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Innovative Transformation in Rehabilitation and Allied Health Sciences in the Post COVID-19 Era

Fariha Ambreen

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Editorial

In the not-so-distant past, not so long ago in our story of human civilization when the field of rehabilitation and allied health sciences operated within the confines of traditional methodologies. Patient assessments, diagnoses, and treatments followed conventional paths, with healthcare professionals relying heavily on hands-on techniques and their practical experience. This traditional mode, deeply rooted in human-centric practices, proved effective for decades until the unforeseen arrival of an unprecedented challenge.

It is said that there is no engaging story without an unforeseen twist and the novel Coronavirus did just that in our story as it put the whole world to a complete halt and shook the entire traditional healthcare system to its very foundations, transforming the healthcare industry for eons to come. In addition to implementing preventative measures to stop the virus from spreading in a highly interactive, multidisciplinary setting, the COVID-19 pandemic has presented significant hurdles for inpatient rehabilitation services in terms of developing a recovery route for patients who have recovered from the virus[(1)]

In response to this crisis, there was a rapid paradigm shift in the fields of healthcare, especially rehabilitation and allied health sectors. Conventional practices gave way to innovative approaches, and the industry witnessed an accelerated adoption of telerehabilitation and allied health services. This sudden transformation marked a turning point, highlighting the significance of adaptability and technological integration in rehabilitation and allied healthcare. The need for remote patient care offered Telehealth services which is an acceptable means to get over physical obstacles and safety concerns so that patients and caregivers could receive the proper medical care.[(2)]

Allied health and rehabilitation professionals embraced digital platforms to ensure continuity of care while minimizing the risk of virus transmission.

However, the most profound impact on rehabilitation and allied health sciences came with the integration of Artificial Intelligence (AI) into the clinical framework. AI, with its ability to process vast amounts of data and identify patterns, revolutionized patient care, or at least transformed the methods of patient care. Assessment, diagnosis, and treatment, once solely reliant on human expertise, became augmented and, in some cases, replaced by AI-driven solutions.[(3)]

This integration of AI into allied healthcare and rehabilitation was not just a response to the immediate challenges posed by the pandemic; it represented a fundamental shift towards a future by doing this, it raises awareness of the importance of AI in healthcare and helps healthcare companies successfully implement AI technologies where AI plays a central role in every aspect of patient care.[(4)] From early detection of conditions through advanced imaging analysis to personalized treatment plans generated by machine learning algorithms, [(5)] AI is in the process of reshaping the landscape of rehabilitation and allied health sciences.

In the realm of diagnostics, AI has demonstrated remarkable capabilities. Advanced imaging technologies, coupled with AI algorithms, can detect subtle abnormalities that might go unnoticed by the human eye. Medical imaging provides complex insights into physiological, anatomical, and molecular disease processes that have a major influence on patient care. These insights enable treatment customization, which enhances therapeutic outcomes and reduces side effects.[(6)] By enabling quicker, more accurate diagnosis and individualized treatment planning, AI-powered diagnostics are transforming the healthcare industry and eventually enhancing patient outcomes and healthcare delivery. [(7)]

Treatment modalities are also undergoing a transformative shift with the incorporation of AI. Robotic-assisted therapies, guided by intelligent algorithms, are becoming increasingly prevalent. These robotic systems not only assist in physical rehabilitation but also provide real-time feedback, allowing healthcare professionals to adjust treatment plans dynamically. Large volumes of patient data can be processed by AI-powered robots, which helps with disease diagnosis and the creation of individualized treatment regimens. These smart technologies are able to recognize patterns in complex medical data, analyze it, and help medical practitioners make well-informed decisions. Robotic systems can help improve the accuracy and efficiency of healthcare procedures by utilizing AI.[(8)]

Looking beyond the clinical setting, AI is influencing the education and training of future allied health and rehabilitation professionals using modern pedagogical approaches embedded with AI, moving away from conventional teaching methods. Open Distance Learning, powered by the aforementioned technology, has become the cornerstone of academic programs, allowing students to access high-quality education remotely. Virtual simulations and AI-driven educational tools offer immersive learning experiences, preparing students for the evolving landscape of healthcare globally. They provide innovative ways to engage students, adapt content, and promote personalized learning.[(9)]

As Charles Darwin says in his book, "The Voyage of the Beagle" – based on what he observed in the beaks of finches at the Galapagos Islands, "It is not the strongest of the species that survive, nor the smartest ones, but those who are most adaptive to change."

In the same manner, as we observe these initial trends of transformation and slight changes, it becomes evident that what may seem like minor changes today will likely transform into major disruptions in the near future, where the integration of AI into the assessment, diagnosis, and treatment processes will gradually render orthodox methods obsolete. The era of solely

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relying on human intuition and experience in healthcare is waning, making way for a future where AI is an indispensable partner in patient care.

The integration of AI in rehabilitation and allied health sciences promises to revolutionize the aforementioned fields, offering innovative solutions that enhance the quality of care, improve patient outcomes, and optimize resource utilization in the healthcare system. However, it is the ongoing and future integration of AI into the clinical and educational realms that holds the promise of reshaping the entire landscape of healthcare.

As we navigate these changes, it is imperative to embrace the potential of AI while remaining vigilant about the ethical and societal implications, ensuring that the future of healthcare is not just technologically advanced but also compassionate and patient-centered.

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Diagnostic Accuracy of Transabdominal Ultrasonography in Urolithiasis, Keeping Ct Kub Gold Standard

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ABSTRACT

Background: Every year, around half a million people visit hospitals for flank pain. According to studies, 1 out of 10 individuals have a risk of forming renal stones in their lifetime
Objective: To find diagnostic accuracy of transabdominal ultrasound in urolithiasis, keeping CT KUB gold standard.
Methodology: A cross-sectional analytical study was performed at University of Lahore teaching hospital, Lahore duration of 4 months. 114 patients included in study with flank pain, Ultrasonography was conducted on all patients with full urine bladders using the Transabdominal technique with comparison comprehensive KUB image of non-enhanced CT (Toshiba 64) was conducted from the abdomen to the pelvis.
Results: Age of the study participants ranged between 12 to 85 years with mean age 39.3 + 16.4 years. Out of 114 participants 53 (46.5%) were females and 61 (53.5%) were males. On CT KUB, total 94 (82.5%) participants were positive for urolithiasis and 20 (17.5%) were negative. On ultrasound, 68 (59.6%) participants were found positive for urolithiasis and 46 (40.4%) were found negative. The sensitivity of ultrasound was 71.28%, specificity was 95%, PPV was 98.5%, NPV was 41.3% and diagnostic accuracy was found to be 75.4%.
Conclusion: In present study, ultrasonography showed good diagnostic sensitivity for detecting urolithiasis. Preliminary diagnosis can be made through ultrasonography, as it is safer than other diagnostic modalities in terms of radiation exposure.

Key words: Non-enhanced Computed Tomography, Ultrasound, Ureteric Calculi, Radiation, Computed Tomography

Original Research Article

Introduction:

The urogenital calculi, also known as stones, are these solid structures that form in the urinary system when minerals in urine crystallize. These stones can be present in different areas of the urogenital system and over time have been a recurrent health problem [1]. Studies showed that the prevalence of urinary tract calculi is higher in men 15% than women 8% with the estimated prevalence to be 131 cases per hundred thousand [2]. Researchers have noted diverse prevalence estimates in different populations showing that urolithiasis has higher incidence in some areas. For example, the prevalence is reported as 29.5% in Egyptian, 24.9% in Pakistan, 23.3% in Indians, 20.5% in Yemeni, 17.6% in Sudan, 16.2% in Bangladesh and 7.4% in Saudi Arabia [3]. Symptoms of urolithiasis can include kidney colic, flank pain, dysuria, or the presence of blood in the urine [4]. These symptoms place it among the top causes for people to seek medical help [5]. After a long period of obstruction and infection due to stone disease, the affected kidneys eventually lead to renal failure. Therefore, timely and precise diagnosis, as well as proper treatment of urolithiasis, are vital to avoid complications and maintain renal function [6].

Ultrasound of the urinary tract (USG) is one of the best screening methods because it is non injurious, fast, comfortable to the patients, and the cost is lesser compared to Intravenous Urography IVU and the computed tomography CT scan [7]. The diagnostic sensitivity of ultrasound in the detection of urinary calculi has varied across different studies, largely depending on the location of the stone, its size, and the patient's condition [8]. Ultrasound studies show varied sensitivities in diagnosis, depending on factors such as stone size and position. Smith et al. introduced non-contrast helical CT scan (NCCT) as a modern imaging technique for renal stones, which later on became a successful alternative to Intravenous Urography [9]. Nowadays, in medical practice, NCCT is recognized as the procedure of the first choice for evaluating this clinical condition [10]. However, the NCCT has some downsides. Issues of radiation dosage are of concern since ionizing radiation increases the potential health risks [12]. Furthermore, there may be varying levels of availability of CT scans and the cost associated with them is generally higher than with ultrasound imaging, which may present potential barriers to acceptance [13].

Materials And Methods:

A cross-sectional analytical study was conducted at University of Lahore teaching hospital, Lahore. Sample size was calculated at 95% level of confidence and 8% margin of error. Total 114 participants were included in the study. All

participants underwent ultrasound. Participants were scanned using Toshiba Xario 5 MHz curve-array transducer. Ultrasonography was conducted on all patients with full urinary bladder using the Transabdominal technique. The kidneys were seen from both the axial and coronal planes. For a comprehensive KUB image, a non-enhanced CT (Toshiba 64) was conducted from the abdomen to the pelvis. Reconstruction of images was done at 1mm for reviewing and 5mm for filming. The size of the stone was determined using the long axis of Non-enhanced CT and ultrasound.

Results:

Participants of age 12 to 85 years with mean age + SD of 39.3 + 16.4 years were included in study. Out of 114 participants 53 (46.5%) were females and 61 (53.5%) were males. On CT KUB, total 94 (82.5%) participants were positive for urolithiasis and 20 (17.5%) were negative. On ultrasound, 68 (59.6%) participants were found positive for urolithiasis and 46 (40.4%) were found to be negative (Table 1).

VARIABLES		FREQUENCY
Age	Minimum Maximum Mean age + SD	12 years 85 years 39.3 + 16.4 years
Gender	Females Males	53 (46.5%) 61 (53.5%)
Site of Pain	B/L Right Left	20 (17.5%) 43 (37.7%) 51 (44.7%)
Hydronephrosis	Absent Mild Moderate Gross	24 (21.1%) 59 (51.8%) 21 (18.4%) 10 (8.8%)
Ultrasound Findings	Stone Present Stone Absent	68 (59.6%) 46 (40.4%)
CT Findings	Stone Present Stone Absent	94 (82.5%) 20 (17.5%)
Size of stone	Minimum Maximum	1.0 mm 8.0 mm

Table 1: Variables

Out of 114 participants, Hydronephrosis was absent in 24(21.05%) participant, Gross hydronephrosis was found in 10(8.77%) participants, Mild in 59(51.75%) and Moderate in 21(18.42%). Out of 114 participants, Calculi was absent in 19(16.67%) participant, 4(3.51%) was found in bladder, 15(13.16%) at distal ureter, 15(13.16%) at lower pole of kidney, 8(7.02%) at mid pole of kidney, 5(4.39) at proximal ureter, 27(23.68%) at PUJ, 7(6.14%) at upper pole of kidney, 14(12.28%) calculi were found at VUJ level. Mean size of stone was found to be 1.28cm. The sensitivity of ultrasound was 71.28%, specificity was 95%, Positive predictive value was 98.5%, Negative predictive value was 41.3% and diagnostic accuracy was found to be 75.4% (Table 2).

