

THE IMPACT OF TC-99M DMSA SCINTIGRAPHY ON DNA DAMAGE AND OXIDATIVE STRESS IN CHILDREN

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Abstract

Background: Tc-99m DMSA scintigraphy is a commonly used imaging modality in the pediatric population. The radiopharmaceuticals which have the effects of ionizing are used in this method. This study aimed to investigate the impact of the Tc-99m DMSA scan on renal oxidative stress and mononuclear leukocyte DNA damage. **Methods:** Twenty-seven patients who performed Tc-99m DMSA scintigraphy were included in this study. Three ml heparinized blood samples were taken just before, during, and after a week from the scintigraphy. Mononuclear leukocyte(MNL) DNA damage, total antioxidant status (TAS), and total oxidant status(TOS) were measured in blood samples. The oxidative stress index (OSI) was calculated. The spot urine samples were taken from each patient before and within three days after performing the scintigraphy. TAS/Creatinine(TAS/Cr), TOS/Creatinine(TOS/Cr), and N-acetyl-glucosaminidase/creatinine(NAG/Cr) levels were measured in urine samples. OSI was calculated. **Results:** There was no statistically significant difference in the values of TAS, TOS and OSI studied in serum samples between controls and study group($p=0.105$, $p=0.913$, and $p=0.721$, respectively). There was no statistically significant difference in the levels of TAS/Cr, TOS/Cr, NAG/Cr, and OSI which were studied in urine samples before and after scintigraphy scan($p=0.381$, $p=0.543$, $p=0.129$ and $p=0.08$ respectively). The levels of DNA damage were increased only after the performance of the scintigraphy scan and decreased a week later($p<0.05$). **Conclusions:** The effect of Tc-99m DMSA scintigraphy is insufficient to create oxidative damage, but it can cause DNA damage via the direct impact of ionizing radiation which can be repaired again in a short time. **Keywords:** Tc-99m DMSA, DNA damage; reactive oxygen species; renal tubular injury; children

TITLE PAGE

The Impact of Tc-99m DMSA Scintigraphy on DNA Damage and Oxidative Stress in Children

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Abstract

Background: Tc-99m DMSA scintigraphy is a commonly used imaging modality in the pediatric population. The radiopharmaceuticals which have the effects of ionizing are used in this method. This study aimed to investigate the impact of the Tc-99m DMSA scan on renal oxidative stress and mononuclear leukocyte DNA damage.

Methods: Twenty-seven patients who performed Tc-99m DMSA scintigraphy were included in this study. Three ml heparinized blood samples were taken just before, during, and after a week from the scintigraphy. Mononuclear leukocyte(MNL) DNA damage, total antioxidant status (TAS), and total oxidant status(TOS) were measured in blood samples. The oxidative stress index (OSI) was calculated. The spot urine samples were taken from each patient before and within three days after performing the scintigraphy. TAS/Creatinine(TAS/Cr), TOS/Creatinin(TOS/Cr), and N-acetyl-glucosaminidase/creatinine(NAG/Cr) levels were measured in urine samples. OSI was calculated.

Results: There was no statistically significant difference in the values of TAS, TOS and OSI studied in serum samples between controls and study group($p=0.105$, $p=0.913$, and $p=0.721$, respectively). There was no statistically significant difference in the levels of TAS/Cr, TOS/Cr, NAG/Cr, and OSI which were studied in urine samples before and after scintigraphy scan($p=0.381$, $p=0.543$, $p=0.129$ and $p=0.08$ respectively). The levels of DNA damage were increased only after the performance of the scintigraphy scan and decreased a week later($p<0.05$).

Conclusions: The effect of Tc-99m DMSA scintigraphy is insufficient to create oxidative damage, but it can cause DNA damage via the direct impact of ionizing radiation which can be repaired again in a short time.

