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Dos and Don'ts that are Issued through Radiolabeling Process of DMSA (Dimercaptosuccinic Acid) by ^{99}mTco4- as ^{99}mTc-DMSA(III), the Gold Standard Radiopharmaceutical for Renal Cortical Scintigraphy

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Abstract

Objective: Since DMSA (Dimercaptosuccinic Acid) is an important and more sensitive kit in the realm of renal scintigraphy, in the course of this enormous study throughout five years, all quality control results from more than 500 Tc-99m radiolabeled DMSA kits and scans were studied to improve radiolabeling method through optimization of all involving parameters.

Methods: Instant thin layer chromatography (ITLC) (support: ITLC-Silicic-acid (SA), solvent: n-Butanol Saturated with 0.3N HCl) were used to calculate %radiochemical purity (%Tc-99m DMSA). Data from ITLC and scans were investigated to obtain the optimized radiolabeling method so as to decrease background uptake particularly liver uptake.

Results: Data suggested that if radiolabeling is performed under optimized involving parameters (Generator used day 2, SA=(15-20 mCi)/ (1cc) radiochemical purity=99.5% (Al_2O_3<5 ppm), generator's pH=4-4.5), the % Tc-99m dimercaptosuccinic acid (DMSA) will be ≥ 90-95%. Moreover, more intricate scans with abnormal biodistribution of radiotracer developed the expanded view on ^{99m}Tc-DMSA(III) renal scan impression with regard to diseases that can affect the ^{99m}Tc-DMSA(III) biodistribution. During this investigation, it was concluded that almost all diseases that influence liver and spleen including fatty liver, mononucleosis and similar illnesses could change routine biodistribution of ^{99m}Tc-DMSA(III).

Conclusion: Finally, we suggested an optimized radiolabeling method (^{99m}Tc-DMSA(III) ≥ 90-95%) to reach a precious imaging as more as we can decrease uncertainties about interpretation of Tc-99m DMSA scan considering patient's background to provide an effective diagnosis.

Keywords: Radiolabeling; ^{99m}Tc- DMSA; Renal scintigraphy; Renal pharmaceuticals

Introduction

Kidney as one of the vital organs participates in homeostasis of human body [1] including production of 1, 25-dihydroxy vitamin D [2], maintenance of pH [3] and electrolytes [4] balance, production of red blood cell [5] and control of blood pressure [6] as well as clearance. Many problems related to renal diseases could affect whole body activities by interrupting biological cycles in either direct or indirect way [7]. Therefore, well-timed diagnosis and efficient treatments play important role in patient's promotion and contraction of long-termed side effects. There is a variety of diagnostic methods applied in this field [8]. Renal scintigraphy is one of the interesting diagnostic methods because of physiological survey, providing dynamic-static renal studies and modified radiation dose in relation to some classic radiological methods [9]. Three different issues (Glomerular Filtration, Tubular Secretion and Cortical Function) are discussed in renal scintigraphy. Therefore, there are supposedly three different series of technetium radiopharmaceuticals with regard to purpose.

Renal cortical radiopharmaceuticals

Renal cortical scintigraphy is one of the important imaging which is performed to study renal morphology because of probability of scars in related patients. Several kinds of renal problems including UTI, pyelonephritis, reflux, etc. lead to cortical renal scar emerging [10,11]. High percentage of glomerulus and proximal convoluted tubules home in cortex, therefore, cortex's deficiencies can hardly decrease renal function [12]. It should be mentioned that if scar is diagnosed as early as it emerges, there is high possibility of reversibility through efficient therapy. Therefore, with a well-timed and certain diagnosis, suffering patient can be promoted. Nuclear medicine provides a special method with renal cortical imaging via reasonable sensitivity and specificity [13]. The radiopharmaceutical used for this purpose should be retained in cortex without remarkable secretion supposedly. Two common positions in cortex, sulfhydryl groups and some cytoplasmic proteins, are the objects to capture renal cortical radiopharmaceuticals [14]. ^{99m}Tc-DMSA(III), as a gold standard to renal cortical radiopharmaceutical, is most likely to maintain in cortex through cytoplasmic protein, approximately 50% of injected dose 2 hours after injection [14]. ^{99m}Tc-glucoheptonate (GHA) as uncommon cortical radioagent is eliminated by glomerulus in sum, with 10%-15% of injected dose captured in

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cortex [15]. Because of low percentage of cortex accumulation, it is not commonly used in this field.

