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P53 expression in colorectal carcinomas study at a tertiary health care center in South Kerala

Alina Ajoy Thomas [1]*, Meeta Thomas [1], Jessy M.M [1]

[1] Department of Pathology, Pushpagiri Institute of Medical Sciences & Research Centre, Thiruvalla, Kerala, India

Abstract

Introduction: Colorectal carcinoma (CRC) ranks as the third most ubiquitous cancer globally and the fourth primary source of cancer-related mortality. Loss of the p53 gene is vital in the conversion of colorectal adenoma into carcinoma. The study aims to evaluate the prevalence of p53 expression and investigate its correlation with diverse clinicopathological parameters, providing valuable insights into the dynamics of colorectal cancer in the specified region.

Methods: A total of 42 CRC cases from tertiary healthcare center in South Kerala, India, were sampled between December 2018 and January 2021. Comprehensive clinical data and clinicopathological parameters were collected, followed by histomorphological and immunohistochemical evaluations. The results were then correlated with clinicopathological variables.

Results: Patients aged 45 to 82 years (mean 63.5) exhibited a predilection for the left colon (57%) and rectum (33%), with symptoms ranging from abdominal pain to weight loss. Histologically, 95.2% were adenocarcinomas, mostly moderately differentiated (57.1%). Tumor extension (T3: 57%) and lymph node involvement (N1: 29%) were prevalent, with Stage II tumors (38.1%) most frequent. P53 immunoreactivity was observed in 83.3% of cases, correlating with moderately differentiated grades, higher tumor extensions (T3/T4), N1/N2 lymph node statuses, and Stage II/III tumors. No significant associations were found with age, sex, lesion site, or tumor type. P53 nuclear positivity, identified through IHC analysis, provides crucial insights into cancer biology, prognosis, and potential therapeutic implications. The finding highlights significant associations between p53 expression and key clinicopathological parameters. P53 positivity is notably higher in moderately differentiated tumors (Grade) and T3/T4 tumor extensions compared to well and poorly differentiated grades and T1/T2 extensions, respectively. Significant links were also observed with lymph node status (N1/N2 > N0) and tumor stage (S2/S3 > S1), indicating a strong correlation between p53 expression and advanced disease characteristics. However, no significant associations were found with age, sex, lesion site, or tumor type. The novelty of our study lies in the focused exploration of p53 expression in colorectal carcinomas. By specifically investigating the correlation between p53 expression and various clinicopathological parameters, we contribute a unique perspective to the understanding of the molecular characteristics of colorectal cancer. This targeted approach enhances the visibility of novel insights that our study brings to the field of p53 expression in the context of colorectal carcinomas.

Conclusion: Our investigation underscores that p53 overexpression is particularly prominent in advanced-stage colorectal cancer cases and those having LNM, further supporting its role as an adverse prognostic marker in this context.

Keywords: Colorectal carcinoma, P53, Immunohistochemistry, Lymph node, Prognosis

Corresponding Authors: Alina Ajoy Thomas

✉ Email: alina.ajoy@gmail.com

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Introduction

Colorectal carcinoma (CRC), a prevalent malignancy worldwide, poses a significant health challenge due to its high ubiquity and associated morbidity and mortality In 2020, it resulted in approximately 1.9 million new cases and 930,000 deaths. Factors such as age, family history, genetics, and lifestyle choices, including diet, physical activity, smoking, and alcohol consumption, influence the risk of developing CRC. Incidence and mortality rates vary significantly worldwide, with Europe and Australia/New Zealand experiencing the highest rates and Africa and Asia the lowest. Projections suggest a 63% increase in incidence and a 73% rise in mortality by 2040 (1), driven by population growth, ageing, and evolving risk factors. While CRC is preventable and treatable, early detection and proper management are crucial, emphasizing the importance of effective strategies for primary prevention, screening, diagnosis, and treatment

Also, it is the third most ubiquitous cancer in men along with the second most ubiquitous cancer in women across the globe. However, in India, the incidence rates of colon cancer are notably lower in comparison to other cancer types (2) In India, the annual incidence of CRC stands at 4/100, 000, with Kerala reporting a slightly higher rate at 5.5/100, 000. In recent years, extensive research has focused on identifying specific biomarkers and clinicopathological variables that can provide valuable insights into the prognosis and management of CRC. One such key molecular player in the context of colorectal cancer is the p53 protein a critical tumor suppressor protein, which plays a fundamental role in preserving genomic stability along with regulating cell cycle progression. Dysregulation of the p53 pathway, often associated with p53 protein overexpression, has been involved in the development along with progression of CRC (3).

CRC stands as a complex malignancy characterized by a spectrum of clinical and pathological features. Our study, encompassing various demographic and tumor-related factors to unravel the intricacies of this disease. This investigation offered insights into key aspects such as age distribution, gender variations, preferred carcinoma sites, histological grading, tumor extension patterns, lymph node involvement, and tumor staging (4). Additionally, our scrutiny extended to the pivotal biomarker, p53, known for its association with cancer development and prognosis. By comprehensively examining these demographic and tumor-related factors, our study adds valuable insights to the intricate landscape of colorectal adenocarcinoma. The focus on p53 expression further contributes to the understanding of molecular markers with potential implications for prognosis and targeted therapeutic interventions (Harris and Hollstein, 2013) (5). The study aimed to determine the correlation between p53 expression and clinicopathological parameters in CRC, focusing on tumor grade, extension, lymph node status, and stage to elucidate the molecular implications for prognosis and therapy.

Methods

This cross-sectional study involved CRC patients' cases presented to the Department of Pathology at Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala, India, between December 2018 and January 2019. The study included a cohort of 42 patients for analysis and relevant clinical data, encompassing variables such as age, gender, colon subsite distribution, clinical presentation at diagnosis, histopathological type, tumor grade, disease stage, and presence of LNM, were collected from medical records for evaluation and correlation analysis.

Inclusion criteria encompassed all histopathologically diagnosed cases of carcinoma in the colon and rectum, comprising both biopsies and resected specimens.

Exclusion criteria

Comprised endoscopic biopsies with corresponding resected specimens of the colon and resection specimens from patients who underwent neoadjuvant chemotherapy.

Immunohistochemical (IHC) Staining for p53

IHC staining for the p53 protein was conducted using 5-micrometer sections acquired from formalin-fixed paraffin-embedded blocks. These IHC-stained sections were evaluated alongside H&E-stained specimens to determine the expression of p53 in CRC . The interpretation of p53 immunostaining was based on whether it was positive or negative. Also, positive

staining was demarcated as the presence of nuclear staining in ≥5% of cells per high-power field.

Data Analysis

The collected data was input into Microsoft Office Excel 2019 spreadsheets and subsequently analysed using SPSS version 16.0 software. Associations between p53 expression and clinicopathological parameters were assessed using Fisher's exact test. A p-value less than 0.05 was deemed to be significant in statistical terms. The study findings were presented in appropriate charts and tables.

Ethical Considerations

All procedures performed in this study received approval from the Institutional Review Board (IRB) with reference number 19666/2018, dated 17/01/2019. The research followed the guidelines established in the Helsinki Declaration of 1964 along with its following revisions. Written informed consent was not required, as determined by the IRB, with a waiver granted for this purpose.

Results

The results demonstrate the patient population and the characteristics of the colorectal adenocarcinoma cases under investigation. In a cohort of 42 cases, we observed a diverse range of demographic and tumor-related factors. As shown in Figure 1, the study group, patients' ages spanned from 45 to 82 years, with the most substantial representation observed in the 61-70 years age category. The mean age at diagnosis was 63.5±10.60 years, and a minority of patients, specifically 11.9% (n=5), were under the age of 50.

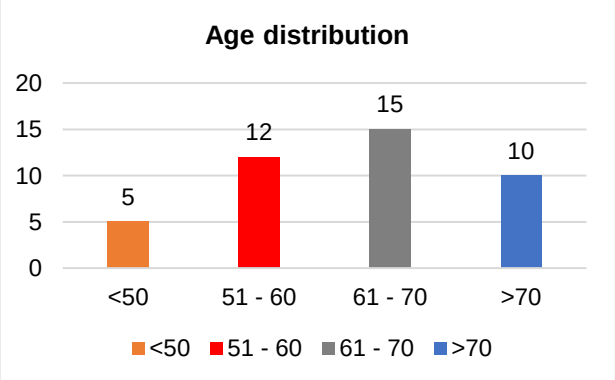

Figure 1. Age Distribution within the study population.

