Original Article

OPEN ACCESS

Metastatic Axillary Lymphadenopathy in Breast Cancer: Diagnostic accuracy of Ultrasound Combined with Ultrasound Guided Fine Needle Aspiration

Sana Shaikh[1], Kashaf Anwar[2], Aiman Rahim[3]

[1]Associate Professor, [2]Senior Lecturer Dept. of Radiology
Sindh Institute of Urology and Transplantation (SIUT), Karachi
[3]Medical Student, Dow University of Health and Sciences, Karachi

Author`s Contribution

[1]Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work,[2]Drafting the work or revising it critically for important intellectual content, [3]Active participation in active methodology

Funding Source: None
Conflict of Interest: None

Received: Dec 10, 2024
Revised: April 18, 2025
Accepted: April 29, 2025

Address of Correspondent
Dr Sana Shaikh
Associate Professor, Radiology department SIUT, Karachi
docsanashaikh@gmail.com

ABSTRACT

Objective: To detect sonographic criteria for detection of metastatic involvement of axillary lymph nodes (ALN) and to detect accuracy of ultrasound combined with ultrasound guided FNAC in staging workup of breast cancer.

Methodology: A prospective cross-sectional study was conducted in the Radiology Department of the Sindh Institute of Urology and Transplantation (SIUT), Karachi, from October 2020 to October 2021. The study included patients diagnosed with either symptomatic or screen-detected breast cancer who underwent preoperative ultrasound evaluation of the breast and axilla. Fine-needle aspiration biopsy (FNAB) was performed on axillary lymph nodes with sonographic features suggestive of malignancy using an 18-gauge needle attached to a 10 mL disposable syringe. The aspirated samples were reviewed by a histopathologist. The diagnostic performance of ultrasound and ultrasound-guided FNAC was compared to the final histopathological findings of surgically excised axillary lymph nodes. Data were analyzed using SPSS version 20.

Results: The mean age of the study participants was 48.05 ± 11.3 years. Ultrasound alone demonstrated a sensitivity of 60.9%, specificity of 39.5%, and overall accuracy of 79%. When combined with FNAC, diagnostic sensitivity increased to 92%, specificity to 85%, and accuracy to 53.1%. Notably, the highest diagnostic accuracy was observed with the combined use of ultrasound and FNAC, yielding a sensitivity of 81.8%, specificity of 62.0%, and overall accuracy of 74.6%. The combined approach also achieved the highest positive predictive value (78.9%) and negative predictive value (66.1%), indicating superior diagnostic performance.

Conclusion: The combined use of ultrasound and ultrasound-guided FNAC proves to be a highly effective and reliable diagnostic modality for detecting metastatic axillary lymphadenopathy in breast cancer patients. This dual approach enhances diagnostic accuracy and supports its integration into the preoperative staging protocol for breast carcinoma.

Keywords: Breast cancer, ALN, Ultrasound, FNAC, Sensitivity, Specificity

Cite this article as: Shaikh S, Anwar K, Rahim A. Metastatic Axillary Lymphadenopathy in Breast Cancer: Diagnostic Accuracy of Ultrasound Combined with Ultrasound Guided Fine Needle Aspiration. Ann Pak Inst Med Sci. 2025; 21(2):430-435. Doi.10.48036/apims.v21i2.1325.

Introduction

Breast cancer is a leading cause of illness and death in women, representing a serious public health concern.[1] In these patient's axillary nodal status determines staging,[2] and remains an important prognostic factor.[3] Various imaging modalities can provide information on axillary nodes preoperatively. Mammography despite being the primary imaging modality does not completely visualize the axilla and is not helpful in staging the axilla. Ultrasound has become an indispensable imaging modality in evaluating various pathological conditions due to its real-time imaging capability, non-invasiveness,

and absence of ionizing radiation. In assessing superficial and deep-seated lesions, particularly in the thyroid, lymph nodes, and breast, US plays a pivotal role in detecting malignancies and guiding further diagnostic interventions.[4,5] However, while US provides crucial morphological information, its specificity remains limited, necessitating additional confirmatory techniques to improve diagnostic accuracy.[6] Fine-needle aspiration cytology (FNAC) is widely regarded as the gold standard for obtaining cytological samples from suspicious lesions, offering a minimally invasive, cost-effective, and highly sensitive approach to tissue characterization.[7]

