

Original Research Article

COMPARATIVE STUDY OF ULTRASONOGRAPHY AND CT SCAN IMAGING IN THE DIAGNOSIS OF ACUTE APPENDICITIS

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ABSTRACT

Background: Acute appendicitis is one of the most common causes of acute abdominal pain requiring surgical intervention. Accurate and timely diagnosis is crucial to reduce complications associated with delayed or unnecessary surgery. Although appendicitis is usually diagnosed on the basis of clinical findings and scoring system such as Alvarado score, as many as 45 percent of patients do not display classic signs of acute appendicitis, making imaging modalities like USG and CT scan a potentially useful tool when the diagnosis is unclear. This study was undertaken to compare the diagnostic accuracy of USG and CT scan in the diagnosis of acute appendicitis. **Objective:** To compare the diagnostic accuracy of graded compression ultrasonography and computed tomography in the diagnosis of clinically suspected acute appendicitis using histopathological findings as the reference standard.

Materials and Methods: This is a cross-sectional comparative study conducted among 100 patients clinically suspected of having acute appendicitis at JNIMS, hospital during the period from august 2022 to July 2024. All eligible patients underwent abdominal ultrasonography and CT scan. The imaging findings were assessed for features suggestive of acute appendicitis. The imaging diagnoses were compared with the final diagnosis based on histopathological findings. Sensitivity, specificity, predictive values and diagnostic accuracy were calculated for both modalities.

Results: Among the 100 patients included in the study, histopathological examination confirmed acute appendicitis in 64.7% patients diagnosed as acute appendicitis in USG and 100% in CT scan. USG as compared to CT scan had sensitivity of 63.5%, specificity of 62.2% and diagnostic accuracy of 63%. CT scan showed higher diagnostic accuracy compared with ultrasonography, particularly in patients with equivocal or atypical clinical presentations.

Conclusion: Both USG and CT scan are useful imaging modalities in the evaluation of suspected acute appendicitis. CT scan demonstrated superior diagnostic performance in this study, while ultrasonography remains a valuable, readily available, and radiation-free initial imaging modality. The choice of imaging technique should be guided by clinical presentation, patient characteristics, availability and the need to minimize radiation exposure. Although CT scan is more accurate and sensitive than USG for diagnosing acute appendicitis, USG can still be used as the primary imaging modality due to its high specificity and excellent positive predictive value. In turn, CT scan may be reserved for the patients who have a clinical diagnosis of acute appendicitis and negative USG results. This approach will be cost effective, safe and may reduce the radiation exposure to ~50% of patients presenting with acute appendicitis.

Keywords: Acute appendicitis, Computed tomography, Diagnostic accuracy, Ultrasonography.

INTRODUCTION

Although acute appendicitis is usually diagnosed on the basis of clinical findings and scoring system such as Alvarado score, as many as 45 percent of patients do not display classic signs of acute appendicitis,^[1] making imaging modalities like USG and CT scan a potentially useful tool when the diagnosis is unclear. Despite being a common clinical problem and the recent advances in the diagnostic imaging including high-resolution USG, CT scan and MRI, diagnosis of acute appendicitis is not always easy and can be challenging even for the best of clinicians. In a healthy young man with right lower quadrant pain, “negative appendicectomy” rate is less than 10 percent, however misdiagnosis is maximal in women of childbearing age with negative appendicectomy reaching up-to 20 percent because of frequent overlap with gynecological conditions that may mimic acute appendicitis clinically.^[1,2] Laboratory investigations such as total leukocyte count, differential leukocyte count, C-reactive protein, etc., though sensitive, lack sufficient specificity.

Although the accuracy of USG for diagnosing appendicitis is considered the same as that of CT scan when done by an experienced radiologist, of late, CT scan has been found to be more accurate than USG. For this purpose, CT scan is being widely advocated and used. In the absence of a clear-cut and well-established guideline for the workup of suspected acute appendicitis, today's clinician is bound to face a dilemma of choosing the most appropriate investigative protocol for successful management of it.^[2] USG with graded compression as popularized by Puylaert, has long been considered as a very useful modality for diagnosing AA.^[3]

Imaging modalities like ultrasonography and CT scan play an adjunctive role in improving the diagnostic accuracy of acute appendicitis. Nevertheless, definite diagnosis can be made only after operation and by histopathology examination of the specimen.

