; it correlates closely with inulin clearance as proved by Brod, but is not as exact as inulin clearance in estimating GFR, since a small quantity of creatinine is secreted by the renal tubules (Brod & Sirota 1948). However, creatinine clearance determines the total GFR and not individual renal function. It is also require an accurate 24 hr. urine collection for precise result which is subjected to error and the reliability of this method is too dependent on accurate urine collection (Aydin et al. 2008).

1.6.3 Prediction Equation

GFR can also be calculated through prediction equations; a number of prediction equations have been described to estimate GFR, by incorporating biometrical variables such as age, height, weight, gender and race with serum creatinine

concentration and other biochemical parameters (Gaspari et al. 2004). MDRD and Cockcroft Gault formulas have been the most frequently used among them (Aydin et al. 2008). However, it has been debated whether the equations accurately predict the GFR, Itoh 2002 *et al*, studied 133 patients with a wide range of renal function and found that the Cockcroft– Gault formula is not accurate for the measurement of GFR because of its worse precision (Itoh et al. 2002). Li reported that MDRD or Cockcroft–Gault formulas tend to overestimate GFR. He also mentioned that the laboratory measurement of serum creatinine is critical when estimating GFR by prediction equations, because a small change in serum creatinine will result in a large change in MDRD or Cockcroft– Gault formulas (Li et al. 2007).

1.6.4 Plasma Sample Clearance Method

The measurements of GFR based on plasma clearance following a single injection of renal markers have been widely used (Li et al. 2007). Many radionuclides have been reported and some of them have been commonly used. Radioactive isotopes like ^{99m}Tc -diethylenetriaminepentaacetic ($^{99m}\text{TC-DTPA}$), ^{125}I -iothalamate, and Chromium 51-ethylenediaminetetraacetic acid ($^{51}\text{Cr-EDTA}$) are used (Gates 1982). These substances harbor several features of inulin and several studies have demonstrated that the above-mentioned methods have high level of correlation with inulin clearance (Prigent et al. 1999). This method, however, is not routinely performed, because of the laborious and cumbersome procedures (Aras et al. 2012). The plasma sample method has been regarded as a reference method, despite that this procedure requires serial blood samples in order to perform the computation necessary to calculate GFR. Thus the time interval needed for acquiring the multiple blood samples, which may be up to 4 hours, combined with sample counting and subsequent back extrapolation of data to determine the radionuclide clearance rate, impose logistical constraints on a busy nuclear medicine department (Aydin et al. 2008).

1.6.5 Gamma Camera Based Method

Renal dynamic imaging with ^{99m}Tc -DTPA is a commonly used method to determine renal blood flow and unilateral kidney function. The timed uptake curves of the two kidneys, especially the comparison, give notable information such as quantitative unilateral renal function and pathophysiological changes in the kidney in renovascular hypertension, hydronephrosis and renal transplant (Ma et al. 2007). The estimation of GFR is a major role of renal scintigraphy with ^{99m}Tc -DTPA which is known as the Gamma Camera-based method GFR, in which the GFR is calculated without blood or urine sampling, offering technical simplicity and relatively quick procedure to perform (Inoue et al. 1998). The in vivo GFR determination with nuclear medical imaging is based on the assumption that there is a certain period of time when a large concentration of the imaging agent accumulates in the kidney and is not discharged, and the renal uptake percentage is linear with GFR, as it was described by Gates 1982 (Gates 1982). This represents the ideal situation, but the true situation varies accordingly. Several Camera Based GFR methods have been applied in clinical practice; some of them were applied for decades like Gates method while others like Inoue method are newly introduced.

1.6.5.1 Gates Method

The Gates Method, introduced by Gary F. Gates is a popular method and has been widely used since 1982. Gates derived his formula by a linear regression analysis between the renal uptake percentage of ^{99m}Tc -DTPA and the 24-h creatinine clearance rate (CCR). The region of interest (ROI) were manually drawn for the kidneys and Background were drawn in the portion infero-lateral to the kidneys, then renal uptake percentage was determined from the renal radioactivity uptake (counts) 2–3 min post-injection (when the imaging agent reaches the kidneys) corrected by the renal depth using Tonnesen's formula (Gates 1982). Many studies have been conducted to test the accuracy of the Gate's method in estimation of GFR. John compared the ^{99m}Tc -DTPA renal dynamic imaging method with ^{99m}Tc -DTPA plasma clearance as the reference

GFR, and found significant difference between the Gate's method and reference GFR (John, Peter & James 1990). Using inulin clearance as the reference standard, Natale *et al*, indicated that the Gate's method tended to overestimate GFR at low levels, and underestimate GFR at high levels of GFR (Mulligan, Blue & Hasbargen 1990). A recent published study also found similar results, regarding the Gate's method, the performance of the renal dynamic GFR method was even worse than creatinine clearance in GFR estimation (Inoue et al. 1998).

1.6.5.2 Inoue Method

Modern, newly developed Camera Based GFR methods are available; however there diagnostic accuracy is still ambiguous, among them is the Inoue Method introduced, by Inoue *et al* 1998. Inoue derived his formula by a linear regression analysis between the renal uptake percentage of ^{99m}Tc -DTPA and the plasma samples taken at 2 and 3 hours post injection, which was used as a reference to measure the actual GFR. The regions of interest (ROI) were manually drawn for the kidneys and perirenal background areas, and then renal uptake percentage was determined from the renal counts at 2-2.5 min after tracer arrival in the kidney. Renal depth was estimated in children using the equation described by Raynaud and in adults using the equation presented by Taylor; both were used as a correction index for attenuation. There result demonstrated a high correlation between the reference and the ^{99m}Tc -DTPA renal uptake in GFR clearance, irrespective of patient's age (Inoue et al. 1998). The accurate results of the Inoue Method renovated the interest in Camera Based GFR estimation, as well as being able to determine split renal GFR which cannot be achieved with plasma sample method (De Santo et al. 1999).

1.7 Region of Interest in Nuclear Medicine

In nuclear medicine, quantitative results are normally obtained from images after the manual drawing of regions of interest (ROIs) about the organ or area being investigated. The reproducibility of these results often depends on an

operator's ability to draw the same region accurately and consistently (Oyamada et al. 1994). Increasingly, the requirements of quality assurance are being brought to bear on diagnostic departments, with a demand for objective quantitative results. Manually drawn ROIs are viewed as subjective, because they depend on an operator's skill in drawing regions accurately (Arteaga de Murphy et al. 1995), so as a result automated processing techniques are proliferating.

Drawing ROIs is a technical problem, which should lend itself to automatic methods. Automatic techniques such as "thresholding" and other edge detection methods have been shown to be successful for comparatively uncomplicated images in which an organ is clearly defined (Todd-Popropek, Kwok & Cavailloles 1981). Many nuclear medicine studies, however, introduce a degree of complexity that precludes the use of these techniques, for reasons including patient or organ movement during the study and the overlaying of different organs in two-dimensional images (White et al. 1999). This is particularly true for dynamic imaging in which the time element is also introduced.

More sophisticated techniques are being developed, including the use of factor and cluster analysis, neural networks, and image registration (Hannequin, Liehn & Valeyre 1990). To assess the ability of these techniques to draw ROIs correctly, a gold standard is needed against which to compare them. The most obvious types of gold standard to test ROI drawing are a phantom or ROIs which is drawn by experienced nuclear medicine professionals. Both have advantages and disadvantages (White et al. 1999). In simple terms, for a phantom, the true answer is known but the model is less complex than a human study, whereas if an expert's ROI is used as the "true" region, the complexity of a human study is accounted for but the real true answer cannot be found.

1.8 Background Subtraction

Background subtraction has long been a major factor in the quantification process in nuclear medicine. Since the introduction of ^{99m}Tc - DTPA

renography, the need for accurate background subtraction has been well documented (Shore et al. 1984). In order to obtain quantitative information from ^{99m}Tc -DTPA renogram it is first necessary to correct for the presence of background activity in the kidney. It is required to assess true renal activity and evaluate renal function by an external counting technique, and inappropriate background subtraction causes errors in estimating renal function (Inoue et al. 1994).

Many methods have been proposed leading to a diversity in derived quantitative values. Perhaps the most frequently used technique involves the use of a bilinear interpolation across the region under scrutiny (Goris et al. 1976). An alternative method, based on Cauchy integral interpolation, has recently been suggested (Nichols et al. 1987). Another method is the linear regression (LR) technique, first described by Thomson et al. for ^{99m}Tc -DTPA (Thomson et al. 1980).

The majority of these techniques depend on the use of a single background region, often normalized to the kidney region size as Middleton reported. A variety of different locations for a background region have also been proposed (Middleton et al. 1989). Perhaps the common used locations are the subrenal background and the perirenal background. Modern, newly developed Nuclear Medicine software have introduced an automatic background drawing feature, nevertheless the clinical utility is still ambiguous. Uses of background estimation in more sophisticated data processing techniques, e.g. as a constraint in FADS (factor analysis of dynamic structures) (Houston 1986), or for artifact removal in SPECT (single photon emission computed tomography) (Gillen et al. 1988), have revived interest in estimating quantitatively the accuracy of background subtraction methods.

1.9 Radiopharmaceutical

Two Radiopharmaceuticals with slightly different renal handling characteristics; chromium-51 ethylenediaminetetraacetic acid (^{51}Cr -EDTA) and technetium -

^{99m}Tc -diethylenetriaminepentaacetic acid (^{99m}Tc -DTPA) have been commonly utilized in clinical practice of GFR (Bhatt. et al. 2011). ^{51}Cr -EDTA is considered to be more reliable, with pure glomerular filtration, a high and stable (both in vitro and in vivo) labeling yield, little protein binding, and as revealed by Chantler, its plasma clearance is nearly identical to that of inulin (Chantler et al. 1969; Hall, Guyton & Farr 1977). Nonetheless this radiopharmaceutical is primarily used in Europe, and it is not commercially available in most of the countries (Gates 1982).

DTPA; is a soluble organic molecule of less than 500 molecular weight, which is stable at physiologic pH. When stannous (Sn^{2+}) ion is mixed with pertechnetate, the chelating properties of DTPA result in technetium stannous DTPA (Gates 1982). ^{99m}Tc -DTPA is considered an acceptable alternative to ^{51}Cr -EDTA as has been shown (Fleming et al. 2004). It has the advantages of being inexpensive and widely available; further, the radiation dose administered to the patient is low. It is also suitable for gamma camera imaging, allowing simultaneous acquisition of a renogram for calculation of differential renal function (Blaufox et al. 1996). This radiopharmaceutical satisfies criteria for the measurement of GFR, since any such agent must be: completely filtered at the glomerulus; not synthesized, destroyed, reabsorbed or secreted by the renal tubule; physiologically inert; and not be bound to plasma protein as mentioned by Braren (Braren et al. 1979). A confirmation that ^{99m}Tc -DTPA satisfies these requirements has been made by Klopper, although they did show minimal protein binding that was considered insignificant (Klopper et al. 1972). By comparing ^{99m}Tc -DTPA with inulin, Gunasekera found that ^{99m}Tc -DTPA is a perfect glomerular tracer (Gunasekera, Allison & Peters 1996). Therefore ^{99m}Tc -DTPA was chosen as the radiopharmaceutical of choice in this study.

1.10 Reference Criterion

Plasma clearance method of ^{99m}Tc -DTPA GFR can be estimated from either one, two or multiple plasma samples. Previous research has proven that the

^{99m}Tc -DTPA multiple plasma method is strongly correlated with the inulin continuous infusion method for the measurement of GFR, Cousins 1999 et al has affirmed that (Cousins et al. 1999). As the two-plasma showed a high correlation, when Waller *et al* compared the correlation of the one-plasma method and two-plasma method with the multi-plasma method, and found that the two plasma method showed a correlation of 0.996 which was the highest (Cousins et al. 1999). While Russell verified that, the two plasma sample method is more accurate in GFR determination than the single plasma sample method (Russell 1987). Moreover, the Nephro-urology Conference held in 1996 in the USA also recommended the adoption of the two-plasma method, especially when a high precision of GFR is needed (Blaufox et al. 1996). Thus, in this research we selected the two-plasma method as the reference criterion.