Among the 42 cases examined (as depicted in Figure 2), 19 were male, while 23 were female, resulting in a male-to-female ratio of 0.8:1.

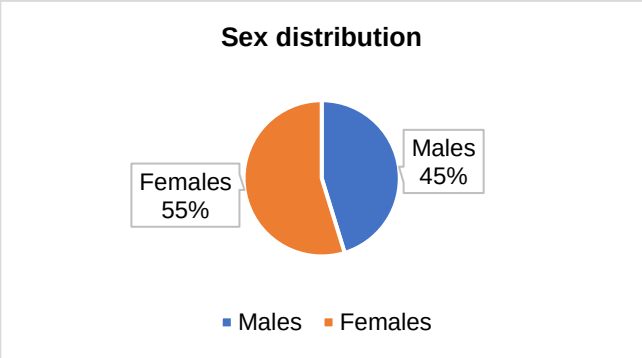

Figure 2. Gender wise distribution among study population.

As illustrated in Figure 3, the observed tumor growth exhibited a predilection for the colon and rectum's left side, with 24 (57%) cases occurring in this region, whereas 33 % cases were observed under rectum site and 10 % were on left side Clinical presentations among these cases varied and included symptoms such as abdominal pain, rectal bleeding, altered bowel habits, signs indicative of intestinal obstruction, weight loss, and anaemia.

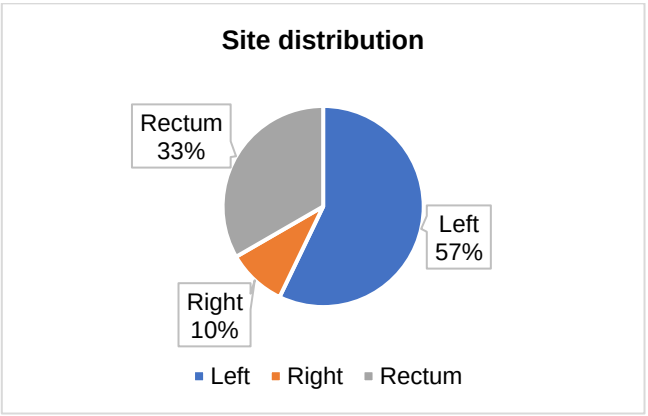

Figure 3. Distribution of Carcinoma Sites within the Study Population.

As depicted in Figure 4, histological grading show vast majority of colorectal carcinomas, accounting for 95.2% (n=40), were categorized as adenocarcinoma NOS. Within this category, 57.1% (n=24) were moderately differentiated, 38.1% (n=16) were well-differentiated, and a smaller proportion, 4.8% (n=2), were poorly differentiated.

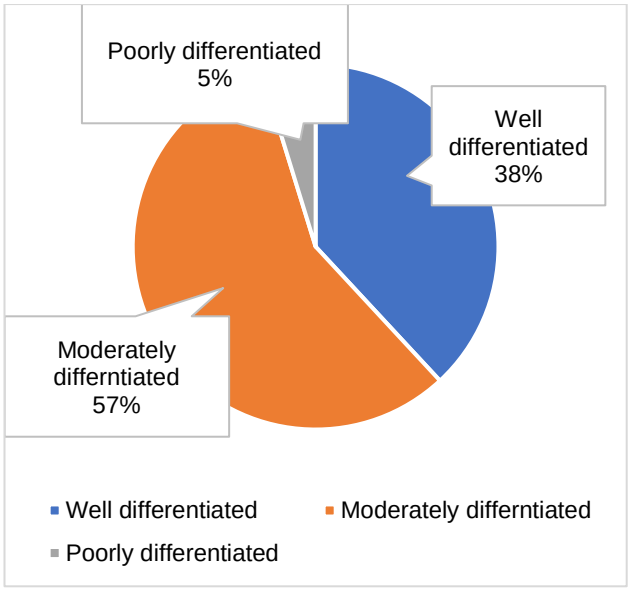

Figure 4. Histological grading among study population.

This histology image (Figure 4.1) demonstrates that moderately differentiated adenocarcinomas were predominantly observed in the left colon within the study population.

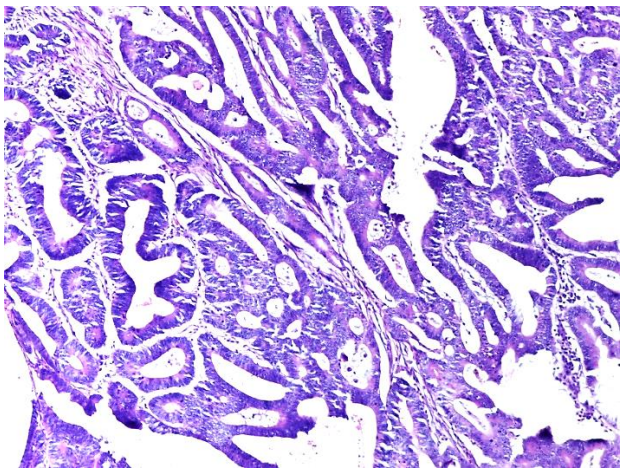

Figure 4.1. Moderately differentiated adenocarcinoma – left colon (H&E).

As shown in Figure 5 the remaining cases comprised two instances of mucinous carcinoma. Among the tumors, 57% (n=24) exhibited infiltration extending beyond the muscularis propria into the adjacent pericolic adipose tissue, designated as T3, while 31% (n=13) were restricted to the muscularis propria, categorized as T2 A smaller subset, 9.5% (n=4), showed infiltration into the visceral peritoneum or adjacent organs, classified as pT4. Additionally, only one case (2.4%) was identified as an early-stage T1 tumor.

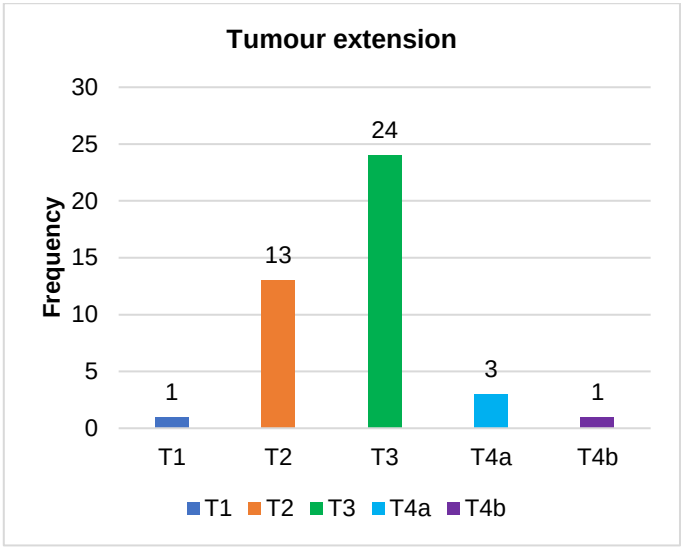

Figure 5. Distribution of tumor extension among study population.

As indicated in Figure 6 in half of the cases, there was no evidence of nodal involvement, whereas 29% were categorized as N1 and 21% as N2 were observed in study population.

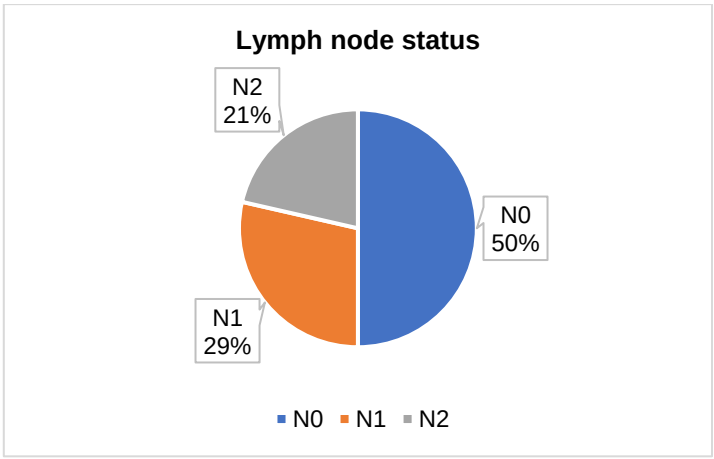

Figure 6. Distribution of Lymph node status among study population.

As demonstrated in Figure 7, the most frequently observed tumor stage was Stage II, with 38.1% (n=16) of cases, followed by Stage I tumours at 33.3% (n=14), and Stage III at 28.6% (n=12).