Kinds of ^{99m} Tc- DMSA complex

Generally, two different types of ^{99m}Tc- DMSA complex, ^{99m}Tc-DMSA(III) and ^{99m}Tc-DMSA(V), had been reported with regard to radiolabeling condition. Due to spatial and geometrical differences, each complex specifically has its own biodistribution that caused two distinct clinical uses [16]. It was proved which complexes will be outstanding hardly depended on pH and ratio of DMSA: $SnCl_2.2H_2O$. At acidic pH 2.5 and excess amount of $SnCl_2.2H_2O$, the ^{99m}Tc- DMSA(III) will be reached that aggregates in renal cortex and prepares the renal cortical scintigraphy. In low concentration of $SnCl_2.2H_2O$ and alkali pH 7.5-8, the ^{99m}Tc-DMSA(V) will be outstanding ones that could be absorbed in carcinoma deficiencies [17]. During a study formation of ^{99m}Tc-DMSA complexes in wide range of pH and DMSA: $SnCl_2.2H_2O$ ratios were investigated in detail and also third form of ^{99m}Tc-DMSA with optimistic potential to clinical uses was reported [18].

Characteristics of ^{99m}Tc-DMSA(III) injection

Up to 3 hours after injection, 40-50% of injected ^{99m}Tc- DMSA(III) dose (adults: ≈80MBq children: 15MBq) [19] retained in renal cortex and about half of that is eliminated by tubules. Without any disruption in vivo and in vitro factors (it will be discussed), 2-3 hours after injection, there isn't any significant background uptake, so well-timed scintigraphy could be performed. Because of both renal aggregation/elimination, kidney is considered as both target and critical organ.

Ingredients of DMSA cold kit

All kit ingredients are categorized into active and inactive moieties (Table 1) (Ingredients of DMSA cold kit). Active moiety is considered as DMSA which is able to bind with Tc-99m technetium as ^{99m}Tc-DMSA(III). Inactive moiety is all excipients and reductants. Inositol is common excipient used in DMSA kit.

Methodology Applied

Materials

Chromatography paper, Silicic-acid (SA), was obtained from Agilent technologies Part No. A120B12. Mixture of solvent (n-Butanol Saturated with 0.3N NaCl), was prepared following USP pharmacopoeia1.

Equipment

Biodex Medical System, UPTAKE STAND 2" CTYSTAL, Model 187-220, No. 120397604. This system has been equipped with well counter.

Active ingredient	Dimercaptocuccinic Acid (DMSA)
Inactive ingredients	Stannous Chloride Dihydrate, Ascorbic Acid and Inositol

Table 1: Ingredients of DMSA cold kit.

Methods

There are two kinds of chromatography systems to assay Tc-99m DMSA kit quality (Table 2) (Various chromatography systems used for calculation of % ^{99m} Tc-DMSA(III)) In this study DMSA kit was labeled by (15-70) mCi of fresh ^{99m} Tc- sodium pertechnetate according to brochure instruction. ^{99m}Tc-sodium pertechnetate added kit was shaken for 2-10 minutes and then left in room temperature. After that, radiochemical purity was calculated by chromatography SYS (1). In this five-year study, all of the parameters involved in radiolabeling process were investigated to figure out how they can shift range of % ^{99m} Tc- DMSA(III). That should be noticed throughout this five-year study more than 500 kits were radiolabeled. Some of the important interests involved in radiolabeling process including vacuum quality of cold kit, dilution, specific activity, generator's involving factors (used day, pH and aluminum oxide releasing) were investigated. To study of each independent parameter, we made an attempt to select kits that all had been radiolabeled in the same condition except under studied parameter.

