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Immunohistochemical analysis of clinicopathological predictors for BRCA1 protein alterations in invasive breast carcinoma; a cross-sectional study

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Abstract

Introduction: Breast cancer is a heterogeneous disease influenced by genetic, hormonal, and molecular factors. Among these, BReast CAncer gene 1 (BRCA1) protein alterations play a critical role in hereditary breast cancers and may also have implications in sporadic cases.
Objectives: This study aims to analyze the immunohistochemical expression of BRCA1 protein and its association with clinicopathological predictors.
Materials and Methods: This cross-sectional study analyzed 100 paraffin-embedded tissue blocks from female breast cancer patients treated at educational and therapeutic hospitals in Isfahan, Iran, between March 2018 and April 2023. Tissue sections underwent immunohistochemical staining to evaluate BRCA1 protein expression. Patient data, including demographic information (age and family history of breast cancer), tumor characteristics (size, grade, and lymph node involvement), hormonal receptor status (estrogen receptor [ER], progesterone receptor [PR], human epidermal growth factor receptor 2 [HER2]) and Ki67 expression were extracted from archived medical records and staining results. Statistical tests were used to assess the correlation between clinicopathological factors and BRCA1 alteration status.
Results: This study identified several clinico-pathological factors significantly associated with BRCA1 alteration status. Advanced tumor grades and lymph node involvement were strongly linked to BRCA1 mutations, with grade II and III tumors showing substantial increases in likelihood compared to grade I. Initially, hormone receptor-negative status also correlated with altered BRCA1. However, after adjusting for confounders, only tumor grade and lymph node involvement remained as independent predictors. Specifically, grade II and III tumors demonstrated significantly elevated odds, while lymph node involvement showed a strong association. Although negative ER still showed an increased likelihood, it did not reach statistical significance in the adjusted analysis, and neither PR nor HER2 status retained predictive value.
Conclusion: The study findings suggest strong relationships between BRCA1 alterations and advanced disease characteristics, particularly the nodal metastasis, hormone receptor negativity, and higher tumor grade. The magnitude of association for tumor grade and nodal metastasis progression highlights their potential clinical relevance in BRCA1-related oncogenesis as independent predictors.

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Introduction

Breast cancer is the most commonly diagnosed cancer worldwide and a leading cause of death among women. Its incidence is increasing rapidly, particularly in urban areas, due to factors such as population growth, aging, and lifestyle changes (1-3). Breast and cervical cancers collectively account for nearly half of all cancer cases among women. The risk of developing breast cancer notably increases with age, highlighting the importance of regular screenings and preventive measures (4). In Iran, the median age at diagnosis for breast cancer is significantly lower, typically falling within a younger age range, whereas in many other countries, it generally occurs between 50 and 60 years of age; this difference is primarily due to the younger demographic of Iranian society. While the growth rate of breast cancer in the West has remained almost constant, in Iran, it is increasing due to aging and rising risk factors (5).

Risk factors influencing the neoplastic transformation process can be categorized into two groups. The first group includes intrinsic factors such as age, sex, race, and genetic structure, which can lead to familial occurrences of neoplastic diseases or benign proliferative lesions in the mammary gland. These are independent parameters that

Key point

In this study, we found that advanced tumor grades and lymph node involvement were strongly linked to breast cancer type 1 (BRCA1) mutations, with grade II and III tumors showing significantly higher odds compared to grade I. While hormone receptor-negative status initially correlated with BRCA1 alterations, only tumor grade and lymph node involvement remained independent predictors after adjusting for confounding variables. The findings highlight the importance of tumor grade and lymph node involvement as critical indicators for identifying BRCA1 alterations in breast cancer patients.

cannot be easily altered during a person's lifetime. The second group consists of external factors such as lifestyle, diet, and long-term medical interventions like hormonal oral contraceptives or hormone replacement therapy. These factors can potentially be modified to some extent to influence the neoplastic process. Identifying modifiable factors can aid in developing prevention strategies to reduce the incidence of breast cancer (6,7). Extensive research has identified several key biomarkers that play a crucial role in the diagnosis and treatment of breast cancer. Among these are BReast CAncer gene 1 (BRCA1) protein, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and the proliferation marker Ki67. These biomarkers provide valuable insights into tumor characteristics, guiding personalized treatment strategies and improving patient outcomes (8,9). Among the genes related to the occurrence of breast cancer, BRCA1 and BRCA2 play a significant role in its pathogenesis (10). Previous studies showed that, BRCA1 and BRCA2 are crucial tumor suppressor genes located on chromosomes 17q21 and 13q12, respectively. The BRCA1 gene codes for a 220 kDa protein composed of 1863 amino acids, while the BRCA2 gene encodes a 384 kDa protein made up of 3418 amino acids. Many tumors with BRCA1 mutations display heterozygosity, consistently losing the BRCA1 wild-type allele, underscoring the gene's role as a tumor suppressor (11).