STATISTIC	VALUE	95% CI
Sensitivity	71.28%	61.02% to 80.14%
Specificity	95.00%	75.13% to 99.87%
Positive likelihood ratio	14.26	2.10 to 96.72
Negative likelihood ratio	0.30	0.22 to 0.42
Disease prevalence	82.46%	74.21% to 88.94%
Positive predictive value	98.53%	90.80% to 99.78%
Negative predictive value	41.30%	33.51% to 49.56%
Accuracy	75.44%	66.49% to 83.02%

Table 2: Statistical analysis of study

Discussion:

In emergency departments, ureteric calculi are frequently detected in participants who complain of side discomfort, blood in urine, trouble or burning when passing urine. According to American criteria, NECT is highly sensitive imaging modality for detecting ureter calculi, whereas according to European recommendation sonography is the first-choice modality for primary screening of Ureteric calculi [14,15]. In this study, 114 participants were taken. Females accounted for 53 (46.5%) of the 114 patients, while males' patients were 61 (53.5 %). Ultrasound revealed that 68 (59.6%) of the patients had diagnosed urolithiasis, while 46 (40.4%) had no calculus. On computed tomography scan, 94 (82.5%) of the patients had diagnosed urolithiasis, while 20 (17.5%) of the patients had no calculus. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 71.2%, 95%, 98.5%, 41.3%, and 75% respectively. In comparable research with 80 patients, Wahab et al, (2019) discovered that ultrasonography sensitivity was 69.64 % and specificity was 66.6 % [16]. Our research were consistent with that of Rao et al, (2021) sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 65.3%, 72.1%, 79.7%, 55.4%, and 67.9%, respectively[17]. In another research, trans-abdominal ultrasound showed the sensitivity, specificity, positive predictive value and negative predictive value were 92%, 44%, 90% and 50% respectively, in the detection of calculi in ureters using computed tomography as the benchmark [18]. According to Faiq SM et al, (2014) [19] Sensitivity of Computed tomography, ultrasound and X-ray for deterring ureter stones is 100%, 53% and 90% respectively. According to Ahmed F et al, (2018) [20] ultrasound has a sensitivity, specificity, positive predictive value and negative predictive value were 75%, 17%, 97.2%, and 1.7%. According to Xia J et al, (2020) [21] individuals with a positive "ultrasonic ureteral crossing sign" had a sensitivity, specificity, positive predictive value and negative predictive value were of 91%, 97%, 98%, and 87% respectively, all of which are relatively comparable to our findings.

In this study, 51 (44.7%) of the individuals had left kidney calculus, 43 (37.7%) had right, and 20 (17.5%) had bilateral stones. Another research found that 30.0 percent of cases had right ureter stone, 38.8 % had left ureter stone, and 8.3 % had bilateral ureter stones 18. The mean age in years of the participants in this study was 39.3 + 16.4, which was similar to the results of Sasui et al, (2021)18 where the mean age of the participants was 36.6 ± 8.5 and Wahab M et al, (2019)16 where the mean age of the study subjects was 36.5 ±8.3. Javed M et al (2018) [22] discovered that the mean age of the patients was 35.7 ± 6 years. According to a study conducted in Iran, Riaz et al, (2022) [23] discovered that the mean age of study participants were 47.7±15.9 years. Males made up 61 (53.5 percent) of the participants in this study, while females made up 53.5%. (46.5 percent). In the Memon et al (202) [24] research, 31 (51.7 percent) of the participants were men and females were 29, but Altaf N et al, (2019) [25] discovered 64.4% males and 35.6 % females [23]. In general view, ultrasound of abdomen has notable benefit as a screening tool for calculi of ureter, especially in gravid women and new born, and also in follow-up screening. New techniques, advanced equipment, and experience of sonographer can provide better information and diagnostic efficacy of ultrasound in detecting ureter stones can be increased as well.

Conclusion:

In present study, ultrasonography showed good diagnostic sensitivity for detecting urolithiasis. Preliminary diagnosis can

be made through ultrasonography, as it is safer than other diagnostic modalities in terms of radiation exposure.

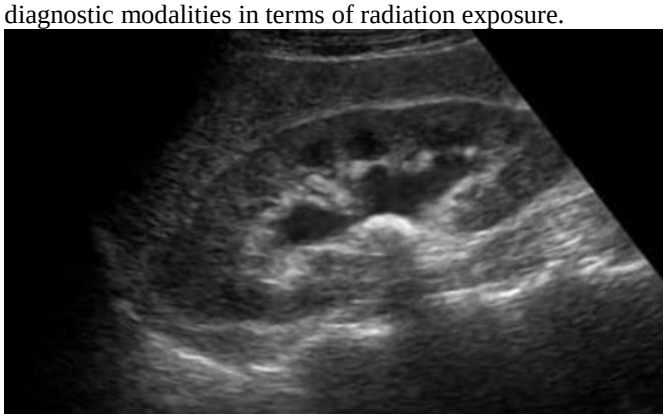

Figure 1: Ultrasound image shows an obstructing Calculus at PUJ

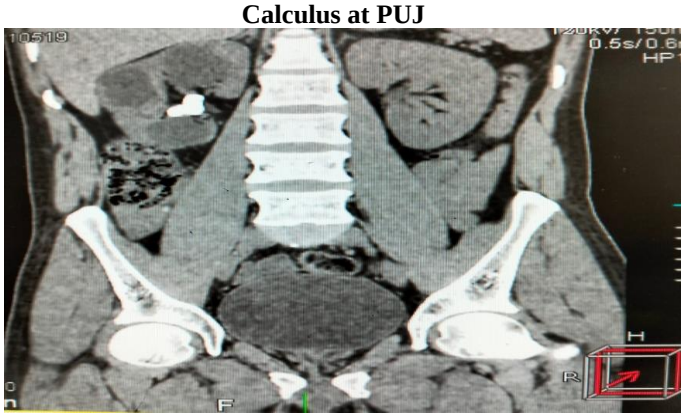

Figure 2: CT coronal image shows a PUJ Calculus

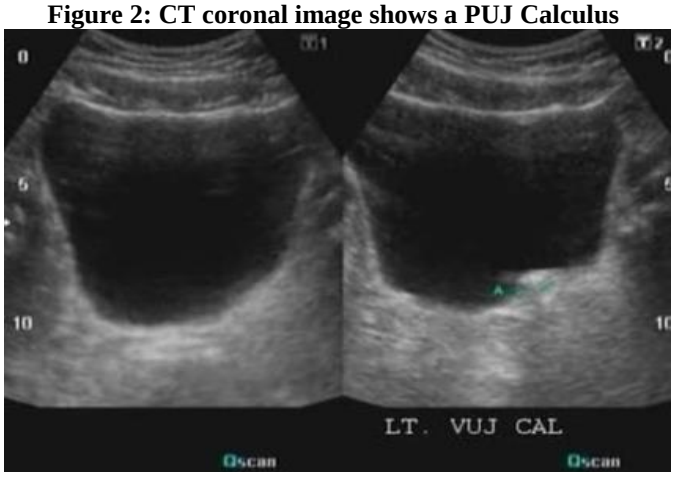

Figure 3: Ultrasound image of calculus at left VUJ

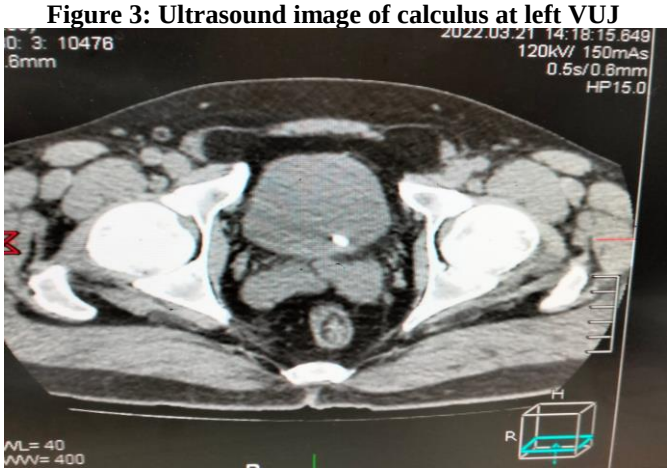

Figure 4: Transverse Plane of CT shows a calculus at left VUJ

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Efficacy Of Ultrasound in Detecting Renal Calculi Keeping Non-Enhanced Computed Tomography as a Reference Standard

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ABSTRACT

Background: Renal calculi, are a prevalent health issue afflicting 10 to 15% of the world's population.
Objective: The objective of this study was to compare ultrasonography and conventional (non-enhanced) CT in diagnosing kidney calculi.
Methods: In 2020 at Gurki Trust Hospital in Lahore, 100 patients suspected of having kidney calculi were enrolled in a cross-sectional study employing ultrasonography and unenhanced CT scans. To assess the sensitivity and specificity of ultrasonography in identifying renal calculi, non-enhanced CT was used as the gold standard, and the presence or absence of renal calculi was recorded for each imaging modality.
Results: Non-enhanced CT detected kidney calculi in 56 of 100 patients, whereas ultrasonography only detected them in 44. With a sensitivity of 78.6% and a specificity of 97.8%, ultrasonography was found to be highly effective at diagnosing kidney calculi. It had a predictive value of 97.7% for the positive and 80% for the negative.
Conclusion: Study concluded that ultrasound is an effective diagnostic tool for detecting renal calculi, with high specificity and moderate sensitivity compared to non-enhanced CT. Our findings suggest that ultrasound may be particularly useful in settings where non-enhanced CT is not readily available or is contraindicated. Further research is needed to determine the diagnosis and management of renal calculi.
Keywords: renal calculi, radiation, ultrasound, non-enhanced computed tomography.

Original Research Article

Introduction:

Renal calculi, affect approximately 10–15% of the global population, these mineral deposits can result in a variety of disagreeable side effects, including obstruction of the urinary tract, renal injury, and even kidney failure [1]. Ultrasonography and non-enhanced computed tomography (CT), in particular, are frequently used to detect kidney calculi [2].

Although ultrasonography is commonly used as a non-invasive diagnostic technique, its ability to detect kidney calculi has been contested, some research suggests that ultrasound may not be as effective as non-enhanced CT in identifying kidney calculi [3]. However, ultrasound has several advantages, including lower cost, a lack of hazardous radiation, and the ability to detect alternative conditions such as hydronephrosis and obstruction [4].4 Due to the importance of a correct diagnosis of kidney calculi, it is essential to compare the efficacy of ultrasound and non-enhanced CT in detecting these stones[5].

Renal calculi must be accurately and promptly identified for the correct treatment and avoidance of negative outcomes but due to its high sensitivity and accuracy, non-enhanced CT is the gold standard for identifying kidney calculi at this time[6]. Non-enhanced CT is preferred, but it is not always available, is inappropriate for all patients, and exposes patients to hazardous radiation [7]. Ultrasound, on the other hand, has several advantages over x-rays: it is simple to obtain, does not require invasive surgery, and does not expose the patient to radiation, in addition, ultrasonography can detect secondary conditions such as hydronephrosis and obstruction, making it a useful screening tool [8].

In light of ultrasound's potential advantages in kidney calculus detection, a comparison to non-enhanced CT is required and in situations where non-enhanced CT is unavailable or prohibited, the findings of this study can inform clinicians about the potential of ultrasound as a screening tool for kidney calculi[9]. In addition, by providing physicians with evidence-based guidance on selecting the optimal imaging procedure for individual patients, this research can facilitate the improvement of the detection and treatment of renal calculi and will assist radiologists in determining which diagnostic technique to employ when detecting renal calculi.