Discussion

Through this study which took as long as five years, all data obtained from radiolabeled DMSA kit were gathered around all involving parameters that might have affected the quality of radiolabeled kit with regard to % ^{99m}Tc-DMSA(III). All variables including temperature, shaking time, specific radioactivity (^{99m}Tc- sodium pertechnetate mCi/ml) and all days that generator if it would be milked to use, were studied in this deliberation in detail. All results depend on $SnCl_2$ as a common reductant in kit and structure of DMSA.

Temperature and shaking time studies

In same condition of all parameters except temperature and shaking time, results from 150 radiolabeled kits performed in range of room temperature (20-25) °C and shaking time (2-10) minutes, showed that there isn't a significant difference between obtained %radiolabeling. It demonstrates that shaking time and mentioned temperature range don't have meaningful effect on radiolabeling process. It should be mentioned that if it is performed under incubation time less than 10 minutes without any shaking it will decrease %radiolabeling.

Vacuum quality of cold kit

At first, it must be underlined vacuum DMSA kits had been used in this investigation (instead of nitrogen filled kits). All data for surveying this matter were fortuitously come from non-vacuum kits during study of other parameters (19 kits). It was concluded that the received data from non-vacuum cold kit were 15-20% (13% in average) decreased in comparison to ideal ones (Figure 1). It was estimated that interaction of

Systems	Solvent	Support	Rf ^{99m}Tc O_4^-	Rf ^{99m}Tc reduced/ hydrolysed	Rf ^{99m}Tc DMSA	%radiochemical purity of ^{99m}Tc DMSA
SYS (1)	n-Butanol Saturated with 0.3N HCl	ITLC-Silicic- acid (SA)	0.8-1.0	0.0-0.15	0.45-0.7	%85 ≤
	MEK or Acetone	TLC-Whatman No.1 or ITLC-SG	0.9-1.0	0	0.0-0.3	
SYS (2)	Solvent	Support	Rf		Rf	%90 ≤
	Glycine 5%	TLC-Whatman No.1	(^{99m}Tc reduced/ hydrolysed) 0-0.2		(^{99m}Tc O_4^- + ^{99m}Tc DMSA) 0.2-1	
	MEK	TLC-Whatman	(^{99m}Tc O_4^-) 0.8-1		(^{99m}Tc O_2^- + ^{99m}Tc DMSA) 0.0-0.8	

Table 2: Various chromatography systems used for calculation of %Tc- 99m DMSA

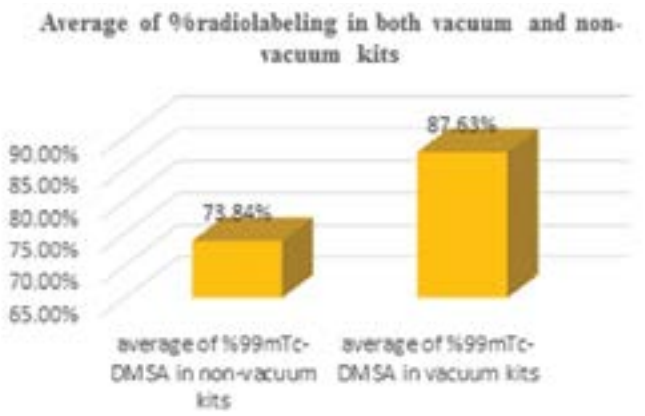

Figure 1: The bar graph illustrates the average of %radiolabeling in both vacuum and non-vacuum DMSA kits. According to the bar chart non-vacuum kits decrease %radiolabeling to 72.84% in average so it shouldn't be radiolabeled.