Sonographic findings include visualisation of an inflamed, enlarged, blind ending, non-compressible, aperistaltic appendix and appendicolith(fig.1). Other secondary signs include mesenteric fat stranding, distal inflammation, fluid filled lumen, peri-appendiceal fluid collection.^[4] CT findings include classic patterns such as concentric thickened appendiceal wall, an appendicolith, fat stranding or other signs of inflammation(fig.2). A phlegmon, abscess, or free air can also be suggestive of appendicitis.

Because both the modalities have advantages and limitations, comparing their diagnostic performance is clinically relevant. Hence, the purposes of this

study were to calculate sensitivity, specificity, positive predictive value and negative predictive value and to compare and justify the utility of USG as a primary imaging modality in acute appendicitis and to establish the role of CT scan as a second-line/problem-solving modality.

Objectives

The present study was done to compare the diagnostic accuracy of graded compression USG and CT in the diagnosis of clinically suspected acute appendicitis of patients attending to JNIMS Hospital and to compare USG and CT findings with intraoperative and histopathological findings of acute appendicitis.

MATERIALS AND METHODS

A hospital-based, cross-sectional study was conducted in the Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur during the period from August 2022 to July 2024. The study participants were patients attending to JNIMS Hospital with clinical suspicion of acute appendicitis. All patients irrespective of age and gender admitted in JNIMS Hospital with clinical suspicion of acute appendicitis during the whole study-period and who underwent conservative management or appendicectomy with preoperative imaging were included. Patients not willing to participate in the study and pregnant women presenting with clinical suspicion of acute appendicitis were excluded.

Participants underwent USG and CT scan by using Phillips clearvue 350 USG Machine and Toshiba Aquilion 64 CT scanner, model TSX-101A, Japan before they were subjected for further management. The findings were recorded in a pre-tested proforma. Later on, the same findings were compared with the HPE reports. All data were entered in Microsoft excel. Analysis was done using SPSSv21 (IBM Corp., Armonk, New York, USA). Descriptive and analytical statistics were done wherever required. P value < 0.05 was considered as statistically significant.

RESULTS

Completed data sets could be collected from 100 study participants. There was no refusal encountered. The mean (SD) age was 32.5 (17.72) years. It ranged from five years to 74 years. A majority (23%) of them belonged to the age-group of 21-30 years. More than half (58%) were females. Most of them (60%) had an average body habitus while the remaining 40% were either slim or obese.

Table 1: Distribution of cases according to USG findings

USG findings		Frequency (n=100)	Percentage
Findings of appendicitis	No	46	46.0
	Yes	54	54.0

Compressibility of appendix	Non compressible	54	54.0
	NA	46	46.0
Diameter (mm)	Mean (SD)	8.78 (2.81)	
Peristalsis	Aperistaltic	54	54.0
	NA	46	46.0
Blind ending	Yes	54	54.0
	NA	46	46.0

USG findings were suggestive/equivocal of appendicitis in 54% cases. Mean (SD) diameter of appendix was 8.78 (2.81) mm. 46% cases were suboptimal/not visualized on USG. (Table 1)

Majority (44%) cases had secondary findings on USG, most common being mesenteric nodes (7%), followed by conglomerated mass (6%), peri appendiceal collection (5%), peri appendiceal free fluid (4%) and excessive bowel gases (3%). Other secondary findings were appendicolith, fat stranding in right iliac fossa, inflamed bowel within bowel structure, intussusception, multiple ileocecal wall cystic lesion, peri appendiceal free fluid with

appendicolith, peri-caecal fat stranding, pelvic inflammatory disease and right renal calculus in 1% cases each.

Regarding diagnosis, USG was suggestive/equivocal of acute appendicitis in 31% cases, followed by ruptured appendicitis (7%), appendicular lump (6%), subacute appendicitis (5%). Appendicitis with abscess, appendicitis with appendicolith, appendicular abscess, rupture appendicitis with peritonitis and Stump appendicitis was noted in 1% cases each. Other findings included Intussusception in 1% and tenderness in RIF and LIF in 1% cases. USG was found to be normal in 2% cases.