HER2 is an oncogene encoding the transmembrane glycoprotein tyrosine kinase p185, which is part of the epidermal growth factor receptor family. Non-BRCA1-associated breast cancer typically has a lower grade, more tubular formation, less nuclear pleomorphism, and fewer mitoses (lower Nottingham scoring) compared to breast cancer associated with BRCA1 or BRCA2 protein expression, or both. Non-BRCA1-associated breast cancers have significantly higher expression of ER, PR, and HER2, and lower expression of p53 and Ki67 (3,12). Given the confirmed role of this mutation in the genome in breast cancer, this study investigated BRCA1 expression and its relationship with various clinical factors. These factors include age, family history, tumor size, side of the involved breast, grade, number of involved lymph nodes, histological subtype, and the simultaneous presence of in-situ component, ER, PR, HER2, and also Ki67.

Objectives

The objective of this study is to investigate the clinico-pathological factors associated with BRCA1 protein alterations in invasive breast carcinoma. By identifying associated risk factors and independent predictors, the study aims to enhance the understanding of BRCA1-related tumor biology and provide insights for improving diagnostic accuracy, risk stratification, and personalized treatment strategies in breast cancer patients.

Materials and Methods

Study design

This cross-sectional study involved the retrieval of 100 paraffin-embedded tissue blocks from female breast cancer patients treated at educational and therapeutic hospitals in Isfahan, Iran. The samples were obtained from the educational and therapeutic hospitals pathology archive between March 2018 and April 2023 and prepared for immunohistochemical staining.

Inclusion and exclusion criteria

The inclusion criteria for this study consisted of confirmed breast cancer diagnoses and the availability of paraffin blocks in the hospital's pathology archive. Patients were excluded if their medical records lacked essential data or if the paraffin blocks were damaged, rendering them unsuitable for immunohistochemical testing.

Sample preparation

The paraffin blocks were sectioned and prepared for staining. The sections were first incubated at 37 °C for 24 hours, followed by placement in an oven at 60 °C for 10 to 15 minutes. Deparaffinization, rehydration, and blocking steps were carried out using xylene, a series of ethanol solutions, and a hydrogen peroxide/methanol mixture, respectively.

Data collection

Patient data, including age, family history of breast cancer, the side of the breast affected, histological subtype, tumor grade, tumor size, lymph node involvement, presence of an in situ component, and the expression levels of ER, PR, HER2, and Ki67 were extracted from medical records archived at educational and therapeutic hospitals pathology department.

Immunohistochemical staining

Before staining, the sections were kept at 37 °C for one day, then placed in an oven at 60 °C for 10-15 minutes. The stages of deparaffinization, rehydration, and blocking were performed using xylene, serial ethanol alcohol, and hydrogen peroxide/methanol solution, respectively. The tissues underwent antigen retrieval for 10 minutes in an autoclave with a suitable buffer (citrate buffer, pH = 6). They were then incubated with the primary antibody (BRCA1 mouse monoclonal MS110, Zeta brand, USA) for one

hour, followed by incubation with the secondary antibody for one hour, and then with the HRP-labeled streptavidin complex for half an hour. For visualization, the prepared solution (DAB) and hematoxylin were employed. The stained slides were interpreted under a light microscope based on the percentage of positive cells and the intensity of the BRCA1 marker. The scoring method categorized the cells based on the staining percentage, with scores ranging from 0 to 4, and the intensity of staining, with scores ranging from 0 to 3 (13). The final score was calculated as follows (Figure 1):

$$Final\ score = \frac{Nuclear\ staining\ percentage \times Intensity\ score}{100}$$