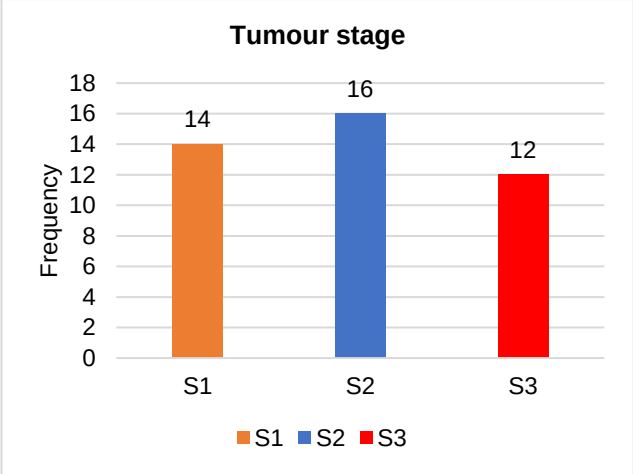

Figure 7. Distribution of tumour staging among study population.

As depicted in Figure 8, p53 immunoreactivity expression was detected in 35 cases of CRC, making up 83.3% of the study cohort. Only seven cases (16.7%) displayed no p53 expression.

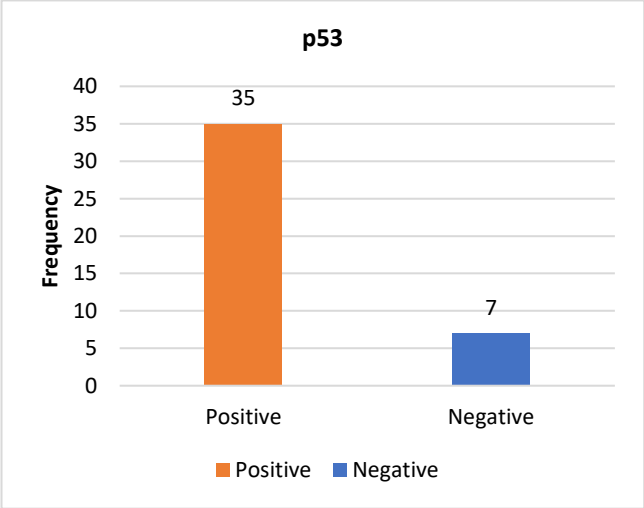

Figure 8. Expression of p53 immunoreactivity among study population.

P53 nuclear positivity, as observed in Figure 8.1 (IHC), signifies the presence of the p53 protein within the cell nuclei. In this context, p53 nuclear positivity suggests that the p53 protein is actively present and localized within the nuclei of the cells in the examined tissue sample. This finding can be significant in cancer research and diagnosis, as alterations or overexpression of the p53 protein are associated with various cancer types and can provide insights into the molecular characteristics of the tumor, its prognosis, and potential therapeutic implications. Therefore, identifying p53 nuclear positivity through IHC analysis is vital in comprehending the biology and behaviour of cancer cells in the context of the studied tissue or tumor specimen.

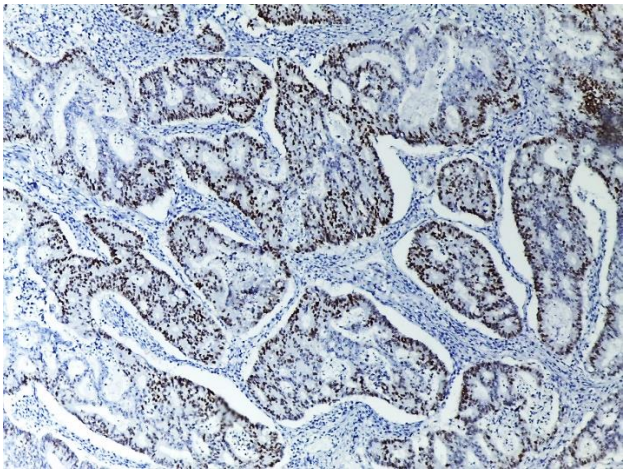

Figure 8.1. p53 nuclear positivity corresponding to Image 1 (IHC).

In table 1, the study revealed a noteworthy association between p53 expression and various clinical-pathological characteristics. Notably, p53 positivity was more frequent in moderately differentiated tumor grades compared to well and poorly differentiated tumors. There was also a significant link between p53 expression and tumor extension, with T3 and T4 tumors showing higher p53 positivity compared to T1 and T2. Similarly, lymph node status and tumor stage exhibited significant associations with p53 expression, indicating that N1 and N2 lymph node statuses and stages S2 and S3 were more likely to be p53-positive. However, no noteworthy links were observed between p53 expression and other clinicopathological factors like age, sex, site of lesion, or tumor type.

Table 1. Correlation of clinicopathologic parameters with the expression of p53.

Clinical pathological Characteristic	Fisher's Exact Value	P-Value	Association with p53 Expression
Grade of the Tumor	7.255	0.024	Significant
			Moderately > Well & Poorly
Tumor Extension	5.355	0.031	Significant
			T3 & T4 > T1 & T2
Lymph Node Status	7.177	0.012	Significant
			N1 & N2 > N0
Stage of the Tumor	14.053	0.000	Significant
			S2 & S3 > S1

Discussion

The findings of our study provide valuable insights into the clinicopathological characteristics and p53 expression in colorectal adenocarcinoma cases within our patient population. The study discusses age distribution in Figure 1 depicts a diverse age distribution, with a significant representation in the 61-70 years age category. The male-to-female ratio of 0.8:1, as illustrated in Figure 2, aligns with existing literature. These demographic observations are consistent with previous studies, mean age distribution in previous studies ranged from 55.23 to 59 years, with varying gender ratios, while our study showed a predominance of females, consistent with study by (Mardi et al., 2017) (4). In this study Figure 3 highlights a predilection for tumor growth on the left side of the colon and rectum, consistent with known distribution. Clinical presentations varied, encompassing symptoms such as abdominal pain, rectal bleeding, altered bowel habits, signs of intestinal obstruction, weight loss, and anemia.

Histological grading, showcased in Figure 4, reveals a predominance of adenocarcinoma NOS, with moderately differentiated tumors being the most prevalent. Figure 5 illustrates diverse tumor extension patterns, with a notable frequency of infiltration beyond the muscularis propria (T3). Figure 6 indicates diverse lymph node status, with notable associations in half of the cases, and Figure 7 portrays a varied distribution of tumor staging, with Stage II being the most frequently observed. In our investigation, colorectal carcinomas were primarily classified as adenocarcinoma NOS, with a predominance of moderately differentiated tumors, followed by well-differentiated and poorly differentiated subtypes. The T staging system revealed diverse tumor extension patterns, ranging from infiltration beyond the muscularis propria (pT3) to confined muscularis propria involvement (pT2), as well as infiltration into the visceral peritoneum or adjacent organs (pT4) and rare early-stage T1 tumors, while lymph node status and tumor stage indicated significant prognostic variability, with Stage II being the most common, followed by Stage I and Stage III.

Moderately differentiated adenocarcinoma displayed a higher frequency of p53 positivity (95.8%) compared to well-differentiated tumors, with a statistically noteworthy link between p53 expression and tumor grade, consistent with findings (Harris & Hollstein, 2013) (5) reported an increased frequency of p53 expression in 95% of moderately differentiated adenocarcinomas, further supporting this correlation.

Our study revealed a predilection for tumor growth on the left side of the colon and rectum, a finding consistent with the known distribution of colorectal cancers. (Fearon & Vogelstan, 2010) (6). reported a comparable histological type distribution to our study, where conventional adenocarcinomas were predominantly located in the left colon, consistent with existing literature, and most cases in our study were moderately differentiated (57.1%), in line with the findings by (Dignam et al., 2016) (7).

The evaluation of lymph nodes continues to be the primary method for determining prognosis and determining the need for adjuvant treatment. We noted LNM in 21 cases (50%) which was comparable to the investigation by (Chithra et al., 2018) (8).

Similarly, Kim et al.,(2022) (9) summarize the role of p53 signaling in colorectal cancer, including the molecular mechanisms, the clinical implications, and the therapeutic strategies results have reported a high frequency of p53 expression in colorectal carcinomas and its association with tumor grade, extension, lymph node status, and stage. Another study by (Cotran et al., 2014) (10) compares the clinical effect of p53

expression and TP53 variation status in colorectal cancer patients, using immunohistochemistry and next-generation sequencing hence finding suggest that p53 expression rather than TP53 variation status has more significant impact on the overall survival of colorectal cancer patients and also suggest However, some studies have also found significant correlations between p53 expression and age, sex, lesion site, or tumor type , which were not observed in our study.