1.11 Problem of the study

Measurement of GFR with plasma sample method is time consuming, invasive, and requires multiple laboratory manipulations. Nonetheless, it has another major limitation, that a single kidney GFR cannot be assessed. On the other hand the Gamma Camera Based Method for GFR omits the need to draw blood which can be traumatic especially for children, takes about 30 min to perform, and can obtain an estimate of a single kidney GFR. Several Gamma Camera Based Methods for GFR have been adopted with varied outcomes. One of the most promising methods is the Inoue method. Although it has been suggested since 1998, it has not gain prominence due to lack of verification, this study intends to assess the Inoue method.

1.12 Objective

1.12.1 Main Objective

The main objective of this study is to assess the Gamma Camera Based GFR method of ^{99m}Tc -DTPA clearance using Inoue method to prove the applicability and importance of using Inoue method.

1.12.2 Specific objectives

- To assess the accuracy of Inoue method in calculating GFR
- To compare the accuracy and reliability of semiautomatic kidneys region to the manual drawing.
- To evaluate the effect of the different background locations in GFR measurement.

1.13 Significance

- If assessed positively; the Inoue method could provide an alternative for GFR measurement; however, the possibility of replacing the current method in certain renal stages is existed.
- In addition, the introduction of the semiautomatic kidneys region is aiming to decrease the inter-subject variability related to the manual procedure, and to reduce the procedure time while maintaining the accuracy of GFR estimation.
- Moreover, background subtraction is a major factor in GFR quantification; perhaps the most frequently used techniques are the subrenal background and the perirenal background, the automatic background proposed could provide the best option for background correction.

Chapter Two

Literature Review

In the literature, there are many studies that compared several methods in the determination of GFR in subjects with various degrees of renal dysfunction. Constant intravenous infusion of inulin and bladder catheterization is the 'gold standard' method in the determination of GFR. This method is seldom applied in clinical practice due to the technical complexity and limited availability. Twenty-four-hour endogenous creatinine clearance, which requires 24-h urine collection, is one of the other methods for the assessment of GFR. However, this method was reported to be less reliable than inulin clearance. The gamma camera method with Tc-99m-DTPA renography is another method for the calculation of GFR in clinical practice, the glomerular filtration rate (GFR) is calculated without blood or urine sampling. Several techniques have been applied in clinical practice, because of technical simplicity and requirement for less time for the patients. GFR can also be calculated through prediction equations using parameters such as age, sex and serum creatinine level. The most widely accepted prediction equations are the modification of renal disease (MDRD) and the Cockcroft–Gault. Measurement of plasma clearance of $^{51}\text{Cr-EDTA}$ and $^{99\text{m}}\text{Tc-DTPA}$ is widely used for the estimation of overall GFR due to its simplicity and accuracy. Measurement of plasma clearances of $^{99\text{m}}\text{Tc-DTPA}$ and $^{51}\text{Cr-EDTA}$ can be performed using a single or multiple plasma samples.

Gates (1981) developed a method to estimate GFR from fractional renal accumulation of $^{99\text{m}}\text{Tc-DTPA}$. The objective of their study was to describe a rapid method for determining GFR, as a total value and individually for each kidney, by the use of $^{99\text{m}}\text{Tc-DTPA}$. Thirty-one patients including and undergone twenty-four hour creatinine clearance and $^{99\text{m}}\text{Tc-DTPA}$ scan. Several variables were tested during this study in order to determine the method by which the radionuclide GFR calculation would have the closest correlation with the 24-h creatinine clearance value. The results revealed that the optimal combination of variables was: (1) depth correction (2) semilunar background area of interest;

(3) analysis performed during the 2-3 min time period after tracer arrival within the kidneys. This combination had the best correlation coefficient ($r > 0.95$) and the lowest standard error (8.62). He concluded that correlation coefficient between DTPA uptake and creatinine clearance is as high as or higher than that reported by others using plasma clearance techniques and the reproducibility of the method is excellent.

Russell et.al (1985) well measured glomerular filtration rate using ^{99m}Tc -DTPA with the gamma camera. Their objective was to compare the variety of methods which have been proposed to estimate the GFR from the renal uptake of ^{99m}Tc -DTPA using a gamma camera. To compare the different methods, the GFR was calculated using algorithms proposed by Piepsz et al. (1977a), Nielsen et al. (1977), Assailly et al. (1977), and Gates (1982), and compared their results with an independent GFR based plasma clearance of ^{169}Yb - DTPA. Data were acquired for 30 min by means of a computer-linked gamma camera; blood samples were collected and were counted in a well-counter. In the result the method of Assailly et.al using correction for blood pool, height, weight and kidney depth, gave significantly better results than the other methods. They conclude that gamma-camera methods can reasonably be substituted for creatinine-clearance in clinical use, especially under circumstances in which reliable 24-h urine collection is difficult.

Moonen et.al (1994) studied three methods for determination of split renal function from gamma camera renography. The aim of the study is to compare the reliability and applicability of three methods based on their different principles. All patients underwent gamma camera renography after a bolus injection of 100 MBq ^{99m}Tc -DTPA, split kidney function was calculated according to the following method; integral method, slope method, and uptake index method. Single kidney glomerular filtration rate (GFR) was assessed by the three methods and ^{51}Cr -EDTA clearance, giving the accuracy of each

method. In the result; the slope and uptake index methods showed high accuracy (0.9-2.0 ml/min), while it is significantly reduced with the integral method (4.6 ml/min), compared ^{51}Cr -EDTA clearance. The reproducibility in calculating the relative renal function was very good with all three methods.

Li et.al (2007) developed a formula for accurate measurement of the glomerular filtration rate by renal dynamic imaging. Their objective was to explore an alternative improved formula to calculate GFR by renal dynamic imaging. 367 patients were selected and their GFR values were measured using renal dynamic imaging and the two-plasma method with $^{99\text{m}}\text{Tc}$ -DTPA. With the two-plasma GFR as reference value, two equations were obtained from linear and non-linear regression analyses between the renal uptake percentage and two-plasma GFR. Their result showed the biases of the GFR values calculated using the linear and non-linear formulae and Gates' formula relative to the two-plasma GFR were $-2.5 \pm 19.1 \text{ ml/min/1.73 m}^2$, $-2.0 \pm 19.3 \text{ ml/min/1.73 m}^2$ and $3.4 \pm 19.4 \text{ ml/min/1.73 m}^2$, respectively. They concluded that the GFR values calculated using their new formulae correlate better with the reference GFR value than does GFR calculated by Gates' formula, and the GFR values measured using the non-linear formula are more accurate than those obtained using the linear

Carlsen (2004) determined glomerular filtration rate from $^{99\text{m}}\text{Tc}$ -DTPA renography using a dual head gamma camera as an absolute measurement device. The objective of the study was to design a general nuclear medicine method for fast, accurate and direct determination of the clearance of a radioactive indicator. The gamma camera method (GCM) was tested in a group of 26 adult subjects in whom GFR was determined simultaneously using the simplified multiple samples method (MSM)

with plasma samples drawn 3–5 h following the injection of $^{51}\text{Cr-EDTA}$. The results for GFR above 30 ml min^{-1} ; the regression line of GFR in the MSM versus GFR in the GCM was not significantly different from the line of identity. The reliability of the GCM alone was about 14%, 11% and 6% for GFR values of 30, 60 and 120 ml min^{-1} . They concluded that; since the recording of the renal count rates in the anterior and posterior views permits an accurate determination of the split renal function; the GCM yields reliable estimates also for the single kidney GFR.

Inoue et.al (1998) evaluated glomerular filtration rate by camera-based method in both children and adults. The purpose of the study was to develop a camera-based method to estimate GFR in both children and adults. Renal scintigraphy with $^{99\text{m}}\text{Tc-DTPA}$ was performed in 40 children and 92 adults with various degrees of renal function. The percent renal uptake at 2-2.5 min after tracer arrival in the kidney was determined with background subtraction and correction for soft-tissue attenuation and was correlated by linear regression analysis with GFR measured from two blood samples. The result of the regression analysis obtained between BSA-corrected GFR and percent renal uptake was closely correlated ($y = 15.958x - 2.94$; $r = 0.939$; $\text{s.e.e} = 13.89$). They concluded that their method allows estimation of GFR in both children and adults and may contribute to the quantitative assessment of renal function, especially in nuclear medicine departments to which both pediatric and adult patients are referred.

Madsen et.al (2013) determined kidney function with $^{99\text{m}}\text{Tc-DTPA}$ renography using a dual-head camera. The aim of the study was to compare single-head versus dual-head in assessment of single kidney function. 34 patients were examined with single-head renography and dual-head renography, acquiring counts from the left ventricle from an anterior projection and kidneys from both anterior and posterior projections using geometric mean values. Single kidney GFR from both models was

estimated and compared with GFR determined with plasma samples of ^{99m}Tc -DTPA. In the results the prediction intervals of single-head and dual-head camera compared with plasma samples GFR did not differ significantly, the corresponding coefficients of variation were 16.5 and 14.7%, respectively. They came to conclude that there is no difference in variance between GFR estimated from single-head renography and that estimated using dual-head renography.

Levey et.al (1999) developed a method to estimate Glomerular Filtration Rate from Serum Creatinine as a new prediction equation. The objective of their study was to develop a more accurate equation to predict GFR from serum creatinine concentration and other factors. 1628 patients enrolled in the baseline period of the Modification of Diet in Renal Disease (MDRD) Study, of whom 1070 were randomly selected as the training sample; the remaining 558 patients constituted the validation sample. The prediction equation was developed by stepwise regression applied to the training sample. The equation was then tested and compared with other prediction equations in the validation sample. The result revealed that the measured creatinine clearance overestimated GFR by 19%, and creatinine clearance predicted by the Cockcroft–Gault formula overestimated GFR by 16%. After adjustment for this overestimation, the percentage of variance of the logarithm of GFR predicted by measured creatinine clearance or the Cockcroft–Gault formula was 86.6% and 84.2%, respectively. They concluded that the equation developed from the MDRD Study provided a more accurate estimate of GFR in their study group than measured creatinine clearance or other commonly used equations.

Li et.al (1997) studied the measurement of GFR with the single sample methods of ^{99m}Tc -DTPA. The purpose was to assess the relative accuracy of the many single-sample methods which are still not clear for clinical practice. 54 GFR studies with ^{99m}Tc -DTPA were performed on 37 adult patients; each study included a UVIP, plasma clearance method (three-sample) and single-sample

methods. The single-sample methods used were those of Christensen and Groth, Constable, Dakubu, Groth and Aasted, Jacobsson, Morgan, Russell and Tauxe. The result showed that; when $GFR > 30$ ml/min, all of the single-sample methods were highly correlated with UVIP, and the correlation of the single-sample method with plasma clearance was higher than with UVIP, whereas most single-sample methods do not perform well with $GFR < 30$ ml/min, and none of them has a good correlation with UVIP or plasma clearance at this level of renal failure. They concluded that the single-sample methods may not correctly predict GFR in advanced renal failure, and that all methods perform acceptably at $GFR > 30$ ml/min.