$SnCl_2$ and trapped air in the kit due to $SnCl_2$ oxidation to Sn^{+4} (Equation 1) that could obviously diminish the %radiolabeling because of usable Sn^{+2} deficiency [20]. Furthermore, oxidized and hydrolyzed stannous as colloidal particles have a tendency to technetium-99m that will lead to liver aggregation of ^{99m}Tc- DMSA(III) [21]. Results suggest that non-vacuum kit should not be radiolabeled with regard to chromatography system SYS (1) that rules %radiolabeling should be more than %85. All non-vacuum Tc99m-kits are not preferable but more decreased %radiolabeling on DMSA estimating interference of ^{99m} Tc-Sn colloid on ^{99m} Tc- DMSA(III) bio distribution.

$$6SnCl_2(aq) + O_2(g) + 2H_2O(l) \rightarrow 2SnCl_4(aq) + 4Sn(OH)Cl(s) \quad (1)$$

Dilution effects

During investigation, it has been concluded that the amount of total volume (V_t) in radiolabeled kit could lead to change in clearance rate of ^{99m}Tc-DMSA(III) from blood pool and soft tissue. Almost 50 ^{99m}Tc-DMSA (III) injected patients, despite high-quality radiolabeled kit and acceptable range of serum BUN and creatinine, associated with more soft tissue retention in delayed imaging step (3 hour after injection) (Figure 2). Detailed studies proved that all ^{99m}Tc-DMSA (III) kits were diluted by normal saline nearly to V_t = 5-7 cc.

Results suggest that if there is necessity, radiolabeled DMSA kit must be diluted up to 4 cc.

$$V_t = V\ (^{99m}TcO_4^-) + V\ (\text{normal saline})_{(\text{for dilatation})} \leq 4cc$$

Specific activity (SA)

All gathered QC (quality control) results from nearly 500 kits that were radiolabeled with wide range of $^{99m}TcO_4^-$ (15-70mCi)/(1-4cc) during 5 years, fortuitously 80 radiolabeled kits performed in same condition except (SA), had been selected to investigate based on the ITLC results. This study suggested that in same conditions, the kits that were performed in $^{99m}TcO_4^-$ (15-20 mCi) / (1cc) caused an increase in % radiolabeling %4 in average. Furthermore, we found that Tc-99m sodium pertechnetate added kits more than 60 mCi were accompanied by free Tc-99m sodium pertechnetate according to SYS (1).

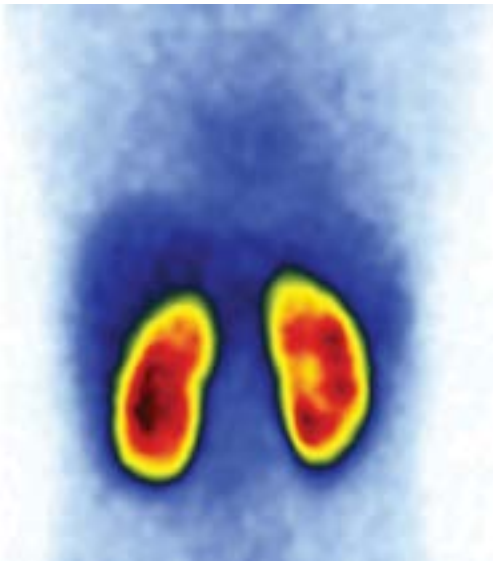

Figure 2: It illustrates 18-year-old man injected by diluted Tc-99m DMSA (V_t=6.5cc). As scan reveals, 3 hours after injection the blood pool clearance isn't satisfactory.

Generator's pH effects

Primarily the generators have been received at pH= (4-7), acidic pH (pH near to bottom of the range (pH 4)) is preferable in order to prevent $SnCl_2$ degeneration (Equation 2) and inhibit Sn-Tc colloid formation. Because of high %Tc-99m DMSA occurs at pH 2-3, pH in DMSA cold must adjust to acidic pH respect to generator's pH. If the ^{99m}Tc-sodium pertechnetate added DMSA kit had been at pH ≥ 4, it would have been associated with liver uptake.

$$\begin{cases} SnCl_2(aq) + 2NaOH(aq) \rightarrow SnO.H_2O(s) + 2NaCl \\ SnO.H_2O(s) + \text{NaOH(aq)} \rightarrow NaSn(OH)_3(aq) \end{cases} \quad (2)$$