Another relevant stud done by Tomicic et al., (2021) (11) which investigates the role of mutant p53 in colon cancer, using human and mouse genetic studies and explain the possible mechanisms and functions of mutant p53 in colorectal carcinogenesis and progression. Additionally, a study done by Scott et al., (2011) (12) explores the epigenetic alterations upstream and downstream of p53 signaling in colorectal cancer, including DNA methylation, histone modifications, and micro-RNAs, insights into the complex regulation of p53 signaling by epigenetic factors and its implications for colorectal cancer diagnosis and therapy.Finally, study by Mizuho et al.,(2019) (13) which examines the correlation between p53 expression and clinicopathological parameters in colorectal cancer, using immunohistochemistry and corroborate with current finding that p53 expression is associated with tumor grade, extension, lymph node status, and stage in colorectal cancer patients.

Similarly, overexpression was observed in cases with LNM (100%), indicating a poor prognosis associated with p53 detection in CRC. A statistically noteworthy link was found between p53 expression, tumor extension, and LNM. In Figure 8, p53 immunoreactivity is detected in 83.3% of cases, with a notable nuclear positivity (Figure 8.1). Table 1 underscores significant associations between p53 expression and clinicopathological characteristics, emphasizing its prevalence in moderately differentiated tumors, advanced tumor stages, and lymph node involvement. Our findings offer a comprehensive understanding of the clinicopathological landscape of colorectal adenocarcinoma, highlighting the significance of p53 expression as a potential prognostic indicator. These insights contribute to the ongoing efforts to unravel the complexities of this malignancy and pave the way for targeted therapeutic interventions. According to study by (Dabiri et al., 2019) (14) contributes to a comprehensive understanding of the clinicopathological landscape of colorectal adenocarcinoma. The prevalence of p53 expression in specific tumor grades and stages emphasizes its potential as a prognostic marker. These insights not only validate prior research but also add nuanced details to the intricate interplay between p53 and the progression of colorectal carcinoma.Similarly (Russo et al., 2012)(15) observed associations underscore the significance of p53 expression as a potential prognostic indicator in colorectal adenocarcinoma. Identification of p53 as a molecular marker holds promise for predicting the behavior of tumors and guiding therapeutic interventions. Given its prevalence in advanced stages and lymph node involvement, p53 expression could aid in risk stratification and decision-making regarding the intensity of therapeutic strategies.

In summary of the discussion, the findings highlight the diversity within CRC cases and underscore the significance of p53 as a molecular marker associated with various clinicopathological parameters. The clinical significance of p53 expression in colorectal carcinomas remains debated, with our study suggesting its potential as a useful biomarker for identifying advanced disease. Recent studies also support the role of p53 signaling in colorectal cancer and its impact on overall survival.

In study provides valuable data on clinicopathological characteristics and p53 expression in colorectal adenocarcinoma, shedding light on potential prognostic markers and guiding further research for a comprehensive understanding of this complex disease

Conclusions

In conclusion, study includes 42 colorectal adenocarcinoma cases providing key insights. Notably, the age span (45-82 years) centers around 61-70 years, with a male-to-female ratio of 0.8:1. Carcinoma growth predominantly occurs on the left side (57%), and most cases are adenocarcinoma NOS (95.2%), with 57.1% being moderately differentiated. Tumor extension, lymph node involvement, and staging patterns exhibit diversity. P53 expression is detected in 83.3% of cases, emphasizing its significant nuclear presence. Clinical-

pathological associations highlight links with tumor characteristics but not with age, sex, site, or tumor type. Hence understanding of colorectal adenocarcinoma, with a particular focus on the prevalent expression of p53 and its clinicopathological implications. This underscores the importance of incorporating p53 status into the comprehensive management strategy for this intricate malignancy.

Future perspectives

Undoubtedly, the reactivation and restoration of p53 function hold significant promise as a novel therapeutic approach for CRC. However, it's worth noting that while several molecules have demonstrated the ability to induce cell cycle arrest and apoptosis in CRC cells, most of these findings originate from cell line and animal model studies and have not yet progressed to clinical trials. Additionally, the diverse oncogenic effects of mutant p53 remain incompletely understood, and the impact of different mutations on p53 function complicates the assessment of small molecule inhibitors targeting mutant p53 in clinical trials. This area of research warrants further exploration. Notably, addressing resistance to treatments and improving the prognosis of CRC patients with new p53 mutations will necessitate the ongoing development of agents specifically targeting these novel mutations.

Author contribution

All authors have contributed equally and read and approved the final draft of the manuscript.

Conflict of interest

The authors report no conflict of interest.

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Harnessing the prognostic potential of CT imaging in pediatric lymphoma: an in-depth analysis of disease evaluation and outcomes

Marya Hameed [1], Saadia Khurshid [1], Fatima Siddiqui [1], Muhammad Khuzzaim Khan [2]*, Lana Akram Alhouri [3], Noor Fatima [1], Sumera Mahar [4], Kelash Kumar [5]

[1] Department of Radiology, National Institute of Child Health, Karachi, Pakistan

[2] Department of Internal Medicine, Dow University of Health Sciences, Karachi, Pakistan

[3] College of Medicine, Alfaisal University, Riyadh, Saudia Arabia

[4] Department of Radiology, National Institute of Rehabilitation Medicine, Islamabad, Pakistan

[5] Department of Radiology, Hamdard University, Karachi, Pakistan

Abstract

Introduction: Pediatric lymphomas are a significant childhood malignancy primarily treated with chemotherapy. While CT imaging is crucial for disease evaluation, its prognostic value remains under-explored. This study investigates the potential of CT characteristics to predict treatment response and clinical outcomes in pediatric lymphoma patients. Investigate the prognostic value of CT characteristics in pediatric lymphoma treated with chemotherapy.

Materials and Methods: Retrospective analysis of 69 patients' medical records and CT scans. CT features (regression, size, nodal appearance, site involvement) were correlated with treatment response (regression, stable disease, progression, relapse, resolution) via univariate analysis.

Results: Most patients (76.8%) achieved good outcomes with tumor regression. However, a subset displayed stable disease (11.6%), progression (7.2%), relapse (1.4%), or resolution (2.9%). CT characteristics associated with poor outcomes ($p < 0.05$) included: multiple site involvement (neck, chest, abdomen), larger tumor size (>3 cm), discrete nodal appearance.

Conclusion: CT features hold promise for prognostication in pediatric lymphoma. Integrating these findings into clinical practice may improve risk stratification and guide personalized treatment strategies.

Keywords: Pediatric lymphoma, Computed tomography, Prognosis, Outcomes, Regression, Risk stratification

Corresponding Authors: Muhammad Khuzzaim Khan

✉ Email: khuzzaimkhan@yahoo.com

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Introduction

Pediatric lymphomas represent a significant portion of childhood malignancies, ranking as the third most common type. They can be broadly classified into Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL). HL further encompasses the classical and nodular lymphocyte-predominant types, while NHL is categorized into B, T, and natural killer (NK) cell lymphomas based on the World Health Organization (WHO) classification. Non-Hodgkin lymphoma (NHL) accounts for approximately 50% of pediatric lymphomas, with the remainder being Hodgkin's lymphoma (HL) (1-3).

In the staging of high-grade lymphomas in children, contrast-enhanced CT studies of the chest, abdomen, and pelvis are the standard imaging modalities. However, it is important to note that extrapolating FDG-PET and PET/CT results from adult NHL to pediatric NHL is not appropriate due to the differences in disease biology, prognostic factors, staging systems, treatment approaches, and outcomes between these two groups (4-7).

Computed tomography (CT) is commonly utilized for evaluating lymphoma patients as it provides valuable information about both the nodal and extranodal components of the disease (8,9). Its accuracy in disease staging and monitoring therapeutic response makes it an indispensable tool in clinical practice (10-12).

While FDG PET/CT has gained worldwide acceptance as a baseline test for staging and prognostic prediction in lymphoma, it is not routinely used in the pediatric age group. Instead, CT remains the preferred modality for staging and predicting lymphoma survival in children (13-15).

To contribute to the understanding of CT's significance in predicting prognosis and outcomes in pediatric lymphoma, we conducted a retrospective study involving 69 known cases of lymphoma in pediatric patients who underwent CT scans at our hospital over a period of two years. The aim of our study was to assess the role of CT in predicting the prognosis and outcome of the disease in this specific population.