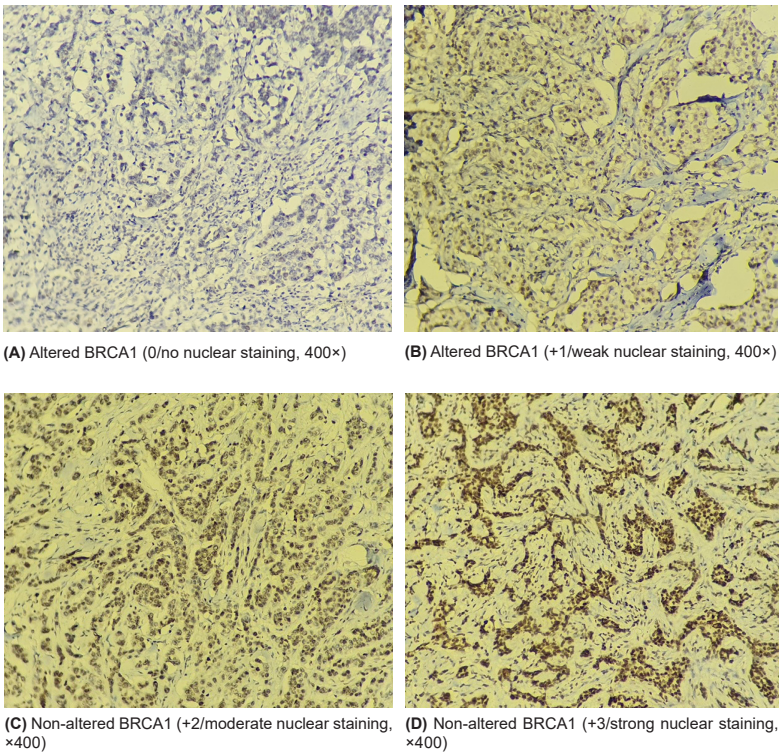
A final score of 0-1 indicated altered BRCA1 expression, while a final score of 2-3 indicated non-altered BRCA1 expression (similar to normal cells) (9). For ER and PR, tumors with more than 1% staining in invasive cells were considered positive. The color intensity was categorized as weak, moderate, or strong. For HER2/neu, the evaluation was as follows: 0 or +1 (negative) when there was no or low membrane staining of the tumor cells, +2 (borderline) when more than 10% of tumor cells had weak to moderate membrane staining, and +3 (strong positive) when more than 10% of tumor cells showed strong and complete brown membranous staining (10). Samples reported as HER2 (2+) were excluded from the study due to the need for fluorescence in situ hybridization (FISH) analysis, a technique used to detect and localize specific DNA sequences on chromosomes (11). For Ki67, cases with a median proliferative index of less than 14% were classified as having a low proliferative index, while those with an index of 14% or higher were considered to have a high proliferative index (14).

Outcomes

The primary outcome of this study is the identification of clinic-pathological factors associated with BRCA1 protein alterations in invasive breast carcinoma, including tumor size, histological subtype, grade, lymph node involvement, and receptor status (ER, PR, HER2, and Ki67). These factors were analyzed to establish their correlation with BRCA1 protein dysfunction and its impact on tumor biology. The secondary outcomes involve evaluating the prognostic significance of BRCA1 protein alterations, aiming to improve diagnostic accuracy and risk stratification while contributing to personalized treatment strategies for breast cancer patients.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 27 (USA). The Kolmogorov-Smirnov test was applied to assess data normality, while Levene's test evaluated the equality of variances. Clinico-pathological data frequency distribution based on BRCA1 protein alteration status was analyzed using chi-square tests and independent t-tests. Univariate and multivariate logistic regression analyses were conducted to identify clinic-pathological factors associated with BRCA1 protein alterations. Statistical significance was determined at a P value < 0.05.



(A) Altered BRCA1 (0/no nuclear staining, 400×)

(B) Altered BRCA1 (+1/weak nuclear staining, 400×)

(C) Non-altered BRCA1 (+2/moderate nuclear staining, ×400)

(D) Non-altered BRCA1 (+3/strong nuclear staining, ×400)

Figure 1. Immunohistochemical staining of breast paraffin block samples.

Results

In this study, out of 100 breast cancer samples from 100 women with a mean age of 50.19 ± 11.85 years, 76 exhibited BRCA1-altered, and the remaining 24 did not show such genetic changes. The results indicated that patients with BRCA1-altered breast cancer exhibited distinct clinico-pathological profiles compared to those without BRCA1 alterations. Significant differences were observed in tumor grade, lymph node involvement, and hormone receptor status. The BRCA1-altered group demonstrated more advanced tumor grades and a higher prevalence of lymph node metastasis. The ER, PR, and HER2 expression were significantly less frequent in tumors with BRCA1 alterations compared to non-altered cases. No significant associations were found between BRCA1 status and breast side, family history, tumor size, histology, in-situ components, or proliferation index (Ki67) (Table 1).