Florijn et.al (1994) measured the glomerular filtration rate by a single-shot injection of inulin. The aim of the study was to define and compare the agreement and reproducibility of the single intravenous injection of inulin with the method using a constant infusion of inulin. The clearance of inulin was measured three times with the constant infusion method and three times with a single intravenous injection technique on separate occasions over the course of six weeks. Every other week one constant infusion and one single injection technique of inulin, in random order, was performed and these values were used for comparison. In the result the mean difference of single injection total body clearance minus urinary clearance during constant infusion, was 13.1 ml/min/ $1.73m^2$. Limits of agreement were -7.7 and 33.9 ml/min- $1.73m^2$. Correlation was high for the constant infusion and the single injection technique, $r = 0.96$. In conclusion they pointed out that; the total body clearance of inulin using the single intravenous injection method has a good reproducibility over a wide range of GFR.

Chapter Three

Materials and Methods

3.1 Subjects

This study was approved by the National Cancer Institute, Gezira University ethical committee. Informed consent was obtained from all study subjects before the participation in the study procedures.

In this study, seventy-five subjects were studied, (50.7% men; 49.3% women). The average age was 53.5 (range 17 – 90 years). Height average was 168.5 (range 146–191 cm); weight average was 80.5 (range 32–129 kg), and the average body surface area (BSA) was 1.82 (range 1.16-2.48 m²). The study subjects include patients with renal diseases, as well as potential donor for renal transplantation. Patients with both normal and with increased plasma creatinine concentrations were included in order to cover a wide range of GFRs. They were referred to the Nuclear Medicine Department of the National Cancer Institute, Gezira University, for renal function evaluation with ^{99m}Tc-DTPA during the period from 2008 to 2014. GFR was determined with renal dynamic imaging and the two-plasma method. Patients were categorized into five stages according to their GFR values: stage 1 [GFR > 90ml/min]; stage 2 [GFR= 60 to 89ml/min]; stage 3 [GFR= 30 to 59 mL/min]; stage 4 [GFR=15 to 29 ml/min] and stage 5 [GFR < 15 mL/min].

3.2 Gamma Camera Based Method

Inoue and Gates methods were the gamma camera based method used in this thesis to estimate the GFR.

3.2.1 Materials

The radioactivity of the pre-injection syringe and the post-injection syringe was measured with the Dose Calibrator (PIW Curiementor 3, Germany). For imaging, a SPECT dual head Mediso Camera (NuclineTM Spirit), equipped with

a low-energy high resolution collimator, interfaced to windows XP 2002 computer was used. Images were processed using windows XP 2002 computer with Interview 71 Build software version 1.04.038.

3.2.2 ^{99m}Tc -DTPA preparation

The DTPA used in this study is a commercial freeze-dried kit (Monrol Nuclear Product INC, Turkey). Each vial contains: Diethylenetriaminepentaacetic acid, Tin (II) chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$), Calcium Chloride Dehydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), Sodium Hydroxide, Hydrochloric acid. ^{99m}Tc is obtained from $^{99}\text{Mo}/^{99m}\text{Tc}$ 15 GBq generator supplied by Monrol Company, upon mixing with the chelator (2-10 ml with a maximum of 18500 MBq); ^{99m}Tc - DTPA is ready for intravenous injection. Using Instant Thin-Layer Chromatography, the radiochemical purity of the labeled was constantly above 95% as recommended, and the same DTPA kit was used for all subjects in order to prevent the effects of different protein binding. The patients doses was calculated on the basis of their body weight using Clark's Rule (Eq.1), and so a dose in range of 111-185 MBq was given for each patient.

$$(\text{Patient weight in kg} \times \text{standard adult dose}) \div 70 \text{ kg} = \text{patient} \quad \text{Eq.1}$$

3.2.3 Renography Imaging Protocol

To provide adequate hydration, subjects were asked to drink 200-500 ml water, 20 minutes before the study, and during waiting, an antecubital vein was cannulated for each subject. Using the SPECT machine, with the low-energy high resolution collimator installed, the patients were then placed on the imaging table in a supine position with their back facing the detector. The ^{99m}Tc - DTPA doses were measured in the dose calibrator, having activity in range of 111–185 MBq (3-5 mCi) and in a volume of 0.5 ml, time of preparation was noted. A posterior renal dynamic imaging was performed immediately after a bolus intravenous injection of the radiopharmaceutical followed by 5 ml normal saline flush through the cannula, and residual radioactivity of the syringe was

measured again, so the injected net dosage of the drug was then calculated. 30 frames for 2 sec/frame were acquired, followed by collection of 180 frames at a rate of 10 sec/frame in a 128x128x16 matrix, with a total of 31 min/scan, and then the image was stored, ready for further image manipulation and processing.

The acquisition conditions were as follow: Collimator; LEHR, Energy Peak; 140 KeV, Window Width; 20%, Flood Correction; Extrinsic, Matrix; 128x128x16, Detector Mask; center 41.6 cm, Orientation; 0 degree, Acquisition Mode; frame mode, Multi Energy; None, Data Collection; Segment (1): 30 frames, time/frame 2 sec. Segment (2): 180 frames, time/ frame 10 sec. Display Mode; detector 2, signal 2.

The following data were recorded: sex, age, height, weight, pre-injection syringe radioactivity (full syringe), post-injection syringe radioactivity (empty syringe), time of measurements, time of injection, time of acquisition, radioactive counts and the collection time of the two blood samples after renal dynamic imaging.

3.2.4 Image Processing

Using the Interview 71 Build software version 1.04.038, and from the planar list of processing, kidney – dynamic – perfusion + renography was selected. In the image-role assignments; perfusion and function were assigned manually, the perfusion image representing the first 30 frames of the dynamic set, while the function image represent the other 180 frames. In parameters setting; 99mTc-DTPA was selected, with LEHR collimator, camera sensitivity was calibrated and found to be 72.11 cps/MBq, motion correction was enabled. Then proceeded to processing configuration; from the list of option, perfusion and split renal function was ticked, 2-6 min summed images were selected for ROI placement. In clearance estimation method (GFR); either Gates or Inoue was selected, for kidney depth estimation; Tonnesen equation was used with Gates method, and Taylor + Raynaud equations were used with Inoue method. Patient's height, weight and age were given, which were used in depth estimation and body

surface area (BSA) calculation. Measured radioactivity; full syringe and empty syringe radioactivity were given in MBq, as well as their time of preparation and the injection time, which were used for physical decay correction and calculation of the net injected dose.

3.2.5 Creation of Regions of Interest and Background

Regions of interest (ROIs) were drawn around the left and right kidney in two ways, firstly with the Manual drawing (free hand drawing, a light pen was used to outline each kidney), secondly with the Semiautomatic drawing (iso-count processing feature), and counts in each ROI were determined 2-3 min after tracer arrival in the kidney. With this two types of kidney ROIs, three types of background for each kidney was used, which are; (1) the subrenal background, (2) the perirenal background and (3) the automatic background (figure 3.1). These have given us six GFR values in ml/min/1.73 sqm; and those were used to fulfill the objectives of the study. The perirenal background ROI was set around each kidney, excluding the area facing the renal hilum to allow the use of the same ROIs in producing renogram. If the area facing the renal hilum is included in the background ROI, a large amount of radiotracer excreted in the renal pelvis may appear in the ROI at the late phase, resulting in overestimation of background activity. The counts per pixel in the background ROI were multiplied by the number of pixels in the corresponding kidney ROI and subtracted from counts in the kidney ROI to obtain the background-subtracted renal count (a process known as normalization). The GFR (global GFR) was then estimated by two gamma camera uptake methods using the formulae proposed by Gates and the one of Inoue.

3.2.6 Calculation of GFR with the Gamma Camera Uptake Method

The Gamma Camera Based GFR methods were programmed in our computer with in the Inter View XP (Build71) that was provided by a commercial company (Mediso Medical Imaging System, Budapest Hungary).

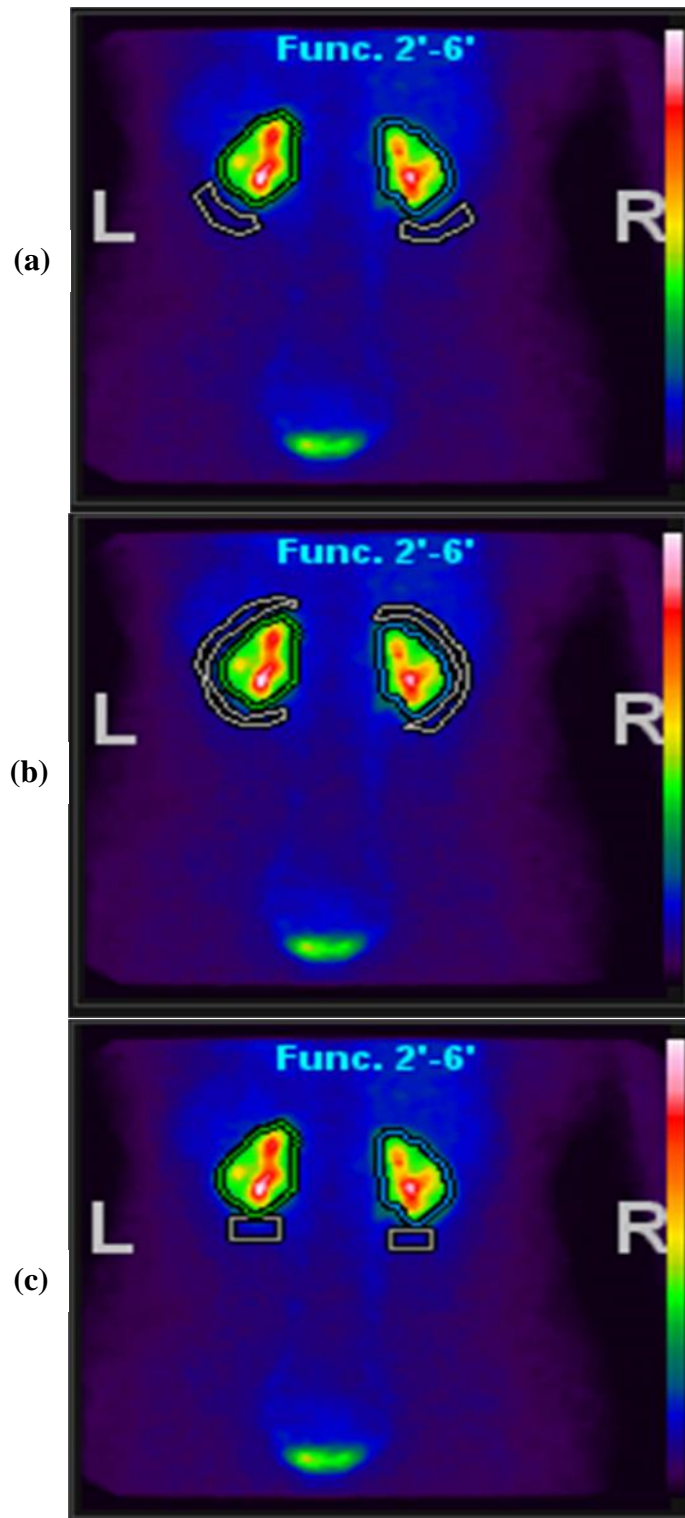


Figure 3.1: Demonstrates regions of interest placed for kidneys and background. (a) Subrenal background. (b) Perirenal background. (c) Automatic background.

3.2.6.1 Calculation of GFR with Gates Method

The formula of Tonnesen was used for determining renal depth in centimeters as follow:

$$\begin{aligned} \text{Right Kidney depth} &= 13.3 (\text{weight in cm/height in kg}) + 0.7 \\ \text{Left Kidney depth} &= 13.2 (\text{weight in cm/height in kg}) + 0.7 \end{aligned} \quad \text{Eq.2}$$

The author's formula for calculation of GFR was derived from the results of the regression analysis: $\text{GFR ml/min} = (\% \text{ total renal DTPA uptake}) (\text{regression coefficient}) + (\text{intercept})$. Substitution into that formula yielded:

$$\text{GFR} = \left(\frac{(\text{RT count} - \text{BG}) / e^{-153(13.3(W/H) + 0.7)} + (\text{LT count} - \text{BG}) / e^{-153(13.2(W/H) + 0.7)}}{\text{Preinjection counts} - \text{Postinjection counts}} \right) \times 100 \times 9.75621 - 6.19843 \quad \text{Eq.3}$$

With W ; as weight, H ; as height, and BG ; as background.