By analyzing the findings and outcomes of our study, we aim to provide valuable insights into the use of CT in pediatric lymphoma management, further improving our understanding of this important field and potentially impacting clinical decision-making for the benefit of young patients. The objectives of this study were to evaluate the prognostic value of computed tomography (CT) imaging in predicting outcomes and prognosis in pediatric lymphoma patients. Specifically, we aimed to assess the correlation between CT characteristics, such as tumor size, nodal involvement, extranodal disease, and clinical outcomes. Additionally, we sought to investigate the association between CT findings and treatment response, including regression, stability, progression, relapse, and resolution of lymphomatous deposits. Moreover, our objectives included identifying specific CT characteristics that are significantly associated with poor clinical outcomes in pediatric lymphoma patients.

Materials and Methods

Study Population and Clinical Data

This retrospective study was conducted with the approval of the Institutional Ethical Review Board Committee at the National Institute of Child Health. Written informed consent was obtained from the legal guardians of all participants.

Inclusion and Exclusion Criteria

Patients diagnosed with lymphoma according to the World Health Organization classification by our hospital pathologists were eligible for inclusion. We included patients who had undergone contrast-enhanced CT scans of the head and neck, chest, abdomen, and pelvis (both unenhanced and contrast-enhanced sequences) within the study period and had visible tumors identified on the CT scans.

Exclusion criteria were:

- Incomplete CT data (missing scans or sequences).
- Underlying medical conditions that could significantly affect CT interpretation (e.g., recent surgery, metal implants).

- Known contraindications to contrast agents used in CT scans.

A computerized search of the hospital database identified 69 patients who met the inclusion criteria during the two-year period from January 2021 to December 2022. Their clinical data, including age, gender, tumor location, stage, treatment received, and clinical outcome, were collected and documented for analysis.

Image Analysis

CT examinations of all patients were conducted using a PQ5000 spiral CT scanner (Picker, New York, NY, USA). The imaging protocol included a series of unenhanced sections followed by intravenous bolus injection of contrast medium (Ultravist 300; Bayer Schering Pharma, Berlin-Wedding, Germany) at a rate of 2.5–3 mL/sec, with a total volume of 75–90 mL. The section thickness for all single spiral CT images was set at 10 mm. For multidetector CT, contiguous axial images and multiplanar reconstructions (MPR) were routinely performed, with a section thickness of 5 mm and a reconstruction interval of 1.25 mm.

To ensure accurate interpretation of the CT findings, a consensus review was conducted by two experienced radiologists (M.H with 8 years of experience in diagnostic imaging, and S.M with 12 years of experience in diagnostic imaging). They were aware that the study population consisted of lymphoma patients; however, they were blinded to the specific pathological type, tumor stage, and survival outcomes. The radiologists assessed various qualitative CT parameters, including tumor location, tumor size, presence of intratumoral necrosis, and lymph node enlargements. In cases where multiple tumors were present, the largest tumor was selected as the representative tumor for each patient. Tumor size was measured in the maximal dimension on the transverse plane. Areas showing reduced or absent contrast enhancement were considered indicative of intratumoral necrosis. Lymph node enlargements were defined as short axis measurements exceeding 1 cm, abnormal round morphology, or the presence of central necrosis.

The rigorous evaluation of the CT findings by experienced radiologists using standardized criteria ensures the reliability and consistency of the image analysis in this study. The blinded assessment prevents bias and enhances the objectivity of the results obtained from the CT scans.

Statistical Analysis

This section details the statistical methods used to assess the prognostic value of CT findings in predicting patient outcomes following chemotherapy for lymphoma. Patient outcomes were categorized into good or poor based on disease status after a 24-month follow-up (no recurrence/stable disease vs. progression during treatment or recurrence within 24 months). Recurrence was further classified as local, distant, or both. To evaluate the relationship between CT characteristics and prognosis, several radiologic variables were chosen based on their established role in lymphoma staging and their potential to influence treatment response and survival. These variables included involvement site (single vs. multiple), tumor size (greater than or equal to 3.0 cm vs. less than 3.0 cm), presence of intratumoral necrosis, lymph node involvement (site and appearance), and involvement of extranodal and extra-intestinal sites. The Chi-square (χ^2) test was used to compare the frequency of these findings between the good and poor outcome groups. A statistically significant difference (p-value < 0.05) would indicate a potential association between the variable and patient outcome. Following the initial analysis, variables with a significant association with outcome (p-value < 0.05) were incorporated into a multivariate logistic regression model. This model allows us to assess the independent contribution of each significant radiologic variable to predicting poor outcomes while accounting for the potential influence of other variables. By employing both univariate and multivariate analyses, this comprehensive statistical approach strengthens our understanding of the relationship between specific CT findings and prognosis in childhood lymphoma.

Results

Patient Characteristics

Our study included 69 patients diagnosed with lymphoma, with a mean age of 7.8 years (range: 4.8-14.2 years). The majority (52, 75.4%) were male with a mean age of 8.1 years (range: 6.7-9.1 years), while the remainder (17, 24.6%) were female with a mean age of 7.6 years (range: 4.8-13.5 years). According to the Ann Arbor Staging system, most patients (63, 91.3%) presented with advanced-stage lymphoma. All patients received cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)-based chemotherapy as part of their treatment regimen (Table 1). The follow-up period ranged from 12 to 36 months, with a mean of 26 months. Treatment outcomes were categorized as follows:

- **Good Outcome (n=63, 91.3%):** No evidence of relapse and stable disease after at least 24 months of therapy.
- **Poor Outcome (n=6, 8.7%):** Progression of lesions during treatment (n=5) or relapse within 24 months after therapy (n=1).

Table 1. Clinical characteristics of included patient sample.

Characteristics	Number of cases	Percentage (%)
Gender		
Male	52	75.3
Female	17	24.6
Age (years)	8.1 (6.7-9.1)	
Ann Arbor Stage		
1-2	6	8.6
3-4	63	91.3
4. Clinical Outcome		
Progression or relapse within 24 months	6	8.6
No evidence of relapse within 24 months	63	91.3

CT Characteristics

Table 2 summarizes the distribution of CT findings in our patient cohort.

- **Site Involvement:** Multiple site involvement (neck, chest, abdomen) was observed in 45 patients (65.2%), while 24 patients (34.8%) had single-site involvement.
- **Tumor Size:** Most patients (63, 91.3%) had tumors less than 3 cm in diameter. Only six cases had tumors larger than 3 cm.
- **Organomegaly:** Hepatosplenomegaly was present in 17 patients (24.6%), splenomegaly in two (2.9%), and hepatomegaly in 17 (24.6%). However, 33 patients (47.8%) did not exhibit organomegaly.
- **Nodal Involvement:** All patients (100%) had nodal involvement, with sites including the neck, chest, and abdomen (anterior/posterior triangles, supraclavicular, axilla, mediastinum, hila).
- **Extranodal Involvement:** Extranodal involvement was identified in 31 cases (44.1%), with sites including the nasal cavity, paranasal sinuses, lungs, liver, spleen, gastrointestinal tract, and musculoskeletal tissues.
- **Intratumoral Necrosis:** Necrosis was present in 13 cases (18.8%).

Table 2. CT findings of included patients.

Characteristics	Number of cases	Percentage (%)
1. Involvement site		
Single	24	34.7
Multiple	45	65.2
2.Tumor size		
<3cm	63	91.3
>3cm	6	8.6
3.Lymph node involvement		
Discrete	58	84.0
Confluent	2	2.8
Both	9	13.0
4.Visceromegaly		
Hepatosplenomegaly	17	24.6
Splenomegaly	2	2.9
Hepatomegaly	17	24.6

Absent	33	47.8
5.Intratumoral necrosis		
Present	13	18.8
Absent	56	81.1
6.Extranodal involvement		
Present	31	44.9
Absent	38	55.0
7.Extraintestinal findings		
Present	9	13.0
Absent	60	86.9

Analysis of Clinical Outcomes

Univariate analysis using the Chi-square test identified statistically significant associations between certain CT features and clinical outcomes (Table 3). These features included:

- **Multiple Site Involvement:** Patients with involvement of multiple sites were more likely to experience poor outcomes ($p < 0.05$).
- **Tumor Size:** Larger tumors (>3 cm) were associated with a higher risk of poor outcomes ($p < 0.05$).
- **Nodal Appearance:** Discrete nodal involvement on CT scans was linked to worse prognosis ($p < 0.05$).