Univariate logistic regression analysis identified several clinico-pathological factors significantly associated with BRCA1 alteration status. Advanced tumor grades demonstrated markedly elevated odds, with grade II and III tumors showing 28.8-fold and 20.25-fold increased likelihood of BRCA1 alterations compared to grade I, respectively. Lymph node involvement exhibited a 5.14-fold higher odds of BRCA1 mutations. Hormone receptor-negative status substantially correlated with genetic alterations of BRCA1, as evidenced by 3.24-fold increased odds for negative ER 3.40-fold for negative PR, and 2.93-fold for HER2 negativity (Table 2).

Multivariate logistic regression analysis with adjustment of confounder variables revealed that tumor grade and lymph node involvement are independent predictors of BRCA1 alterations, while hormone receptor status lost significance when adjusted for other variables. Advanced tumor grades showed markedly elevated odds ratios, with grade II tumors demonstrating a 37.79-fold increased likelihood and grade III a 12.39-fold higher probability of BRCA1 alterations compared to grade I. Lymph node involvement remained strongly associated with genetic changes, exhibiting a 8.16-fold increased odds. Although negative ER showed a 3.04-fold elevated odds, this association did not reach statistical significance in the adjusted model, and neither PR nor HER2 status retained predictive value (Table 3).

Discussion

The current study identified altered BRCA1 protein expression in 76% of breast tumor samples, a frequency markedly higher than rates reported in prior studies such as Kumar et al (48.2%) (15) and Juneja et al (42.52%)

Table 1. Frequency distribution of patients› demographic and clinic-pathological characteristics between the two groups with and without BRCA1 alterations

Clinicopathological data		BRCA1 alteration status				*P* value
		No (n = 24)		Yes (n = 76)		
		No.	%	No.	%	
Side	Right	10	41.7	44	57.9	0.161*
	Left	14	58.3	32	42.1	
Family history	Negative	23	95.8	63	82.9	0.111*
	Positive	1	4.2	13	17.1	
Tumor size (cm)	< 2	10	41.7	17	22.4	0.168*
	2 - 5	9	37.5	41	53.9	
	> 5	5	20.8	18	23.7	
Tumor grade	I	6	25	1	1.3	<0.001*
	II	10	41.7	48	63.2	
	III	8	33.3	27	35.5	
Histology	Ductal carcinoma	19	79.2	66	86.8	0.359*
	Ductal & lobular	5	20.8	10	13.2	
Lymph node involvement	No	18	75	28	36.8	0.001*
	Yes	6	25	48	63.2	
In-situ	Negative	11	45.8	36	47.4	0.895*
	Positive	13	54.2	40	52.6	
ER	Positive	16	66.7	29	38.2	0.014*
	Negative	8	33.3	47	61.8	
PR	Positive	15	62.5	25	329	0.010*
	Negative	9	37.5	51	67.1	
HER2	Positive	11	45.8	17	22.4	0.026*
	Negative	13	54.2	59	77.6	
Ki67 (%)	< 14	8	33.3	35	46.1	0.273*
	≥ 14	16	66.7	41	53.9	
Variable		**Mean**	**SD**	**Mean**	**SD**	***P* value**
Age (year)		51.17	13.39	49.88	11.40	0.646**

SD; Standard deviation, ER; Estrogen receptor, PR; Progesterone receptor, HER2; Human epidermal growth factor receptor 2.
*Chi-square, **Independent *t* test.

Table 2. The correlation of Clinico-pathological data with BRCA1 alteration status using univariate logistic regression (unadjusted model)

Clinicopathological data		BRCA1 alteration status (changed)			
		P value	OR	95% CI	
				Lower	Upper
Tumor grade	I		Ref (1)		
	II	0.003	28.8	3.11	266.21
	III	0.009	20.25	2.11	193.91
Lymph node involvement		0.002	51.4	1.82	14.47
Negative ER		0.017	3.24	1.23	8.52
Negative PR		0.012	3.40	1.30	8.83
Negative HER2		0.029	2.93	1.11	7.72

CI, Confidence interval; OR, Odds ratio; Ref, Reference; ER, Estrogen receptor; PR, Progesterone receptor; HER2, Human epidermal growth factor receptor 2.