Radiochemical purity

All generators must be controlled in terms of releasing alumina to perform in high radiochemical purity. The limited concentration of Al_2O_3 was reduced to 10 µg/ml in United States Pharmacopeia to prevent probability of Tc-99m Al_2O_3 formation [22]. Generator quality control test showed that cholorometry results in range of Al_2O_3<5 ppm causes radiochemical purity in range (99.5-99.69) % [23]. Assuming Al^{+3} (as lewis acid) affinity to DMSA (Dimercaptosuccinic Acid), it is most likely to radiolabel of DMSA kit in Al_2O_3 <5 ppm (Figure 3). The more amounts of alumina, the more liver uptake.

Generator used days

For assaying of generator' variations effect on %radiolabeling along the week, we had approximately selected ITLC results of 100 kits that were undergone radiolabeling in the same condition of other interfered mentioned parameters expect the generator used days. According to generator program, one passed days after production of generator, it has been daily milked during 7 days (Table 3) (The program of generator used days as long as a week). After each milking, the generator has been surely dried by vacuum kit, therefore, it will be ready to use in good condition. It is worth mentioning, the generator will be available to us one day after production because of distance. We don't use on

received day in order to probability of high concentrated radiolysis production, so it would be just undergone milking and drying without contribution in radiolabeling process. All 100 kits had been labeled on different days from Day 2 to Day 8 (latest used day), in same condition of batch number, specific activity, etc. results showed that the kits radiolabeled on Day 2, raise % ^{99m}Tc-DMSA(III) up to 5% (in average) in comparison to other days. Minimum amounts of % ^{99m}Tc-DMSA(III) have been recorded on Day 8 (Figure 4). It would be guessed that an increase in % ^{99m}Tc-reduced/ hydrolyzed in generator along the week can slightly cause %radiolabeling to go down. It should be regarded all parameters that can increase colloids, decrease quality of kits, but could affect DMAS more than others in order to DMSA structure. Two critical days after generator production (2th, 8th), were more focused in this investigation.

Other Factors That Could Affect Kit Quality or Change Routine Biodistribution of ^{99m}Tc- DMSA(III)

The amount of excipient in the cold kit

During this study we rarely received scans associated with mild bone up take particularly in spinal column and hip (Figure 5). More investigations developed proof that related to imbalanced ratio of kit ingredients. Generally, the cold kit of DMSA is promoted by several excipients as productive agents. Inositol is a well-known agent of excipients ilk in this kit. It would mind that the ratio of Inositol/DMSA should be indeed managed because of competition on radiolabeling with 99m-Technetium (Figure 6). If the amount of inositol is more than optimized range, the bone uptake will be appear caused by tendency of radiolabeled inositol to hydroxyapatite's phosphate groups (Figure 6).

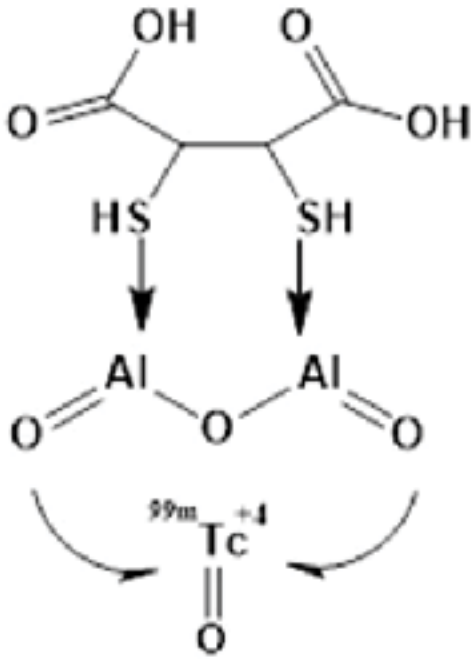

Figure 3: Affinity of Al^{+3} as Lewis acid for DMSA.

Days after production	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Used days	Non-used	Non-used	used	used	used	used	used	Non-used	used

Table 3: The program of generator used days as long as a week.