These findings suggest that multiple site involvement, larger tumor size, and discrete nodal characteristics on CT may be potential prognostic indicators for lymphoma patients. Further investigation using multivariate models is warranted to assess the independent predictive value of these features while accounting for other factors.

Table 3. Summary of univariate analysis.

	Regression	Stable	Progression	Relapse	Resolution	p-value
Patient n (%)	53 (76.8)	8 (11.6)	5 (7.2)	1 (1.4)	2 (2.9)	
Age (years)	7.41 ± 3.68	7.87 ± 3.18	10.2 ± 3.83	9	5.5 ± 0.70	0.719
Male: Female	2.53	All male	1.5	All male	All male	0.329
Multiple involvement site, n (%)	31 (58.5)	8 (100)	5 (100)	1 (100)	2 (100)	0.033
Tumor size, n (%) <3 cm >3 cm	51 (96.2) 3 (5.7)	7 (87.5) 1 (12.5)	1 (20) 4 (80)	1 (100) 0	1 (50) 1 (50)	0.036
Nodal appearance, n (%) Discrete Confluent Both	44 (83) 1 (1.9) 8 (15.1)	7 (87.5) 0 1 (12.5)	5 (100) 0 0	1 (100) 0 0	1 (50) 1 (50) 0	0.024
Extra nodal involvement, n (%)	26 (49.1)	1 (12.5)	3 (60)	0	1 (50)	0.202
Intratumoral necrosis, n (%)	10 (18.9)	3 (37.5)	5 (100)	0	0	0.01
Tumor type, n (%) Hodgkin's lymphoma Non-Hodgkin's lymphoma	30 (56.6) 23 (43.4)	7 (87.5) 1 (12.5)	2 (40) 3 (60)	0 1 (100)	1 (100) 0	0.096
Advanced disease, n (%).	48 (90.6)	7 (87.5)	5 (100)	1 (100)	2 (100)	0.061

Detailed Outcomes Analysis by Category

We further analyzed the data by categorizing patients based on treatment outcome (regression, stable disease, progression, relapse, resolution).

- **Regression:** The majority of patients (76.8%) demonstrated regression of lymphoma. Analysis of CT characteristics within this group revealed:
- Multiple site involvement: 58.5%
- Tumor size < 3 cm: Majority
- Discrete nodal appearance: Majority

Stable Disease: Eleven patients (11.6%) exhibited stable disease. Here, the findings were:

- Multiple site involvement: 100% ($p = 0.033$)
- Tumor size > 3 cm: Majority ($p = 0.036$)
- Discrete nodal appearance: Majority ($p = 0.024$)

Progression/Relapse: A small number of patients experienced progression (7.2%) or relapse (1.4%). The distribution of CT features did not show significant trends within these categories.

Resolution: Two patients (2.9%) achieved complete resolution.

Discussion

Lymphoma represents a significant global health burden, accounting for a substantial portion of childhood malignancies (16, 17). This study aimed to investigate the potential role of computed tomography (CT) in predicting prognosis and outcomes for pediatric lymphoma patients. By analyzing various CT characteristics and their association with clinical outcomes, we sought to gain insights into the utility of CT for assessing disease progression and treatment response.

Treatment Response and Heterogeneity

Our findings revealed a positive treatment response, with the majority of patients (76.8%) experiencing lymphoma regression following chemotherapy. This aligns with established knowledge regarding the effectiveness of chemotherapy in reducing lymphoma tumor burden (18). Our study further emphasizes the importance of chemotherapy as a cornerstone treatment for pediatric lymphoma, corroborating its efficacy demonstrated in prior research (19, 20).

However, a subset of patients exhibited stable disease (11.6%), progression (7.2%), relapse (1.4%), or resolution (2.9%). These observations highlight the heterogeneity of lymphoma and the variable treatment responses observed in clinical practice. Identifying factors associated with poor clinical outcomes remains crucial for refining treatment strategies and optimizing patient management (21).

CT Characteristics and Prognostic Value

Our study identified several CT characteristics with significant associations to clinical outcomes. Multiple site involvement, tumor size, and discrete nodal appearance emerged as factors linked to poorer prognosis. Patients with involvement of multiple sites displayed a higher likelihood of unfavorable outcomes. Similarly, larger tumor size was associated with a greater risk of poor outcomes. Discrete nodal appearance on CT scans, potentially indicative of a more aggressive disease process, was another factor associated with a worse prognosis.

These findings align with existing literature that emphasizes the role of CT imaging in lymphoma prognosis and outcome prediction. The Lugano Classification, a pivotal contribution to the field, established recommendations for initial lymphoma evaluation, staging, and response assessment (23). This influential work underscores the importance of CT imaging in accurate lymphoma staging and treatment response evaluation. By providing standardized guidelines, the Lugano Classification facilitates consistent interpretation and reporting of CT findings, recognizing CT as a vital tool for assessing disease extent, nodal involvement, and extranodal disease.

Imaging in Lymphoma Management: A Broader Perspective

The study by Cao et al. (2022) further emphasizes the consensus within the International Conference on Malignant Lymphomas Imaging Working Group regarding the significance of imaging techniques like CT for lymphoma staging and treatment response assessment (25). This international effort highlights the need for standardized imaging protocols and interpretation criteria to ensure reliable and reproducible results, ultimately serving as a guide for clinicians and radiologists to optimize CT use in lymphoma management.

While CT offers valuable information for prognosis and treatment planning, advancements in imaging modalities like 18F-FDG PET/CT have revolutionized lymphoma management (24). The expert consensus from the LYSA/LYSARC/ILSG International Expert Meeting, as outlined by Xie et al. (2019), underscores the value of PET/CT in providing metabolic information that complements anatomical details provided by CT. PET/CT helps evaluate the metabolic activity of lymphoma lesions, offering insights into treatment response and guiding crucial treatment decisions, particularly for DLBCL patients.

Limitations and Future Directions

This study has limitations inherent to its retrospective design, including potential selection bias and incomplete data collection. The relatively small sample size of 69 patients restricts the generalizability of findings and increases statistical variability. Additionally, conducting the study at a single center limits the external validity and generalizability of results. Our focus on CT imaging potentially overlooks contributions from other modalities like PET/CT. Furthermore, the lack of long-term follow-up and survival data limits our understanding of the prognostic value of CT over time. Finally, the study did not account for potential confounding factors that may influence treatment response and outcomes.

Future research should address these limitations by employing larger, multicenter, prospective studies to enhance generalizability and reduce selection bias. Additionally, incorporating PET/CT data alongside CT findings could provide a more comprehensive picture of lymphoma characteristics and improve prognostic accuracy. Long-term follow-up data on patient survival would further strengthen the understanding of the prognostic value of CT in pediatric lymphoma. Moreover, future studies should account for potential confounding factors such as patient demographics, treatment variations, and underlying genetic mutations to provide a more holistic view of factors influencing treatment response and prognosis..

Conclusion

This study explored CT features for prognosis in pediatric lymphoma treated with chemotherapy. While most patients responded well, CT characteristics like multiple site involvement, larger tumor size, and discrete nodal features linked to poorer outcomes. These findings suggest CT's potential role in prognosis, but future research with larger, prospective designs and long-term follow-up is needed for further validation.

Conflict of interests

The authors declare that they have no competing interests.

IRB approval

The study was approved for publication by the National Institute of Child Health’s Institutional Review Board. The IRB number is NICH/23/0110.

Ethics Statement

The manuscript complies with the ethical recommendations of the Declaration of Helsinki of the World Medical Association (WMA).

Authors contributions

MH, **FS**, **SK**, **LAA**, **SM**, and **NF** contributed to the conception and design of the manuscript. **MH**, **LAA**, **SK**, **SM**, and **NF** supervised the project. **MH**, **MKK**, **NF**, **SM**, and **LAA** provided the materials and contributed to data collection and processing. **FS**, **MKK**, **KK**, and **SK** contributed to the interpretation and analysis of the project. **FS**, **MKK**, **KK**, **SM**, and **SK** contributed to the literature review and writing of the manuscript respectively. **MH**, **FS**, **MKK**, **KK**, and **LAA** critically revised the manuscript.