Methodology:

The cross-sectional study took place in 2020 at Gurki Trust Hospital in Lahore and received prior approval from the Institutional Review Board (IRB). From September to

December 2022, the research involved the analysis of 100 individuals who had undergone both ultrasonography and non-enhanced CT scans for the detection of renal calculi. All participants in this study provided informed consent for their inclusion. Exclusion criteria encompassed individuals with a history of kidney surgery, nephrostomy tubes, or ureteral catheters.

From the medical records of all eligible patients, information on patient information, complaints, test results, and imaging investigations was compiled. Ultrasound and non-enhanced CT images were independently analyzed by two radiologists who were oblivious to the results of the other imaging technique. The detection or absence of kidney calculi by each modality was noted and statistical analysis, IBM SPSS Statistics 25.0 was used to determine the sensitivity, specificity, PPV, and NPV of ultrasonography for identifying kidney calculi using non-enhanced CT as the gold standard.

Results:

A total of 100 individuals fulfilled the participation criteria for the study (55 men and 45 women). The average age of participants was 43.60 years old (range: 18-76 years). Hematuria (n=53, 53%) and abdominal pain (n=82, 82%) were the most common initial symptoms among those who obtained medical attention. (See Table 1). Using ultrasound, calculi were detected in 72 patients, whereas non-enhanced CT detected calculi in 85 patients. (See Table 2). In detecting renal calculi, ultrasound had a sensitivity of 84.7% and a specificity of 100% when compared to the gold standard of non-enhanced CT. The ultrasonography had a 100% PPV and a 70% NPV. (See Table 3). In a subgroup study, ultrasonography detection of kidney calculi was found to be more accurate for stones >5mm (92,3%) compared to stones 5mm (81,1%). In addition, the ultrasonic sensitivity was the same for both sizes of stone. Sonography had a 100% PPV for both small and large stones, but a lower NPV (50%) for stones 5mm than for stones >5mm (80%). (See Figure 1 & 2)

Characteristics	Value
Total number of patients	100
Male, n (%)	55 (55%)
Age (years), mean ± SD	43.6 ± 12.5
Presenting symptom, n (%)	
- Flank pain	82 (82%)
- Hematuria	53 (53%)
- Abdominal pain	12 (12%)
- Other	3 (3%)

Table 1: Baseline characteristics of study participants

Diagnostic test	Positive	Negative
Ultrasound	72	28
Non-enhanced CT	85	15

Table 2: Ultrasound and non-enhanced CT results for detecting renal calculi

Diagnostic performance	Value
Sensitivity	84.7%
Specificity	100%
Positive predictive value	100%
Negative predictive value	70%

Table 3: Diagnostic performance of ultrasound in detecting renal calculi compared to non-enhanced CT

Figure 1: Ultrasound of left kidney shows moderate hydronephrosis.

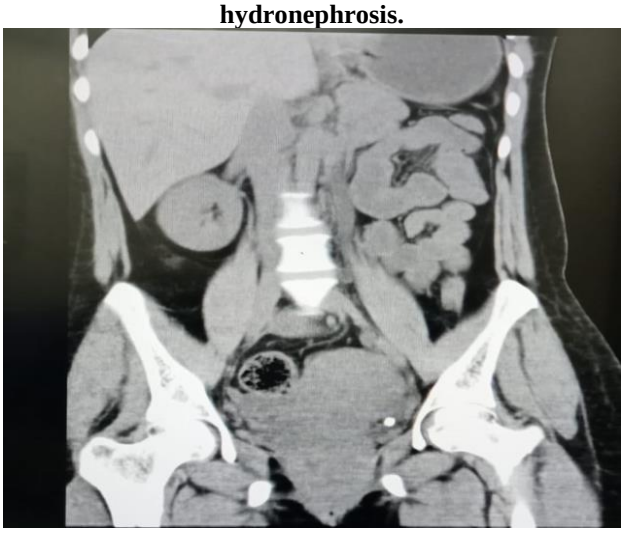

Figure 2: Coronal CT image Stone is seen in VUJ.

Discussion:

Prompt and accurate identification of kidney calculi is required for effective treatment and to reduce the risk of adverse effects [10]. Due to its high sensitivity and accuracy, non-enhanced CT is the gold standard for identifying kidney calculi at this time.11 Non-enhanced CT is preferred, but it is not always available, is not appropriate for all patients, and exposes patients to hazardous radiation [11]. Ultrasound, on the other hand, has several advantages over x-rays: it is simple to obtain, does not require invasive surgery, and does not expose the patient to radiation, in addition, ultrasonography can detect secondary conditions such as hydronephrosis and obstruction, making it a useful screening tool[12].

Study discovered that ultrasound, in comparison to non-enhanced CT, the current gold standard, had a sensitivity of 84.7% and a specificity of 100% for identifying kidney calculi. Due to its high precision, ultrasound appears to be a reliable instrument for determining whether or not kidney calculi are present [14]. Ultrasound may be less effective at detecting small stones than unenhanced CT because its accuracy is inferior[15]. However, a subgroup study revealed that ultrasound has a higher sensitivity for larger stones (>5mm), suggesting that it may be useful for identifying larger stones.

Previous research on the ability of ultrasound to detect kidney calculi has yielded similar results to our own. Maryam et al.

found the diagnosis rate to be 67.83%, the PPV to be 79.66%, the NPV to be 55.36 %, and the sensitivity to be 65% [16]. According to the study by Aqsa Rao et al., 121 individuals were examined, and our findings are in complete agreement with theirs. 82 (67.2%) of the group's 121 individuals were female, while 39 (32.8%) were male. 60 patients (40.6%) did not have ureteric calculi based on ultrasonographic findings, while 61 patients (50.4%) did. Even though ureteric calculi have been reported (90.9%), CT only detected them in 11% of individuals. ($p<0.05$). Accuracy for transabdominal ultrasound was 58.68%, sensitivity was 58.62%, specificity was 56.76%, PPV was 51.52%, and NPV was 63.64% [17].

One of the benefits of our research is the use of non-enhanced CT as the reference standard, which is currently the gold standard for identifying kidney calculi. Additionally, we increased the transferability of our findings by evaluating ultrasound's ability to detect kidney calculi in a clinical setting. However, our investigation is not without faults. Our results may not be pertinent to a larger population because our sample size is so small. Our research was limited in its applicability because it was conducted at a single institution.

Conclusion:

Study concluded that ultrasound is an effective diagnostic tool for detecting renal calculi, with a high specificity and moderate sensitivity compared to non-enhanced CT. Our findings suggest that ultrasound may be particularly useful in settings where non-enhanced CT is not readily available or is contraindicated. Further research is needed to determine the diagnosis and management of renal calculi.

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Frequency of Hemorrhagic and Ischemic Patients of Cerebrovascular Accident (Stroke), Diagnosed on CT-Scan

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ABSTRACT

Aim: To evaluate the frequency of ischemic and hemorrhagic stroke on computed tomography and evaluate clinical manifestation related with Hemorrhagic stroke and ischemic stroke.

Methodology: A retrospective study was Performed in which 77 patients were taken to get required results. After informed consent the entire patient's data had been collected from the indoor, outdoor, and emergency department of DHQ Hospital of Okara, data was collected through the Toshiba Aquilion 16 multi detector CT scanner.

Results: Total of 77 patients diagnosed with stroke were included, 40 patients were male and 37 patients were female. Most of the patients were of age 50 to 70. Results concluded that patients with ischemic stroke were 25 in which female patients were 10, and 15 male. Furthermore, 17 patients were diagnosed with hemorrhagic stroke, in which 8 patients were female, and 9 male. Study excluded 35(45.45%) patients who were classified as stroke from other causes. In stroke, the rate of Ischemic stroke was significantly higher than hemorrhagic stroke (p=0.039).

Conclusion: In current study numerous clinical indications of different types of cerebrovascular accident were assessed in order to distinguished hemorrhagic and ischemic stroke. This comparative study was to be in the favor of Ischemic stroke as compared to hemorrhagic stroke (Ischemic stroke>Hemorrhagic stroke). Though these result provided trustworthy signs for differentiation of stroke types. For diagnosis of stroke imaging modality is still the gold standard.

Key words: CT, Hemorrhagic stroke, Ischemic Stroke, Stroke, Cerebrovascular accident.

Original Research Article

Introduction:

Stroke patients with hemorrhage (HS) and ischemia (IS) were measured on the basis of stroke extremity, seriousness, mortality and cardiovascular causes.(1) In United State, Stroke is 3rd leading death causing disease after other major diseases like heart attacks and cancers (2) Stroke is common cause of dysfunction in survivors, where twenty percent of patients need medical treatment in few months after cerebrovascular accident occur. Beside this, CVA use a major percentage of health care system budget (3). In 1994, Matchar and Duncan declared that every year residents of Americans undergo 550000 strokes, which leads to 150000 demises and leaving 300000 survivors paralyzed. (4) Stroke and heart diseases, a statistical updates of the American Heart Association published in which they said 500000 Americans undergo firstly or repeated stroke every year. These two reports were derived from the white cohort study of Framingham, Mass (5).

According to the institute of health metrics, stroke is leading cause of death in Pakistan, where socioeconomic and health facilities services is becoming better there should be decrease in this cause but it is increasing with increasing number of patients with hypertension and diabetes.(6) Stroke is common in world including 7-8% of American Indians, 2.8 percent in Hispanics, and 1.5 percent in Asians. Furthermore, as compared to Europe, Asia has a higher incidence of stroke-related disabilities. Ischemic stroke occurs 75% to 80, whereas 8% to 20 of time hemorrhagic stroke happens (7).

Cerebrovascular accidents are divided into two main classifications hemorrhagic and ischemic. The Ischemic strokes are triggered due to blockage of blood supply and the hemorrhagic strokes are initiated by blood vessel rupturing or due to abnormal vascular structure. (8) Approximately 87% cerebrovascular accidents are caused by Ischemia, and the remainders are caused because of hemorrhage. It is seen that some of the hemorrhages progress inside areas of ischemia ("hemorrhagic transformation"). (9) Blood supply to the portion of brain is reduced in an ischemic stroke which leads to brain tissue dysfunction in that zone. (10) Cerebrovascular accident without recognizable explanation is known as "cryptogenic". This is about 30-40 percent of all ischemic strokes. (11) Hemorrhagic stroke may happen after blood vessels in brain erupt thus causing bleeding. Subarachnoid hemorrhage and intracerebral hemorrhage are the two types of hemorrhagic stroke. (12). Most important type of extra axial hemorrhage is

subdural hematoma, epidural hematoma and subarachnoid hemorrhage.

Cerebrovascular Accident is speedy damage to brain normal role because of disruption in brain blood supply. Brain cell necrosis afterward the occurrence of stroke is considered as unexpected cause of clinical indications of important loss of cerebral activity that lasts more than 24 hours. [13] It also causes demise of patient with no clear cause other than vascular reason (ischemia and hemorrhagic. [14, 15] Symptoms may depend on brain area damaged. If wider region of the brain is damaged by CVA, it is likely that more functions of body are lost. In some conditions of the strokes can cause other symptoms. It is seen that many forms of stroke are not linked with headache except cerebral venous thrombosis and subarachnoid hemorrhage and frequently intracerebral hemorrhage. [16]

Strokes are identified through several different methods: Computed tomography scans (mostly without contrast enhancements), neurological examination mainly the NIHSS, or Magnetic resonance imaging scans. Imaging techniques also helps in defining the different types or causes of stroke. There are however no blood tests for the stroke identification, however blood tests are helpful in finding out the likely reason of stroke. [17] CT scan is very accurate for the representation of hemorrhagic lesions. Important role of non-contrast Computed tomography is the detection of hemorrhage or other possible cause which may mimic stroke that might be cause of the brain insufficiency. [18]. Furthermore, it is non-invasive modality and it is under practice to diagnose the cause of hemorrhage, also help to measure the size of hematoma and involvement of subarachnoid, or subdural, intra-ventricular fluid, [19].