Despite its advantages, conventional FNAC has limitations, including inadequate sampling and operator dependence, which may lead to false-negative or inconclusive results.[8] The combination of ultrasound with ultrasound-guided FNAC (US-FNAC) has significantly enhanced the precision of cytological sampling by ensuring accurate needle placement, particularly in small or non-palpable lesions.[9] Several research studies have proven that sensitivity and specificity for diagnostic of lesions are higher with US-FNAC than with that of FNAC palpation only, and the rate of non-diagnostic or insufficient samples is lower.[10,11] The most prominent benefit of this technique is in assessing thyroid nodules, cervical lymphadenopathy and breast lesions where differentiating benign from malignant pathology is important for the clinical practice.[12,13] Though its increasing implementation, there remains concern regarding experiences of the operators, characteristics of the lesions and adequacy of the samples, which requires further definition of its diagnostic performance across anatomical sites and the clinical situations.[14,15] This study was conducted to evaluate the diagnostic efficacy of ultrasound and ultrasound-guided fine-needle aspiration cytology (FNAC) in detecting metastatic axillary lymphadenopathy, with the goal of promoting a non-invasive approach to diagnosing axillary lymph node involvement in breast cancer patients. By comparing cytological findings with final histopathological results, the study aims to advocate for this combined method as a high-accuracy, cost-effective, and minimally invasive alternative to surgical biopsy. The findings may aid in the early and accurate detection of axillary metastasis, potentially reducing unnecessary surgical procedures and contributing to improved clinical decision-making. Such an approach is particularly advantageous in resource-limited settings, where it could enhance oncology care and optimize patient outcomes.

Methodology

This prospective cross-sectional study was conducted in the Radiology Department of the Sindh Institute of Urology and Transplantation (SIUT), Karachi, from October 2020 to October 2021, following approval from the Institutional Ethical Review Committee (Ref No. SIUT-ERC-2020/A-233). The study included patients with both symptomatic and screen-detected breast cancer who underwent preoperative ultrasound examination of the breast and axilla.

Exclusion criteria included patients in whom axillary FNAC was not performed due to small lymph node size, those who received neoadjuvant chemotherapy, patients who underwent FNAC for primary tumors other than breast cancer, and those who declined to participate.

Ultrasound examinations of the breast and axilla were performed using a 14 MHz linear array transducer on a Xario 200 ultrasound system. All scans were conducted by a radiologist with over five years of clinical experience. Axillary lymph nodes were assessed and reported as abnormal based on the following sonographic criteria: size, morphology, long and short axis diameters, aspect ratio (long-to-short axis), cortical thickness (concentric or eccentric), presence or absence of a central echogenic fatty hilum, and vascularity (abnormal blood flow). Tumor burden was evaluated by determining the number and ratio of involved to uninvolved lymph nodes.

Ultrasound-guided FNAC was performed using an 18-gauge needle attached to a 10 mL disposable syringe. In cases with multiple abnormal lymph nodes, the most suspicious node was selected for aspiration. The needle was introduced into the thickest part of the cortex for sample collection.

All breast tumor and lymph node specimens were reviewed by a dedicated histopathologist. The results of ultrasound and ultrasound-guided FNAC were compared to the final histopathological findings from surgically removed axillary lymph nodes obtained via sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND). Data were collected regarding the total number of lymph nodes removed, number of nodes with metastatic involvement, size of metastatic deposits, and presence or absence of extranodal extension.

In cases where cytological samples were deemed insufficient for diagnosis, a repeat ultrasound-guided FNAC was performed. For this reason, cytological results in the study were reported only as either positive or

negative. Additional pathological and prognostic factors—such as histologic type and grade of the breast tumor, estrogen and progesterone receptor status, and Ki-67 expression—were also recorded.

Data entry and statistical analysis were performed using SPSS version 20. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation. Diagnostic parameters including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Associations were assessed using the Chi-square test, with a p-value of <0.05 considered statistically significant.

