Study of 99m-Tc DMSA (Dimercaptosuccinic acid) Scintigraphy in children with urinary tract infection

Shenoy B.1, Prasad M.2, Dodderi S.K.3

1Dr. Bhaskar Shenoy, 2Dr. Prasad Mruthyunjaya, Department of Pediatrics, Dr. B.R. Ambedkar Medical College, Bangalore, 3Dr. Sunil Kumar Dodderi, Department of Community Medicine, Akash Institute of Medical Sciences and Research, Centre, Bangalore.

Corresponding Author: Dr. Bhaskar Shenoy, Assistant Professor, Department of Pediatrics, Dr. B.R. Ambedkar Medical College, Kadugondanahalli, Bangalore. Email- bshenoy@gmail.com

Abstract

Introduction: DMSA scan is superior to ultrasonography in detecting cortical scars, although the later identifies dilatation of the collecting systems and renal swelling. This study was conducted to know the role of 99m-Tc Dimercaptosuccinic acids (DMSA) scan in children with UTI, its role in the early detection of renal scar.

Materials and Methods: A study was conducted among 125 children at Dr. B.R. Ambedkar Medical College, Bangalore from August 2015- October 2017. The study group included both inpatient and outpatient children with urinary tract infection. The patients with culture positive urinary tract infections during first and recurrent attacks were studied and followed from 3 months to 2 years. On follow-up 99m-Tc DMSA scan was conducted, if needed USG abdomen and MCU scan were conducted.

Results: 44% of the children showed abnormal features both by DMSA scan and USG and 21.6% of the children had normal scan but found to have abnormality in DMSA scan. On follow-up of the children, it was noticed that in 56% of the DMSA scan was normal, in 6% of children who initially had renal scar, on follow up did not have renal scar. 24% of the children persisted to have renal scar on follow up and 12% who initially had normal DMSA scan, on follow up had scar. In that 12 children with renal scar, all the children had VUR grade 4-5.

Conclusion: 99m-Tc DMSA scintigraphy is the gold standard for early identification and evaluation of acute renal inflammation and subsequent renal scarring. This study recommends both renal ultrasound scanning and 99m-Tc DMSA scintigraphy to be routinely performed in infants and children with a first febrile UTI because ultrasonography alone as low sensitivity for the detection of renal cortical abnormalities.

Keywords- DMSA Scan, USG, Urinary Tract Infection, Children

Introduction

Urinary tract infection (UTI) is a leading cause of childhood morbidity and is one of the commonest renal diseases in childhood [1-3]. UTI is the most common bacterial infection in developed countries among infants and children. The risk of renal damage secondary to UTI is highest in children below 2 years. However, it is precisely this group where diagnosis is most difficult since clinical features are often subtle and nonspecific and proper urine sample are the hardest to obtain.

The estimate of true incidence of UTI depends on rate of diagnosis and investigation. About 90% of first symptomatic UTI and recurrent infection are due to E. Coli, Proteus more common in boys. Other organism including Klebsiella, Staphylococcus epidermidis and Streptococcus and Streptococcus faecalis are occasionally responsible. Proteus and Pseudomonas are associated with recurrent UTI, instrumentation and nosocomial infection. Though, UTI may be the first symptom of obstructive uropathy or bladder dysfunction, the most common abnormality heralded by UTI is vesico-ureteral reflux [4].

Predisposition to UTI include Obstructive uropathy, stones in urinary tract, incomplete emptying of bladder with residual urine, constipation and threadworm infestation. UTI is 10 times more common in non-circumcised infants. Vesicoureteral Reflex (VUR) is an important predictor of renal damage in children. It is one of the commonest urological anomalies in Children [5]. The exact incidence of VUR is not known, because...
it is not feasible to do voiding cystourethrogram (VCUG) in a large cohort of healthy children. Its prevalence varies from 1.3% of healthy children [6] to 8-50% of children evaluated after UTI [7]. In newborn and infants, the incidence of VUR after diagnosis of UTI is 36-49% [8].