Table 2: CT abdomen findings in cases with clinically suspected acute appendicitis

CT scan findings		Frequency (n=100)	Percentage
Findings of appendicitis	No	37	37.0
	Yes	63	63.0
Concentric thickened wall	No	37	37.0
	Yes	63	63.0
Diameter (mm)	Mean (SD)	8.17 (2.73)	
Appendicolith	Yes	5	5.0
	NA	95	95.0
Fat stranding	Yes	63	63.0
	NA	37	37.0

CT scan was suggestive of appendicitis in 63% cases and the features suggestive of appendicitis were Concentric thickened wall and fat stranding in all the cases. Mean (SD) diameter of appendix on CT was 8.17 (2.73) mm and 5% cases had appendicolith. (Table 2)

Other associated findings on CT were presence of mesenteric nodes in 11% cases, followed by conglomerated mass and peri appendiceal free fluid in 5% cases each. Subhepatic cecum and appendix was noted in 2% cases. About 55% cases had no associated findings on CT scan.

CT scan was diagnostic of acute appendicitis in majority (37%) cases, followed by retrocecal appendicitis in 7%, appendicular lump in 6% and

ruptured appendicitis in 5% cases. Acute appendicitis in subhepatic location and with abscess collection was found in 2% cases each whereas Appendicolith in normal appendix, appendicular abscess, ruptured appendicitis with appendicolith and stump appendicitis were features in 1% cases each

Intraoperatively, acute appendicitis was noted in 34% cases, whereas 7% cases had retrocecal acute appendicitis, 6% had perforated appendicitis and 3% cases had appendicular abscess. Acute appendicitis with necrosed base, appendicolith in normal appendix, hollow viscus perforation with reactive appendicitis and stump appendicitis was noted in 1% cases each.

Table 3: Distribution of cases according to findings on Histopathology

HPE impression	Frequency (n=100)	Percentage
Inflamed and necrosed appendix	12	12.0
Inflamed appendix	33	33.0
Perforated inflamed and necrosed appendix	5	5.0
Perforated inflamed appendix	1	1.0
Normal appendix	2	2.0
Desmoid	1	1.0
GIST	1	1.0
Not done	45	45.0

Histopathology was done in 55% cases, it revealed inflamed appendix in 33% cases, inflamed and necrosed appendix in 12% cases, perforated, inflamed and necrosed appendix in 5% cases,

perforated inflamed appendix in 1% cases and normal appendix in 2% cases. Desmoid and GIST was noted in 1% cases each. (Table 3)

Table 4: Comparison of USG findings with CT scan findings

CT diagnosis	n	USG diagnosis	n	Concordance (%)
Acute appendicitis	37	Acute appendicitis	27	73
		Appendicular lump	1	2.7
		Intussusception	1	2.7
		Probe tenderness in RIF	7	18.9
		Tenderness in RIF and LIF	1	2.7
Acute appendicitis in subhepatic location	2	Probe tenderness in RIF	2	100
Acute appendicitis with abscess collection	2	Appendicitis with abscess	1	50
		Subacute appendicitis	1	50
Appendicolith in normal appendix	1	Normal study	1	100
Appendicular abscess	1	Appendicular abscess	1	100
Appendicular lump	6	Acute appendicitis	1	16.7
		Appendicular lump	2	33.3
		Probe tenderness in RIF	2	33.3
		Rupture appendicitis with peritonitis	1	16.7
Retrocecal appendicitis	7	Probe Tenderness RIF	7	100
Ruptured appendicitis	5	Probe Tenderness RIF	2	40
		Ruptured appendicitis	3	60
Ruptured appendicitis with appendicolith	1	Appendicitis with appendicolith	1	100
Stump appendicitis	1	Stump appendicitis	1	100
IBD	1	Ruptured appendicitis	1	100
Koch's abdomen	4	Appendicular lump	1	25
		Probe Tenderness RIF	2	50
		Ruptured appendicitis	1	25
Lipomatosis of ileocecal valve	1	Acute appendicitis	1	100
Steatohepatitis	1	Probe Tenderness RIF	1	100
Hollow viscus perforation with peritonitis	1	Rupture appendicitis with peritonitis	1	100
Reactive mesenteric nodes with normal appendix	2	Probe Tenderness RIF	2	100
Desmoid	1	Appendicular lump	1	100
GIST	1	Appendicular lump	1	100
Normal appendix	25	Acute appendicitis	2	8
		Probe Tenderness RIF	17	68
		Ruptured appendicitis	1	4
		Subacute appendicitis	4	16
		Normal study	1	4

As observed from the Table No. 4, USG was concordant with CT scan for Appendicular abscess and stump appendicitis in 100% cases, acute appendicitis in 73% cases, ruptured appendicitis in 60% cases Appendicitis with abscess in 50% cases, appendicular lump in 33.3% cases.

