Objective: To evaluate the role of mean density value in computed tomography (CT) and grading of twinkling artifact in colour Doppler analysis in urolithiasis.

Material and Methods: A total of 320 patients who had undergone both abdominal non-contrast CT and colour Doppler ultrasonography examinations were included in the study and the correlations between calculated mean density values and twinkling artifact grading were investigated for each calculus.

Results: 689 calculi were detected by non-contrast CT, in which 407 (59%) were in the kidneys, 263 in ureter (38.1%), and 19 (2.7%) were in bladder. The density of calculi on CT with HU values are categorized into groups: Less than 200 HU consists of 80 calculi, 201-500 HU consists of 316 calculi, 501-1000 HU consists of 153 calculi, and more than 1000 HU consists of 140 calculi. Among the calculi with HU value less than 200, 46% no twinkling artifact (Grade 0) seen, with HU value more than 1000, 70.9% with grade 3 twinkling artifact. The calculi with HU 201-500, majority showed grade 1 and 2 twinkling artifact. Among the calculi with HU value ranging between 501-1000, all grades of twinkling artifact are almost equally seen.

Conclusion: Twinkling artifact is not constantly seen in urolithiasis. High HU value calculi showed higher grading of Twinkling artifact. Twinkling artifact can help in predicting the calculi with higher HU values which in turn is helps in segregation of patients for medical and surgical management.
INTRODUCTION:
In India, kidney stone disease is prevalent, with about 12% of the total population reported to be prone to urolithiasis [1]. Of this 12%, 50% of the population is severely affected by renal damage, which even leads to decrease in renal function [1]. Urolithiasis can have varied clinical manifestations, depending on their position, size, surface, and number. Clinical manifestations vary from typical renal colic with acute lumbar pain to asymptomatic cases with unexplained recurrent urinary infections [2]. Therefore, their identification is mandatory in order to confirm the diagnosis and for proper treatment. Ultrasound (US) is an inexpensive, non-radiation, non-invasive primary imaging modality. Multiple imaging modalities are available, but widespread clinical use is currently limited to CT, ultrasonography, and kidney ureter bladder (KUB) plain film radiography. On plain ultrasound the sensitivity to detect calculi mainly in the kidney is difficult owing to the echogenicity of the renal sinus fat. On application of the colour doppler the twinkling artifact can be used to detect the occult calculi in the kidneys. The twinkling artifact (TwA) is a complex phenomenon, where an intense colour signal produced by calcifications in different organs [3-4]. The artifact persists even after increasing pulse repetition frequency (PRF) to higher levels. In pulsed wave (PW) doppler, only linear lines with a disturbing audio-signal are seen without flow. The use of helical non-contrast computed tomography (CT) in patients with urolithiasis has increased. Hounsfield units (HU) is a parameter generated from CT, which is related to density of the calculus. Hounsfield units are named after Sir Godfrey Newbold Hounsfield the inventor. HU value corresponds to the amount of X-rays that pass through the structure and can be measured. When the Hounsfield unit of water is defined as 0, fat has a negative HU, and blood and other tissues have a positive HU. It is possible to differentiate 256 shades of gray which are indistinguishable to the naked eye [5]. HU is also used to assess the CT density of urolithiasis. Hounsfield unit has become an important diagnostic tool, for predicting the type of stone and also for determining the appropriate mode of treatment. In the this study, we evaluated the role of mean density value (HU) in CT imaging and grading of twinkling artifact seen in colour Doppler of urolithiasis. Also the presence of any correlation between stone density and intensity of the twinkling artifact has been evaluated.

MATERIAL AND METHODS
Ethical committee approval - Institutional Review Board approval is obtained. This is a retrospective study so written informed consent forms from the patients were not taken.

STUDY POPULATION:
Patient data is collected from the Department of the Radio-Diagnosis, Rajarajeswari Medical college and hospital, Bengaluru between April 2021 to March 2022. Total 320 patients with 173 males and 147 females are included in this study.