Renal scarring is associated with its fatal consequence like chronic kidney disease (CKD) in children. Children with higher grade of VUR have an increased likelihood of developing renal scarring [9]. The International reflux study reported that renal injury is more frequent in children less than two years with high grade VUR [10]. Therefore, evaluation of reflux and associated scarring is of paramount importance, particularly in younger age, who are more prone to develop such fatal consequences. Ultrasonography (USG) is the initial modality for the evaluation of post-natal hydronephrosis and UTI in children [4].

USG has been used to detect VUR successfully in high grades of VUR in several studies, but it failed to detect lower grades in many studies [11]. That is why VCUG is the primary diagnostic modality for identifying VUR. For diagnosing renal scar, Dimercaptosuccinic acid renal scan (DMSA Renal Scan) is currently the accepted gold standard [12]. Although most of the patients with UTI have a good prognosis, there is a serious complication in a group of them. In up to 40% of the cases of infection of the upper urinary tract, pyelonephritis, renal scar develops and the scarring process may occasionally lead to chronic renal insufficiency. Moreover, UTI has a high tendency of recurrence and recurrent UTI’s even increases the risk of renal scarring.

Renal scars occur in children within 1 year of their first diagnosis of UTI. In most cases, scars are noted at the time of initial assessment, suggesting high level of pre-existing scarring perhaps caused by renal scarring and acute pyelonephritis but acute pyelonephritis doesn’t necessarily need VUR for its development. The earliest detection of VUR is particularly important since the presence of severe VUR may lead to recurrent UTI. This results in parenchymal scarring leading to long term complication like hypertension and chronic renal failure.

In view of very high incidence of abnormalities of the kidney, urinary tract are associated with UTI, it is essential that imaging studies are done to exclude them. Posterior urethral valves are commonly detected in male infants with UTI. Age beyond which there is no further risk of developing first scar is uncertain. Renal cortical scintigraphy is used for the detection of the cortical defects of acute pyelonephritis and scarring related to chronic pyelonephritis.

Cortical scintigraphy is able to detect twice as many defects as intravenous and four times as many defects as intravenous urography, so 99mTc Dimercaptosuccinic acids (DMSA) scan is used for detection of renal scars and anatomic details of the kidneys. DMSA scan is superior to ultrasonography in detecting cortical scars, although the later identifies dilatation of the collecting systems and renal swelling. 99m-Tc DMSA scintigraphy is the agent of choice for planar scintigraphy or single photon emission computer tomography (SPECT).

Most of the studies on 99mTc DMSA were from western world. Thus, it was decided to study the role of 99mTc DMSA in a hospital setting in India. This study was conducted to know the role of 99m-Tc Dimercaptosuccinic acids (DMSA) scan in children with UTI, its role in the early detection of renal scar, thus preventing the complications like hypertension and chronic renal failure.

Materials and Methods

Place of study: Dr. B.R. Ambedkar Medical College, Bangalore

Type of study: A Prospective Study.

Study Period: August 2015- October 2017

Sample collection: Urine routine and culture was done by suprapubic aspiration or midstream clean catch in older children.

Inclusion Criteria: All children with 1 to 10 years of age with fever 5 days or more, with urine routine microscopy showing more than 10 pus cells/cu.mm and urine culture positive (10^5 CFU / ml).

Exclusion Criteria: Age less than 1 month, children without clear laboratory evidence, major congenital anamoly, urine culture negative.
Statistical analysis: Data was entered in MS Excel, analyzed using SPSS version 24.

Descriptive statistics (percentage, mean) were used to summarize baseline characteristics of the study subjects. Sociodemographic variables were denoted in terms of percentage. An association between two categorical variables was analysed by using Chi-square test and p<0.05 was considered to be statistically significant.

A prospective study was undertaken who were diagnosed to have urinary tract infection at Dr.B.R. Ambedkar Medical College, Bangalore between August 2015 to October 2017. Accordingly, 125 children fulfilling inclusion criteria were selected for the study. Ethical approval was obtained from Institutional Ethical committee of the Institution prior to the start of the study.

Objective: To study the 99m-Tc DMSA scintigraphy in children with urinary tract infection in Bangalore.