The epidemiology of ischemic and hemorrhagic stroke is an ongoing investigation to identify risk factors, which continues to expand with technological advances and preventative medical practices. A critical step in determining stroke type is identifying risk factors that can or cannot be modified. Once the risk factors are identified, modifiable risk factors can be addressed to lower the risk of stroke. Although ischemic or hemorrhagic events in Cerebrovascular accident patients are common, they can be fatal. There is little information available about the characterization of this complication. This study will aid in differentiating the cause and risk factors of CVA, as well as evaluating the frequency of hemorrhagic or ischemic patents in CVA and providing information on disease progression, complication development, and intervention effectiveness. All this information is particularly useful in assessing the physiological effect of acute ICH, and it is not available from a clinical examination alone. Because of lack of resources, CT scans cannot be repeated in rural locations. For doctors estimating physiological result from CT scan of brain done during patient sign and symptoms is important task

Methodology:

Observational descriptive study was carried out in radiology department of DHQ Hospital of Okara on frequency of hemorrhagic and ischemic patients of cerebrovascular accident (stroke), diagnosed on Ct-scan. Duration of the study was 4 months (From November 2022 to February 2023 after the approval of synopsis, Ethical Letter from intuitional review board issued with reference number AAI/OSA/2022550) in which data is collected by using specially created Performa. In which total of 77 patients were included using non convenient sampling technique, of both genders presenting with stroke. Inclusion criteria for sampling was defined such as patients presenting with the history of hypertension, diabetes and headache were selected for study Patients presenting with the clinical symptoms of aphasia, fits and altered state of conscious were also included in study

But patients presenting with the symptoms of Parkinson disease, chronic cerebral disease and other neurologic defect were not included and patients presenting with not well-defined symptoms of stoke were also exclude from study. Examination and demographic data were recorded after taking permission of patients on written consent forms. All patients were scan on CT scanner using Toshiba Aquilian 64 slice model, brain normal and contrast study carried out. Data was arranged and evaluated with the help of SPSS version 21 and MS excel 2012. Data was described using descriptive statistics and chi square test.

Results:

Total 77 patients with Stroke were involved in this research. Age of the majority of the patients were 50 to 70 years. The mean age of the patients were 55.87 ± 20.98 SD. Out of 77 patients, there were 40(51.9%) males and 37(48.1%) were females. Percentage of patients with hypertension were 57.1%, in which female were more than male and patients with diabetes mellitus were 46 %.23% patients had dyslipidemia and 22 percent patients were chain smoker. 36.3 % patients were present with symptoms of altered state of conscious and 29.8 % patients had headache. Percentage of patients with aphasia was 16.8% and with seizure 10.3 %. It was diagnosed that; hemorrhagic stroke was present in 17 patients (22.07%) and ischemic stroke in 25 patients (32.46%) as shown in table 4. And 35 patients (45.45%) were classified as stroke from other causes.

In 77 patients, Mean age of the patients was 55 years with Standard deviation 20.9, minimum age of the patient was 32 year and maximum age was 79 year. Previous research on the ability of ultrasound to detect kidney calculi has yielded similar results to present research. Maryam et al. found the diagnosis rate to be 67.83%, the PPV to be 79.66%, the NPV to be 55.36 %, and the sensitivity to be 65% 16. According to the study by Aqsa Rao et al., 121 individuals were examined, and research findings were in complete agreement with theirs. 82 (67.2%) of the group's 121 individuals were female, while 39 (32.8%) were male. 60 patients (40.6%) were not diagnosed ureteric calculi based on ultrasonographic findings, while 61 patients (50.4%) were. Even though ureteric calculi have been reported (90.9%), CT only detected them in 11% of individuals. ($p<0.05$). Accuracy for transabdominal ultrasound was 58.68%, sensitivity was 58.62%, specificity was 56.76%, PPV was 51.52%, and NPV was 63.64%. [17]

One of the benefits of research was the use of non-enhanced CT as the reference standard, which is currently the gold standard for identifying kidney calculi. Additionally, study increased the transferability of findings by evaluating ultrasound's ability to detect kidney calculi in a clinical setting. However, study investigation was not without faults. Results may not be pertinent to a larger population because sample size was so small. Research was limited in its applicability because it was conducted at a single institution.

Statistics		
Age		
N	Valid	77
	Missing	0
Mean		55.8700
Std. Deviation		20.988
Minimum		32.00
Maximum		79.00

Table 1: Age of Patients

The below mentioned cross-tabulation and Bar-Graph table illustrated the distribution of common risk factors among patients categorized by gender. Notably, hypertension and diabetes mellitus were more prevalent in females, with 23 females having hypertension and 20 having diabetes compared to 21 males with hypertension and 16 with diabetes. Percentage of patients with hypertension was 57.1%, in which female was more than male and patients with diabetes mellitus were 46 %. 23% patients had dyslipidemia and 22 percent patients were chain smoker. (Table 2)

Risk Factors			
	Present in Male	Present in Female	Total
Hypertension	21	23	44
Diabetes Mellitus	16	20	36
Dyslipidemia	5	13	18
Smoking	17	0	17

Table 2: Presence of risk factors in patients

Below mentioned bar-graph of clinical symptoms showed that 36.3 % patients were present with symptoms of altered state of conscious and 29.8 % patients had headache. Percentage of patients with aphasia was 16.8% and with seizure 10.3 %. Some patients had multiple risk factors. This tabular presentation provided the distribution of clinical symptoms based on gender, aiding in the identification of potential gender-specific patterns in the manifestation of these symptoms within the studied patient population. (Table 3)

Clinical Symptoms			
	Present in Male	Present in Female	Total
Seizure	7	1	8
Altered state of conscious	15	13	28
Headache	9	14	23
Aphasia	5	8	13

Table 3: Clinical Symptoms in Patients

The below presented cross-tabulation and bar-graph revealed the association between gender and the occurrence of infarction and hemorrhage in a patient population. Out of 77 patients, 25 patients (32.46%) had infarction and 17 patients (22.07%) had hemorrhage. Among males, 15 individuals exhibit infarction and 9 show hemorrhage, contributing to a total of 25 cases. In contrast, among females, 10 individuals had infarction and 8 have hemorrhage, resulting in a total of 18 cases. This breakdown suggested a potential gender-related difference in the prevalence of these cerebrovascular events. Analyzing the table, it appeared that infarction was more prevalent in males, while hemorrhage occurs at a slightly higher rate in females. In patients with cerebrovascular accident, the rate of Ischemic stroke was significantly higher than hemorrhagic stroke (p=0.039). IS>HS (Table 4)

Association b/w Gender and Infarction, Hemorrhage			
	Present in Male	Present in Female	Total
Infarction	15	10	25
Hemorrhage	9	8	17

Table 4: Association b/w gender and infarction, hemorrhage

Below mentioned table showed 37 cases where both infarction and hemorrhage were not present, 15 cases where infarction was not present but hemorrhage was, 23 cases where infarction is present but hemorrhage was not, and 2 cases where both infarction and hemorrhage were present. This showed that only 2 patients had infarction out of total of 17 hemorrhagic. (Table 5)

Infarction and Hemorrhage Cross tabulation				
		Hemorrhage		Total
		Not Present	Present	
Infarction	Not Present	37	15	52
	Present	23	2	25
Total		60	17	77

Table 5: Association b/w infarction and hemorrhage

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.265a	1	.039		
Continuity Correctionb	3.139	1	.076		
Likelihood Ratio	4.877	1	.027		
Fisher's Exact Test				.044	.033
Linear-by-Linear Association	4.209	1	.040		
N of Valid Cases	77				
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.52.					
b. Computed only for a 2x2 table					

Table 6: Findings

The table illustrated the counts of cases based on the presence or absence of infarction and hemorrhage. Various statistical tests were employed to assess the significance of the association. The Pearson Chi-Square test yielded a value of 4.265 with a corresponding p-value of 0.039, suggested a statistically significant association between infarction and hemorrhage. Additional tests, such as Fisher's Exact Test, also supported this association with a p-value of 0.044. Symmetric measures, specifically Pearson's R and Spearman Correlation, indicated a negative correlation of -0.235 between interval and ordinal variables, respectively. These findings collectively suggest a significant relationship between infarction and hemorrhage.

Discussion:

In a cross-sectional study involving 77 patients diagnosed with stroke, 51.9% were male, and 48.1% were female. The majority of patients were in the 50 to 70 years age range. The study identified that 32.46% had ischemic stroke, 22.07% had hemorrhagic stroke, and 45.45% were classified under other stroke causes and were excluded. The analysis revealed a significantly higher rate of ischemic stroke compared to hemorrhagic stroke in patients with cerebrovascular stroke (p=0.039).

Abdul Sahto et al. in his cross sectional study, about 160 patients with hypertension were included and frequency of hemorrhagic stroke and ischemic patients and risk factors associated with them are rule out. Previous research findings, hemorrhagic stroke were detected in 55 (34%) patients and ischemic stroke were identified in 66% (105) patients. Hypertension was present in both types of stroke. Symptoms such as dyslipidemia, diabetes, and smoking were ominously high in hemorrhagic stroke as compared to in ischemic stroke. It was identified that Prevalence of ischemic stroke is high in population as compared to hemorrhagic stroke which support the study that the hypertension is main the risk factor and percentage of ischemic stroke is higher than hemorrhagic stroke [(20)]. This research results are also supported by another retrospective research which was conducted by Hassen Abdu et al. in 2021. In which compared the ischemic and

hemorrhagic stroke in 312 patients, according to the study 65.4% (204) patients had ischemic stroke [21].

PK Chhetri et al. in 2012 conduct research in which he evaluated the patients with cerebrovascular stroke on Computed Tomography. In his study numbers of patients were 100 in which 64 patients had ischemic infarction and other patients had intracranial hemorrhage. 77 percent of patient with stroke had risk factors. Among these risk factors, most common was smoking which was seen in 38 cases (38%), followed by hypertension in 28 cases (28%), This study finding was also in line with previous findings of Pk Chhetri et al. findings, in our study 57% patients present with hypertension and 22 % patients was chain smoker [22]. In general, the estimated overall findings in the current study are considered within the range of several recent studies

Conclusion:

In this study, the aim was to differentiate between hemorrhagic and ischemic stroke by excluding other types of cerebrovascular accidents. The comparative analysis was designed to favor ischemic stroke over hemorrhagic stroke (IS > HS). While these findings offer significant indications for distinguishing between the two types of strokes, it is important to note that imaging modalities, particularly computed tomography, serve as the gold standard for stroke diagnosis.

Conflict of Interest:

There is no conflict of interest

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Carriage of Enterotoxin Genes by Methicillin-Resistant Staphylococcus aureus and Anti-Staphylococcal Activity of Local Citrus Fruits

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ABSTRACT

Background: Staphylococcus aureus (S. aureus) is one of the leading causes of skin infections and food poisoning worldwide. Citrus fruits enriched with phytochemicals can exhibit anti-staphylococcal activity.
Objective: The study aimed to investigate in vitro susceptibility of citrus fruits against S. aureus harboured enterotoxin genes among food handlers, personnel, and the environment.
Methods: S. aureus was isolated from food handlers, personnel, and the environment. Methicillin-Resistant Staphylococcus aureus (MRSA) screening was performed using cefoxitin antibiotic discs. The antimicrobial activity of citrus fruits, including Citrus limon (Lemon), Citrus pseudolimon (galgal), Citrus tangerine (fruiter), Citrus sinensis (malta), and Citrus paradisi (grapefruit), was analyzed by performing well-diffusion assay and broth microdilution method. Genes for toxins production, sea and seg were detected using a Polymerase Chain Reaction (PCR).
Results: Out of 200 swab samples, 50 (25%) S. aureus were isolated using the biochemical battery. Antibiotic susceptibility testing (AST) and cefoxitin disc revealed 12 (24%) MRSA strains. Most strains were sensitive to Citrus limon, followed by Citrus pseudolimon, Citrus tangerine, Citrus sinensis, and Citrus paradisi. PCR results detected carriage of the sea gene in 4 (8%) isolates, and 20 (40%) isolates harboured the seg gene.
Conclusion: The prevalence of S. aureus, mainly MRSA, was high in the environment. These resistant strains' carriage of sea and sea enterotoxin genes increases their virulence and leads to untreatable infections. The antibacterial activities of citrus fruits should be explored using adavance methods.