In present study, the USG findings were concordant with intraoperative findings for Hollow viscus perforation with reactive appendicitis and stump appendicitis in 100% cases, acute appendicitis in 64.7% cases, perforated appendicitis in 50% cases and appendicular abscess in 33.3% cases.

A total of 12 cases had inflamed and necrosed appendicitis on histopathological examination. Out of these 12 cases, 75% cases had acute appendicitis. Similarly, out of 33 cases with inflamed appendix, 48.5% cases had acute appendicitis. Out of 5 cases with perforated, inflamed and necrosed appendix,

60% cases had ruptured appendicitis. Desmoid and GIST were diagnosed as appendicular lump on USG. The CT scan was concordant with intraoperative findings in 100% cases for appendicolith in normal appendix and stump appendicitis, 85.3% cases for acute appendicitis, 57.1% cases with retrocaecal appendicitis, 33.3% cases for appendicular abscess and 16.7% cases for ruptured appendicitis.

Out of 12 cases with inflamed and necrosed appendix on HPE, 83.3% cases had acute appendicitis on CT scan. Similarly, out of 33 cases with inflamed appendix, 69.7% cases had acute appendicitis. Out of 5 cases with perforated, inflamed and necrosed appendix, 80% cases had ruptured appendicitis.

CT scan revealed appendicitis in 64 cases, and of them 41 cases were diagnosed correctly by USG. Similarly, out of 36 cases with clinical features of appendicitis, 23 cases were correctly diagnosed as negative.

Table 5: Comparison of CT scan and USG with intraoperative findings for diagnosis of appendicitis

Appendicitis		Intraoperative Appendicitis (excluding non-operative cases)			HPE Appendicitis (excluding HPE not done cases)		
		Positive	Negative	Total	Positive	Negative	Total
USG	Positive	35	4	39	33	3	36
	Negative	19	0	19	18	1	19
	Total	54	4	58	51	4	55
CT	Positive	53	1	54	51	2	53

	Negative	1	3	4	0	2	2
	Total	54	4	58	51	4	55
		Intraoperative Appendicitis (including non-operative cases as negative)			HPE Appendicitis (including HPE not done cases)		
USG	Positive	35	19	54	33	21	54
	Negative	19	27	46	18	28	46
	Total	54	46	100	51	49	100
CT	Positive	53	10	63	51	12	63
	Negative	1	36	37	0	37	37
	Total	54	46	100	51	49	100

A total of 54 and 51 cases of acute appendicitis diagnosed intraoperatively and HPE, USG identified acute appendicitis in 35 and 33 cases whereas CT scan diagnosed acute appendicitis in 53 and 51 cases respectively. [Table 4]

Table 5: Diagnostic accuracy of USG and CT scan for diagnosis of acute appendicitis

Diagnostic accuracy	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
USG vs CT	63.5	62.2	74.1	50.0	63.0
USG vs Intraoperative (excluding non-operated cases)	64.8	0.0	89.7	0.0	60.3
USG vs Intraoperative (including non-operated cases)	64.8	58.7	64.8	58.7	62.0
CT vs Intraoperative (excluding non-operated cases)	98.1	75.0	98.1	75.0	96.6
CT vs Intraoperative (including non-operated cases)	98.1	78.3	84.1	97.3	89.0
USG vs HPE (excluding non HPE cases)	64.7	25.0	91.7	5.3	61.8
USG vs HPE (including non HPE cases)	64.7	57.1	61.1	60.9	61.0
CT vs HPE (excluding non HPE cases)	100.0	50.0	96.2	100.0	96.4
CT vs HPE (including non HPE cases)	100.0	75.5	81.0	100.0	88.0

Based upon the Table No. 4 and 5, sensitivity, specificity and diagnostic accuracy was calculated. The sensitivity and negative predictive value of CT scan as compared to HPE was 100% whether HPE not done cases were included or excluded. CT scan as compared to intraoperative finding had sensitivity of 98.1%, specificity of 75% and diagnostic accuracy of 96.6% when non operated cases were excluded whereas considering non operated cases as negative, CT scan had sensitivity of 98.1%, specificity of 78.3% and diagnostic accuracy of 89%. USG as compared to CT scan had sensitivity of 63.5%, specificity of 62.2% and diagnostic accuracy of 63%. Similarly, USG as compared to intraoperative and HPE findings had sensitivity of 64.8% and 64.7% respectively.