The study group included both inpatient and outpatient children with symptoms and signs of urinary tract infection. In children who presented with symptoms and signs of UTI, microscopic examination of urine was done. If urine microscopy showed more than 10 pus cells per high power field (HPF) in uncentrifuged sample, then urine culture was sent, and child was started on oral or intravenous antibiotics such as cephalosporin, penicillin group of drugs or aminoglycosides. Urine culture was done by suprapubic aspiration or midstream clean catch in older children. If urine culture showed more than 10⁵ colony count of organism then those children were included in the study group and after 7-10 days, ultrasonography of kidney and urinary tract and 99m Tc- DMSA scan was done , data was analyzed. According to Indian Pediatric Nephrology group criteria, in children less than 2 years of age, ultrasonography of abdomen and 99m Tc- DMSA scan was done between 7-10 days.

After prophylactic antibiotic, MCU scan was done between 4-8 weeks. 99m Tc DMSA scan was done for demonstration and monitoring of renal scarring. Based on Pediatric committee of the European Association of Nuclear Medicine guidelines, 0.5 MBq- 1.0 MBq/kilogram of DMSA isotope is given intravenously and serial images with gamma camera were taken after 2-3 hours after injection and the data was analyzed. The patients with culture positive urinary tract infections both during first and recurrent attacks were studied and followed over a period of 2 years. The children in the study group were regularly followed between 3 months to 2 years after the diagnoses of UTI and on follow-up 99m Tc DMSA scan were done, if needed USG abdomen and MCU scan were done and results were analyzed.

Results

A total of 125 children with the diagnosis of urinary tract infection constituted the study sample.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male</th>
<th>Percentage (%)</th>
<th>Female</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month – 1 year</td>
<td>31</td>
<td>40.8</td>
<td>13</td>
<td>26.5</td>
</tr>
<tr>
<td>1 -5 years</td>
<td>37</td>
<td>48.7</td>
<td>21</td>
<td>42.9</td>
</tr>
<tr>
<td>5 – 10 years</td>
<td>8</td>
<td>10.5</td>
<td>15</td>
<td>30.6</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>100</td>
<td>49</td>
<td>100</td>
</tr>
</tbody>
</table>

X² = 8.471     DF = 2     P value = 0.014
Out of 125 children studied, about 26.5% of the children were between 1 month and 1 year, 42.9% of children were between 1 year to 5 years and 30.6% were between 5 to 10 years of age. Maximum children in this study were aged between 1 year to 5 years (48.7%), followed by 1 month to 1 year of age. The sex-wise distribution has shown that 60.8% of children were males and 39.2% were females. Male to female ratio was 1.5:1. This shows the prevalence of UTI is more in the male than in the female children. The prevalence of UTI was more in the age group of 1 month to 1 year in boys. The results also show that prevalence of UTI (41%) was more in the boys below 1 year of age and the prevalence is same both in the boys and the girls (48%) between 1-5 years and after 5 years, girls (30%) are more commonly affected. The chi square test is 8.47 and p-value is 0.014. There was a statistically significant difference between the sex and age group since “p” value is significant.

Table-2: Comparison of ultrasonography versus 99m Tc DMSA scan of the study group.

<table>
<thead>
<tr>
<th>Imaging</th>
<th>Abnormal n (%)</th>
<th>Normal n (%)</th>
<th>X²= 26.51</th>
<th>p Value = 0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal</td>
<td>55 (44)</td>
<td>27 (21.6)</td>
<td></td>
<td>(Significant)</td>
</tr>
<tr>
<td>Normal</td>
<td>8 (6.4)</td>
<td>35 (28)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Out of 125 children in this study, 44% of the children showed abnormal features both by DMSA scan and ultrasonography and 21.6% of the children having normal scan found to have abnormality in DMSA scan. There was a statistically significant difference between the two modalities of diagnosis. Compared to DMSA scan the sensitivity of ultrasonography was 87.30%, the specificity was 56.45%. The abnormal findings on USG were hydronephrosis, ureterocele, Hydroureteronephrosis, Pelviectasia, Smaller and shrunken kidney and bladder wall thickening. This shows that USG abdomen detects structural abnormality of kidney than renal scars.

Table-3: Renal scarring following UTI.