3.2.6.2 Calculation of GFR with Inoue Method

As was implemented by the author; renal depth was estimated in children using the equation described by Raynaud et al, and in adults using the equation presented by Taylor et al:

0-9 years	$d = 2.366 + 0.083 \cdot BW - 0.00281 \cdot BH$	Eq.4
10-15 years	$d = 3.686 + 0.028 \cdot BW - 0.00248 \cdot BH$	
16 years	(right) $d = 15.13 \cdot BW/BH + 0.022 \cdot A + 0.077$	
	(left) $d = 16.17 \cdot BW/BH + 0.027 \cdot A - 0.94$	

Where's d ; is renal depth in cm, A ; is age in years, BW ; is body weight in Kg and BH ; is body height in cm.

Based on the calculated renal depth, the background-subtracted renal count (C_b) was corrected for soft- tissue attenuation assuming the attenuation coefficient to be 0.12/cm:

$$C_a = C_b / \exp(-0.12 \cdot d) \quad \text{Eq.5}$$

Where C_a ; is renal counts after attenuation correction.

The percentage renal uptake at 2-3 min (GFR) was computed as follows:

$$\% \text{ uptake} = (rCa + lCa)/Ci \quad \text{Eq.6}$$

Where *rCa* and *lCa* are attenuation-corrected renal counts for the right and left kidneys, respectively, and *Ci* is injected count.

The percentage renal uptake (% uptake) was then corrected (normalized) for body surface area to obtain GFR in ml/min/1.73:

$$\text{GFR (ml/min/1.73)} = \% \text{ uptake} \times 1.73 / \text{BSA} \quad \text{Eq.7}$$

The body surface area (BSA) was calculated using the Du Bois formula:

$$\text{BSA} = 0.007184 \times \text{weight}^{0.425} \times \text{height}^{0.725} \quad \text{Eq.8}$$

3.3 The Two Plasma Samples Method; Gold Standard

The GFR was also measured with a dual plasma samples using the Slope-Intercept method, which was used as the reference for GFR values.

3.3.1 Material

The dual plasma sample radioactivity (cpm) together with a standard dilution of the dose was measured with Na (TI) single-function well counter (Mini-Assay type 6.2, Mini instruments LTD Company, England). A Centrifuge model EC-800 (Jasan Group International Div, Hong Kong) was used to separate the plasma from the blood component.

3.3.2 Procedure

Two blood samples were taken from the patients, whom who had undergone ^{99m}Tc-DTPA renal dynamic imaging. The radioactive dose had already been given, which was measured in the dose calibrator and the time of preparation was noted for decay correction. The residual radioactivity of the syringe has previously been measured, and the injected net dosage of drug was then calculated. The same dose of ^{99m}Tc-DTPA was prepared and measured in the dose calibrator; this standard activity was then diluted into volumetric flask to 1000 ml. Following ^{99m}Tc-DTPA injection, venous blood samples (3ml) were

collected from the contralateral arm at 60, and 180 post injections. The two blood samples were transferred to dry tube and then centrifuged, and one ml of each plasma sample was counted in triple in a well gamma counter for one minute (cpm). Also one ml of the standard solution in duplicate was counted in the same well counter for one minute at the same time as the plasma.

3.3.3 Calculation of GFR with the Two Plasma Samples Method

The clearance method using blood samples taken at 2 and 3 hours post-injection was used as a reference to measure the actual GFR. Using the two plasma samples counts obtained from the well counter in cpm, along with their time of collection, so as the GFR was calculated by the following equation;

$$Cl_1 = \frac{D \ln (P_1/P_2)}{T_2 - T_1} \exp \frac{(T_1 \ln P_2) - (T_2 \ln P_1)}{T_2 - T_1} \quad \text{Eq.9}$$

Where, D is total counts of injected dose (cpm), P_1 is plasma activity (cpm/ml) at the time of T_1 , P_2 is plasma activity (cpm/ml) at the time of T_2 .

Cl_1 (ml/min) is a preliminary estimate of GFR, which is corrected for body surface area as:

$$Cl_2 (\text{ml/min}/1.73) = Cl_1 \times 1.73 / \text{BSA} \quad \text{Eq.10}$$

The body surface area (BSA) was calculated using the Du Bois formula as in equation 8 (Eq.8).

3.4 Hypothesis

In this study, we have hypothesized that:

- The Gamma Camera GFR method will provide better result in donors, stage 1, 2 and 3 compare to stage 4 & 5 kidney disease.

- The semiautomatic kidneys region will give accurate results in normal kidney function and stage 1 kidney disease, while the manual drawing will give accurate result in stage 2 & 3 kidney disease.
- The automatic background is the most satisfactory for quantification in renal scintigraphy.

3.5 Statistical Analysis

The statistical analyses were performed using commercially available Statistical Package for the Social Sciences (IBM SPSS ver.21) and Office Excel 2010 (Microsoft, Inc.). Using the normal Q-Q plot with Blom's estimation method, the normality of distribution was statistical verified. A scatter plot analysis was made and the correlation coefficient was obtained, determining the degree of scatter along the trend line. The sex independence of the results was also tested with the independent sample T-test, assuming both equal variants and non-equal variants. As well as that, the difference in mean between the GFR estimation methods was compared using the student paired sample t-test and P values of less than 0.05 were considered significant.

Chapter Four

Result

An assessment of the Inoue Gamma Camera 99mTc-DTPA GFR method were performed in this study against the Two Plasma Sample 99mTc-DTPA GFR reference method (TPS) and the result of which are presented with tables and figures. This study included 75 cases, 38 males (50.7%) and 37 females (49.3%) with a mean age of 44.3 ± 17.5 and range of 18-85 years; the anthropomorphic data are shown in table 1. This study contained patients with different renal diseases (85.3%) as well as potential kidney donors with normal renal function (14.7%). The patients were categorized with respect to their GFR values and according to the National Kidney Foundation into five renal stages; Stage 1 with GFR ≥ 90 ml/min (20%), Stage 2 with GFR = 60 to 89 ml/min (20%), Stage 3 with GFR = 30 to 59 mL/min (20%), Stage 4 with GFR =15 to 29 ml/min (10.6%), Stage 5 with GFR ≤ 15 mL/min (14.7%). Table 4.1 shows the classification of the patients GFR values with the renal diseases stages.

Table 4.1: The anthropomorphic data and the percent of cases in each stage.

Age	Mean \pm SD	Years 44.3 \pm 17.5
	Range	18 - 85
Sex	Male	Number (%) 50.7 (%)
	Female	49.3 (%)
Stage		Number (%)
	0 Donor [with normal GFR values]	14.7 (%)
	1 [GFR ≥ 90 ml/min]	20 (%)
	2 [GFR = 60 to 89 ml/min]	20 (%)
	3 [GFR = 30 to 59 mL/min]	20 (%)
	4 [GFR =15 to 29 ml/min]	10.6 (%)
	5 [GFR ≤ 15 mL/min]	14.7 (%)

Preliminary; two models of region of interest (ROI) drawing were tested in this thesis; the Manual and the Semiautomatic drawing. With each of those, three types of background subtraction were applied; the Automatic, the Subrenal, and the Perirenal background. Along with the Reference GFR values (62.8 ± 34.8 SD), the combination of the two ROI models with the three backgrounds types gave six GFR values, as follow; Manual ROI + Automatic background (62.5 ± 35.2 SD), Manual ROI + Subrenal background (67.0 ± 38.3 SD), Manual ROI + Perirenal background (54.3 ± 34.8 SD), Semiautomatic ROI + Automatic background (60.7 ± 37.1 SD), Semiautomatic ROI + Subrenal background (67.5 ± 41.9 SD), Semiautomatic ROI + Perirenal background (55.6 ± 37.5 SD), table 4.2 shows the mean, standard deviation and the standard error in mean.

Table 4.2: Mean and Standard deviation.

GFR Estimation Method	Mean \pm SD ml/min/1.73 m²
Reference	62.8 ± 34.8
Manual ROI with Auto background	62.5 ± 35.2
Manual ROI with Subrenal background	67.0 ± 38.3
Manual ROI with Perirenal background	54.3 ± 34.8
Semiautomatic ROI with Auto background	60.7 ± 37.1
Semiautomatic ROI with Subrenal background	67.5 ± 41.9
Semiautomatic ROI with Perirenal background	55.6 ± 37.5

The normality of distribution was statistical verified using normal Q-Q plot with Blom's estimation method for all GFR methods and had found to be acceptable for parametric analysis, Figure 4.1 verify the normal distribution of GFR values using normal Q-Q plot with Blom's estimation method. The sex independence of the results was tested with the independent sample T-test, and the difference in results between male and female were negligible. Table 4.3 demonstrates the results of the independent sample T-test when assuming that two sexes are equal variants and vice-versa.

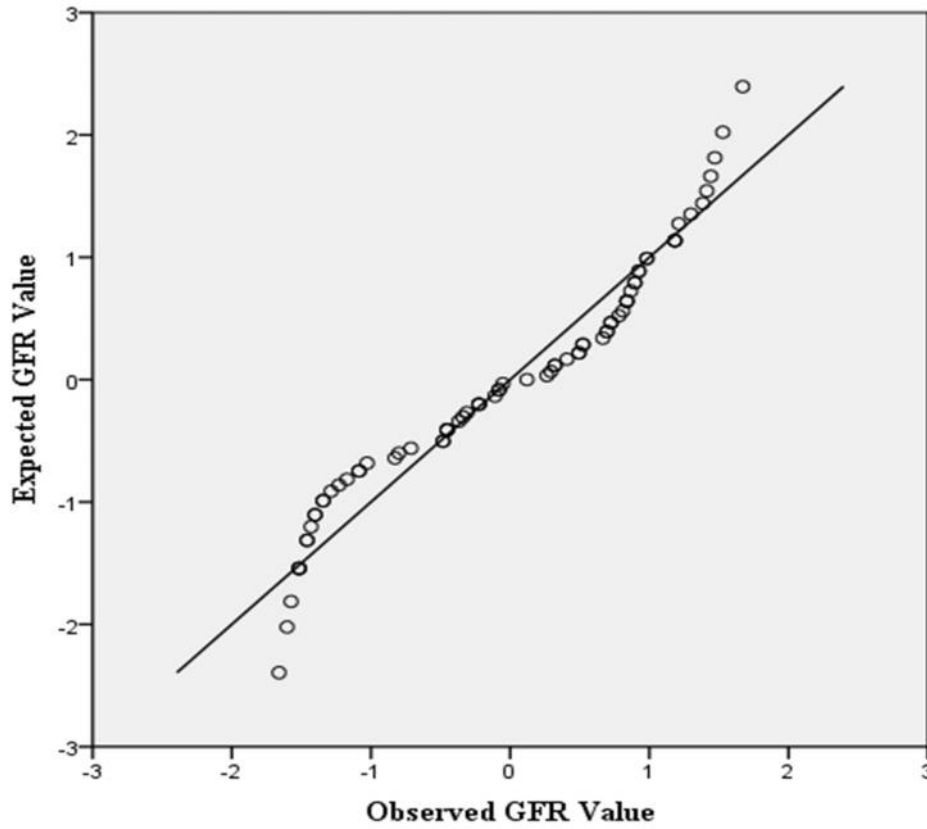


Figure 4.1: The normal distribution of the Q-Q plot with Blom's estimation

Table 4.3: Result of Sex independent sample T-test.