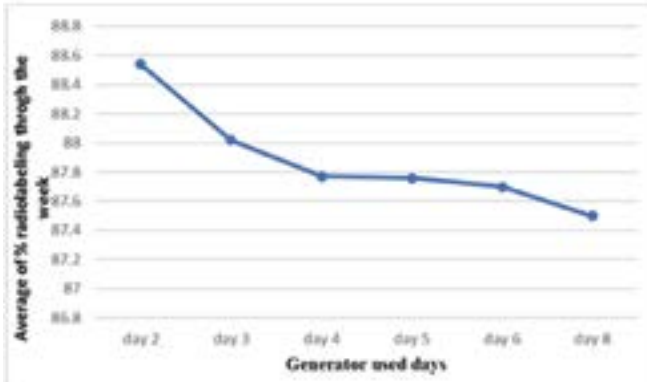

Figure 4: The graph illustrates the alternation of %radiolabeling through the week.

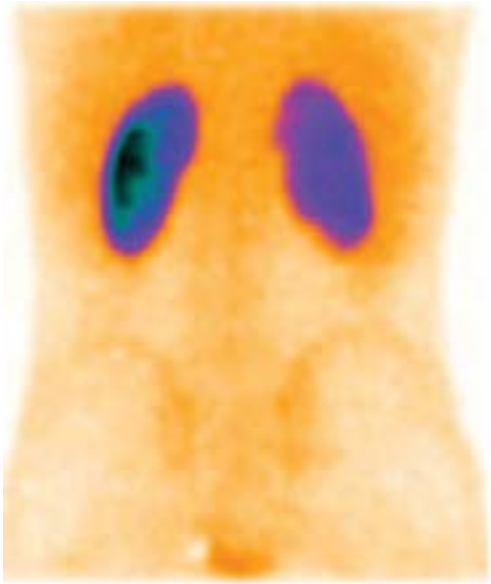

Figure 5: The ^{99m}Tc-DMSA scintigraphy reveals the bone marrow uptake. As it is illustrated the kidneys are in acceptable conditions. Further studies proved that it backs to amount of inositol as excipient which is a bit more than ideal concentration.

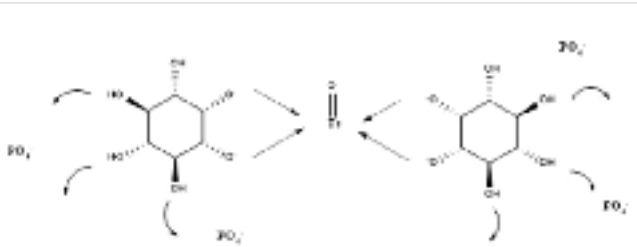

Figure 6: Tendency of inositol to technetium and phosphate (hydroxyapatite).

External disrupting materials

Recently, it was reported that if antiseptic added chlorhexidine

is employed, it could significantly interfere in DMSA radiolabeling process. It backs to past that chlorhexidine accelerates colloid complex in technetium added DMSA kit [24].

Some Diseases would Determine to Change ^{99m}Tc-DMSA(III) Biodistribution

Fatty liver

Our studies demonstrated that in patients with high blood LDL, would cause fatty liver, the liver uptake of ^{99m}Tc-DMSA(III) will be considerable but the liver uptake race depends on fatty liver grade (Figure 7).

High acidosis and alkaline condition

According to mechanism of renal cortical retention which performed by cytoplasmic protein of cortical cell, high concentration of H^+/OH^- in plasma disrupts ^{99m}Tc-DMSA(III) cortical retention in order to OH^-/H^+- saturated proteins in charge for renal cortical retention of ^{99m}Tc- DMSA(III). In the mentioned condition, in spite of the high-quality radiotracer, liver uptake will be considerable (Figure 8).

Dehydration effects

It was found that patients under dehydration lead to decreased uptake of ^{99m}Tc-DMSA(III) because of decreasing capacity of kidney

Acute renal failure (ARF)

Generally, in ARF patients, all agents that should be interfered by kidney tend towards liver because of renal function deficiencies in comparison to normal cases. In these patients, liver will be basically appeared however low cortical retention of 99mTc-DMS1A (Figure 9). Results showed that there is a correspondence between creatinine levels and kidney up take. The high ranges of creatinine will lead to more liver uptake.