<table>
<thead>
<tr>
<th>DMSA scan</th>
<th>First UTI</th>
<th>Recurrent UTI</th>
<th>Total</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Scar</td>
<td>58 (46.4)</td>
<td>28 (22.4)</td>
<td>86 (68.8)</td>
<td>x²=3.98</td>
</tr>
<tr>
<td>Renal Scar</td>
<td>19 (15.2)</td>
<td>20 (16.0)</td>
<td>39 (31.2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>77 (61.6)</td>
<td>48 (38.4)</td>
<td>125 (100)</td>
<td></td>
</tr>
</tbody>
</table>

A total 61.6% of the children had UTI for the first time and 38.4% had more than one episode of UTI in the past. The prevalence of renal scar in our study was 31.2%. The prevalence of renal scar after recurrent attacks of UTI are 41.6% and after first UTI is 24.7%. Of the study group, 46% of children after first attack of UTI did not have renal scar. However, this difference was statistically significant.

Table-4: Distribution of study group showing relationship of UTI and VUR

<table>
<thead>
<tr>
<th>VUR</th>
<th>First UTI</th>
<th>Recurrent UTI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflux</td>
<td>28 (31.8)</td>
<td>32 (36.4)</td>
<td>X²=6.21</td>
</tr>
<tr>
<td>No Reflux</td>
<td>21 (23.9)</td>
<td>7 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49 (55.7)</td>
<td>39 (44.3)</td>
<td></td>
</tr>
</tbody>
</table>

Among the study group 70% of the children underwent MCU scan, of them 31.8% of the children had reflux after first attack of UTI and 36.4% children had reflux after recurrent UTI. The difference between the reflux and occurrence of UTI was statistically significant.

Table-5: Comparing the risk of VUR and renal scar.

<table>
<thead>
<tr>
<th>DMSA Scan</th>
<th>Grade 1 n (%)</th>
<th>Grade 2 n (%)</th>
<th>Grade 3 n (%)</th>
<th>Grade 4 n (%)</th>
<th>Grade 5 n (%)</th>
<th>No Reflux n (%)</th>
<th>Renal Units n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scar</td>
<td>2 (18.2)</td>
<td>10 (34.5)</td>
<td>22 (66.7)</td>
<td>11 (33.3)</td>
<td>6 (18.2)</td>
<td>15 (25)</td>
<td>79 (48)</td>
</tr>
<tr>
<td>No Scar</td>
<td>9 (81.8)</td>
<td>19 (65.5)</td>
<td>11 (33.3)</td>
<td>1 (8.3)</td>
<td>5 (15.8)</td>
<td>45 (75)</td>
<td>84 (52)</td>
</tr>
</tbody>
</table>

X² = 44.597

p VALUE = 0.0001
The MCU scan conducted upon 88 children in the study group and 68% had VUR. VUR grading was done and results were analyzed. 37% of the children had no reflux, 7% of the children had grade 1, 18% had grade 2, 20% of the children had grade 3, 7% children had grade 4 and 11% of the children had grade 5 VUR. The statistical significance between the grading of VUR and Scarring was significant.

Table-6: Relationship between VUR and pathological DMSA scan.

<table>
<thead>
<tr>
<th>DMSA Scan</th>
<th>VUR (%)</th>
<th>No VUR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal</td>
<td>26 (84)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Normal</td>
<td>27 (47)</td>
<td>30 (53)</td>
</tr>
</tbody>
</table>

$\chi^2 = 11.2 \quad p \text{ VALUE} = 0.001$

Of the study group, 84% of the children with VUR had renal scarring and 16% of the children with renal scarring did not have VUR. There was a statistically significant difference between the pathological DMSA scan and presence of VUR. Asymptomatic VUR was present in 47% of children.

Table-7: Outcome of Renal scar on follow-up

<table>
<thead>
<tr>
<th>Number (n =125)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Scar on Follow Up</td>
<td>8</td>
</tr>
<tr>
<td>Normal</td>
<td>71</td>
</tr>
<tr>
<td>New Scar on Follow Up</td>
<td>15</td>
</tr>
<tr>
<td>Persistent Scar</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
</tr>
</tbody>
</table>

On follow-up of the children, the author noticed that in 56% of the DMSA scan was normal, in 6% of children who initially had renal scar, on follow up did not have renal scar. 24% of the children persisted to have renal scar on follow up and 12% who initially had normal DMSA scan, on follow up had scar. In those12 children with renal scar, all the children had VUR grade 4-5.