Method	Sig. (2-tailed)	
	Equal variances assumed	Equal variances not assumed
Reference	0.301	0.302
Manual ROI with Auto background	0.271	0.272
Manual ROI with Subrenal background	0.265	0.266
Manual ROI with Perirenal background	0.325	0.325
Semiautomatic ROI with Auto background	0.318	0.318
Semiautomatic ROI with Subrenal background	0.322	0.323
Semiautomatic ROI with Perirenal background	0.247	0.247

Having to establish a relation between the Two Plasma Sample GFR Reference method and the Inoue Gamma Camera GFR method, a scatter plot analysis was done. For each of the two model of ROI drawing with the three types of background a separated analysis was performed. The extent to which each camera GFR model agreed with the Reference is represented graphically as a scatter plots in figure 4.2, showing a trend line and analyses of correlation between the reference GFR and with each of the GFR models being investigated. The Correlation between the GFR estimated by the Camera using the different models and the Reference was high in overall ($r = 0.93$). The correlation coefficients in the Manual ROI + Automatic background and the Semiautomatic ROI + Automatic background were the highest ($r = 0.99$; $r = 0.98$: respectively). Both models showed the highest linear relation ($y = 1.0075x - 0.7605$; $y = 1.0607x - 5.9338$; respectively) and the degree of scatter around the trend line was negligible. Consequently the Manual ROI + Subrenal background and the Semiautomatic ROI + Subrenal background, a good correlation exists ($r = 0.95$). They demonstrated good linear relation ($y = 1.0769x - 0.6318$; $y = 1.1778x - 6.5202$: respectively), and the degree of scatter around the trend line was acceptable. While the Manual ROI + Perirenal background and the Semiautomatic ROI + Perirenal background had the lowest correlation coefficient compared to the others ($r = 0.93$). The two of them had a considerable amount of scatter but the close linear relation was still evident ($y = 0.9682x - 6.5215$; $y = 1.0437x - 9.9827$: respectively). In overall the two model of ROI drawing (Manual and Semiautomatic) with the three types of background (Automatic, Subrenal and Perirenal background), correlated very well with the reference, and the linear relation between the three of them and the Reference were clear. In general the amount of scatter for the Automatic background was too slight and was considered non-existent, on the other hand the scatter on the Subrenal background increased considerable, while the Perirenal background demonstrate the highest amount of scatter.

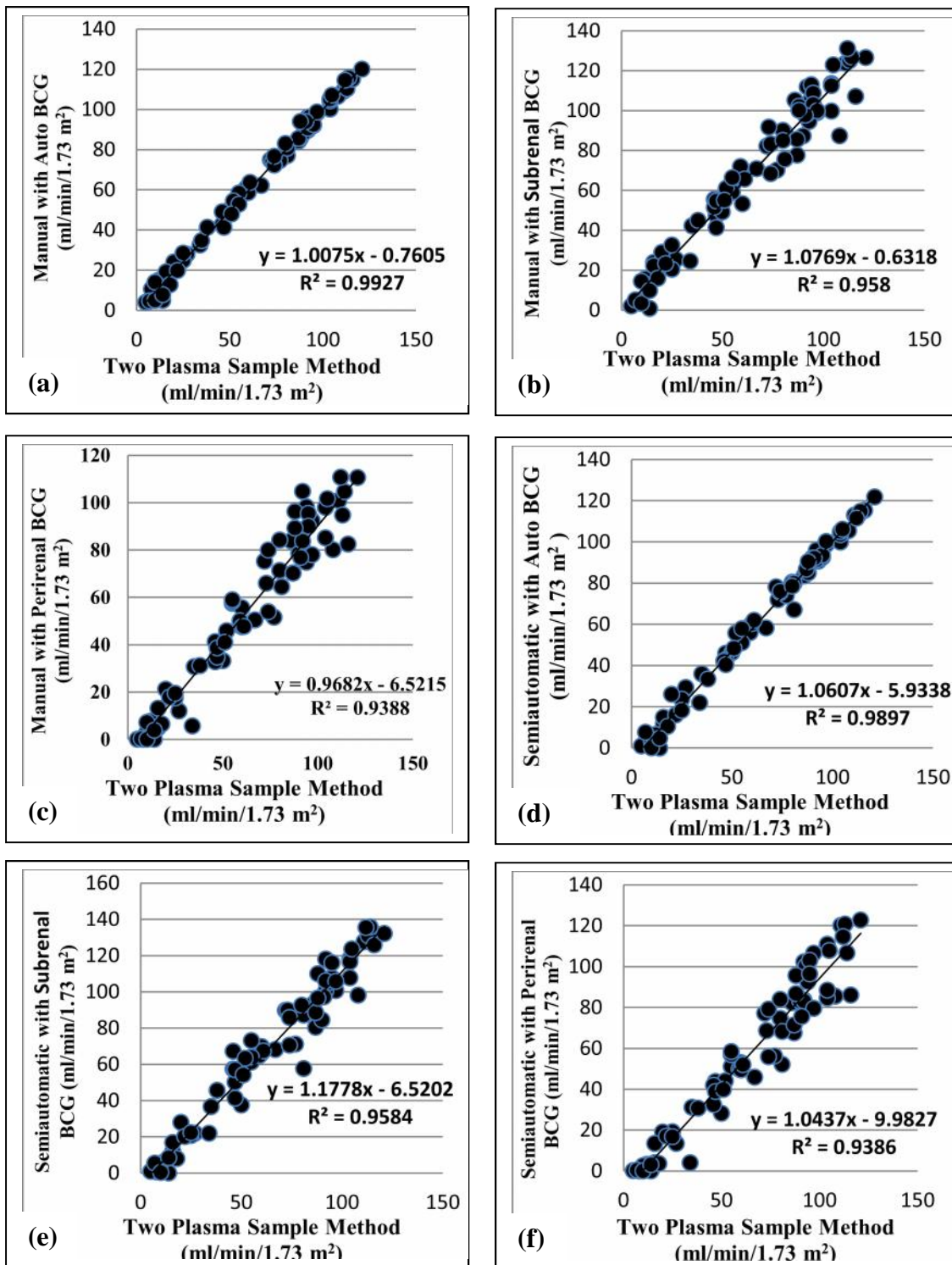


Figure 4.2: A scatter plot of the Two Plasma Reference method versus the Inoue Camera method with different models shows a linear relation and a tight correlation between them.

In addition, the difference in mean between the GFR estimation methods was compared using the student paired sample t-test. All cut-off values were determined before the statistical procedures. Demarcation criteria were set at < 0.05 and P values for all analysis were two-tailed. When conducting the t-test, the means of the Reference and the Manual ROI + Automatic background were not statistically significantly different ($t = 0.8$; $P = 0.406$; 95% confidence interval -.4 to 0.9). On the other hand the means of the Semiautomatic ROI + Automatic background, the Manual ROI + Subrenal background, the Semiautomatic ROI + Subrenal background, the Manual ROI + Perirenal background and the Semiautomatic ROI + Perirenal background were highly statistically significantly different from the Reference ($P < 0.000$). Table 4.4 shows the result of the t-test with two-tailed significance and the 95% Confidence Interval of the Difference.

Table 4.4: Result of the Paired Sample t-test.

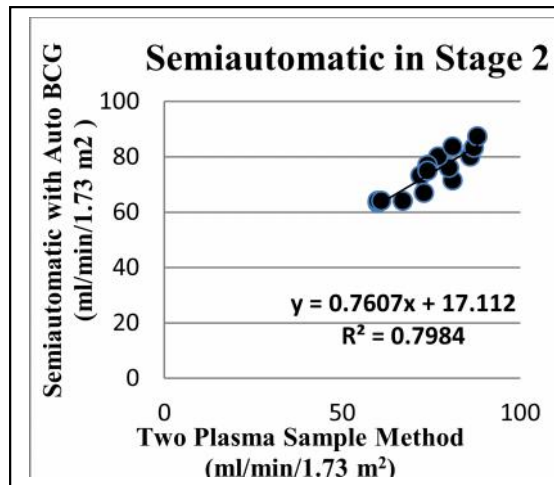
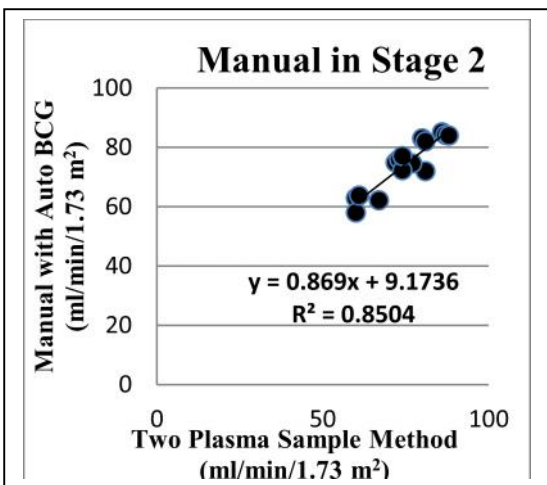
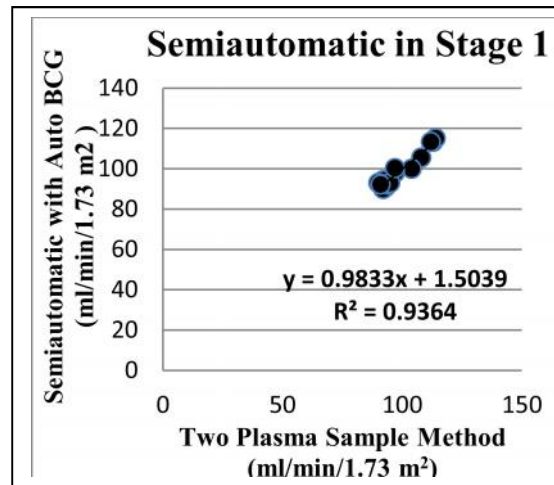
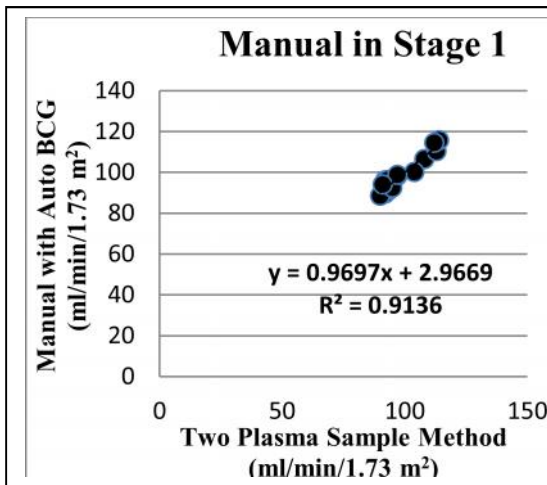
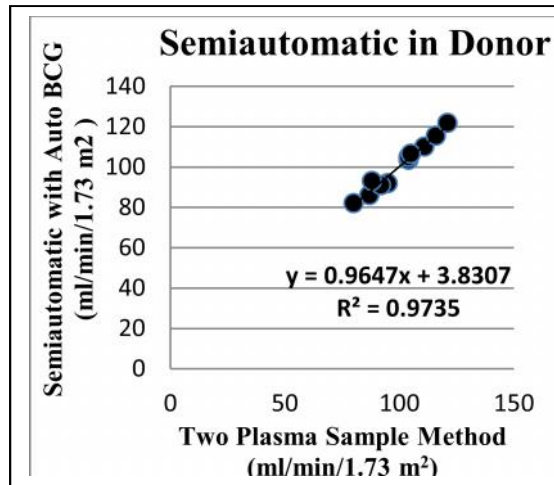
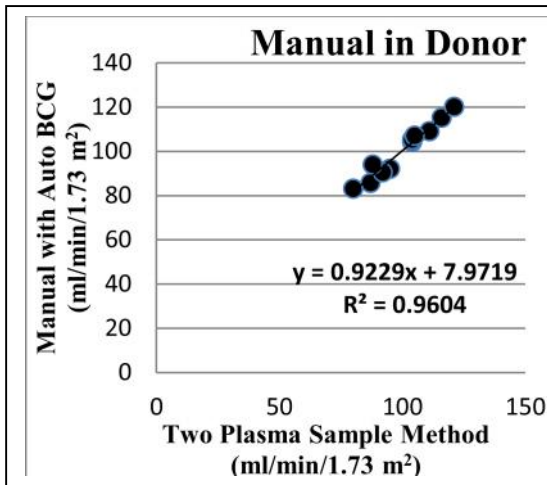
GFR Estimation Method vs the Reference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
			Lower	Upper
Manual with Automatic background	0.8	0.406	-.4	0.9
Manual with Subrenal background	-4.4	0.000	-6.1	-2.3
Manual with Perirenal background	8.5	0.000	6.5	10.5
Semiautomatic with Automatic background	4.3	0.000	1.1	3.1
Semiautomatic Subrenal background	-3.8	0.000	-7.1	-2.2
Semiautomatic with Perirenal background	6.6	0.000	5.1	9.4