INCLUSION CRITERIA:
Patients with urolithiasis who underwent both Noncontrast CT and Ultrasound of abdomen and pelvis.

EXCLUSION CRITERIA:
- Renal calculi less than 3 mm and staghorn calculus are not included in this study.
- If the interval between Ultrasound and CT is more than 2 weeks.
- Patients with double J stent.

STUDY DESIGN: This is a Cross-sectional Retrospective study performed at our institution. Among the USG performed in the department, reports describing the renal calculus and presence of renal twinkling artifact between the study periods were indentified. Patients who did not undergo non-contrast spiral computed tomography within 2 weeks after sonography were excluded. Ultrasound of the abdomen and pelvis were performed using SAMSUNG RS 80 equipped with a 2–5 MHz convex probe. Both gray scale and colour Doppler ultrasonography were performed in all patients. Ultrasound examinations are reviewed for location and size of renal calculus with twinkling artifacts were documented.
Signal intensities of the twinkling artifacts were classified as follows:  
Grade 0: twinkling artifact not observed,  
Grade 1: focal and hardly observed twinkling artifact,  
Grade 2: strong signal intensity observed on only some part,  
Grade 3: all over the stone.

Non-contrast spiral CT examination was performed with SIEMENS 128-slice multi-detector row CT scanner, from the upper poles of the kidneys down to the base of the urinary bladder, with the following parameters: 5mm collimation, 120 kV, 200 mAs with reconstruction at 3 mm intervals. Patients should have a full bladder for the examination by drinking about one liter of water over a period of 60 min before the scheduled exam. Image analysis was performed at a workstation with reconstruction processing. All examinations were non-enhanced and no intravenous contrast was administered. Sonographic findings were correlated with non-contrast CT for Urolithiasis and other causes of twinkling artifact. The location, size and HU of each calculus is documented on CT.

For each calculus mean (± SD) density values were measured in HU. Density measurements were measured in conventional soft tissue window using region of interest (ROI). The entire calculus were included in axial plane in the ROI without extending ROI into the surrounding soft tissue. Calculi smaller than 3 mm and staghorn calculus were not included in the study so as to avoid any erroneous density measurements. Calculi were categorized based on their locations as renal, ureteral, and bladder calculi.

### RESULTS

A total of 320 patients were examined in the present study which include 173 (54%) males and 147 (46%) females. Each patient had at least one calculus in the kidney, ureter or bladder. The age range was 21–69 years. The detected stones measured in length from 4 to 20 mm in their long axis in CT images. The stones were categorized into groups based on density values consisting of less than 200 Hounsfield units (HU), 201 to 500 (HU), 501 to 1000, more than 1000 HU. 689 stones were detected by non-contrast spiral CT, in which 407 (59%) were in the kidneys, 263 in ureter (38.1%), and 19 (2.7%) were in the bladder.

<table>
<thead>
<tr>
<th>Table I: Location of calculus and their numbers.</th>
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<tbody>
<tr>
<td>Location of calculus</td>
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<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Renal</td>
</tr>
<tr>
<td>Ureteric</td>
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<tr>
<td>Urinary bladder</td>
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<td>Total</td>
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Of the total 689 calculi identified in 320 patients, 589 calculi had a positive twinkling artifact in ultrasound while the remaining 100 had no artifact seen (Grade 0). Among 589 calculi Grade 1 was seen in 136 calculi, Grade 2 in 343 and Grade 3 in 110 calculi. So in our study most of the calculi were exhibiting Grade 2 twinkling artifact i.e. strong signal intensity in some part of calculi. Location wise majority are renal calculi.

<table>
<thead>
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<th>Table II: Location of the urinary calculi with grading of twinkling artifact</th>
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<tbody>
<tr>
<td>Grade of twinkling artifact</td>
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<tr>
<td>Renal</td>
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<tr>
<td>Ureteric</td>
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<tr>
<td>Urinary bladder</td>
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<tr>
<td>Total</td>
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</tbody>
</table>
The density of calculi on CT with HU values are categorized into groups: Less than 200 HU consists of 80 calculi, 201-500 HU consists of 316 calculi, 501-1000 HU consists of 153 calculi, more than 1000 HU consists of 140 calculi.