Key Words: Staphylococcus aureus, MRSA, Enterotoxin, Citrus fruits, Anti-staphylococcal activity.

Original Research Article

Introduction:

Citrus fruits are enriched sources of nutrients and bioactive elements such as essential oils, phenolics, alkaloids and vitamins [1]. Phytochemical constituents of citrus fruits have antioxidant, anti-inflammatory and antimicrobial properties that protect against many diseases [2]. Pakistan is blessed with an ideal climate for growing a wide range of vegetables and delicious fruits [3]. However, very few studies have discussed the antimicrobial activities of citrus fruits in detail regarding their principal bioactive constituents and antimicrobial interactions.

S. aureus is one of the significant causes of skin infections and leads to high rates of death in the world [4,5]. S. aureus produces staphylococcal enterotoxins (SEs), which are pyrogenic toxins of the superantigen family. SEs and SE-like toxins (SEls) are the most notable virulence factors associated with food poisoning [6]. Staphylococcal food poisoning (SFP) results in nausea, diarrhea, emesis, and prostration [7]. SEA is a potent gastrointestinal toxin; a minimal amount of 100ng is enough to cause toxicity [8]. The situation has worsened due to antibiotic resistance which makes treatments difficult.

MRSA causes many infections, including skin and soft tissue infections and invasive infections like pneumonia, meningitis, and lung abscess. Several S. aureus strains have developed resistance against both β-lactam and non-β-lactam antibiotics. Infections due to these strains confer a significant public health threat in the healthcare system [9]. S. aureus acquires multiple genes by mobile genetic elements; the mecA gene responsible for methicillin resistance and SEA and SEG for enterotoxin secretions [10]. Previous literature revealed that the genetic elements that encode virulence factors may be the culprit of antibiotic resistance in S. aureus strains. In this scenario, when the world faces the challenge of antimicrobial resistance, plant-based antimicrobials can be promising therapeutic options with no side effects, unlike other synthetic drugs. Thus, this study is aimed to investigate the antimicrobial effects of citrus fruits in MRSA isolated from environment and genetic analysis of their enterotoxin genes.

Materials and Methods

Collection and Identification of Staphylococcus aureus

This cross-sectional study was conducted at the Institute of Microbiology and Molecular Genetics, University of Punjab, Lahore, Pakistan. Two hundred samples were collected from June 2019 to December 2019; using sterile cotton swabs moistened in saline water. Sampling sites included the hands and nares of food handlers 80, students 100 and the environment 20. Samples were cultured on Mannitol Salt Agar (MSA) media, and routine bacteriological analysis was performed to identify S. aureus.

Antibiotic Susceptibility Testing

The antibiotic susceptibility testing was performed according to the Kirby-Bauer Disc Diffusion method. Eight antibiotics were used; Penicillin (P 10U), Amoxicillin (AMC 10µg), Tetracycline (TE 30 µg), Cefoxitin (FOX 10 µg), Gentamycin (CN 10 µg) and Ceftriaxone (CRO 30 µg). All the zone diameters were interpreted per the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2018).

Phytochemical Analysis of Fruit

Juices were sterilized by using 0.45µm sterile syringe filters. The phytochemical constituents of the raw juices were determined by treating them with different reagents. Alkaloids, carbohydrates, coumarins, flavonoids, steroids, and glycosides were tested [11-13]. To test alkaloids, 1 ml extract juice was treated with Dragendroff's reagent and 1% HCL. The appearance of a red precipitate confirmed its presence. Fehling A and B were treated with juice in 1:1 proportion and heated in a flame, establishing the carbohydrates' presence by the appearance of a brick-red precipitate. 10% sodium hydroxide (NaOH) and 1 ml chloroform were treated with 1 ml juice extract. The appearance of the yellow colour showed the presence of coumarins. Production of yellow colour after adding a few drops of ferric chloride solution 1ml juice extract indicated the presence of flavonoids. To confirm the glycosides presence, 1 ml of the extract was mixed with 0.5 ml chloroform and 0.5 ml conc. and heated. A red-brown colour developed in the presence of glycosides. Mixing 1 ml extract with 0.5 ml chloroform and heated. The red colour indicated the presence of steroids.

Well Diffusion Assay

Screening of citrus juice for antibacterial activity against S. aureus isolates was performed by agar well diffusion assay. Using sterile swabs, culture broth diluted to match 0.5 McFarland standards was streaked onto Muller Hinton Agar (MHA). Wells were made by using a sterile cork borer, and 20 µl of each juice was diffused into separate wells. The plates were incubated at 37^{o} C. After 24 hrs, zones of inhibitions were measured for each of the juice in millimetre (mm).

Minimum Inhibitory Concentration

The broth micro-dilution method evaluated the Minimum Inhibitory Concentration (MIC) of all the S. aureus isolates. A 96-well microtiter plate was used, and all the samples were treated in triplicates. The nutrient broth was taken as sterility control, raw juice as a negative control, while a bacterial inoculum was considered as a positive control. Five different concentrations (60 µl/ml, 70 µl/ml, 80 µl/ml, 90 µl/ml and 100 µl/ml) of the juices were added for each bacterial isolate. The micro-titer plates were incubated at 37 °C for 24 hrs. Interpretation of MIC was done by naked eyes and measuring optical density (OD).

Genetic analysis of resistance and Enterotoxin causing elements

DNA extraction was done through the Cetyltrimethylammonium bromide (CTAB) method [14]. Molecular detection of methicillin resistance was carried out by mecA gene detection [15]. The mecA gene was amplified using the following thermocycler conditions: pre-denaturation at 94 °C for 5 minutes, 35 cycles each of denaturation at 94 °C, annealing at 52 °C and extension at 72 °C for 30 seconds each, followed by a single cycle of post-extension at 72 °C for 10 minutes. Enterotoxin genes, including sea and seg, were also detected [16]. The reaction mixture contains 0.5 µl of each primer, 10 µl of Master Mix (Taq, dNTPs, and Buffer included), and 7 µl RNase free water. The total volume was made up to 20 µl. A known control for each gene was used in all reactions. The annealing temperature for the sea and seg multiplex PCR was optimized at 47 °C. Amplified products were visualized in 2% agarose gel on a UV illuminator. PCR product of 552 bp for sea gene and 331 bp for seg gene were confirmed.

Results

Bacterial Identification and Antibiotic Susceptibility Testing

A total of 200 samples were collected to determine the anti-staphylococcal activity of citrus fruits against non-clinical isolates of S. aureus. 160 (80%) swab samples were grown as positive cultures on mannitol salt agar media. Out of 160 isolates of Staphylococcus spp, 50 (31.25%) were identified as S. aureus. Among these 50 strains, 19 (38%) were isolated from food handlers, 23 (46%) personnel, and 8 (16%) environments (tabs and door locks). AST results revealed the highest resistance against penicillin 41 (82%), followed by amoxicillin 39 (78%) and tetracycline 23 (46%). Vancomycin, ceftriaxone, cefoxitin, and gentamycin were susceptible to S. aureus (Table 1). Based on cefoxitin susceptibility, 12 (24%) strains were screened as MRSA.

Table I Antibiotic susceptibility against non-clinical Staphylococcus strains

Antibiotics	Concentration	Resistance n	Resistance %	Sensitive (N)	Sensitive (%)
Amoxicillin	30 µg	39	78	11	22
Tetracycline	30 µg	23	46	27	54
Vancomycin	30 µg	0	0	50	100
Cefoxitin	30 µg	12	24	38	76
Gentamycin	30 µg	15	30	35	70
Penicillin	10 U	41	82	9	18
Ceftrioxone	30 µg	6	12	44	88

Phytochemical Screening

Alkaloids were present in all five fruits. High amounts of alkaloids were detected in Citrus tangerina and Citrus sinensis. Carbohydrates were also present in all fruits. Coumarins were present in four juices of citrus fruits except for Citrus pseudolimon . Citrus sinensis and Citrus paradisi contained a high amount of coumarins. Citrus sinensis , Citrus tangerina , and Citrus paradisi had many flavonoids. It was absent in Citrus pseudolimon . Red-brown precipitates of glycosides were only seen in the juices of Citrus pseudolimon and Citrus paradisi . Steroids were slightly detected in Citrus paradisi.

Well Diffusion Assay

Citrus limon 45 (90%) and Citrus pseudolimon 34 (68%) showed the best antibacterial activity among citrus fruits. At

the same time, more than half of S. aureus were resistant against the Citrus paradisi, Citrus tangerina, and Citrus sinensis juices, with percentages of 30 (60%), 28 (56%), and 28 (56%) of the isolates, respectively.

Table II Percentages of resistant/sensitive strains for fruit juices by well diffusion assay

Fruits names	Resistance (n)	Resistance (%)	Sensitive (n)	Sensitive (%)
Citrus pseudolimon	16	32	34	68
Citrus limon	5	10	45	90
Citrus sinensis	28	56	22	44
Citrus tangerina	28	56	22	44
Citrus paradisi	30	60	20	40

Minimum Inhibitory Concentration

Citrus paradisi juice was most sensitive at the lowest concentrations, inhibiting growth at 60 µg/ml of 7 (35.0%) strains. Then, Citrus tangerine juice at 70 µl/ml showed maximum sensitivity against 7 (31.81%) strains. Citrus pseudolimon juice inhibited maximum bacterial growth at 80 µl/ml. MIC of both Citrus sinensis and Citrus limon was highly sensitive at µl/ml 8 (36.36%) and 16 (35.56%) strains, respectively (Table 3).

Table III Minimum inhibitory concentrations for citrus fruit juices

Citrus Fruits (n)	60 µg/ml	70 µg/ml	80 µg/ml	90 µg/ml	100 µg/ml
Citrus pseudolimon (34)	7 (20.5%)	9 (26.47%)	10 (29.41%)	5 (14.70%)	3 (17.64%)
Citrus limon (45)	7 (15.56%)	7 (15.56%)	14 (31.11%)	16 (35.56%)	1 (2.23%)
Citrus sinensis (22)	4 (18.18%)	3 (13.63%)	7 (31.81%)	8 (36.36%)	1 (4.54%)
Citrus tangerina (22)	5 (22.72%)	7 (31.81%)	5 (22.72%)	2 (9.09%)	3 (13.63%)
Citrus paradisi (20)	7 (35.0%)	6 (30.0%)	5 (25.0%)	1 (5.0%)	1 (5.0%)

Sea and Seg Enterotoxin Genes

PCR results expressed the presence of enterotoxin genes in 50 S. aureus, including MRSA and MSSA (Table 4). Among 12 MRSA, 2 (16.67%) strains with sea and 5 (41.60%) with seg genes were detected. However, MSSA strains had a high frequency of seg gene 15 (39.70%). The coexistence of sea/seg genes was detected in only one strain (Table 4 and Figure 1).