Discussion

This study was conducted with the aim of studying the role DMSA scan in the Urinary tract infections in children which are most frequent infections. At about 8% of girls and 2% of boys will have a urinary tract infection (UTI) in childhood and between 30% and 40% will have another episode within 2 years. Boys are more susceptible to urinary tract infection (UTI) than girls before the age of 6 months; thereafter, the incidence is substantially higher in girls than in Boys [13].

An estimate of the true incidence of UTI depends upon the rates of diagnosis and investigation. Observational studies have found that UTIs have been diagnosed in Sweden in at least 2.2% of boys and 2.1% of girls by the age of 2 years in 7.8% of girls and 1.7% of boys by the age of 7 years [13] and in the UK in 11.3% of girls and 3.6% of boys by the age of 16 years [14].

In India, the risk of developing symptomatic UTI before 14 years is 1 to 2% in boys and 3- 8% in the girls. The incidence of UTI in the term neonates is approximately 1 % and the preterm 3%[15].

DMSA scintigraphy is currently considered as the imaging agent of choice for estimating the presence and extension of acute parenchymal changes as well as the development of permanent renal scarring. Acute pyelonephritis may resolve completely, and the scar may return to normal within 3 months or it may become into a permanent damage or scar formation. The ultimate goal of treatment for UTI in children is prevention or reducing the morbidity and long term clinical sequelae of renal scarring including hypertension, proteinuria and chronic renal failure. This 2 years study of 99m- Tc DMSA scan in children with UTI has made an effort to show that early detection and treatment of UTI as lead to decreased incidence of renal scarring. UTI is common in childhood. In our study, 36.8% children were between the age group of 1 month to 1 year, 44.8% were in the age group of 1year to 5 years and 18.4% were in the age group of 5year to 10 years. This study corroborates with findings of other studies conducted by Manish D. Simha et al at District General Hospital [16] and B.R. Nammalvar and Vijaykumar et al. [17].
A slight male preponderance of UTI in this study was also found by another study done by Dr. C.B. Fong and Wong in Hong Kong [18]. The results from several studies of acute pyelonephritis using DMSA scintigraphy reveal that 50% to 90% of children with febrile UTI have abnormal DMSA renal scan findings. In our study group, 31.2% had features of renal scars on DMSA scan of which only 64% had renal scarring on USG. In a study by Nammalvar et al it was shown that USG was normal in half the number of kidneys with abnormal DMSA and indicated that USG is a poor investigation to diagnose APN [17]. A study in the UK has shown that 78% of girls and 71% of boys presenting with UTI within the first year of life experienced recurrence, and that 45% of girls and 39% of boys presenting after their first year of life developed further infections [14]. A systematic review of imaging in childhood UTI suggested that renal scarring occurs in 5–15% of children within 1–2 years of their first diagnosed UTI. Between 32–70% of these scars were noted at the time of initial assessment, suggesting a high level of pre-existing scarring, perhaps caused by previously unrecognized infection.

99m Tc DMSA scan was normal in 65% of the children and showed scarring in 31.2%. The occurrence of renal scarring following UTI in our study is 31.2% which is lower than other studies. Nammalvar et al showed the occurrence of renal scarring of 35.3% in children with UTI[17]. Hewitt et al found the occurrence of renal scarring to the extent of 31%[19]. Howard et al study on Chinese children showed occurrence of renal scarring of 23%[20]. The occurrence of renal scarring after first episode of UTI is 24.7% and after recurrent attack of UTI is 41.6%. Nammalvar et al showed the prevalence of renal scarring of 35.3% in children with first episode of UTI and 70% in children with recurrent UTI [17]. The prevalence of scarring was more in the children older than 1 year (74%) than in the infants less than 1 year of age group (26%). Benador et al, in a randomized controlled trial and showed prevalence of renal scar is 24% in children less than 1 year and 42% in children older than 1 year [21].