Table III: Calculi with HU values and grading of twinkling artifact

<table>
<thead>
<tr>
<th>Grade of twinkling artifact</th>
<th>CT HU of the calculus</th>
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<tbody>
<tr>
<td></td>
<td>Less than 200</td>
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<tr>
<td>Grade 0</td>
<td>46</td>
</tr>
<tr>
<td>Grade 1</td>
<td>4</td>
</tr>
<tr>
<td>Grade 2</td>
<td>27</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
</tr>
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</table>

In our study, majority of calculi showed HU values of 201 to 500 followed by 501 to 1000, then more than 1000 and least calculi showed HU less than 200. Among the calculi with HU value less than 200, approximately 46% didn’t show twinkling artifact (Grade 0). Among the calculi with HU value more than 1000, approximately 70.9% showed grade 3 twinkling artifact. The calculi with HU value between 201-500, majority showed grade 1 and 2 twinkling artifact. Among the calculi with HU value ranging between 501-1000, all grades of twinkling artifact are almost equally seen.
Figure I (A, B): US (grey scale and colour doppler) as well as non-contrast CT showing calculus in the right vesico-ureteric junction which shows grade 0 twinkling artifact on doppler with mean HU of 199.

Figure II (A, B): US (grey scale and colour doppler) showing calculus in the distal left ureter with grade 1 twinkling artifact. Same patient CT performed one day later showed calculus in left vesico-ureteric junction with mean HU of 770.
Figure III (A, B): US (grey scale and colour doppler) as well as non-contrast CT showing calculus in the left renal pelvis which shows grade 2 twinkling artifact on doppler with mean HU of 611.
Figure IV (A, B): US (grey scale and colour doppler) as well as non-contrast CT showing calculus in the right proximal ureter which shows grade 3 twinkling artifact on doppler with mean HU of 327.

DISCUSSION

Ultrasound of the kidneys and bladder reliably characterizes hydronephrosis and does not involve ionizing radiation. It is the preferred initial imaging modality in these patients especially presented in emergency department with acute flank pain. Both CT and ultrasound can be used to evaluate urinary tract calculi. Computed tomography is considered as reference standard, but ultrasound has lower cost than CT, can be performed portably and no exposure to ionizing radiation. One of the major limitations of gray-scale ultrasound for identifying renal calculi is the presence of small echogenic areas at tissue interfaces without posterior acoustic shadowing. These interfaces may be false-positively misinterpreted as renal calculi or may lead to obscuration of visualization of small nearby calculi, which in turn leads to false-negative results. In such patients twinkling artifact helps in identifying calculi without shadowing.

Previous studies stated that the twinkling artifact depends on many factors like machine settings, biochemical composition and surface of the calculi. Few studies have been carried out regarding the dimensions of the renal calculi and they included a small number of patients [6].

The twinkling artifact is a complex phenomenon was first described by Rahmouni et al in 1996, which consists of an intense alternating colour signal between red and blue behind some structures [6].
There are two theories proposed to explain the twinkling artifact. The first one was described by Rahmouni [6] et al., which states that this artifact is due to strongly reflecting medium with a rough interface. They concluded that, when an incidental ultrasound beam is reflected by a flat interface, the sound waves are reflected which results in the production of short-wave sound signals. With reflection on a rough interface, the acoustic wave is split into a complex beam pattern caused by multiple reflections in the medium in turn resulting in prolonged pulse duration of the transmitted sound signal and the Doppler interpret this result as movement and thus assign it different colours.

Also, Hirsh et al. performed several experiments, which explained the twinkling artifact behind granular structures such as sodium chloride, iron filings, emery paper and ground chalk [7]. The second theory was described by Kamaya et al., who states that the twinkling artifact is caused by a narrow band, intrinsic sonographic machine noise called as phase or clock jitter, which may be generated by slight random time fluctuations in the path lengths of both transmitted and reflected acoustic sound waves [8].