Keywords: Tc-99m DMSA, DNA damage; reactive oxygen species; renal tubular injury; children

Static renal scans using the radioisotope Tc-99m DMSA provide visualization of focal renal parenchymal abnormalities, assessment of a difference in kidney function and detection of renal scars. This scan has considerable amount of radiation.

In our study we showed that Tc-99m DMSA scan doesn't create oxidative stress but by analyzing mononuclear DNA damage we demonstrated that it can cause DNA damage via ionizing radiation which can be repaired in a short time

Introduction

The use of diagnostic radiologic imaging has increased over the last decades, especially computed tomography (CT) and nuclear medicine technology [1]. These procedures deliver high dose of radiation and epidemiologically linked with radiation-induced cancers [2]. Also, there is an emerging evidence that early life diagnostic radiation exposure is associated with childhood malignancies [3, 4]. Free radicals induced by ionizing radiation (such as superoxide, hydrogen peroxide and hydroxyl radicals) react with macromolecules, impair oxidant-antioxidant balance over time and lead to the development of oxidative stress [5]. Oxidative stress is described as the disruption of the balance between free radical production and antioxidant levels in favor of free radicals. This causes molecular damage, which accumulates over time in tissues and cells. DNA damage can lead to mutations, an essential step in carcinogenesis [6]. It is estimated that 2% of future cancers will result from current diagnostic imaging use [2].

The diagnostic nuclear medicine procedures that are widely used in the pediatric population are Technetium-99m-dimercapto succinic acid (Tc-99m DMSA), Technetium-99m-diethylenetriamine pentaacetic acid (Tc-99m DTPA) and, Technetium-99m-mercaptoacetyltriglycine (Tc-99m MAG-3) (7). DMSA plays an essential role in the assessment of renal scars in febrile urinary tract infections and differential function of kidneys. Vesicoureteral reflux along with recurrent urinary tract infections are important risk factors for renal damage [8]. The cortical uptake decrease on DMSA is considered as a marker of upper urinary tract infection and an indirect indicator of vesicoureteral reflux and renal scarring [9]. DMSA imaging involves the intravenous administration of radiolabeled tracer that binds to proximal convoluted tubules. The radiation dose delivered from the procedure changes from one institution to another. Michaud et al. demonstrated that the mean cumulative radiation dose of DMSA scan per patient approach to those of pediatric chest computed tomography [10].

Compared to adults, nationwide survey data on radiation-induced risks are limited in pediatric population. The radiation-related harmful effects of DMSA scan is not well known. Salmanoglu E et al. showed impaired oxidative and nitrosative balance in adult patients who undergone DMSA scintigraphy. Malondialdehyde which is an oxidative stress biomarker, was significantly increased in patient group compared to controls [11]. Up to our knowledge, there is no study performed in pediatric population investigating the oxidative stress and DNA damage of DMSA scan. Also, there is no study on possible tubular injury of the radiopharmaceutical used for the scan. N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme found in many tissues and, is a widely used urinary biomarker for the diagnosis of renal tubular injury [12]. Urinary NAG is used to detect renal tubular damage in cases such as acute pyelonephritis, vesicoureteral reflux, hydronephrosis, nephrotoxic drug or heavy metal exposure, nephrolithiasis, proteinuria, hyperglycemia and, hypertension [13].

This study aimed to determine the possible harmful effects and cellular damage of Tc-99m DMSA scintigraphy in the pediatric population by determining radiation-induced renal oxidative stress and mononuclear leukocyte (MNL) DNA damage, and renal tubular injury by urine NAG level measurements.

Methods

This prospective cohort study involved 27 pediatric patients who were followed up at the outpatient pediatric nephrology clinic and Tc-99m dimercapto- succinic acid (DMSA) scintigraphy was planned between April 2015-January 2016. The indications were recurrent urinary tract infections and detection of renal scars with the suspicion of vesicoureteral reflux. Patients with nephrolithiasis, history of premature birth, and who had recent urinary tract infection six month prior to scintigraphy or used antibiotics in last one month were excluded from the study. Power calculation was done with the Gpower analysis [14]. A previous study with Tc-99m pertechnetate thyroid scintigraphy was used and a calculation with the data of mean and standard deviation of dependent variables (92.06 ± 13.38 and 79 ± 9.43) was performed [15]. As a result of the Gpower analysis, the effect size was found to be 0.8. The determined study sample size was 23 if confidence level was 95%, power was 95%, and the effect size was 0.8. We included 27 patients with the probability of 20% missing patient.