Out of 29 children with VUR, 26 children (89.6%) had DMSA finding of APN. Nammalvar studies showed 96.6% had DMSA finding of APN (17). The possibility of VUR is high if there is abnormal DMSA scan and absence of VUR does not rule out UTI. The association of UTI, as suggested by DMSA scan and the presence of VUR can be value to prognosticate for subsequent scarring. The prevalence of renal scarring with VUR has been reported to vary from 23- 62%. The prevalence of VUR was higher in our study, probably because ours being a referral hospital MCU scan is done for most patients, so VUR is picked up early. The correlation between reflux and scarring in our study was higher in patients with higher grades (4-5) VUR when compared with lower grades (1-3). This is in accordance with the finding that the risk of scarring is higher in patients with major degrees of reflux who develop UTI, when compared with lower grades.

The renal scarring was present in 31% of the cases in our study and the results was similar to the study done by Fong and Wong in Hong Kong children (18). The occurrence was lower than that of Nammalvar study probably due to early identification of UTI and treating it appropriately.

Our study showed that children with grade 4-5 VUR went for renal scarring in 91%. Filho et al in Brazil over a period of 30 years following UTI, showed prevalence of renal scarring of 19.2% at the time of diagnosis. The renal scarring was significantly more frequent in children with VUR grade 4 and 5 (48.6% and 87% respectively)[22].

Thus, the presence of VUR and acute pyelonephritis is necessary to prognosticate the risk of scarring would help in planning for the prevention of parenchymal damage in terms of efficient control of infection, prevention of progression of parenchymal damage in terms of efficient control of infection, prevention of relapses and correction of VUR. The management strategy for children with VUR has been the avoidance of UTI induced damage by surgical correction of VUR or long-term antibiotic prophylaxis or both.

Most patients are managed conservatively on long-term antibiotic prophylaxis until spontaneous resolution of VUR. The main drugs used were Nitrofurantoin, Nalidixic acid, Cephalexin and Cotrimoxazole. No patients in the study group had any major adverse reactions to drugs e.g. hypersensitivity, peripheral neuropathy or benign intracranial hypertension, but a few experienced minor side effects like anorexia and nausea.

A systemic review of trials comparing long-term prophylaxis antibiotic use with placebo does not show any major side effects. Studies have shown that reflux disappears without any permanent renal impairment in a vast number of children on prophylaxis. In a cohort study by Conway et al involving 611 children who had first episode of UTI, antimicrobial prophylaxis was not associated with decreased risk of recurrent UTI (HR, 1.01;95% CI, 0.50- 2.02) [23].
Similar conclusion has been drawn by Garin et al, where in 218 children with pyelonephritis were randomized to receive antibiotic prophylaxis or not [24]. The distribution of reflux was similar. Follow up after one year revealed that there were no statistically significant differences among the groups with respect to rate or type of UTI recurrence or development of renal parenchymal scars.

**Conclusion**

This study provides a useful data on childhood UTI in terms of demographical data, imaging abnormality and confirms the importance of 99m-Tc DMSA scintigraphy in the assessment of UTI.

UTI is a common infection in childhood and UTI must be highly suspected in children with unexplained fever. The prevalence of urinary tract infection is common in the boys of less than 1 year of age and in girls of more than 5 years of age which is similar to other Indian studies. 99m-Tc DMSA scintigraphy is the gold standard for early identification and evaluation of acute renal inflammation and subsequent renal scarring.

Almost all in these study group children have undergone ultrasonography, MCU and DMSA scans, so our data provides more accurate information on renal scarring: this study shows that renal scarring after acute pyelonephritis is considerably more common than was thought previously.

This study recommends both renal ultrasound scanning and 99m-Tc DMSA scintigraphy to be routinely performed in infants and children with a first febrile UTI because ultrasonography alone as low sensitivity for the detection of renal cortical abnormalities.

A high degree of suspicion, Early detection and treatment, Identification of risk factors and Prevention of recurrences are important to prevent permanent renal damage in children with UTI.

**Value edition of the study**- This study recommends both renal ultrasound scanning and 99m-Tc DMSA scintigraphy to be routinely performed in infants and children with a first febrile UTI because ultrasonography alone has low sensitivity for the detection of renal cortical abnormalities.

**Authors Contribution:** Concept, design and drafting the Manuscript: Bhaskar Shenoy and Sunil Kumar Dodderi, Datacollection: Bhaskar Shenoy, Analysis: Sunil Kumar Dodderi, Critical review: Prasad Mruthyunjaya. All authors have read and approved the final version.

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**References**


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