Table 3. The predictive power of clinico-pathological data in the BRCA1 alteration diagnosis in the adjusted model of logistic regression (multivariate)

Clinicopathological data		BRCA1 alteration status (changed)			
		P value	OR	95% CI	
				Lower	Upper
Tumor grade	I		Ref (1)		
	II	0.006	37.79	2.83	503.35
	III	0.049	12.39	1.01	155.60
Lymph node involvement		0.003	8.16	2.03	32.77
Negative ER		0.167	3.04	0.62	17.70
Negative PR		0.777	0.79	0.15	3.98
Negative HER2		0.924	1.07	0.26	4.32

CI, Confidence interval; OR, Odds ratio; Ref, Reference; ER, Estrogen receptor; PR, Progesterone receptor; HER2, Human epidermal growth factor receptor 2.

(16) in India, and Hussein et al (20.5%) in Iraq (8). These inter-study disparities may reflect geographic and ethnic variations in BRCA1 mutation prevalence, as well as methodological inconsistencies in immunohistochemical staining protocols (e.g., antibody specificity, antigen retrieval techniques) or divergent scoring criteria for classifying expression loss. Such technical and population heterogeneity underscores the need for standardized biomarker assessment frameworks to ensure comparability across diverse cohorts.

In our study, although patients with altered BRCA1 expression tended to be younger on average, no statistically significant correlation was found between BRCA1 expression and patient age. This observation aligns with previous research by Juneja et al, who noted that altered BRCA1 expression was more prevalent among patients under 45 years old, whereas non-altered expression was more common in those over 45 (34%) (16). This pattern suggests that ER negativity, often linked to younger age, may be an intrinsic characteristic of BRCA1-related cancers (17). Consistent with this, Tung et al found that patients under 50 years were significantly more likely to have ER-negative tumors compared to older individuals (18). Hussein et al also found no significant correlation between BRCA1 protein immunoexpression and patients' age (8). These findings collectively indicate that younger age and ER negativity are closely associated with BRCA1 alterations, potentially reflecting distinct biological features of BRCA1-related breast cancers.

Our study revealed a significant correlation between altered BRCA1 expression and negative ER, PR, and HER2 status. This finding aligns with previous research by Ashraf et al, who observed lower ER/PR expression in tumors lacking BRCA1 protein expression during immunohistochemical analysis (19). Similarly, Kumar et al found that altered BRCA1 gene expression was associated with negative ER and PR status, which correlated with a poor prognosis (15). These observations suggest that BRCA1 protein expression may serve as a prognostic factor for predicting treatment response to hormone therapy. Further support for the relationship between BRCA1 mutations and negative ER/PR status comes from studies by Juneja et al (16) and Amirrad et al (20). However, Ansquer et al noted no association between the lack of BRCA1 expression and HER2 status (21), while Hussein et al reported a high incidence of BRCA1 cases in ER-negative and PR-negative tumors without a significant relationship between BRCA1 status and ER/PR expression (8). Importantly, Verma et al highlighted the potential of combining immunohistochemical expression of BRCA1, ER, PR, and HER2/neu with clinical data to identify individuals at risk of carrying BRCA1 mutations, thereby aiding in the selection of candidates for genetic screening (22). Variability in study findings may stem from the absence of standardized assessment protocols for ER and BRCA1 status. For example, traditional definitions of ER/PR negativity often rely on a threshold of less than 10% stained tumor cells, whereas newer guidelines propose a more stringent criterion of less than 1% (23). This discrepancy highlights the need for consistent

methodologies to ensure accurate and comparable results across studies, ultimately enhancing the reliability of breast cancer diagnostics and treatment planning.