Results

Overall mean age of the patients was 48.05±11.37 years. The most common presenting complaint was a breast lump, reported by 91% of patients, followed by pain in 5.6%, while 1.7% had both lump and pain, and another 1.7% reported nipple discharge. The disease affected the right breast in 51.4% of cases and the left in 48.6%. A family history of breast cancer was positive in 34.5% of patients. Regarding menopausal status, 51.4% were postmenopausal. Only 20.3% had a history of lactation. Mammographic findings were positive in 98.9% of patients. Based on histopathological luminal classification, 44.6% were Luminal B, 21.5% Luminal A, 18.1% triple-negative, and 15.8% HER2-neu positive. (Table I)

Table I: Clinical characteristics of the patients. (n= 354)

Variables		N	%
Presenting complaint	Lump	322	91.0%
	Pain	20	05.6%
	Lump and pain	06	01.7%
	Nipple discharge	06	01.7%
Site	Right	182	51.4%
	Left	172	48.6%
Family history	Positive	122	34.5%
	Negative	232	65.5%
Postmenopausal status	Yes	182	51.4
	No	172	48.6
Lactating history	Yes	72	20.3
	No	282	79.7
Mammogram	Positive	350	98.9
	Negative	04	01.1
HP Luminal classification	Liminal A	76	21.5
	Luminal B	158	44.6
	Triple -ve	64	18.1
	her2 neu	56	15.8

Ultrasound identified a lump in 98.9% of cases, while only 1.1% presented with pain. Ultrasound assessment of axillary lymph nodes showed that 40.7% were benign, 41.8% malignant, and 17.5% had intermediate suspicion. Histopathological analysis revealed malignant cells in 62.1% of patients, whereas 37.9% showed no malignancy. Regarding tumor grade, 48.6% of tumors were grade III, 43.5% were grade I, and 7.9% were grade II. Additionally, 42.4% of patients had a positive breast mass or ductal carcinoma in situ (DCIS), while 57.6% tested negative. (Table II)

Table II: Diagnostic characteristic of the patients. (n= 354)

Variables		**N**	**%**
Ultrasound	Lump	350	98.9
	Pain	04	01.1
USAL presence	Benign	144	40.7
	Malignant	148	41.8
	Intermediate suspicion	62	17.5
HP Cells	Malignant	220	62.1
	No malignant cells	134	37.9
Grade of tumor	I	154	43.5
	II	28	07.9
	III	172	48.6
Breast Mass and DCIS	Positive	150	42.4
	Negative	204	57.6

US alone showed a sensitivity of 60.9%, specificity of 39.5%, PPV of 63.7%, NPV of 36.7%, and an overall accuracy of 53.1%. In contrast, the combination of ultrasound with FNAC demonstrated much better performance, with a sensitivity of 81.8%, specificity of 62.0%, PPV of 78.9%, NPV of 66.1%, and accuracy of 74.6%, indicating that the combined approach significantly improves the diagnostic accuracy compared to ultrasound alone. (Table III)

Table III: Diagnostic accuracy of Ultrasound combined with Ultrasound guided FNAC taking histopathology as gold standard.

US findings + Combined findings		**Histopathology**		**Total**
		Negative	**Positive**	
US findings	Negative	51	88	139
	Positive	78	137	215
Total		129	225	354
Combined findings	Negative	80	41	121
	Positive	49	184	233
Total		129	225	354

US findings	Sensitivity	Specificity	PPV	NPV	Accuracy
	60.9%	39.5%	63.7%	36.7%	53.1%
Combined findings	81.8%	62.0%	78.9%	66.1%	74.6%

Discussion

Breast cancer is the most common malignancy among females, with axillary lymph node involvement recognized as a critical prognostic factor. For many years, axillary lymph node dissection has been the standard procedure for assessing nodal metastasis and guiding

systemic treatment decisions.[16,17] The present study was conducted on 354 patients to evaluate the diagnostic accuracy of ultrasound combined with ultrasound-guided fine-needle aspiration (FNA) in detecting metastatic axillary lymphadenopathy in patients with breast cancer. The overall mean age of the participants was 48.05 ± 11.37 years. This is slightly lower but comparable to the findings of Kane G et al.[3], who reported a mean age of 55 years, and Gouveia MC et al.[16], who noted a mean age of 56 years. Variations in the reported mean age across studies may be attributed to differences in study populations, geographic distribution, and access to healthcare services, all of which can influence the age at diagnosis and clinical presentation of breast carcinoma.