Moreover, the effect of the renal disease stage on the performance of the Camera GFR method was assessed for the models that gave the best agreement, which are the Manual and the Semiautomatic ROI with the Automatic background. The tight correlation provided by those two methods was not maintained in the different stages of renal diseases with the wide range of GFR values, (table 4.5 shows the mean \pm SD). In potential kidney donors (referred to as stage 0) and in stage1; the Semiautomatic drawing gave a strong correlation ($r = 0.97$ and $r = 0.93$ respectively), and the Manual drawing also showed a strong correlation but lower than the Semiautomatic ($r = 0.96$ and $r = 0.91$

respectively). For stage 2 and 3; the Manual drawing exhibit a good correlation ($r = 0.85$ and $r = 0.83$ respectively), while with the Semiautomatic a lower correlation were provided ($r = 0.79$ and $r = 0.76$ respectively). On the other side in stage 4 and 5; with the Manual drawing a poor correlation was observed in stage 4 and in stage 5 there was almost no correlation ($r = 0.64$ and $r = 0.07$ respectively), for the Semiautomatic a worse correlation was obtained in stage 4 and there was no correlation in stage 5 ($r = 0.53$ and $r = 0.01$ respectively). A scatter plot of the Manual and Semiautomatic against the Reference with in the different renal stages are presented graphically in figure 4.3.

Table 4.5: Results of the Mean \pm SD for each GFR stage.

Stage	GFR Method	Mean \pm SD
0	Reference	100.3 \pm 12.9
	Manual with Automatic background	100.5 12.2
	Semiautomatic with Automatic background	100.6 \pm 12.7
1	Reference	99.1 \pm 8.7
	Manual with Automatic background	99.0 \pm 8.8
	Semiautomatic with Automatic background	98.9 \pm 8.8
2	Reference	74.7 \pm 9.5
	Manual with Automatic background	74.1 \pm 8.9
	Semiautomatic with Automatic background	74.0 \pm 8.1
3	Reference	47.8 \pm 7.4
	Manual with Automatic background	48.2 \pm 8.4
	Semiautomatic with Automatic background	45.2 \pm 12.5
4	Reference	21.1 \pm 4.3
	Manual with Automatic background	21.9 \pm 5.5
	Semiautomatic with Automatic background	18.8 \pm 6.9
5	Reference	10.5 \pm 2.9
	Manual with Automatic background	8.3 \pm 4.1
	Semiautomatic with Automatic background	3.1 \pm 2.4



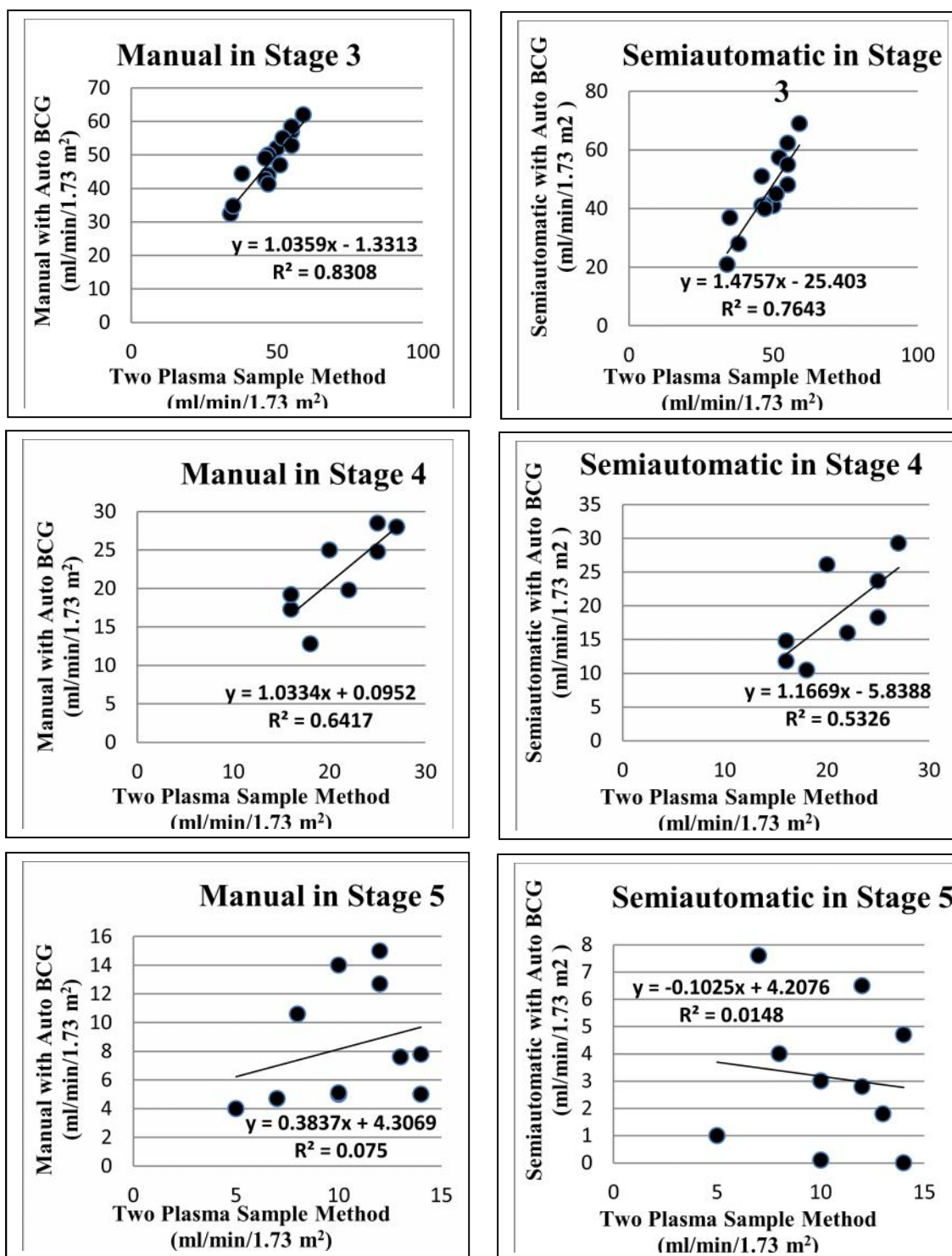


Figure 4.3: A graphical representation of the relation between the Manual and the Semiautomatic with the three backgrounds and the GFR values in the different renal stages. It shows with the decrease of the GFR; a decrease in correlation coefficients, a less degree of linearity and an increase in scatter.

For Gates Gamma Camera GFR method, one of the methods has been widely used; which is known to underestimate GFR at all levels. Verification of the literature for Gates method was implemented. The GFR for 41 cases were estimated with the Two Plasma Sample Reference method and with the Gates method using a manual drawing and the infero lateral background. Figure 4.4, display a scatter plot of the Gates method versus the reference revealing a moderate correlation ($y = 0.6867x - 7.6108$ and $r = 0.88$) and striking amount of scatter. The mean of the Gate's method (31.4 ± 27.7 SD) was much lower than the Reference method (56.9 ± 37.9 SD). And apparently the mean of the Reference GFR and the mean of the GFR estimated by Gates method was statistically significantly different ($P < 0.000000$). There for it has been excluded from the scope of this study.

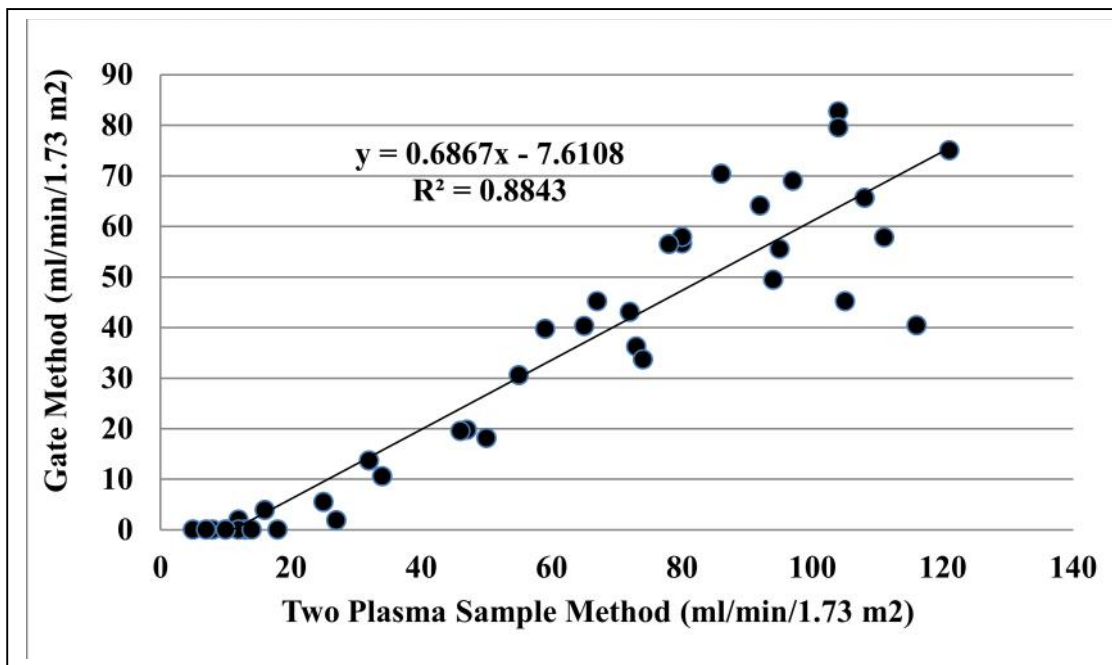


Figure 4.4: A scatter plot of Two Plasma Sample Reference method against the Gates method. It shows a weak correlation and a remarkable scatters.

Chapter Five

Discussion

The goal of nuclear medicine in Nephro-urology is to yield functional and structural information that is reliable and acquired non-invasively, and which provides the clinician with both diagnosis and prognosis (Woolfson & Neild 1997). Nuclear medicine renography is frequently used in the investigation of renal function, renovascular disease, outflow obstruction, reflux nephropathy, renal transplantation and acute renal failure (Woolfson & Neild 1997). However, the measurement of Glomerular Filtration Rate (GFR) remains the standard of excellence for evaluation in renal performance (Prigent et al. 1999; Holness et al. 2013). There are numerous procedural methods available to obtain GFR and, if properly performed, each method will provide a comparable result. Despite superior accuracy, precision and reproducibility, these diagnostic measurements are underutilized both in research and in clinical medicine (Macunluoglu et al. 2011).