	Sea	Seg	Sea/Seg +	Sea/Seg -
MRSA (n=12)	2 (16.66%)	5 (41.60%)	1 (8.33%)	4 (33.33%)
MSSA (n=38)	2 (5.26%)	15 (39.70%)	0	21 (55.26%)

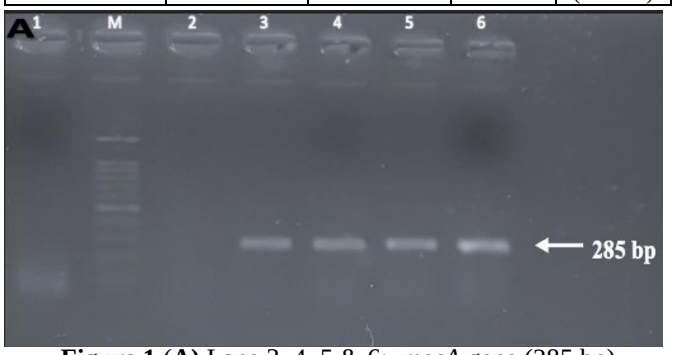

Figure 1 (**A)** Lane 3, 4, 5 & 6: *mecA* gene (285 bp)

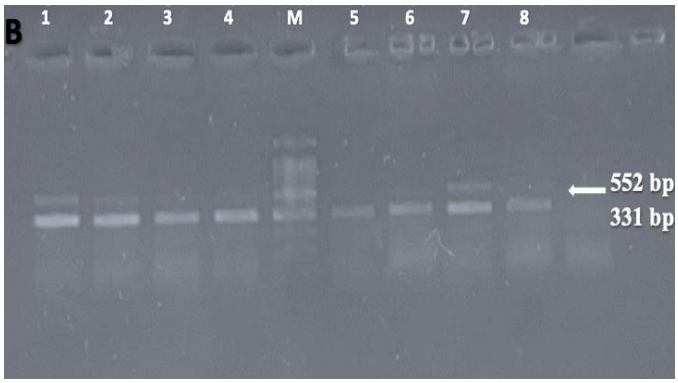

Figure 1 (B) Lanes 1, 2, 4, 7: *sea* (552bp) and Lanes: 1, 2, 3, 4, 5, 6, 7, 8 *seg* (331 bp)

Discussion

Citrus fruits are a well-appreciated therapeutic option due to their anti-inflammatory, antioxidant and antimicrobial properties. In contrast, the antibacterial effects of these fruits against pathogens have not been extensively studied [17]. S. aureus, as an opportunistic pathogen, can cause severe invasive infections, including bacteraemia, pneumonia, osteomyelitis and septic arthritis [18]. This study investigated the anti-staphylococcal activity of different citrus fruits and the carriage of enterotoxin genes by S. aureus strains isolated from non-clinical samples.

Out of 200 swab samples, 25% of cultures were identified as S. aureus in this study. These percentages vary in different studies. Sexton et al., isolated 70% S. aureus strains from patients and their isolation rooms. He also observed that the strains isolated from patients and their environment were closely related. This indicates environmental strains' contribution to infection spread [19].

In this study, amoxicillin and penicillin exhibited maximum resistance against S. aureus. Vancomycin, followed by ceftriaxone, were the most susceptible drugs. In Nigeria, ceftriaxone was also the least resistant antibiotic to S. aureus isolated from students' mobile phones [20]. Out of 50 S. aureus, 24% were identified as MRSA by cefoxitin sensitivity, a reference method for MRSA detection. Cefoxitin resistance results from acquiring the mecA gene by horizontal transfer for modified target proteins and inactivating ß-lactams drugs [21]. In this study, MSSA were higher in number 38 (76%) than MRSA 12 (24%). A systematic review of food contamination in Iran calculated the prevalence of MRSA, ranging from 0% to 32% [22]. In contrast to our results, another study on environmental samples of football team locker rooms found MRSA were more prevalent at 33% than MSSA at 24% [23]. The differences in percentages of MRSA might be due to the sampling surfaces in this study, which were the hands and nasal swabs of food handlers, personnel, and the environment.

Biochemical tests detected phytochemicals like alkaloids, carbohydrates, coumarin, flavonoids, glycosides and steroids in raw juices of citrus fruits. In previous literature, functional groups of these compounds have been detected by advanced techniques like Fourier-Transform Infrared (FTIR) Spectroscopy [24, 25].

Among the citrus fruits, the highest antibacterial activity was observed in the case of Citrus limon juice, inhibiting 90% S. aureus growth. A study reported the antimicrobial properties of Citrus limon and found remarkable antimicrobial properties against S. aureus and Pseudomonas aeruginosa, Escherichia

coli, and Candida Albicans [24]. Afroja also supported the findings by reporting Citrus limon juice was highly sensitive at MIC 12.5 µg/ml concentrations [2].

Over half of the strains were sensitive to Citrus pseudolimon. Citrus sinensis and Citrus tangerina had the same sensitivity values. While most of the strains 60% were resistant to Citrus paradisi juice. On the other hand, Citrus paradisi juices primarily inhibited bacterial growth at its minimum concentrations of 60 µg/ml as compared to other citrus fruits. This showed the solid antimicrobial effects of Citrus paradisi juice. Kim et al. described the antibacterial activity of Citrus paradisi extract by interrupting the glycolysis reactions in bacterial cells. He reported the combination of Citrus paradisi seed extract with melic acid could be an antibacterial agent [26]. However, studies are insufficient to describe the main constituent responsible for the antimicrobial activity of citrus fruits. This needs further investigation to explore the antimicrobial potentials of citrus fruits worldwide, where resistant pathogens and new antibiotics discovery are challenging.

Staphylococcal enterotoxins' (SEs) are one of the significant causes of food poisoning worldwide. Enterotoxins encoded by sea and seg genes can cause severe immunological responses and tissue damage in host. Both toxins show super antigenic and emetic activity. In the present study, the enterotoxigenic genes were detected in 48% of strains more prevalent in MRSA than MSSA (Table 4). Similar results were obtained for toxins genes in 19.12% MRSA isolated from Karachi [27-29]. In Iran, the estimated prevalence of SEs types SEA and SEG in food resources ranged from 13% to 83.3% [22]. This number poses a risk in terms of public health and food safety. Carriage of antibiotics resistance and enterotoxins enhances MRSA's virulence and enables them to cause particular clinical outcomes compared to MSSA strains. Poor hygiene practices cause its dissemination in the environment, consequently, untreatable infections of MRSA. Food handlers with poor personal hygiene are a potential source of infection and serve as a bridge in the journey of enterotoxigenic MRSA in food. The dissemination of these virulence traits further exaggerates the problem. These findings emphases to raise awareness about food-borne diseases and personal hygiene.

Conclusion:

The carriage of both antimicrobial resistance and enterotoxin genes is a growing problem and a threat to a healthy community. Citrus limon juice has the best antibacterial activity against S. aureus among all the tested citrus fruits. Citrus limon could be considered a lead for discovering antimicrobial substances.

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Conflict of Interest

The authors declare that there is no conflict of interest.

MHS: Literature search, data collection, experimental work and first draft

AA: Experimental work, write up, study design, data anaylysis

KN: Write up, data analysis, proof reading

SM: Write up, data analysis, data interpretation

MAR: data analysis, conceptualization of study design

SR: conceptualization of study design, write up, proof reading, supervisor/PI

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Emerging Candida Species Isolated from Tertiary Care Hospital, Lahore: Species Distribution and Susceptibility Profile

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ABSTRACT

Objective: In the last five years, invasive Candida infections have become more prevalent. Along with Candida albicans, non-albicans candida (NAC), infections are also increasing. The study aimed to identify the emerging candida species from clinical samples and to estimate their susceptibility profile.

Materials and Methods: All Candida suspected samples were collected from Tertiary Care Hospital, Lahore. Samples were processed on sabouraud and blood agar. Wet prep was done for confirmation of Candida. Germ tube testing was done to differentiate C. albicans from NAC. Specie identification and antifungal susceptibility were performed by VITEK 2.0 compact system. 18sr RNA Sequencing was performed for unidentified Candida species.

Results: Out of 86 confirmed Candida samples, 41 were identified as C. albicans by germ tube testing, and 45 were NAC. VITEK 2.0 results indicated that C. tropicalis was isolated from a maximum of 33.3% of samples among NAC. There were 5 cases of candida co-existing with bacterial pathogens, while more than half (58%) were obtained from the Intensive Care Unit (ICU) patients. The antifungal susceptibility pattern of NAC species indicated that most NAC was susceptible. Out of 15 isolates of Candida tropicalis, 11(73.0%) were susceptible to fluconazole, and susceptibility against the other antifungals was 100%. All of the isolates of C. glabrata were 100 % susceptible to micafungin, amphotericin B, and flucytosine. All C. krusei, and C. guilliermondii isolates were susceptible to all the tested antifungals except fluconazole, and amphotericin B, respectively. C. ciferrii showed 100% susceptibility to all antifungals.

Conclusion: All ten unidentified isolates showed 100% susceptibility to all the drugs except fluconazole. This study has shown increased NAC and high susceptibility to antifungals.

Keywords: Candida albicans, non albicans Candida, susceptibility testing, VITEK

Original Research Article

Introduction:

Candida species are well-known opportunistic fungi that can cause fungal infections in immunocompromised individuals [(1)]. Candida species rank seventh in the United States as the most common cause of bloodstream infections. These infections contribute to a significant number of morbidities and mortalities worldwide. Being an opportunistic pathogen, it affects the subjects with predisposing risk factors. Among these risk factors are immunosuppression, exposure to broad-spectrum antimicrobials, cytotoxic drugs, catheterization, malignancies, diabetes myelitis, chronic kidney disease, post-surgery, hemodialysis, neutropenia, prolonged hospitalization, organ transplantation, parenteral feeding, intensive care setting, and elderly age [(2)].

Candida species are typically found as part of the normal flora in healthy individuals, residing in various body sites such as the skin and mucosal linings of the oral cavity, vagina, and gastrointestinal tract [(3)]. While numerous species are within the Candida genus, only a few are known to cause infections in humans. The most prevalent species responsible for most cases is C. albicans, accounting for approximately 80% of candidiasis cases. However, there is also a growing trend in cases of non-albicans Candida (NAC) infections. [(4)]. The worldwide studies showed a grave danger of increasing infections from the NACs. Multiple reasons for this sudden rise of NACs have been proposed, including excessive use of azoles and polyenes, improved diagnostic technologies, geographical factors, increased life expectancy and malignancies [(5)]. Among these NACs, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C. dubliniensis, C. kefyr and C. guilliermondii are said to be associated with most of the infections (10). Among these, C. glabrata, C. krusei, C. parapsilosis and C. tropicalis are more common [(6)].

The incidence of different NAC species varies geographically and depends upon multiple factors. Geographically, C. tropicalis is more prevalent in the Middle East and Africa while

C. glabrata is common in the European Union (EU) and Asia-Pacific. Most cases of C. parapsilosis are reported in Latin and North America. As far as the physiological factors are concerned, most Candida spp. especially C. glabrata infect older adults (>60 years). However, C. parapsilosis affects infants less than one year of age [7]. These Candida species have been documented as emerging human pathogens in global surveillance programs such as SENTRY and ARTEMIS. NACs accounted for 10-40% of all candidiasis cases during 1970-1990, but Krcmery et al reported 2002 that this percentage has increased to 35%-65% [8]. Therefore, these NACs are emerging as serious public health risks and must be given equal attention to C. albicans to minimize the incidence rate.

Exceptionally high antifungal resistance among the NACs mentioned above is a severe concern. Commonly used antifungals belong to four classes: azoles, echinocandins, polyenes and pyrimidine analogues. Antifungal resistance can be intrinsic or acquired [9]. Compared to C. albicans, NACs exhibit varying degrees of acquired resistance to most antifungals. Intrinsic resistance is also quite common among them. This study aimed to identify emerging Candida species from clinical samples and estimate antifungal susceptibilities.