In the present study, luminal classification revealed that 21.5% of patients were categorized as Luminal A, 44.6% as Luminal B, 18.1% as triple-negative, and 15.8% as HER2-positive. These findings indicate that Luminal B was the most prevalent subtype, suggesting a higher proportion of tumors with more aggressive features and potential resistance to hormonal therapy. When compared to previous studies, notable variations are observed. Henna N et al[18] reported a higher prevalence of Luminal A (37.2%) and a lower frequency of Luminal B (12%), while triple-negative and HER2-enriched subtypes were reported at 30.1% and 20.5%, respectively, highlighting geographical or ethnic differences in breast cancer subtypes. Similarly, Millar et al[19] showed a predominance of Luminal A (79.1%) and only 4.6% Luminal B, further underscoring regional variation. On the other hand in a study reported 32.8% Luminal A, 27.9% Luminal B, 13.3% HER2-positive, and 26.7% triple-negative cases, showing a distribution more balanced across subtypes.[20] Another study found Luminal B to be most common (43.73%), followed by Luminal A (27.97%), HER2-enriched (20.9%), and triple-negative (7.4%), closely aligning with our current findings.[21] The differences across studies may be because of genetic diversity, environmental factors, diagnostic criteria, and population-specific risk factors.

In this study US alone showed a sensitivity of 60.9%, specificity of 39.5%, PPV of 63.7%, NPV of 36.7%, and an overall accuracy of 53.1%. In contrast, the combination of ultrasound with FNAC demonstrated much better performance, with a sensitivity of 81.8%, specificity of 62.0%, PPV of 78.9%, NPV of 66.1%, and accuracy of 74.6%, indicating that the combined approach significantly improves the diagnostic accuracy compared to ultrasound alone.

In alignment with the present study, a related investigation was conducted by Singh R et al.[22], who evaluated the diagnostic accuracy of ultrasound (US) and ultrasound-guided fine-needle aspiration cytology (FNAC) in axillary assessment among 100 breast cancer patients. In their study, 58 patients had non-suspicious lymph nodes, while 42 presented with suspicious nodes on axillary ultrasound. They reported 61.5% sensitivity, 75.6% specificity, 69.5%PPV, and 68.5% NPV of ultrasound, while in comparison, USG-FNAC demonstrated a higher diagnostic performance, with sensitivity of 83%, specificity of 100%, PPV of 100%, and NPV of 72.6%.[22] Additionally, they reported an overall diagnostic accuracy of 69% for ultrasound alone and 88.1% for USG-FNAC, supporting the current study and emphasizing the superior effectiveness of combining ultrasound with guided needle aspiration in detecting metastatic axillary lymphadenopathy, particularly for preoperative staging and treatment planning.[22] In the study by Oz A et al[23], ultrasound-guided FNAB demonstrated a sensitivity of 88.46% for detecting axillary metastases, which declined to 76.66% when cases with inadequate sampling were included. Importantly, both specificity and positive predictive value (PPV) were 100%, as all cytologically positive cases were confirmed by pathological examination. Similarly, Feng Y et al[24] highlighted that US-guided FNAC is a practical and dependable approach for triaging patients during axillary staging in newly diagnosed breast cancer.

Gouveia MC et al[16] also reported high specificity and PPV (both 100%) for US-guided FNAC, with moderate overall accuracy (83.5%) but a low negative predictive value (NPV) of 53.6%, while axillary palpation showed a slightly better NPV of 59.7%. Supporting these findings, Yıldırım E et al[25] reported that ultrasound alone had a sensitivity of 36.36%, specificity of 87.10%, PPV of 40%, NPV of 85.26%, and an overall accuracy of 77.39%. In contrast, US-guided FNAB showed a specificity of 100%, NPV of 68%, and an accuracy of 68%. Above studies collectively emphasize the superior diagnostic reliability of US-guided FNAC over ultrasound alone in evaluating axillary lymph node metastases. Ultrasound combined with US-guided FNAC proved highly specific and effective tool for axillary staging in breast cancer, though its sensitivity and negative predictive value may vary due to sampling limitations and different operator dependencies. Hence,

there is still a need for future prospective, multi-centered research, including the use of core needle biopsy and the integration of advanced imaging techniques, to improve diagnostic accuracy and consistency.