The need for simplified methods to measure renal function has been stressed by the Committee on Renal Clearance, which has led to the introduction of the ^{99m}Tc -DTPA gamma camera GFR with external counting procedures, that offer both simplicity and the estimation of separate kidney function as part of an imaging study (Galli et al. 1997). It has been developed in order to determine GFR both as a total value as well as individually for each kidney (Separated kidney GFR). The in vivo GFR determination with nuclear medical imaging is based on the assumption that there is a certain period of time when a large concentration of the imaging agent accumulates in the kidney and is not discharged, and the renal uptake percentage is linear with GFR, as it was described by Gates 1982 (Gates 1982). This represents the ideal situation, but the true situation varies accordingly. Russell et al 1985, conclude that gamma camera methods can be reasonably for GFR determination in clinical use. And it allows the separation of the GFR into components from the right and left kidney, moreover, revealing morphologic or urodynamic abnormalities (Russell et al. 1985a). Moonen et al 1994 performed a survey on the gamma camera renography with ^{99m}Tc -DTPA and the uptake index calculation method, and he concluded that, it offers a reliable estimation of GFR with precision of 1.3% which corresponds to 1.2 ml min^{-1} and accuracy of 2.00 ml min^{-1} (Moonen et al.

1994). In one part of this thesis we performed an assessment of Inoue et al 1998 gamma camera ^{99m}Tc -DTPA GFR method. As described by Inoue, we used a manual region of interest drawing (ROI) for the kidneys and a perirenal background subtraction. We applied their equation to obtain the percent renal uptake (GFR): $\% \text{ uptake} = (r\text{Ca} + l\text{Ca})/C_i$, where $r\text{Ca}$ and $l\text{Ca}$ are the attenuation corrected renal counts for the right and left kidneys, respectively, and C_i is the injected count (Inoue et al. 1998). Inoue calculated the percent renal uptake at 2-2.5 min; we calculated it at 2-3 min, because a .5 min is inapplicable in our software. Using the two plasma ^{99m}Tc -DTPA clearance as the reference method, he found a close correlation between the percent renal uptake and the reference ($r = 0.939$; $y = 15.958x - 2.94$). In our result (Figure 2), the reference and the percent renal uptake were also closely correlated ($r=0.938$; $y = 0.9682x - 6.5215$), this relation seemed to enable estimation of GFR with acceptable accuracy. As well as that, the renography provided notable information such as quantitative individual renal function and pathophysiological changes of the kidney in renovascular hypertension, hydronephrosis, and renal transplant. It worth mentioning, that in our samples all the patients were adults (age ≥ 18 years) due to the lack of pediatric patients referral, which could be a drawback, indicating that Inoue's method need to be asserting in pediatric patients, thus it should be assessed in future.

There are diverse methodologies for estimating GFR with the gamma camera which has been described throughout the literature. Piepsz et al 1977 reported a method to estimate GFR based on the cardiac curve and renal curve after the administration of ^{99m}Tc -DTPA, and used it to evaluate renal function in both children and adults. They assessed plasma concentration using the cardiac curve and a blood sample taken at 20 min post injection. Their technique seems to be more theoretical than Inoue's; however, the cardiac curve is affected by interstitial activity and does not reflect plasma activity accurately. In addition, calibration of the cardiac curve to quantitatively assess the plasma curve is troublesome, requiring blood sampling and conversion of plasma activity measured with a well counter to counts on a gamma camera (Piepsz, Dobbeleir & Erbsmann 1977). Inoue's method appears to offer more convenience and to be better suited for routine use. Shore et al

1984, also described a method to predict GFR in children using ^{99m}Tc -DTPA renography. Their technique takes into consideration the effect of body size on fractional renal accumulation and was found to provide accurate estimates in children. They, however, commented that their method was unsuccessful in calculating GFR in adults (Shore et al. 1984). Body weight was used as an indicator of body size in their technique, whereas body surface area (BSA) was used in this study, this fact may explain, at least in part, the differences in conclusion between their study and this one. In addition to those, Rehling et al 1985, have also described a promising method for the rapid determination of the single-kidney glomerular filtration rate (SKGFR) by ^{99m}Tc -DTPA gamma camera renography that required neither the determination of the injected dose nor the collection of urine or blood samples (Rehling et al. 1985). Nonetheless his method is not widely applied and not commercial available with nuclear medicine software packages.

The GFR values are used to make critical clinical management decisions and, so that, their reliability needs to be quality assured. There are potential general pitfalls in the camera technique used for GFR estimation including; error in the injection dose assaying or recording, incorrect recording of the injection time, error in weight, height or age recording or measurement (Prigent et al. 1999; Holness et al. 2013). These pitfalls can lead to a serious error in the measured GFR values; up on which a faulty clinical decision could be made, consequently, their accuracy must be assured (Prigent et al. 1999; Bhatt et al. 2011). The consistently high correlation coefficients in our dataset indicate that our technique in this study was robust. In addition, the labeling efficiency of the ^{99m}Tc -DTPA has to be checked. A low labeling efficiency lead to increase in amount of free $^{99m}\text{TcO}_4^-$ in the injected dose, which will in turn bind to the red blood cells (RBC) forming ^{99m}Tc -RBC, raising the total activity in the liver, spleen, and great vessels, whom are the main background areas in the renal images. This increase in the background will reduce the target-to-non-target-ratio, leading to a falls lower result in the quantification of the renal function. In the dataset of this research, a 96 % labeling efficiency was always maintained.

The technicalities of GFR renography have always been a source of debate in nuclear medicine, which is reflected by the fact that a consensus could simply not be reached on a number of issues. GFR estimation is a technically challenging procedure, regardless of the calculation process used, there are several technical problems in estimating GFR from renal scintigraphy, these include; the system dead time, type of DTPA preparation, renal depth estimation, region of interest drawing and background subtraction (Russell & Dubovsky 1989; Prigent et al. 1999; Bland & Altman 1999). The system dead time effect can lead to huge losses in count per time; Inoue in his study used the gamma camera to image the full and empty syringes, which could increase the possibility of dead time and may require the use of dead time correction factor (Inoue et al. 1998). To avoid this we performed our activity measurement using the dose calibrator, so as to reduce the likelihood of the dead time effect. Nevertheless, the measurement of the injection dose with a gamma camera is often performed without the imaging table between the syringe and gamma camera. This causes overestimation of the injected dose because the counts in the patient suffer from the table attenuation. This overestimation may be avoided by introducing the attenuation factor of the table as mentioned by Taylor et al 1995; however, this factor should be determined for each table (Taylor et al. 1995). All these complication could simply be avoided by measuring the injection doses with the dose calibrator instead of the gamma camera as we have proved.

To address the influence of the ^{99m}Tc -DTPA preparations on the GFR measurement, a few reports have been published on some of the commercially available DTPA preparations. To determine if their preparation is suitable for GFR measurement, Hilson et al 1976, have compared the plasma clearance of ^{99m}Tc -DTPA (Na₅-DTPA was the used chemical formula) with that of ^{51}Cr -EDTA (Hilson, Mistry & Maisey 1976). In order to obtain identical results they had to use a correction factor of 0.964 for DTPA (the clearance of ^{99m}Tc -DTPA have been about 11% lower than their reference). Likewise, Rossing et al 1978, compared the clearance of ^{99m}Tc -DTPA (CaNa₃-DTPA was the used chemical formula) with that of ^{51}Cr -EDTA, and found that their clearances was identical (Rossing, Bojsen & Frederiksen 1978). In a similar manner, to find a commercially

available DTPA preparation that could be used for the GFR, Carlsen et al 1980, compare the plasma clearances of four different $^{99m}\text{Tc}-(\text{Sn})\text{DTPA}$ preparations with that of $^{51}\text{Cr}\text{-EDTA}$. One preparation yielded results identical to those obtained with $^{51}\text{Cr}\text{-EDTA}$, whereas the others underestimated the GFR to a varying degree (Carlsen et al. 1980). Our preparation which has been used in this study, is an instant kit of Diethylenetriamine pentaacetic acid (DTPA; 7.8 mg) with Tin(II)chloride ($\text{SnCl}_2\cdot\text{H}_2\text{O}$; 0.4 mg), provided commercially by Monrol Company for preparation of $^{99m}\text{Tc}\text{-DTPA}$. To our knowledge there is no published data regarding the utilization of this preparation for GFR measurement, however our result indicates that it is suitable for such purpose. Disregarding which preparation is used, bearing in mind that there are different $^{99m}\text{Tc}\text{-DTPA}$ preparations could be used for GFR estimation; even so, their accuracy varies considerable. It should be noted that whenever a new DTPA preparation is introduced to any Nuclear Medicine department, their reliability for GFR measurement must be verified, otherwise it's highly advised to stick on one preparation for the sake of reproducibility.

An accurate estimate of renal depth is required to correct for soft-tissue attenuation. The equations of Tonnesen et al 1974, used by Schlegel, Gates, Yap and Zubal are a popularly equation used to calculate renal depth (Tonnesen et al. 1974; Schlegel, Halikiopoulos & Prima 1979; Gates 1982; Yap et al. 1985; Zubal & Caride 1992). The formulas were derived from ultrasound measurements obtained from a posterior oblique angle in the sitting position, whereas renal scintigraphy is usually performed in the supine position at present. Calculation using the equations of Tonnesen is found to underestimate renal depth in the supine position in children as reported by Maneval et al 1990, and in adults as reported by Taylor et al 1993 (Maneval et al. 1990; Taylor et al. 1993). We used the formulas described by Raynaud et al 1974, in pediatric patients and those reported by Taylor et al 1993, in adult patients (Raynaud, Jacquot & Freeman 1974; Taylor et al. 1993). Taylor et al 1993 measured the renal depth by transmission CT with the patient in the supine position. The formulas of Raynaud et al were developed using lateral scintigraphy and validated by CT in the supine position. These

equations appear to give more accurate estimates of renal depth compared with the equations of Tonnesen. Although the right-to-left difference in renal depth is neglected in the equations of Raynaud, however the difference is usually small in children as mentioned by Maneval, but ignoring it may cause substantial error in relatively large children (Maneval et al. 1990). Moreover, the application of different equations to younger children, older children and adults may induce some discontinuity in estimating renal depth in long-term observations (Inoue et al. 1998). Further sophistication of the method used in renal depth estimation to calculate renal function may improve the reliability of GFR estimation.

The linear attenuation coefficient for ^{99m}Tc in water is 0.153cm^{-1} , and some authors like Gates, Yap and Zubal have used this value to correct for soft tissue attenuation (Gates 1982; Yap et al. 1985; Zubal & Caride 1992). However, the effective attenuation coefficient is lower because of the presence of scattering photons. The values of the effective attenuation coefficient reported by Bratt et al 1981 and Cosgriff et al 1990, range from 0.10 to 0.14 cm^{-1} (Bratt, Larsson & White 1981; Cosgriff & Brown 1990). We took the coefficient to be 0.12 on the basis of the reported values, which have been applied in this thesis for soft tissue attenuation correction in our software program.

The assignment of the kidney region of interest is accentuated in gamma camera GFR measurement, which calculated the ^{99m}Tc -DTPA counts included in the kidneys, currently, most gamma cameras are equipped with a commercial ROI tool for GFR measurement (Lin, Huang & Chen 2011). Although, ROI determination techniques have been studied extensively and have advanced greatly in the last decade or so, it remains normal practice that ROIs are drawn manually by human operators (Houston et al. 1998), which is tedious. This is so for a variety of reasons, one of which is the noisy nature of nuclear medicine images (Houston et al. 1998). Another reason is the overlapping between the liver and right kidney, which is common and is intensified by the effect of scatter and respiratory movement, this overlapping makes appropriate separation of the liver from the right kidney difficult (Inoue et al. 2000). The problem with the manual ROI drawing is inter-operator and inter-institutional variability, which is a potential source of

non-reproducibility and the subsequent effects on GFR quantification (Jackson et al. 1985). Furthermore, operator dependency can cause a substantial trouble, especially in institutes with no expertise in nuclear nephrology, and it may impair the feasibility and reliability of renal function estimation (Inoue et al. 2000).