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Diagnostic value of peritoneal lavage fluid cytology findings of peritoneal invasion in patients with gastric cancer

Moein Moghadam Ahmadi [1], Fatemeh Nejatifar [2], Habib Eslami Kenarsari [3], Saeed Soroush [4], Amineh Hojati [5]*

[1] Clinical Research Development Unit of Poursina Hospital, Guilan University of Medical Sciences, Rasht, Iran

[2] Department of Hematology and Medical Oncology, Guilan University of Medical Sciences, Rasht, Iran

[3] Clinical Research Development Unit of Poursina Hospital, Razi Hospital, Guilan University of Medical, Sciences, Rasht, Iran

[4] Student Research Committee, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

[5] Gastrointestinal and Liver Disease Research Center, Guilan University of Medical Science, Rasht, Iran

Abstract

Introduction: Currently, patients diagnosed with gastric cancer typically undergo surgical or laparoscopic examination to assess the presence of metastasis.

Methods: This study involved 35 candidates for gastric adenocarcinoma surgery, consisting of 21 males and 14 females from medical centers in Rasht, Iran, in 2021. Patients reported initial complaints such as abdominal pain, nausea, weight loss, loss of appetite, and anemia. All data was analyzed using SPSS version 21.

Results: Peritoneal lavage cytology results indicated 14 positive cases and 21 negative cases for peritoneal metastasis, while laparoscopic examination during surgery showed 12 positive cases and 23 negative cases. There was concordance between the two methods in 23 cases regarding the presence or absence of peritoneal metastasis, while 12 cases showed inconsistency. Specifically, five cases had negative peritoneal lavage cytology and positive laparoscopic examination, and seven had positive peritoneal lavage cytology and negative laparoscopic examination. Although peritoneal lavage cytology aligned with intraoperative findings regarding patient feasibility.

Conclusion: The study illustrated that solely on peritoneal lavage cytology results is not enough for determining peritoneal invasion in patients with gastric cancer.

Keywords: Gastric Cancer, Peritoneal Lavage, Laparoscopy, Cytology

Corresponding Authors: Amineh Hojati

✉ Email: amineh_hojati@yahoo.com

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Introduction

Gastric cancer is a significant global health concern, accounting for a substantial proportion of cancer-related morbidity and mortality. It is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths (1). The incidence of gastric cancer varies across different regions, with higher rates observed in Eastern Asia, Eastern Europe, and parts of South America (2). Several risk factors contribute to the development of gastric cancer, including Helicobacter pylori infection, a family history of gastric cancer, smoking, and an everyday diet (3). Peritoneal involvement in cancer is usually manifested by abdominal distension, changes in bowel habits, feeling full after eating, and pain secondary to ascites accumulation (4,5). The value of peritoneal lavage cytology in stomach, colon, and pancreatic cancers has always been one of the topics of interest for study, and the relationship between positive peritoneal lavage cytology and worsening prognosis has been confirmed (6).

One critical aspect of managing gastric cancer is accurately assessing the presence of peritoneal invasion, as it significantly impacts prognosis and treatment decisions (7). Currently, surgical or laparoscopic examination is commonly used to investigate the presence of peritoneal metastasis in patients diagnosed with gastric cancer. However, these methods can be invasive and may carry certain risks. Therefore, there is a need for less invasive diagnostic approaches that can provide reliable information about peritoneal invasion in gastric cancer patients (8–11). Peritoneal lavage cytology involves collecting and examining fluid samples from the peritoneal cavity and has emerged as a potential diagnostic tool for peritoneal invasion in gastric cancer (12,13). This study aimed to evaluate the diagnostic value of peritoneal lavage fluid cytology findings in determining peritoneal invasion in patients with gastric cancer.

Methods

In this cross-sectional study, a total number of 35 patients diagnosed with gastric cancer, including those undergoing chemotherapy and those not receiving chemotherapy, were recruited from medical centers in Rasht, Iran, in 2021. Eligibility for inclusion in the study was determined based on CT scan findings, following the guidelines outlined by the National Comprehensive Cancer Network (NCCN). Specifically, patients with minimum involvement of the submucosa (T1b, T2, T3, T4a, and T4b), as indicated by the T score corresponding to tumor growth rate through the stomach wall, were considered suitable candidates for laparoscopy. Patients were provided with detailed information about the study and asked to complete a consent form. Demographical data and clinical characteristics of the patients were recorded. Diagnostic peritoneal lavage was performed immediately before the laparoscopy. The peritoneal lavage fluid was collected, and cytological evaluation was applied to identify the malignant cells' presence or absence. Subsequently, laparoscopy was performed on each patient, and tissue samples were obtained for pathological examination to diagnose peritoneal metastasis. Patients with liver and other organ metastasis were excluded from the study. All data was analyzed using SPSS version 21 and reported by numbers and percentages.

Results

According to the results, about 60% of the patients were males, and most were aged 70-80 (table 1). About 17, 5, 4, and 6 patients had pain, early saturation, vomiting, weight loss, anemia, or stomachache, respectively. Out of three detected tumors, 23 were located distal, 10 were proximal, and two were located in the stomach's body, of which 11, 1, and 5 were well, moderate, and poorly differentiated, respectively. About 16 patients had no lymph node involvement, 11 had one reactive lymph node, 7 had two, and one had three lymph nodes. Cytology results were positive in 14 samples while the laparoscopy findings were positive for 12. About 16 findings were not in agreement with laparoscopy and cytology findings, in which seven samples were diagnosed as positive for gastric cancer by cytology missed from laparoscopy findings; and five samples were diagnosed as positive by laparoscopy findings not confirmed by cytology examination. Only seven samples were confirmed as positive by both laparoscopy and cytology findings. According to our results, the cytology and diagnostic laparotomy agreement rate was 65.7% (Table 2).

Table 1. Demographical findings of the patients with gastric cancer.

variables		Frequency n (%)
Gander	Male	21 (60.00)
	female	14 (40.00)
Age	< 50 year	3 (8.50)
	50-60 year	5 (14.28)
	60-70 year	7 (20.00)
	70-80 year	12 (34.2)
	>80 year	8 (22.8)

Table 2. Diagnostic value of peritoneal lavage fluid cytology in identifying peritoneal invasion compared to intraoperative findings as the gold standard.

Variables	Value	Confidence interval
Sensitivity	0.58	0.0-35.78
Specificity	0.69	0.0-49.9
Positive news value	0.5	0.0-2.7
Negative news value	0.76	0.0-.94
Positive agreement	1.87	1.2-28.88
Negative agreement	0.6	0.0-36.8

Discussion

The value of peritoneal lavage cytology in determining the prognosis of stomach, colon, and pancreatic cancers has been studied before, and the relationship between positive peritoneal lavage cytology and poor prognosis has been confirmed. Also, this method has been used to determine the recurrence of abdominal cancers, which has yielded beneficial results (14). The study's findings indicate that cytology and laparoscopy findings showed some discrepancies, with positive results in both modalities for a limited number of samples. The agreement rate between cytology and diagnostic laparotomy was reported as 65.7%.

Higaki et al. reported that the outcomes of gastric cancer patients with positive peritoneal lavage cytology findings vary due to the diversity of cancer cells. This study aimed to establish diagnostic criteria for curative resections based on peritoneal lavage cytology. The presence of specific cytological features, such as signet ring cells, cell clusters, and isolated cancer cells, predicted poor prognoses. Patients with these high-risk positive peritoneal lavage cytology findings criteria had significantly worse survival rates, even without macroscopic peritoneal metastasis (15). The evaluation of cytological examination of peritoneal fluid in patients with gastrointestinal cancers, including stomach and cardia cancer, showed low sensitivity, specificity, positive predictive value, and negative predictive value, which indicated that peritoneal lavage fluid cytology is not reliable for determining operability in gastrointestinal cancer cases. Therefore, alternative indicators should be explored for accurate diagnosis.

In a study conducted by Abolghasemi Fakhri et al., the sensitivity, specificity, positive predictive value, and negative predictive value of the cytological examination of peritoneal lavage fluid in comparison with the findings during the operation as a diagnostic standard were 59%, 57%, 52%, and 64%, respectively (16). In another study by To et al. (12) to evaluate the diagnostic power of peritoneal fluid cytology in diagnosing peritoneal involvement in 65 patients with gastric cancer, the sensitivity was reported as 51.1%, and there were no false positive cases (6). A study by Wilkimir et al. on 40 patients with gastric or esophageal cancer illustrated that positive cases in laparoscopy were significantly more than positive cases in cytology, and false negative cytology was reported in 45% of cases. They reported that diagnostic laparoscopy in these patients was sufficient to confirm or reject peritoneal involvement, and there was no need for cytological examination of peritoneal lavage fluid (17).