Our study revealed a significant correlation between altered BRCA1 expression and more aggressive tumor characteristics, including higher tumor grade and increased lymph node involvement. However, no statistically significant association was observed between BRCA1 alterations and a family history of breast cancer. Tumors without BRCA1 alterations generally exhibited less aggressive features, such as lower Nottingham scores, characterized by lower grades, more tubular formation, less cellular pleomorphism, and fewer mitoses. These findings align with Hussein et al (8), who also reported a significant association between BRCA1 protein expression and higher tumor grade and stage. In contrast, Duzkale et al found a significant relationship between altered BRCA1 expression and a family history of breast cancer, highlighting the complexity and variability in the clinical implications of BRCA1 alterations across different studies (24). Ansquer et al observed that only 26% of sporadic breast cancer tumors expressed BRCA1, with its absence correlating with lymph node metastases and decreased ER levels, suggesting a potential link between BRCA1 expression and tumor aggressiveness (21). Furthermore, Juneja et al emphasized the utility of BRCA1 immunohistochemistry as a diagnostic tool, particularly in triple-negative breast cancers (ER/PR/HER2-negative), highlighting its potential to inform treatment strategies and improve patient outcomes (16). These findings underscore the importance of BRCA1 in the clinical management of sporadic breast cancer, particularly in guiding targeted therapies and prognosis.

Overall, the study's findings underscore a significant correlation between BRCA1 alterations and aggressive disease features, notably nodal metastasis, hormone receptor negativity, and advanced tumor stage. This association suggests that BRCA1 plays a pivotal role in the progression and clinical behavior of breast cancer, potentially serving as an independent predictor for tumor stage and nodal metastasis. The clinical relevance of these findings lies in their ability to inform risk assessment and treatment planning, particularly for patients with BRCA1-related breast cancers. By identifying BRCA1 alterations as markers of aggressive disease, clinicians can tailor more targeted and intensive therapeutic strategies, ultimately improving patient outcomes. In conclusion, these results contribute to a deeper understanding of BRCA1's role in breast cancer oncogenesis and highlight the importance of integrating genetic biomarkers into clinical decision-making processes.

Conclusion

This study demonstrates that advanced tumor grade and lymph node involvement serve as strong independent predictors of BRCA1 protein alterations in invasive breast carcinoma. While hormone receptor status initially showed associations with BRCA1 alterations, these relationships were largely attenuated when controlling for other clinico-pathological factors. Our findings contribute to the evolving understanding of BRCA1-related tumor biology by identifying key clinical and pathological features that may signal underlying genetic vulnerabilities. These results have important implications for clinical practice, potentially enhancing risk stratification approaches and supporting more targeted screening strategies for patients who might benefit from genetic testing. Furthermore, the established connections between specific tumor characteristics and BRCA1 status may inform the development of personalized treatment algorithms, particularly as therapeutic options targeting BRCA-mutated cancers continue to expand. This research underscores the importance of comprehensive clinico-pathological assessment in identifying patients who may harbor BRCA1 alterations, ultimately advancing precision medicine approaches in breast cancer management.

Limitations of the study

The cross-sectional design restricts the ability to establish causality between BRCA1 expression and clinical outcomes, necessitating longitudinal studies for confirmation. The relatively small sample size may limit the generalizability of the findings, and larger studies are needed for validation. Conducting the study at a single center may introduce selection bias, so multi-center studies could provide more comprehensive insights. Technical variability in immunohistochemical staining techniques and interpretation may affect the consistency of BRCA1 expression results. Lastly, the study may not have accounted for all potential confounding factors, and future research should include a broader range of variables to control for confounding effects.

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Authors' contribution

Conceptualization: Azar Naimi and Faezeh Saberi.
Data curation: Faezeh Saberi and Azar Naimi.
Formal analysis: Elham Amjadi.
Investigation: Azar Naimi and Elham Amjadi.
Methodology: Faezeh Saberi and Elham Amjadi.
Project management: Faezeh Saberi.
Resources: All authors.
Supervision: Azar Naimi
Validation: Azar Naimi.
Writing-original draft: All authors.
Writing-review and editing: All authors.

Conflicts of interest

The authors declare no conflict of interest.

Declaration of generative artificial intelligence (AI) and AI-assisted technologies in the writing process

During the preparation of this work, the authors utilized AI (Perplexity.ai and grammarly.com) to refine grammar points and language style in writing. Subsequently, the authors thoroughly reviewed and edited the content as necessary, assuming full responsibility for the publication's content.

Ethical issues

The research was conducted under the principles of the Declaration of Helsinki. This study resulted from the pathology residency thesis of Faezeh Saberi (Thesis #340195), with the ethical code IR.MUI.MED.REC.1401.094; https://ethics.research.ac.ir/EthicsProposalView.php?id=261275), approved by Isfahan University of Medical Sciences, Isfahan, Iran. Additionally, the authors have fully adhered to ethical standards, including issues such as plagiarism, data fabrication, and double publication.

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