Aiming to decrease the inter-subject variability related to the manual procedure, and to reduce the procedure time while maintaining the accuracy of GFR estimation, several semiautomatic algorithms for ROI processing have been proposed; hence, fully automatic methods are ineffective in Nuclear Medicine practice (Tian et al. 2013). The single-threshold method introduced by Halker et al 1996 was the first semiautomatic method to be used; however, with this method, it is difficult to delineate the kidney area even in normal subjects due to the poor contrast between the kidney and the background (Halker et al. 1996). Inoue et al 2000 proposed a semiautomated approach for renal scintigraphy with ^{99m}Tc -mercaptoacetyltriglycine (MAG_3), and evaluated its clinical applicability for the estimation of renal function by camera-based method (Inoue et al. 2000). In their method, an operator placed a large rectangular ROI over each kidney, a circular ROI within the liver, and a rectangular ROI between the kidneys. Using these ROIs, semiautomated renal ROIs were determined on the basis of the temporal changes in counts, in addition to the absolute counts. The relative function of the right kidney determined by the semiautomated method was compared with that determined by the manual method. The clearance was successfully predicted with the semiautomated ROIs ($r = 0.93$), despite this good correlation, it should be noted that the kidney size is variable; hence, the rectangles may not match the kidneys for each patient. In addition, setting the ROI manually based only on visual inspection of the operators can cause artificial intra-operator and inter-operator variations. On the other hand, the applicability of this method with ^{99m}Tc -DTPA needs to be evaluated. Another semiautomated method was developed by Tian et al 2013; he delineated the renal ROI areas by his proposed multi-step semiautomated method. In which it integrates the temporal/morphologic information via visual inspection and computer-aided calculations. The total GFR was estimated using the proposed method performed by 2 junior clinicians and compared with the

reference total GFR estimated by a senior clinician with 20 years of experience, who manually delineated the kidney ROIs (Tian et al. 2013). Their proposed approach correlated well ($r = 0.95$), nevertheless in their data sample, 10 patients with unilateral or non-functioning kidneys were excluded from the analysis, indicating its non-effectiveness in such patients; notwithstanding, their reference criterion was not a gold standard method.

In a second part of this dissertation we have made a modification on Inoue's gamma camera method, an isometric count Semiautomatic processing for delineating the kidney ROI have been applied. This isometric count (iso-count) algorithm based on the contour thresholding, where the operator selects a point on a composite image and specifies a value for the percentage of the maximum count in the image. Provided that the selected point exceeds this value, a contour corresponding to this value and surrounding the point is displayed. The operator may then choose a different value or use the contour to define the periphery of the ROI (kidneys). We performed the calculation of the renal uptake percentage between the second and the third minute, as the perfect time to measure the uptake percentage is when a large amount of imaging agent has accumulated in the kidney and has not been discharged, which has been approved (Gates 1982; Li et al. 2007). The result of our proposed Semiautomatic and the Manual processing for the kidney ROI, with three types of backgrounds (the Automatic, the Subrenal, and the Perirenal background) were compared against the reference. The Semiautomatic processing showed a good correlation and the Manual processing continued to be exploited properly ($r = 0.98$ for the Automatic, $r = 0.95$ for the Subrenal, and $r = 0.93$ for the Perirenal background). We could say that, our proposed semiautomated approach is faster and easier compared to the commonly used Semiautomatic ROI delineation procedure. The time consumed on ROI delineation using this proposed method is approximately one third shorter than that with the Manual method. Moreover, the software based on the proposed method is user friendly, which does not require much training or operator experience. However, its performance with in the wide range of GFR values will be further discussed, nonetheless, its reproducibility still needs to be judged.

The problem of background subtraction is one of the immense practical difficulty in ^{99m}Tc -DTPA camera based GFR. The counts recorded in the kidney ROI include not only the accumulated activity of the tracer filtered in the glomeruli using DTPA, but also an extra renal background activity (Houston et al. 1998). The liver, spleen, and great vessels are the main background areas in the renal imaging with ^{99m}Tc -DTPA; this radiotracer commonly accumulates in these structures as well as in the kidneys. Thus, it is important to remove the extra renal background counts from the kidney ROI counts for accurate GFR measures (Tian et al. 2013). The impact of different background sites on the GFR was evaluated in a third part of this thesis using the Inoue method. We compared the influence of a three types of background on the estimated camera GFR values against the reference GFR values. The three types of background were a Subrenal, and a Perirenal background that were manually drawn, where an Automatic background was automatically placed 1 pixel below the inferior angle of each kidney with a size of 6x3 pixels. In our result the Perirenal background with the Manual and the Semiautomatic renal ROI gave a correlation coefficient of 0.93, This good correlation is consistent with the Granerus and Moonen 1991 results, which they reported that the background ROI closely surrounding each kidney was shown to be proper for estimation of the extra-renal background level, as it adapts itself to both the shape and location of the kidneys (Granerus & Moonen 1991). When they compared the Subrenal and the Perirenal background, they found a correlation coefficient of 0.95 (Granerus & Moonen 1991), in our study the correlation with the reference GFR and the Subrenal background were higher than the Perirenal background ($r = 0.95$). This privilege of the Subrenal over the Perirenal in this study is contradicted by Itoh 2003, as they pointed out that the Semilunar (Perirenal) background is much better than the Inferior (Subrenal) for the correction of background radioactivity (Itoh 2003). On the other hand Dopuda 2008 et al also found that the GFR calculated by the use of a region under the lower pole (subrenal) is statistically significantly higher than GFR calculated using a region by the lateral side and around the whole kidney ($p < 0.0001$) (Dopuda et al. 2008), which is in harmony with our findings. When drawing a Perirenal background, parts of the liver and the spleen could be involved within the background ROI, which is additive and will

increase the background counts especially in less functioning renal patients, resulting in falsely reducing the measured GFR, this maybe the reason for the Perirenal background to have a lower correlation than the Subrenal background. The placement of the background ROIs manual needs to be carefully done and imposes a substantial burden on the operator. The success factor for background correction seems to be a combination of accuracy and precision which is clearly provided by the Automatic background. Inoue et al 2000 used an automatically assigned background, which was placed in the subrenal area for each renal, and found a correlation of 0.96 (Inoue et al. 2000), we managed to obtain a higher correlation with our proposed Automatic background ROI. Using this Automatic background with the Manual renal ROI the correlation coefficient was 0.99, and with the Semiautomatic renal ROI the correlation coefficient was 0.98. It is obvious that the selection of background activity region has a significant influence on GFR measured by the Inoue method, and as our study found that the Automatic background provide the best option for background subtraction.

The ideal method for the assessment of the GFR should be feasible with no limitation in any clinical situation and adaptable regardless to the value of the GFR. Previous researches have proven that, at moderate GFR values, there is no obvious difference between the various methods, whereas, at low GFR level (i.e. < 30 ml/min) the difference is clear (Mulligan, Blue & Hasbargen 1990), on the other hand a few researches have been performed on the difference at high GFR level (Li et al. 2007). Thus there is paucity in data comparing the performance of Gamma Camera GFR method with respect to the GFR level (Bhatt et al. 2011), however, we observed an obvious dissimilarity in the performance of the Inoue camera method within the different renal stages when we assessed the models that gave the best agreement, which were the Manual and the Semiautomatic ROI with the Automatic background. The correlation coefficient was above 0.91 for high GFR (> 90 ml/min), due to the clarity of the kidney outline which lead to a great precision in ROI placement indicating its suitability for such GFR level, as in consistent with Russell et al. 1985 as they mentioned that; for a patient with renal function of a GFR of 100 ml/min, the gamma camera methods is accurate and better than

the creatinine clearance (Russell et al. 1985a). For moderate GFR (59 - 89 ml/min) Russell et al also reported that; the relative error of the camera method increases within this GFR level (Russell et al. 1985a), we managed to obtain a reasonable correlation in range of 0.83 to 0.85 for the Manual drawing, while for the Semiautomatic the correlation was lower in range of 0.76 to 0.79, but still fair because the renal function and outline are still preserved in this GFR level. And as Russell et al mentioned that; in the moderate GFR the noise comprised in the renogram is probably the main reason for the slightly lower precision of the camera method (Russell et al. 1985a), which explain the correlation declension in our results. When coming to the low GFR level (GFR < 30 ml/min) discrepancies between Inoue camera method and the reference method was clear, with a poor correlation of less than 0.65 and almost no correlation of less than 0.08 for GFR below 15 ml/min. When GFR is low, errors always exist because of the minor difference between the kidney and background (Li et al. 2007), causing uncertainty in kidneys region placement.

A verification of the literature for Gates method, which has been widely used since 1982, was implemented lastly in this thesis. It has been debated whether the Gates' method is accurate for predicting the GFR, as it was reported in some previous validation studies, that it tended to overestimate the GFR (Fawdry et al. 1985; Mulligan, Blue & Hasbargen 1990). This overestimation could be attributable to insufficient background correction in the kidney, as it's suggested that the Gates with a simple background correction is less accurate than the methods with more sophisticated background correction for the calculation of GFR (Russell 1987; Gunasekera, Allison & Peters 1996; Itoh et al. 2002). It may also be due to under estimation of plasma concentration for patients with small distribution volumes, resulting in overestimation of GFR in small patients, therefor the method should not be used to estimate GFR in children (Mulligan, Blue & Hasbargen 1990; Inoue et al. 1998). Relatedly, there seems to be inter-individual differences in distribution volume even in adults, although they are smaller than in children (Inoue et al. 1998), but it has been found that adjusting Gates' method for the effect of distribution volume improves its accuracy in adults (Mulligan, Blue & Hasbargen 1990). Our findings on working with the Gates method gave an underestimated GFR, as in agreement with

Natale when he used inulin clearance as the reference standard; he concluded that the Gate's method tended to underestimate GFR (De Santo, 1999 #36). This underestimation could be imputed to the renal depth estimated by Tonnesen's formula which is subject to underestimation, and to the inaccuracy of the reference GFR measured by the creatinine clearance rate (CCR), and because of these two reasons Mulligan reported that the Gates' method may lead to an underestimation of GFR, especially when the true GFR is high (Mulligan, 1990 #44). Moreover, the Gates was proved to be inaccurate and less precise than the creatinine clearance in predicting the GFR (De Santo et al. 1999). The result of implementing Gates' method was very disappointing, although modifications to the procedure have shown a marked improvement in the result obtained (Russell et al. 1985b; Maneval et al. 1990), our specific aim was to assess the method as it is practiced in many centres. In line with Galli, Russell and Dubovsky (Russell & Dubovsky 1989; Galli et al. 1997), the unquestioning use of software supplied by manufacturers ought to be discouraged.

5.1 Conclusion

It has been concluded that the studied method does not meet the final goal, which is to establish a simple and accurate radionuclide method that can easily be performed in routine clinical practice to estimate renal function of any severity. Indeed, the Inoue gamma camera method with ^{99m}Tc -DTPA, found to be more-useful to evaluate the GFR for patients in a subclinical state usually with high GFR and without clinical symptoms.

It is also concluded that the automatic background is the best choice for background correction, which provide accurate and precise results and totally eliminate the non-reproducibility of the others; therefore, it should be used when performing any renal quantitative measurements.

5.2 Recommendations

Further studies in the areas of renal depth estimation and region of interest detection are needed to overcome the current limitation(s) embedded in the methodology of Inoue gamma camera method for GFR estimation. This will certainly improve the accuracy and reliability of the above-mentioned method.

Although, in the current literature efforts to implement Gates' method were without marked success, our specific aim was to assess this method as it is practiced in many centres, which bring us in line with the above-mentioned literature. Thus we discourage the use of this method as supplied in the software(s) by manufacturers.

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