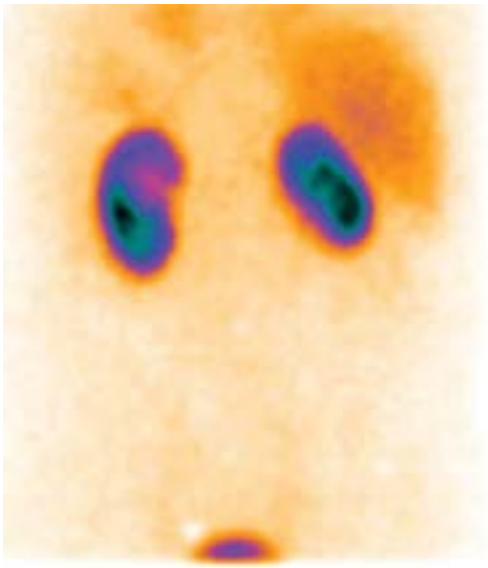

Figure 7: The Tc-99m DMSA scintigraphy of 28-year-old man illustrates obvious left kidney scar and liver uptake. Because right kidney is in good condition, under injection of high quality Tc-99m DMSA, liver uptake related to grade (II) fatty liver (Patient had suffered from fatty liver due to high blood LDL).

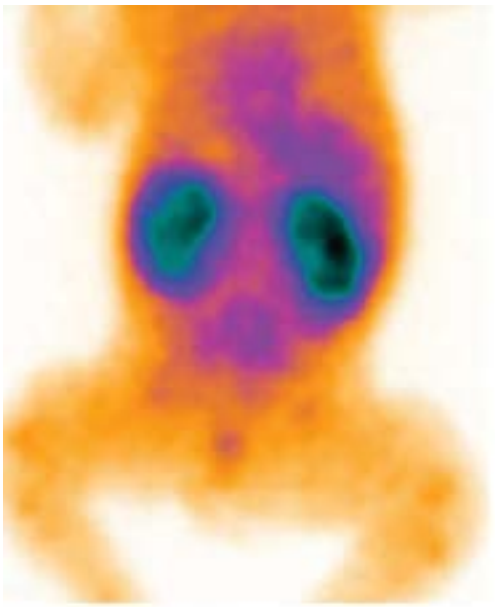

Figure 8: An 8-month-old patient suffering from metabolic acidosis was injected by ^{99m}Tc-DMSA. The scan had revealed mild decreased renal cortical uptake associated with liver uptake in spite of high-quality ^{99m}Tc-DMSA.

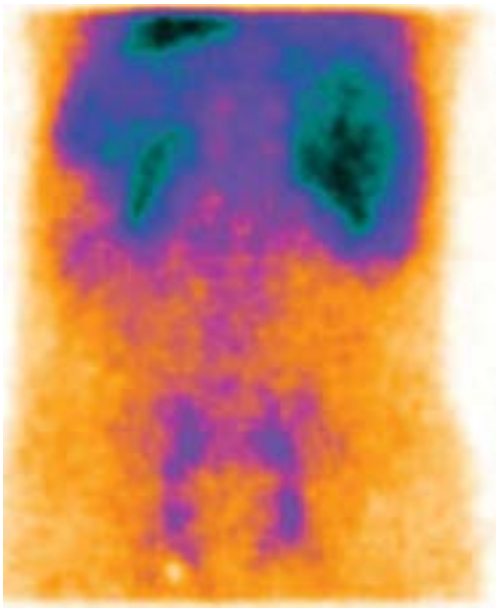

Figure 9: The Tc-99m DMSA scintigraphy of 28 year-old-man with an acute renal failure illustrates high background uptake including liver, spleen, heart and blood pool 3hours after injection.