Methodology

This cross-sectional study was conducted in the Microbiology laboratory of a tertiary care hospital in Lahore in collaboration with the Institute of Microbiology and Molecular Genetics, University of the Punjab Pakistan, from September 2020 to April 2021. All Candida-suspected samples were received in a microbiology laboratory and passed through the inclusion and exclusion criteria. All of the suspected Candida species isolated from these clinical samples were included in the study. However, duplicate samples from the same patient during the same episode of illness were excluded.

Identification of Candida spp.

All the Candida suspected clinical samples were processed as the standard operating procedure of the lab. All the samples were inoculated on Sabouraud agar and blood agar to confirm Candida. They were incubated for 18 hours at 37℃ aerobically. Colonial growth was subjected to wet prep and gram staining. Candida spp. was preliminary identified based on cultural and microscopic morphologies. Germ tube testing and Sabouraud agar Candida were used to differentiate C. albicans from non-albicans Candida (NAC) species.

Germ tube testing

Germ tube testing were performed for C. albicans. The germ tube was the filamentous extension of the yeast cells. In Candida cells, some proteins and ribonucleic acid production are enhanced upon incubation at 37℃ in human or sheep serum for 3-4 hours. For this, colonies are incubated at 37℃ for 3 hours in sheep or human serum. In a test tube, 2 ml of pooled serum was added and inoculated with 1-2 colonies of suspected Candida species. Tubes were incubated at 37℃ for 3-4 hours. After incubation, one drop of suspension on a glass slide was placed with a Pasteur pipette and covered through a cover slip. The slide was examined microscopically for yeast cells with filamentous extension. C. albicans ATCC 10231 was used as the positive, and C. krusei ATCC 6258 was used as negative control.

Specie identification via VITEK 2.0. compact and Antifungal susceptibility testing of non-albicans Candida species

All isolates identified as NACs in the previous step were simultaneously subjected to species identification and antifungal susceptibility testing through VITEK 2.0. compact. In this system, the VITEK YST card was employed for specie identification, and the AST-YS08 card was used to measure MICs against fluconazole, voriconazole, flucytosine, amphotericin B and caspofungin. So, 3ml saline solution was taken in a tube and mixed with a pure bacterial culture. Mcfarland standard (0.50-0.63) was made. For gram-negative antibiotic susceptibility (GN-AST), a 145(l from GN-ID Mcfarland solution was mixed in another tube containing 3ml saline solution. For gram-positive antibiotic susceptibility (GP-AST), a 280 (l from GP-ID Mcfarland solution was mixed in another tube containing 3ml saline solution and different VITEK cards, i.e., GN-ID, GN-AST, GP-ID, and GP-AST, were placed in the tubes, respectively. The racks containing tubes in biomerieux VITEK 2 compact for loading samples were placed. When the samples were loaded, the racks were transferred to the other side to remove the cards and press start. Took out the racks after 5-7 minutes. Logged in to VITEK 2 software on the system. Clicked on cassette view, then clicked on samples showing incomplete status and entered the accession numbers of the samples and linked the AST of samples with their IDs. The results were noted after 18 hours of incubation.

Ribotyping

Two Isolated colonies of unidentified Candida spp. were sent for 18S rRNA sequencing. The sequencing results were subjected to nucleotide BLAST (Basic Local alignment search tool). The isolates were identified as species with the maximum similarity percentage in the database. The evolutionary history was inferred using the Neighbor-Joining method [10]. The optimal tree is drawn to show the rate of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) next to the branches [11]. The evolutionary distances were computed using the Maximum Composite Likelihood method [12] and are in the units of the number of base substitutions per site. The evolutionary analyses were conducted in MEGA11 [13].

Results

Among all the suspected clinical samples that were received within the study duration, 86 were identified as positive for Candida. Out of 86, 41(47.8%) isolates were identified as C. albicans by germ tube development, and the rest of the 45(52.3%) isolates were of non-albicans-Candida (NAC). These 45 NACs were considered emerging Candida spp. and further processing through VITEK 2.0. compact yielded the results of NAC. Candida glabrata was isolated from 8(17.8%) samples, C. tropicalis was also yielded from 15(33.3%) samples, C. krusei from 4(8.9%) and frequencies of C. ciferrii, C. famata, C. guilliermondii, Trichosporon asahii and unidentified Candida species were 3(6.7%), 1(2.2%), 1(2.2%), 3(6.7%) and 10(22.2%) respectively.

In this study, 27(60%) NAC species were male, and 18(40%) belonged to female samples. Out of 45, 39 (87.0%) NACs were isolated from urine samples, 4(9.0%) from blood, and in the rest of the cases, 2(4.4%) were isolated from pus and tracheal secretion. There were 5(11.1%) cases of co-existing bacterial pathogens. A single Enterococcus feacalis and 2, 2 (4.4%) cases of E. coli and Klebsiella pneumoniae were reported.

Out of 45, 2(4.4%) cases of Candida infection were reported in <1 year old and 4(9.0%), 0(0.0%), 8(17.8%), 11(24.4%) and 20(44.4%) was reported from 1-15, 16-30, 31-45, 46-60, >60 years old respectively (Fig. 1).

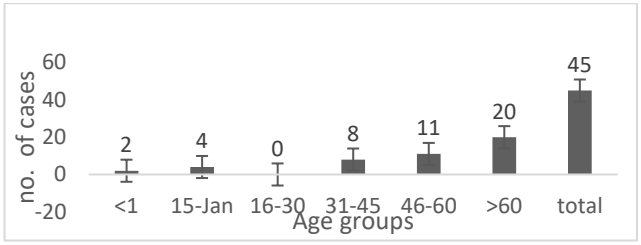

Fig-I Distribution of different NACs in different age groups

Out of 45 isolates, more than half 26(58%) isolates were from patients in the Medical ICU. And 9(20%) from Outpatient, 6(13%) from medical unit and 2(4%), 2(4%) from ICU-N and paediatrics department respectively (Fig. 2).

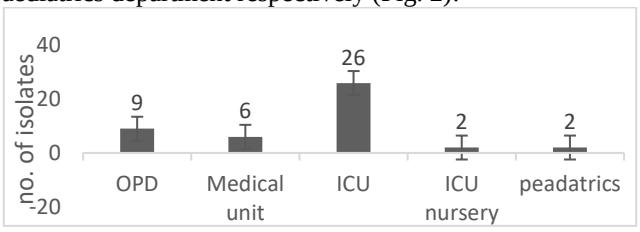

Fig-II Ward-wise distribution of NACs

The antifungal susceptibility pattern of NAC species indicated that out of 15 isolates of *Candida tropicalis*, 11(73.0%) isolates were susceptible to fluconazole and susceptibilities against the other antifungals were 100%. Out of 8 *C. glabrata* isolates, no one was susceptible to fluconazole, 6(75.0%) were susceptible to voriconazole, and 7(87.5%) were susceptible to caspofungin. The susceptibility of the isolates against micafungin, amphotericin B and flucytosine was 100%. All the isolates of *C. krusei* (4 out of 4) were susceptible to all the tested antifungal agents except fluconazole (because of intrinsic resistance pattern) and flucytosine (0.0% susceptible). A single isolate of *C. guilliermondii* was susceptible to all antifungals except amphotericin B. Out of 3 isolates of *Trichosporon asahii*, 2(66.7%) were susceptible to fluconazole, 3(100.0%) were susceptible to voriconazole none was susceptible to caspofungin and micafungin and 1(33%) isolate was susceptible to amphotericin B and flucytosine. All ten isolates of other *Candida species* (unidentified) showed 100% susceptibilities to all drugs, except susceptibilities to fluconazole were 0%. *C. ciferrii* showed 100% susceptibility to all antifungals but susceptibility to flucytosine was reduced to 66.7%. A single isolate of *C. famata* was susceptible to all tested antifungals (Fig. 3).

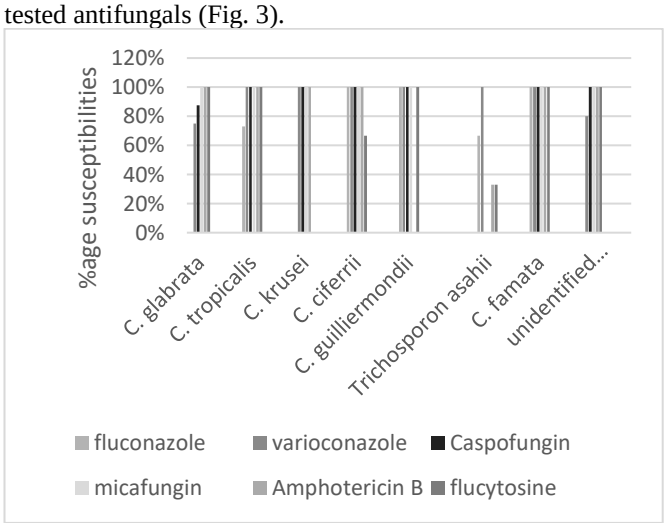

Fig-III Percentages of antifungal susceptibility patterns of NACs

Two isolates were randomly subjected to 18S rRNA sequencing for species identification among unidentified Candida species. Nucleotide BLAST (Basic local alignment search tool) of the 18S rRNA identified one isolate as Candida glabrata (with a percent similarity of 98%) with the accession number MZ562722.1. While the other was identified as *Saccharomyces boulardii* (similarity 99%) with accession number KT000033.1. The neighbour-joining phylogenetic tree was constructed based on the GenBank data of these two strains with similar strains, which was drawn to scale with the same branch length units as the evolutionary distances [13]. The maximum composite likelihood method was used to measure the evolutionary distances. The analysis involved ten nucleotide sequences, in which our strains showed close similarity (Fig. 4)

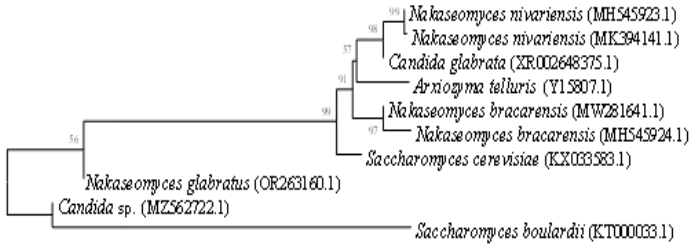

Fig-IV Molecular phylogenetic analysis by neighbour-joining method using MEGA 11

Discussion

In this study, the percentage of non- albicans *Candida* species (NACs) was higher than that of C. albicans and was isolated from more than half (52.3%) of the clinical samples. The frequency of *C. tropicalis* (33.3%) was the highest, followed by Unidentified *Candida* species (22.2%), *C. glabrata* (17.8%), *C. krusei* (8.9%), *Tricosporon asahii* (6.7%) and *C. ciferrii* (6.7%). Giacobbe *et al*. conducted a metanalysis of the data on NACs from 44 countries published between 1971 to 2018 to estimate the pooled prevalence of NAC [14]. This metanalysis revealed that in 49.5% ± 1.5 cases, NACs were responsible for candidiasis, which corresponds with our results. *C. tropicalis* was the most frequent NAC in Asian studies, with a pooled prevalence of 21.3%. We also report similar results. *C. tropicalis* remained to be the most frequent isolate. However, its prevalence is 33.3 % which is higher than that of the metanalysis conducted by Daniele.