Figure 1: USG findings of acute appendicitis showing thickened wall

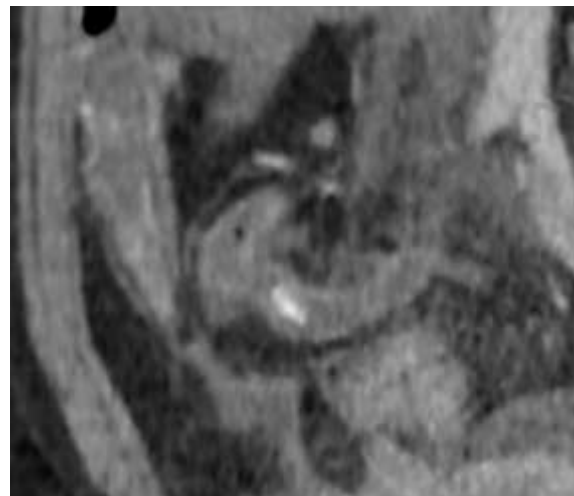


Figure 2: CECT findings of a cute appendicitis with appendicolith and periappendiceal inflammatory fat stranding

DISCUSSION

Abdominal imaging is currently indicated in all but the most straightforward cases of appendicitis. However, the choice of which study to use – either US or CT – remains a point of contention. In children, ultrasound is a viable and commonly used choice, though in adults, the choice is less clear.

In this cross-sectional study, unenhanced or enhanced CT, had sensitivity, specificity, positive predictive value, and negative predictive value performed comparatively better than that of USG. According to Wise et al, CT is significantly better than USG.[5]

For the continuous effort to reduce the incidence of perforation and negative findings at appendectomy, CT and USG are potentially beneficial in the diagnosis of acute appendicitis. Several studies have shown the negative appendectomy rate to fall from over 20% to less than 9% with the use of imaging.^[6,7] According to Jeffry et al, accuracy of USG has brought down the negative laparotomy rate at approximately 10%.^[7]

Generally, the normal appendix is not seen on USG. However, other investigators in 5-50% of patients have reported seeing even normal appendix on USG.^[5,7] The normal appendix is compressible with wall thickness of ≤ 3 mm.^[8]

USG is a rapid, non-invasive, radiation free, relatively inexpensive and requires no special patient preparation or contrast material administration. But graded compression USG is operator dependent as it requires a high level of skill and expertise.

Prints of USG images cannot be always reliably reassessed, as USG is a dynamic procedure. Another important limitation of USG is that the sensitivity and specificity for perforated appendicitis are lower than for nonperforated appendicitis.^[9] Obese patients and patients with a retrocecal appendix or variation in appendix position, with severe abdominal pain and excessive bowel gases are difficult to examine using USG. These patient-related factors limited the diagnostic capability of USG.

In our study, seven cases missed on USG were retrocaecal appendicitis, and three were perforated appendix. Another seven cases were missed on obese patient due to limited visualization of the appendicular region; three cases were missed on USG due to excessive bowel gases and 2 cases due to variation in position of appendix (subhepatic location).

Pitfalls leading to a false positive USG examination include hollow viscus perforation with peritonitis, lipomatosis of ileocaecal valve, ileocaecal TB, epiploic appendagitis, mass mimicking appendicular lump and inflammatory bowel disease.

In our study, the rate of accuracy of diagnosing acute appendicitis on USG were concordant with intraoperative findings for hollow viscus perforation with reactive appendicitis and stump appendicitis in 100% cases, acute appendicitis in 64.7% cases, perforated appendicitis in 50% cases and appendicular abscess in 33.3% cases. Of the 64 cases diagnosed as acute appendicitis by CT, 41 cases were correctly diagnosed in USG. An alternative diagnosis was made on CT in 10% cases, which initially was given as appendicitis or its complication in USG. The sensitivity and negative predictive value of CT scan as compared to HPE was 100% whether HPE not done cases were included or excluded. CT scan as compared to intraoperative finding had sensitivity of 98.1%, specificity of 75% and diagnostic accuracy of 96.6% when non operated cases were excluded whereas considering non operated cases as negative, CT scan had sensitivity of 98.1%, specificity of 78.3% and diagnostic accuracy of 89%. USG as

compared to CT scan had sensitivity of 63.5%, specificity of 62.2% and diagnostic accuracy of 63%. Similarly, USG as compared to intraoperative and HPE findings had sensitivity of 64.8% and 64.7% respectively. Overall, CT scan had superior diagnostic accuracy as compared graded compression USG for diagnosis of acute appendicitis. Factor that likely accounts for the wide range in reported sensitivity and specificity of graded compression USG for the diagnosis of appendicitis across various series is that in some reports all patients with suspected appendicitis were examined on USG, whereas in others only patients with an equivocal clinical diagnosis underwent imaging.^[10,11] The sensitivity of US was not found to be very high because of several factors: (1) difficulty scanning an obese patient; (2) increased gut air reducing the sensitivity; (3) difficult identifying and diagnosing appendicitis in patients who have a retrocecal appendix; (4) variations in position of appendix; and (5) the lack of operator experience may also play a main role in the lower sensitivity of US.