DMSA scans were performed on a dual-head gamma camera (Symbia E, Siemens Medical Solutions, Hoffman Estates, IL, USA) with a low-energy high-resolution collimator. Images were acquired with anterior-posterior, lateral and oblique projections in 128x128 matrix, 300.000 counts or 5 minutes per image. In our institution, an intravenous hydration, bladder Foley catheter or sedation were not routinely applied for DMSA scans. Oral hydration was encouraged. Images were acquired 2 to 3 hours after radiotracer injection. Late images were taken as needed. For the pediatric population, an activity of 1.85 MBq/kg (0.05 mCi/kg) Tc-99m-DMSA was injected, and the minimum administered activity was 18.5 MBq (0.5 mCi) [16].

DNA Damage (Comet assay)

Three milliliters of heparinized venous blood samples were taken from each patient just before (group 1), immediately after (Group 2), and one week after the scan (group 3) from all patients. The groups were involved from the same patients, and they were analyzed three times. MNL isolation from the heparinized blood for the comet assay was performed by density gradient separation (Histopaque 1077; Sigma-Aldrich, Inc., St. Louis, MO, USA). For this purpose, 1mL of blood was carefully layered over 1mL of Histopaque and centrifuged for 35 min. at 500 x g and 25°C. The interface band containing mononuclear leukocyte was washed with phosphate- buffered saline (PBS) and then collected by 15 min. centrifugation at 400 x g. The resulting pellets were resuspended in PBS to obtain 20,000 cells in 10 mL. MNL DNA damage was evaluated by modifying the alkaline single cell gel electrophoresis (comet assay) method whose principle is based on the different migration of DNA molecules with different molecular weights and different electrical charges at the alkaline pH. After electrophoresis, when DNA molecules are stained with DNA- specific fluorescent dyes and examined under a fluorescent microscope, stained DNA can be evaluated by eye or kinetic reading programme [17]. In this study, the images of 100 randomly chosen nuclei (50 nuclei from each of two duplicated slides) were analyzed visually for each subject. Each image was classified according to the intensity of the fluorescence in the comet tail and was given a value of 0, 1, 2, 3 or 4 (from undamaged, class 0, to maximally damaged, class 4) and total score of a slide was between 0 and 400 arbitrary units. Samples from each volunteer were displayed on duplicate slides. The imaged DNAs were at least 50 DNA on each slide, a total of DNA images were recorded and scored on the computer.

Oxidative Stress Markers and NAG

Blood samples were taken into biochemistry contains tubes and were centrifuged at 3000 g for 15 minutes to obtain serum. All serum samples stored at -80 °C until experiments got started. Total antioxidant status(TAS) and total oxidant status(TOS) levels were measured using commercially available kits (Relassay, Turkey) according to Erel's assay. OSI was defined as TOS to TAS ratio was calculated as follows:

OSI (arbitrary unit) = [(TOS, $\mu\text{mol H}_2\text{O}_2$ equivalent) / (TAS, $\mu\text{mol Trolox}$ equivalent) $\times 100$]. [18,19] Urine samples from each patient were collected just before scintigraphy and within three days after scintigraphy. Urinary NAG (N-acetyl-glucosaminidase) was analyzed using a photometric method (Cobbas 8000 auto-analyzer, Diazyme Laboratories, Poway, CA). TAS, TOS and, creatinine levels of urine samples were also studied, OSI was calculated. The ratio of NAG, TAS, and TOS values in spot urine to urine creatinine values were used to avoid any effect on dilution and concentration.