Mononucleosis infection

Through our investigation, as an interesting result it was proved that patient bearing mononucleosis must shift biodistribution of ^{99m}Tc-DMSA(III) toward liver and spleen. ^{99m} Tc- DMSA(III) scintigraphy of mentioned patients showed that scan was accompanied with high aggregation of ^{99m}Tc-DMSA(III) in reticuloendothelial organs despite high quality of radiopharmaceutical, there isn't just kidney retention (Figure 10).

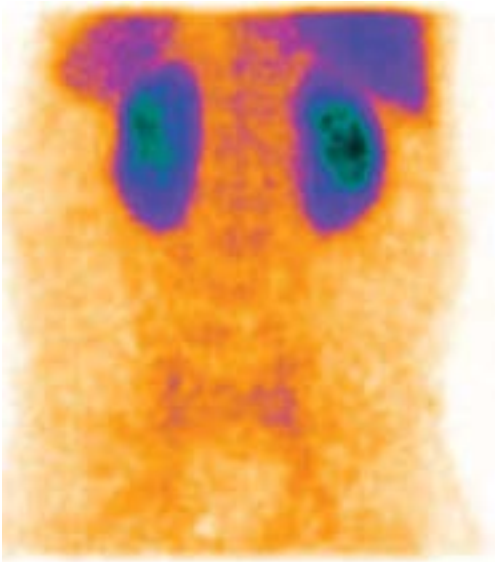

Figure 10: The 65-year-old women injected by high-quality Tc-99m DMSA (%Radiolabeling=91.23% in SYS (1)). The posterior view illustrates liver and spleen uptake despite high quality of injected radiopharmaceutical. A survey of patient's background demonstrated that she was suffering from mononucleosis infection. Similar scans demonstrated that the patients suffering from liver and spleen involvements especially mononucleosis would change routine biodistribution of Tc-99m DMSA. It was concluded from 8 patients who were suffering from mononucleosis. All of them had similar biodistribution of Tc-99m DMSA.

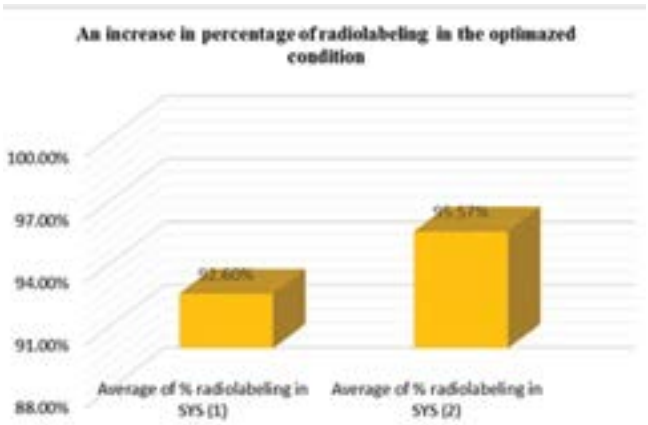

Figure 11: Radiolabeling of 20 DMSA kits with ^{99m}Tc-pertechnetate under optimized condition caused raising of %radiolabeling about 7% and 5% in SYS (1) and SYS (2) respectively.

Throughout this investigation with the aim of improving renal cortical scan by ^{99m}Tc- DMSA(III), has developed some modified approaches to raise % radiolabeling. The more performing under optimized of each parameter, the more raising % radiolabeling. It was concluded that radiolabeling on Day2, SA=(15-20 mCi)/(1cc), radiochemical purity = 99.5% (Al_2O_3 <5 ppm), generator's pH =4-4.5, diluted radiolabeled kit up to 4cc, raise percentage of % ^{99m}Tc-DMSA(III) more than 90% and 95% in ITLC done by chromatography SYS (1) and SYS (2) (Table 2) respectively (Figure 11). Moreover, it was concluded that some diseases with liver and spleen involving could affect ^{99m}Tc-DMSA(III)'s routine biodistribution. So, performing renal cortical scintigraphy by high-quality radiopharmaceutical as well as considering patient's background prepares a precise scan interpretation that leads to accurate diagnosis and patient promotion.

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