A number of large prospective trials have demonstrated that CT is a highly accurate test for confirming or excluding acute appendicitis¹¹ but radiation hazards have to be kept in mind in younger age group. CT clearly has its advantages, with sensitivity approaching 100% and the ability to perform the study in a way that is not operator dependent, and in patients in which ultrasound is difficult to perform, such as those who are obese. However, the risks of contrast administration, exposure to ionizing radiation, and cost are all limiting factors.

Although various authors debate the figure, a normal appendix is seen more frequently at CT than USG, thus CT carries a better true negative rate. CT scanning with oral contrast and/ colonic contrast is more accurate than without contrast especially to exclude other differential diagnosis.

Despite the established superiority that CT has over ultrasound for the diagnosis of appendicitis, recent studies have advocated for a first-line ultrasound approach with adult patients presenting with possible appendicitis.^[12] Lameris et al recommended a conditional CT strategy, with initial US in adult patients presenting with acute abdominal pain, including suspected appendicitis, and CT only after negative or inconclusive US.^[13]

Debate still raises over the use of USG and CT in the setting of suspected acute appendicitis, and local practice will depend on the surgeons, expertise of radiologists and availability of imaging facilities at short notice but in our view CT imaging should be done if USG is equivocal or negative with a high clinical suspicion.

Wilson et al were the first to design a prospective study to determine whether CT and USG affect a surgeon's decision-making process in acute appendicitis.^[14] We believe that further large multicentre prospective studies are still needed to offer a foolproof final diagnostic pathway in which

USG, CT, and clinical observation can be valuable measures in managing acute appendicitis.

In this study we found that CT is superior to US for the diagnosis of acute appendicitis especially in adults. CT sensitivity was higher than US (100% vs. 63.5%). Negative appendectomies after positive scanning with CT was only 1%. These findings are not surprising and are similar to those in the previous literature.

In our opinion, the introduction of CT and USG as a standard procedure in the workup of acute appendicitis can be worthwhile only if the surgeon can rely fully on CT and USG performed in the hospital. Concern still exists that the overuse or reliance of radiologic diagnosis may distract from careful and timely clinical evaluation and not add significantly to establishing the diagnosis. How high should accuracy of CT and USG in acute appendicitis be, to convince the surgeon not to operate? Is still a question.

The present study had certain limitations. First, the study was conducted at a single institution, which may limit generalizability. Secondly, USG is operator-dependent and its diagnostic performance may vary according to the experience of the examiner. Lastly, the patient populations that were compared had wide range of different age. A prospective study, of CT and US performance within the same comparable age group would be ideal.

CONCLUSION

USG is a rapid, non-invasive, radiation free, relatively inexpensive and requires no special patient preparation or contrast material administration. Limitation of USG exist in that there are patient-related factors limiting the diagnostic capability of USG.

CT is a highly accurate modality for confirming or excluding acute appendicitis. CT clearly has its advantages, with sensitivity approaching 100% and the ability to perform the study in a way that is not operator dependent, however, the risks of contrast administration, exposure to ionizing radiation, and cost are all limiting factors.

Although CT is more accurate and sensitive than US for diagnosing acute appendicitis, US can still be used as the primary imaging modality due to its high specificity and excellent positive predictive value. In turn, CT may be reserved for the patients who have a clinical diagnosis of acute appendicitis and negative USG results. This approach will be cost effective, safe and may reduce the radiation exposure to ~50% of patients presenting with acute appendicitis. Therefore, imaging selection should be individualized according to the patient's age, clinical

presentation, diagnostic probability, availability of imaging, and the need to minimize radiation exposure.

Ethical approval: This study was done after obtaining the approval from the Institutional Ethical committee, JNIMS, Imphal.

Informed Consent: Written informed consent was obtained from all participants in accordance with institutional policy.

Conflict of Interest: The authors declare that there is no conflict of interest.

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