Statistical Analysis

SPSS 20.0 was a program utilized for statistical analysis of collected data. Categorical measurements were determined as number and percentage and numerical computations as mean and standard deviation. The Shapiro-Wilk test was used for the analysis of compliance with the normal distribution. Continuous variables were summarized by mean \pm standard deviations whereas non-parametric variables were shown as mean value \pm standard deviation median (interquartile range). The Wilcoxon signed-rank test was used to compare two related samples. Variance analysis was performed for comparing the three times repeated measurements with normal distribution and Friedman analysis was done for parameters with non-normal distribution. In these tests, as there are three different groups, $p < 0.0167$ is considered as statistically significant. Wilcoxon signed ranks test was also used for the comparisons of the subgroups. According to these comparisons, p values were done as follows: P1, group 1 vs. group 2, P2, group 1 vs. group 3, P3, group 2 vs. group 3.

All statistical tests were two-sided. Results were evaluated at 95% confidence interval, and a $p < 0.05$ was considered statistically significant except in comparison of three subgroups.

Results

Our study included 22 girls and 5 boys between 3 months and 15 years of age (mean age 6.9 ± 3.6 years). The mean MNL DNA damage levels were analyzed in 24 children, and the results of the first group, second group, and third group were; 2,125 arbitrary units (AU), 4,222 AU, and, 1,885 AU, respectively (Table 1). There was a statistically significant difference between the first (before scan) and second groups (immediately after scan) ($p < 0.05$) and the second and third group (one week after the scan) ($p < 0.05$) (Figure 1, 2). Overall, the mean DNA damage increased just after the scan and decreased one week after the scan. Power analysis was done by the Gpower statistical program to detect the power of the study by investigating groups with the statistically significant difference in DNA damage levels, between the first and second group and between the second and third group. The effect size was 5.94 for the difference between first and second group; 95% confidence level, power was 1. The effect size was 6.54 for the difference between the first and second group; 95% confidence level was 1.

There were no statistically significant differences between groups in terms of serum TAS, TOS, and OSI values ($p > 0.05$) (Table 1). Due to a storage problem in laboratory, serum TAS levels were evaluated in 18 children and TOS in 15 children.

TAS/Cr, TOS/Cr, NAG/Cr ratios, and OSI levels were performed to all children, and results are illustrated in Table 2. There were no statistically significant differences between the groups (before and after scan) in terms of urine TAS/Cr, TOS/Cr, NAG/Cr ratios, and OSI levels (Table 2).

Discussion

To the best of our knowledge, this is the first study investigating the possible harmful effects of Tc-99m DMSA administration in children in terms of oxidative stress, DNA damage and renal tubular injury. We evaluated the levels of ROS in both urine and serum samples of pediatric patients, serum MNL DNA damage undergoing Tc-99m DMSA scintigraphy and, a urinary biomarker of acute tubular damage (NAG). Our results showed that MNL DNA damage immediately increased after the scan, but measured values decreased to baseline after one week. There were no differences between serum and urine oxidative stress levels before and after the DMSA scan and also, urinary NAG levels did not change.

Our study showed that radiopharmaceuticals might exhibit adverse effects on DNA through the direct impact of ionizing radiation, not by oxidative damage. The effect of Tc-99m DMSA radiopharmaceutical is not sufficient enough to create oxidative damage. Although DNA damage has been repaired, there is no information on the impact of repetitive Tc-99m DMSA or other scintigraphic images on DNA in children. Also we are not sure about some subtle permanent changes in DNA occurred.