However, the most accepted one is the presence of a narrow-band noise due to fluctuations in the circuits of Doppler Ultrasound equipment is the underlying cause of this sign. It is mainly seen on rough, hyperechoic, irregular surfaces with multiple cracks causing strong reflection of the incident ultrasound waves and multiple internal reflections which widen the spectrum. The appearance of the Twinkling artifact is correlated with the roughness of the calculi so greater the surface roughness, the greater the artifact [9]. This can correlate why in 100 cases we did not find the twinkling artifact may be due to smooth, regular interface of the stones surface. The twinkling artifact is dependent on the machine parameters and the Ultrasound frequency; hence, the same settings were used in all cases [10]. One of them which may impact the twinkling artifact is the location of the focal zone: focal zone seen below a rough reflecting surface, the twinkling artifact seen more obvious comparing with the position of the focal zone above.

In one of the in vitro study Shabana et al., describes that renal calculi associated with the twinkling artifact which demonstrated high contrast-to-noise ratio when compared with gray-scale posterior acoustic shadowing [11]. Few other authors have described use of twinkling artifact should increase the detection of renal calculi in comparison with only gray-scale ultrasound. In practice sometimes becomes difficult in detecting stones with gray-scale imaging because of the bowel gas obscuring the field of view. But the twinkling artifact accelerates stone detection and presence of artifact strongly aids in diagnosis of ureteral stone. Twinkling artifact can be regarded as a significant parameter for urolithiasis [12] and a major diagnostic sonographic finding [13]. Especially twinkling artifact seen in colour doppler ultrasound is preferable for the sensitive detection of very small nephrolithiasis [14-15].

The appearance of the twinkling artifact depends on the hardness of the stone. The harder the stone, the larger the twinkling artifact will be. [6] In the study Gliga et al. [16] also credited the lack of artifacts in 10 cases in their study due to the smooth surface of the stone similar to in our study in which 100 calculi showed no twinkling artifact. Understanding the composition of urinary system stones is important for determining the choice of mode of treatment. Previously, in one of the in vitro study, Hassani et al[17] studied both density value of the stone in Hounsfield Units (HU) using non-contrast helical CT with twinkling artifact seen on colour Doppler ultrasound, and evaluated the predictive value of combined use of both imaging techniques in the determination of the mineral composition of urinary stones.

They concluded that the HU was a predictive factor of the composition of all types of calculi - calcium oxalate monohydrate calculi, calcium oxalate dihydrate calculi, uric acid calculi, calcium phosphate calculi and cystine calculi. Twinkling artifact will not help to differentiate between calcium and non-calcium calculi. The absence of a twinkling artifact is a prognostic factor for the presence of calcium oxalate monohydrate stones and so the association of non-contrast CT and Doppler enables the precise classification of the five types of stones in vitro.
Chelfouh N et al [15] found calculi of calcium oxalate dehydrate and calcium phosphate always produced a grade I or grade 2 twinkling artifact. Absence of artifact grade 0 was noted only for calcium oxalate monohydrate and urate stones. Though in our study the chemical composition of the calculi was not evaluated we observed that calculi with higher HU values showed high grade of twinkling artifact. Our study has few limitations, First it is a retrospective study. Second, since the upper limit for interval gap between US and CT is 2 weeks there might be change in location of calculi within the collecting system. Third is we did not correlate the grading of twinkling artifact with CT HU and chemical composition of calculi.

CONCLUSION
Twinkling artifact is not constantly seen in urolithiasis. In our study majority of the high HU value calculi showed higher grading of Twinkling artifact. HU value of calculus is one of the important parameter in treatment of urolithiasis. Hence in non-availability of CT or in contra-indicated patients, B-mode ultrasound with grading of twinkling artifact can help in predicting the calculi with higher HU values which in turn is helps in segregation of patients for medical and surgical management.

REFERENCES