A study conducted by Jeon JS and Kim JK in South Korea from 2014 to 2018 examined the prevalence of Candida species [15]. Similar to our research, they utilized VITEK 2.0 for species identification. Their study reported that NAC species were the most frequently isolated from clinical samples. The percentage of NAC isolation was 59.5%, slightly higher than what we observed in our study. It is important to note that their research specifically focused on Candida species isolated from blood samples. However, *Candida* species isolated from all types of clinical samples were included. Out of all NACs, 87.0% were isolated from urine samples. Satyendu Saha concluded that maximum NACs were isolated from urine samples followed by vaginal swabs [16]. However, in the study, after urine samples, most of the isolation was from blood samples, and no NAC was isolated from vaginal swabs.

A study from Taiwan reported a higher incidence of NACs (54.2%) candidemia in children than that of *C. albicans* [17], which also agrees with the findings of our study. Pfaller *et al* reported in their research that NAC mostly affects people in the

age group >60, which corresponds to our research. Here the most affected age group was also >60 [18]. Kothalawala *et al* from Sri Lanka also associated 69% of cases of candidemia with NACs using VITEK 2.0 for identification [19]. A 6-year surveillance in Korea also concluded that *C. tropicalis* was the most prevalent NAC and constituted 36.4% of all NACs, approximating our results [20].

Chakrabarti *et al* conducted a prospective study and concluded that *C. tropicalis* (42.1%) was the most frequent NAC with azole resistance of 10.2%-13.6% [21]. While in our study, *C. tropicalis* showed 0% resistance against voriconazole and 27% resistance against fluconazole. In the present study, no NAC showed resistance against echinocandins such as caspofungin and micafungin except one *C. glabrata* isolate resistant to caspofungin but susceptible to micafungin. The same results were obtained for echinocandin susceptibility in a study by Yenisehirli *et al* [22]. Many other studies also concluded that echinocandin was active against NACs [23]. In our study, the gene sequencing and phylogenetic analysis by MEGA 11 suggested a close relation of *C. glabrata* with *S. boulardii*, which may be the reason this *Candida* spp resists echinocandin as described by Vermitsky *et al*., 2006 [24].

Resistance to amphotericin B was also rare, and only 4.4% of isolates showed resistance against it including *C. guilliermondii* and *Trichosporon asahii*. Hitkova *et al* also revealed that resistance against amphotericin B was uncommon [25]. In this study, only 5.97% of isolates resisted it, which is very close to our findings. El-Ganiny *et al* also discussed this uncommon amphotericin B resistance in NACs [26]. Shawky *et al* reported that out % of all *C. krusei* isolates, 84.6% were resistant to flucytosine [27]. Resistance to azoles is increasing among *Candida* species, especially in the NACs; 60% of NAC showed resistance to fluconazole, while only 8.9% were resistant to voriconazole in the present study. None of the *C. glabrata* and *C. krusei* were susceptible to fluconazole, while resistance to voriconazole was 25% and 0%, respectively.

The percentage of resistance against fluconazole showed by *C. tropicalis*, and *Trichosporon asahii* was 27% and 33.3%. While the *C. krusei* showed 100% resistance to fluconazole which may be due to its intrinsic resistance pattern and following the results obtained by Satyendu Saha [16]. In contrast to the finding of these studies, 60%-69% isolates of *C. glabrata* were susceptible to fluconazole. However, another study conducted by Mashaly and Shrief from Egypt also reported 0% susceptibility of *C. glabrata* to fluconazole [28]. A study from US reported that no isolate of *C. glabrata* showed susceptibility to fluconazole while using E-test. For *C. tropicalis*, Sadeghi *et al* also observed the emergence of antifungal resistance to fluconazole with the percentage of 14.3% which is slightly lower than our findings [29].

Interestingly, ten isolates remained unidentified in our study on VITEK 2.0; to our surprise, no one was susceptible to fluconazole. These were supposed to be the *C. auris* which cannot be identified on VITEK 2.0 and shows a high resistance to fluconazole [30]. Owing to our limited resources, we could not identify these isolates. Only two isolates were processed. In conclusion, non-albicans *Candida* species are emerging as dominant pathogens, surpassing *C. albicans*, and showing significant resistance to antifungal treatments.

Author contribution

NS: experiment, data and strain collection; FR: strain collection and demographic data; ANS and SR: designing the experiment, data analysis, and manuscript writeup.

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Conflict of interest

None to declare

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Tip-Toe Walk After Acute Viral Illness: a pediatric case report

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ABSTRACT

A school going, 5 years old vaccinated baby boy presented in Outdoor Patient Department with complaints of tip-toe walk and difficulty in walking associated with pain in both lower legs since morning; after having an acute viral illness 4 days back. Neurological examination was unremarkable without any significant positive findings. Lab investigation was advised in the form of serum creatine kinase level which was significantly elevated leading to diagnosis of Acute Viral Myositis. Oral Ibuprofen was advised. The child was kept under observation to document rhabdomyolysis; a complication of acute viral myositis. Signs and symptoms were resolved within 48 hours without any specific treatment.

KEYWORDS: Acute Viral Myositis; Serum Creatine Kinase; Rhabdomyolysis.

Original Research Article

Introduction:

Myositis comes from the Greek words myo ("muscle") and itis ("inflammation"). The cause of infectious myositis include: viral, bacterial, fungal and parasitic agents. Common viral agents are; Influenza virus (causing benign myositis and acute rhabdomyolysis) and Coxsackie virus B (causing pleurodynia). Bacterial agents are summarized as Staphylococcus aureus, group A Streptococcus, group B Streptococcus and Clostridium difficle. Fungal agents affect the immunocompromised hosts causing self-limiting myositis. Common parasitic agents that cause acute benign myositis are Trichinosis and Cysticercosis.

Benign acute childhood myositis (BACM) is an acute transient onset of lower limbs pain documented during or after recovery from a viral illness. Clinical picture may vary but may involve tiptoe gait or difficulty in walking that settles down itself very quickly in three to four days. Common lab finding is highly elevated serum creatine phosphokinase. A rare documented complication is rhabdomyolysis followed by myoglobinuria. Management includes rest and analgesia [2].

Differential diagnoses to BACM (benign acute childhood myositis) include: Trauma, Guillain-Barré syndrome, Osteomyelitis, Deep vein thrombosis, Juvenile rheumatoid arthritis, Malignancy, Dermatomyositis, Polymyositis, Muscular dystrophy and Intracranial pathology [2].

Any virus can cause an acute myositis, yet influenza virus and enterovirus are the most notorious. Chronic viral myositis is associated with retroviruses and hepatitis viruses.

Creatine kinase (CK) also labeled as Creatine phosphokinase (CPK) or Phosphocreatine kinase; is an enzyme that catalyzes the reversible conversion of creatine to phosphocreatine (PCr) and adenosine diphosphate (ADP) by using adenosine triphosphate (ATP).

PCr (phosphocreatine) acts as an energy source for cells and tissues like brain, smooth muscle, skeletal muscle, retina (photoreceptor cells), spermatozoa and inner ear (hair cells) through RCr shuttle.

Clinically, creatine kinase is labeled as a marker of tissue damage in myocardial infarction and rhabdomyolysis. Isoenzyme of creatine kinase may vary in different tissues. CK-MM (98%) and CK-MB (1%) are expressed by skeletal muscles while CK-MM (70%) and CK-MB (25-30%) are expressed by cardiac muscles. CK-BB is expressed by brain and smooth muscles.

Case Presentation:

Muhammad Ahmad, 5 years old vaccinated boy with appropriate height and weight with respect to his age and normal developmental milestones, resident of Kot Radha Kishan (district Kasur) presented in OPD with complaints of tip-toe walk and difficulty in walking associated with pain in both lower legs since morning. Patient was alright 4 days back when he developed complaint of fever with cough and sore throat. Fever was continuous and high grade 103F documented at home, not associated with rigors and chills and relieved by taking oral antipyretics. Medication was taken at that time from local physician in the form of oral antipyretics, antitussives and antiallergy. Fever had been settled then the child was afebrile for last 2 days. Since morning the child had difficulty in walking associated with tip-toe walk and pain in both lower legs without swelling/ edema, petechia or skin temperature change. On neurological examination: child was fully alert and conscious with respect to time, space and person with normal Power and Tone in all 4 limbs with intact superficial reflexes and deep tendon reflexes. Rest of cardiovascular, respiratory and gastrointestinal examination was unremarkable. Serum creatine kinase (CK) was done which was highly raised

9868U/L (normal value: male 24-195U/l female 0-174U/L) with normal inflammatory markers i.e., CRP which was 0.43mg/dl (normal value: <5mg/dl) and ESR 10mm/1st hour (normal value: 0-10mm/1st hour). Complete blood picture showed Hb 14.1g/dl TLC 6.0*109/l Platelets 306*109/l with predominant lymphocytes 65% and neutrophils 27%. Child was labeled as a case of Acute Viral Myositis on the basis of history, clinical presentation, examination findings and lab values. Syp. ibuprofen was advised only. The signs and symptoms were resolved within 48 hours without any documented evidence of complication of the disease.

Discussion:

Case reports and articles have been reported and published from countries all over the world on benign acute childhood myositis (BACM) including Portugal[1], Canada[2], Switzerland[4], Scotland[6], Brazil[7], India[8], United States[9,12], Iran[10] and Greece[11]. Numerous viruses have been found responsible for such sudden, self-resolving and benign inflammatory condition including most commonly Influenza virus [3,4,5] Dengue [8], Covid-19 [12] and other respiratory viruses [7].

A retrospective study were conducted in Portugal from 2015 to 2019 in pediatric emergency department illustrated that benign acute childhood myositis was found to be a rare, self-limiting and acute complication after an viral illness irrespective of the virus isolation due to limited availability of laboratory facilities and cost-effective treatment. The most prevalent mycotrophic virus documented during outbreak of 2019/2020 and flu season was Influenza B virus[1].

Influenza-associated myositis is a sudden and rare complication of influenza virus infection in school-going children affecting the males two times more than the females[3,4]. Influenza B virus is isolated in 76% of cases while Influenza A virus is isolated in 24% of cases. Unilateral or bilateral involvement of the calf muscles is documted. [4,5] Labs investigations show elevated creatine phosphokinase (CPK) level.4 Patients are improved without specific treatment in three to five days[4,5,6]. A rare complication is Rhabdomyolysis (3%) that is more common in girls (80%). Rhabdomyolysis is more common with influenza A virus (86%) leading to renal failure in (80%) patients [4].

Dermatomyositis should not be confused with influenza-associated acute myositis of childhood [5].

Seasonal influenza virus or other respiratory viruses may present with sudden myotropic inflammatory condition with elevated CPK [7].

Dengue virus also causes acute myositis involving difficulty in walking with lower limb muscle weakness but having normal power, tone and deep tendon reflexes which may be investigated by elevated CPK [8].

Community-acquired MRSA is now-a-days documented as an alarming cause of clinically documented pyomyositis and myositis in children [9]. Lab investigations are not mandatory to diagnose and treat myositis. General practitioners and family physicians can diagnose the case and treat by himself or herself on the basis of history, clinical picture, general physical examination and neurological findings [10, 11].

Rhabdomyolysis and elevated CK have been reported as complications of COVID-19 in pediatrics. Pediatric clinicians should be aware of this complication [12].

Conclusion:

Acute viral myositis is an acute and self-limiting inflammatory process; documented as a complication after a preceding viral illness. It usually affects preschool and school going aged children presenting pain in the muscle groups of the both lower limbs mainly affecting the gait of the patient without any significant positive findings in the neurological examination. Rhabdomyolysis, a rare complication of the disease, is investigated by serum creatine kinase level and myoglobinuria. We are presenting the case of a school-going child diagnosed as a case of acute viral myositis. As it is not a usual presentation after acute viral illness, we consider it significant to report the case and review the topic, as it may be helpful for the pediatricians and general practitioners to manage these cases without hospitalization of the patient by using only oral paracetamol or ibuprofen.

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