DMSA scans are frequently used in the evaluation of recurrent febrile urinary tract infections in the pediatric population [7]. The pediatric nephrologists and urologists carefully follow up the children with renal scars and recurrent urinary tract infections in terms of chronic kidney disease, hypertension and investigate the possible underlying vesicoureteral reflux and try to preserve long term renal function [10]. DMSA scintigraphy administer the considerable amount of radiation. The radiation dose in children aged 1-15 years varies between 0.68 to 1.22 mSv (depending on guidelines used). The dose is approximately 1 mSv/examination regardless of the age of the child, adapted according to body surface compared to a pediatric chest CT which is 2-5mSv. [20-22]. Cellular exposure to ionizing radiation leads to oxidizing events, chronic inflammation and has long term effects on genomic instability [23]. It is well known that radiation has carcinogenic effects and pediatric exposure to radiation causes increased lifelong risk for the solid cancers and leukemia [22].

In recent years, interest in ROS and oxidative stress have increased. ROS has both mutagenic and carcinogenic consequences, affecting many cell components and causing oxidative stress and DNA damage [6]. Studies investigating the oxidative stress and ROS after DMSA scan are limited. Salmanoglu showed that oxidative and nitrosative stress was increased in adult patients who were administrated with 99mTc-DMSA

compared to the control group [11]. Studies with adult patients who had undergone different scintigraphic techniques demonstrated that antioxidant enzymes decreased after the injection of radiopharmaceuticals [15, 24]. In our research, we showed that DMSA administration had no effect on the oxidative stress of children.

DNA is considered to be the primary target for the direct and indirect effects of ionizing radiation. The absorption of ionizing radiation can cause disruption of atomic structures, radiolysis of water and subsequently causing damage to nucleic acids, proteins and lipids [23]. The majority of DNA damage is repaired within minutes or hours after induction. However, sometimes, repair cannot be performed or may be incorrect. Both conditions are considered to be a significant risk for carcinogenesis [25]. For these reasons, it is important to investigate possible DNA damage when examining the effects of nuclear imaging techniques using ionizing radiation. Some clinical studies have shown that a low dose of iodine-131 might cause chromosomal damages [26, 27]. Dantas et al. [28] also demonstrated an increased DNA damage in up to two hours following Technetium-99m-labelled radiopharmaceutical injection, which is subsequently reduced to zero after 24 hours, consisted with the results of our study. Researchers of the same study interpreted this as the effect of DNA repair mechanisms. In our research, we evaluated DNA damage one week later and found that DNA damage have been reduced, which initially have increased just after radiopharmaceutical administration

In recent years NAG and similar proteins were commonly discussed in the literature. These proteins can be used as biomarkers for the early diagnosis of acute kidney injury [12, 29-30]. Several studies in the literature demonstrated that urinary NAG excretion is a very sensitive parameter in determining renal tubular damage, and levels can change when exposed to hypoxic, toxic, and even radioactive substances [12, 29-31]. It has been affirmed that increased ROS levels are associated with high urinary NAG excretion and renal tubular damage [32]. In our study, we analyzed the possibility of renal tubular influence in addition to oxidative damage in the early period by evaluating urinary NAG excretion. Urinary NAG, TAS, TOS, and OSI levels did not change after the administration of DMSA radiopharmaceutical. The absence of oxidative damage in the urine samples is a consistent finding along with no increase in urinary NAG levels. It also supports the absence of oxidative damage in serum examination. Based on these results, there was no short- or long-term renal tubular damage after the first DMSA scintigraphy. This might be due to the short-acting and low-dose radiation received from Tc-99m DMSA.

There are some limitations to the study. First, the study sample size is small and had a wide age range distribution. Another limitation, due to technical reasons, we were not able to analyze the serum levels of TAS, TOS, and OSI for all children. Also, for ethical concerns we did not include a control group without previous urinary tract infection. Our study group was free of infection for at least six months but the previous infection itself may have influenced renal tubular function and possible confounding if DMSA showed renal scarring, which may reflect kidney fibrosis and/or altered cellular structure/metabolism. Other limitations were; we did not directly quantify the radiation dosage from the DMSA scan and lack of study of other potential imaging studies that might cause similar oxidative stress injury or DNA damage, like CT scans. Lastly, we did not determine the exact time of the recovery of DNA damage and study the effect of repetitive DMSA scans.