Conclusion

This revealed that the ultrasound combined with ultrasound guided fine needle aspiration cytology observed to be a simple, un-expensive, easy to perform and the reliable diagnostic tool for cells that metastasis to axillary lymphadenopathy, with a higher diagnostic accuracy in preoperative staging and clinical management. Incorporating FNAC with ultrasound can therefore improve clinical decision-making, reduce unnecessary surgeries, and optimize patient management in breast cancer care. However due to certain study limitations, further large scale studies are recommended to validate the findings.

References

1. Du J, Mo H, Fan L, Jiang J. Robot-assisted internal mammary lymph chain excision for breast cancer: a case report. Medicine (Baltimore). 2017;96:e7894. https://doi.org/10.1097/MD.0000000000007894
2. Iwamoto N, Aruga T, Horiguchi S, Asami H, Saita C, Onishi M, et al. Ultrasound-guided fine-needle aspiration of axillary lymph nodes in breast cancer: Diagnostic accuracy and role in surgical management. Diagn Cytopathol. 2019 Apr 30. https://doi.org/10.1002/dc.24203
3. Kane G, Fleming C, Heneghan H, McCartan D, James P, Trueick R, et al. False-negative rate of ultrasound-guided fine-needle aspiration cytology for identifying axillary lymph node metastasis in breast cancer patients. Breast J. 2019 Jun 13. https://doi.org/10.1111/tbj.13402
4. Zheng H, Zhao R, Wang W, Liu X, Wang X, Wen C, Ren Y.. The accuracy of ultrasound-guided fine-needle aspiration and core needle biopsy in diagnosing axillary lymph nodes in women with breast cancer: a systematic review and meta-analysis. Front Oncol. 2023;13:1166035. https://doi.org/10.3389/fonc.2023.1166035
5. Tarigan TJE, Anwar BS, Sinto R, Wisnu W. Diagnostic accuracy of palpation versus ultrasound-guided fine-needle aspiration biopsy for diagnosis of malignancy in thyroid nodules: a systematic review and meta-analysis. BMC Endocr Disord. 2022;22(1):181. https://doi.org/10.1186/s12902-022-01085-5
6. Lan L, Luo Y, Zhou M, Huo L, Chen H, Zuo Q, Deng W. Comparison of diagnostic accuracy of thyroid cancer with ultrasound-guided fine-needle aspiration and core-needle biopsy: a systematic review and meta-analysis. Front Endocrinol (Lausanne). 2020;11:44. https://doi.org/10.3389/fendo.2020.00044
7. Balasubramanian I, Fleming CA, Corrigan MA, Redmond HP, Kerin MJ, Lowery AJ. Meta-analysis of the diagnostic accuracy of ultrasound-guided fine-needle aspiration and core needle biopsy in diagnosing axillary lymph node metastasis. Br J Surg. 2018;105(10):1244–1253. https://doi.org/10.1002/bjs.10920
8. Pyo JS, Jung J, Lee SG, et al. Diagnostic accuracy of fine-needle aspiration cytology and core-needle biopsy in the assessment of the axillary lymph nodes in breast cancer—a meta-analysis. Diagnostics (Basel). 2020;10(9):717. https://doi.org/10.3390/diagnostics10090717
9. Xu Q, Wang J, Wang J, Guo R, Qian Y, Liu F. The effectiveness of ultrasound-guided core needle biopsy in detecting lymph node metastases in the axilla in patients with breast cancer: systematic review and meta-analysis. Clinics (Sao Paulo). 2023;78:100207. https://doi.org/10.1016/j.clinsp.2023.100207
10. Nell S, Kist JW, Debray TP, de Keizer B, Van Oostenbrugge TJ, et al. Qualitative elastography can replace thyroid nodule fine-needle aspiration in patients with soft thyroid nodules: a systematic review and meta-analysis. Eur J Radiol.2015;84(4):652–661. https://doi.org/10.1016/j.ejrad.2015.01.003
11. Suh CH, Baek JH, Lee JH, Choi YJ, Kim KW, Lee J, Chung KW, et al. The role of core-needle biopsy in the diagnosis of thyroid malignancy in 4580 patients with 4746 thyroid nodules: a systematic review and meta-analysis. Endocrine. 2016;54(2):315–328. https://doi.org/10.1007/s12020-016-0991-9
12. Jiang L, Wang H, Ma Y, et al. Diagnostic accuracy of ultrasound-guided fine-needle aspiration and core-needle biopsy for axillary lymph nodes in breast cancer: a meta-analysis. PLoS One. 2020;15(10):e0240645. https://doi.org/10.1371/journal.pone.0240645
13. Kim JH, Lee HK, Park SY, et al. Diagnostic performance of ultrasound-guided fine-needle aspiration and core needle biopsy in thyroid nodules: a systematic review and meta-analysis. Ultrasonography. 2022;41(2):278–289.
14. Ha EJ, Suh CH. & Baek,JH. The role of core-needle biopsy for thyroid nodules with an indeterminate fine-needle aspiration result: a meta-analysis. Thyroid. 2018;28(2):166–178. https://doi.org/10.1007/s00330-018-5367-5
15. Lee HY, Shin JH, Han K, et al. Comparative diagnostic accuracy of fine-needle aspiration versus core needle biopsy for axillary lymph nodes in breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat. 2021;187(1):155–171.
16. Gouveia MC, Santos CL, Pereira IC, Souza AI. Accuracy of ultrasound-guided fine needle aspiration cytology (US-FNAC) to detect axillary involvement in breast cancer. Braz J Oncol. 2021;17:1–9. https://doi.org/10.5935/2526-8732.20200044
17. Del Riego J, Diaz-Ruiz MJ, Teixidó M, et al. The impact of axillary ultrasound with biopsy in overtreatment of early breast cancer. Eur J Radiol. 2018;98:158–164. https://doi.org/10.1016/j.ejrad.2017.11.018
18. Henna N, Shafqat F, Rehman MU, Fakhr SA, Anjum S, Sameen S, Nagi AH. Clinicopathological characteristics of molecularly classified groups of invasive ductal breast carcinoma in Pakistani women. Pak J Med Dent. 2022;11(2):3–8.

19. Millar EK, Graham PH, McNeil CM, Browne L, O'Toole SA, et al. Prediction of outcome of early ER+ breast cancer is improved using a biomarker panel, which includes Ki-67 and p53. Br J Cancer. 2011;105(2):272–280. https://doi.org/10.1038/bjc.2011.228
20. Liu X, Guan Y, Zhang W, Liu S, Liu J, Wang L, Niu Y.. Predictors of recurrence in breast cancer subtypes with negative lymph node in a Chinese population. Int J Clin Exp Pathol. 2014;7(6):3202–3212.
21. Mohammed AA. The clinical behavior of different molecular subtypes of breast cancer. Cancer Treat Res Commun. 2021;29:1–6. https://doi.org/10.1016/j.ctarc.2021.100469
22. Singh R, Deo SV, Dhamija E, et al. To evaluate the accuracy of axillary staging using ultrasound and ultrasound-guided fine-needle aspiration cytology (USG-FNAC) in early breast cancer patients—a prospective study. Indian J Surg Oncol. 2020;11:726–734. https://doi.org/10.1007/s13193-020-01222-3
23. Oz A, Demirkazik FB, Akpinar MG, Soygur I, Baykal A, Onder SC, et al. Efficiency of ultrasound and ultrasound-guided fine needle aspiration cytology in preoperative assessment of axillary lymph node metastases in breast cancer. J Breast Cancer. 2012;15(2):211. https://doi.org/10.4048/jbc.2012.15.2.211
24. Feng Y, Huang R, He Y, Lu A, Fan Z, Fan T, Qi M, et al. Efficacy of physical examination, ultrasound, and ultrasound combined with fine-needle aspiration for axilla staging of primary breast cancer. Breast Cancer Res Treat. 2015;149:761–765. https://doi.org/10.1007/s10549-015-3280-z
25. Yıldırım E, Pelen Z, Keğin M, Uçar N, Kayadibi Y, Gündoğar Ö. Evaluation of the reliability of preoperative ultrasonography and ultrasonography-guided fine needle aspiration biopsy in axillary staging in patients with breast cancer. J Acad Res Med. 2021;11(3). https://doi.org/10.4274/jarem.galenos.2021.73745