In conclusion, the mononuclear leukocyte DNA damage has increased just after the administration of Tc-99m DMSA radiopharmaceutical in children and normalized after one week. This finding suggest that DNA damage caused by radiopharmaceuticals are reversible. However, this does not exclude smaller permanent changes, which may be demonstrated by other techniques. DMSA scan was not associated with an increased oxidative stress or renal tubular damage in this study. Further researches with larger sample sizes are warranted in the future.

Acknowledgments

Not applicable

Authors' contributions

SV, FO, and ADD conceived the present idea. SV carried out the experiments and sample collection and

prepared the first draft of the manuscript. AK and EK performed the laboratory assay, and analysis AK performed the statistical analyses. MA performed nuclear medicine procedures. TI reviewed the manuscript. SV, FÖ, ADD, and NG participated in the study design, interpretation of data, drafting of the manuscript, and approved the final version of the manuscript. NG contributed to the interpretation and critically revised the text. All authors have read and approved the manuscript.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethics declarations

Informed consents were obtained from all parents. This study was approved by the Clinical Research Ethics Committee of Bezmialem Vakif University (no.71306642-050.01.04-6/10).

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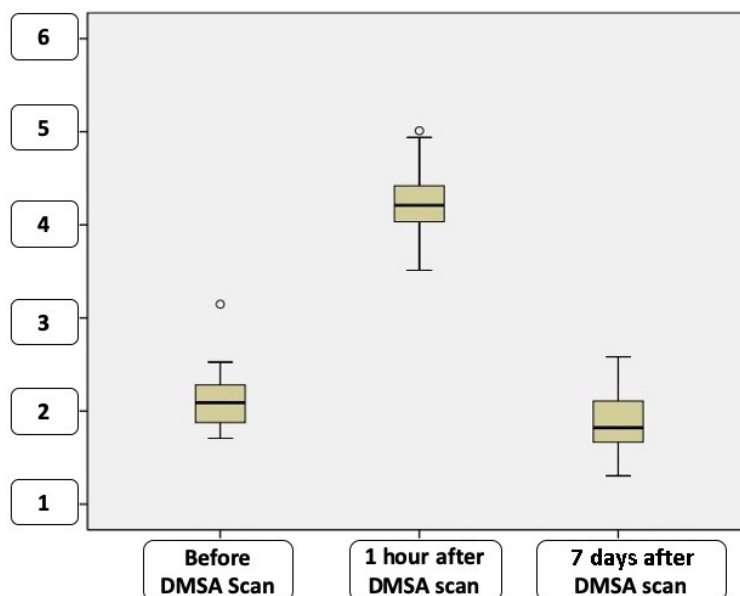
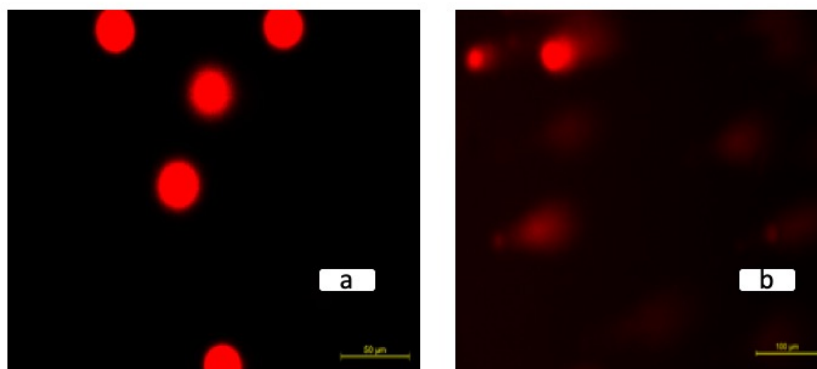
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Figure Legends

Figure 1: a) Image of normal DNA b) Image of damaged DNA

Figure 2: Comparison of DNA damage levels. DNA damage occurred just after the DMSA scan and subsequently normalized one week later.



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