Mozhir et al. studied 27 patients with gastric adenocarcinoma. They performed diagnostic peritoneal lavage before laparoscopy, and a lavage fluid sample was taken for cytology. A successful diagnosis was reported in 22 patients with peritoneal lavage, of which 54.5% had a positive cytology result. Compared with the cytology results of diagnostic laparoscopy samples, the sensitivity of diagnostic peritoneal lavage was calculated to be 100%, and its specificity was 92%. Compared with the results of direct vision with laparoscopy, the sensitivity and specificity of diagnostic peritoneal lavage were reported as 54.5% and 100%, respectively. They reported that in patients with gastric cancer with metastasis, it is possible to

predict the presence of metastasis only based on the cytological findings of peritoneal lavage (18).

Based on the studies, the presence of cancer-free cells in the peritoneal cavity during surgery can predict the outcome of patients. However, whether or not it is helpful for the operability of these patients is still not agreed upon (19,20). Benevolo et al. demonstrated that the immunohistochemical method exhibited a 14% higher rate of detecting free cancer cells than cytology. Additionally, when considering patients identified solely through the immunohistochemical method, they observed comparable rates of recurrence and distant survival compared to the group of patients with a positive cytological examination (21). Other studies have also confirmed these findings, reporting an incidence of free cancer cells ranging from 21.4% to 30% (22–24). The existence of free cancer cells in the peritoneal lavage of patients with gastric cancer carries significant negative implications for their prognosis. The survival advantage of radical surgery in individuals with free cancer cells in the peritoneal lavage is limited, indicating the importance of including peritoneal lavage examination in the preoperative assessment for appropriate surgical planning. The presence of free cancer cells is closely associated with the stage and type of gastric cancer, and their identification can contribute to better categorization of patients. This approach aids in identifying individuals who would benefit the most from aggressive surgical interventions, ultimately leading to improved long-term survival rates.

Conclusion

Based on the findings of the current study, the examination of peritoneal lavage fluid cytology in patients with gastric cancer is consistent with the intraoperative findings of whether the patient is operable or inoperable. However, these results are not significant enough to rely on peritoneal lavage fluid cytology to determine the patients' operability alone.

Conflict of interests

The authors declare that they have no competing interests.

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Consent

This study was approved by the ethics committee of the Guilan University of Medical Sciences [IR.GUMS.REC.1399.537].

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None.

Authors contributions

MMA, **FN**, **HEK** and **SS** did this research, data collection, analysis and wrote the manuscript, **AH** guidance and assisted in data collection and analysis of the results.

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Psychometric properties of the Persian version of the pain beliefs and perceptions inventory (PBPI) in individuals with chronic low back pain

Sarvenaz Karimi-GhasemAbad [1,2] *, Behnam Akhbari [3], Saeed Talebian Moghaddam [4], Ahmad Saeedi [5]

[1] Razi Hospital, School of Medicine, Guilan University Medical Sciences, Rasht, Iran

[2] Physiotherapy Department of University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

[3] Physiotherapy Department of University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

[4] Physiotherapy Department of Tehran University of Medical Sciences, Tehran, Iran

[5] Department of Statistical Research and Information Technology, Institute for Research and Planning in Higher Education, Tehran, Iran

Abstract

Introduction: This study constitutes a methodological investigation aimed at scrutinizing the validity and reliability of the Persian version of the Pain Beliefs and Perceptions Inventory (PBPI) in individuals afflicted with chronic low back pain.

Methods: To gauge reliability, both the test-retest and internal consistency methods were deployed. Furthermore, the correlation coefficient was utilized to assess discriminant validity among 118 individuals suffering from chronic low back pain. The questionnaire's construct validity was ascertained by probing the correlation between the subscales of pain persistence in the future, pain stability in the present, self-blame, and the mysteriousness of pain, with the constructs of pain catastrophizing, disability, pain-related anxiety, coping strategies, quality of life, and pain intensity.

Results: Statistical analysis using the Shapiro-Wilk test revealed a non-normal data distribution. Consequently, the non-parametric Spearman's correlation coefficient was used to scrutinize construct and discriminant validity. The intraclass correlation coefficient (ICC) ranged from 0.58 to 0.78 for the subscales of pain persistence in the future, pain stability in the present, self-blame, and the mysteriousness of pain. Additionally, Cronbach's alpha coefficient ranged from 0.74 to 0.88. With the exception of the self-blame subscale, the other subscales exhibited significant positive correlations with constructs of pain catastrophizing, disability, anxiety, coping strategies, and pain intensity, as well as significant negative correlations with quality of life (correlation coefficient ranging between 0.19 and 0.49).

Conclusion: The outcomes about test-retest reliability, construct validity, and discriminant validity collectively suggest that the Persian version of the PBPI possesses robust psychometric properties.

Keywords: Pain Beliefs and Perceptions Inventory, Chronic Low Back Pain, Validity, Reliability

Corresponding Authors: Sarvenaz Karimi-GhasemAbad

✉ Email: s_karimi@gums.ac.ir

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Introduction

Chronic back pain is one of the most common musculoskeletal disorders with a prevalence of 10-20%. Evidence reveals the influential role of socio-demographic, psychological, and clinical characteristics in the chronicity of back pain (1). Examining psychological risk factors, in addition to the biomechanical approach, aids us in our understanding of the persistence and spread of back pain (2).

Back pain is not always associated with movement disorders and abnormalities. Sometimes, there is an association with negative effects on social relationships, life satisfaction, and psychological disorders such as depression and anxiety. The profile of psychosocial performance in people suffering from back pain is related to their type of pain perception, coping strategy and level of social support (3).

The biopsychosocial model of pain considers the type of pain perception and coping strategies as two factors that can explain the difference between individuals with chronic pain. A person's belief toward pain and the way they perceive it, along with their coping strategies can differ, depending on the situation and culture (4, 5). Research suggests that unfavorable attitudes about pain have an impact on how well chronic pain is treated. Unfavorable attitudes can also turn acute pain into chronic pain and have a detrimental effect on a patient's overall health, self-efficacy, and performance (5, 6). It is recommended that individuals with chronic pain use a variety of cognitive-behavioral techniques to address maladaptive beliefs (6). Different tools were designed to evaluate and determine the beliefs related to pain. The Pain Beliefs and Perceptions Inventory (PBPI) is one of them. Quick and easy identification of cognitive factors is one of the reasons for choosing this scale. This 16-item instrument was designed by Williams and Thorn in 1989. Each of its statements is rated, using a 4-point Likert scale including options of strongly agree, agree, disagree, strongly disagree (7). The PBPI evaluates emotions, behavior, and pain-related perceptions. Strong relationships have been found between this tool and personality traits, physiological processes, coping mechanisms, and feelings of anxiety, depression, and pain (8).

The original version of the questionnaire is composed of three factors namely, time (belief in the stability and continuity of pain), mysteriousness (belief in the mysteriousness and unknowingness of pain) and self-blame (self-guilt and blaming oneself for the pain). The study found that the internal consistency coefficients for the time and mysteriousness of pain subscales as well as self-blame were 0.65 and 0.80, respectively (7). According to a study by Turner et al. (2000) on patients with chronic pain, those who believe in persistence of their pain in the present and continuation of it in the future are more likely to experience physical disability and depression with more severity. The lack of repetition of the time factor, and the emergence of two factors of belief in pain permanence and pain constancy led to the design of a four-factor model (9). Asghari et al. (2005) investigated the psychometric properties of this questionnaire among 232 patients with cancer pain. In this study, the construct validity of the questionnaire was tested using the factor analysis method, and the fourth statement (pain confuses me) was removed from the factor analysis due to a very strong positive bias (10).

The first factor is belief in Pain Permanence with a score between 8 and -8. A positive score indicates a deeper belief in the continuation of pain in the future. The second factor is self-blame. Its score is between 6 and -6, with a positive score suggesting a deeper belief in self-blame. Pain Constancy is stated as the third factor. Its score ranges from 8 to -8. A positive score in this situation expresses a deeper belief in the stability of pain. The fourth and final factor is Mysteriousness, scoring between 8 and -8. A higher score shows a deeper belief in the unknowability of pain and a person's attitude towards pain as an ambiguous phenomenon. The internal consistency coefficients of these four factors varied between 0.70 and 0.77. Persian version of PBPI questionnaire has a significant correlation with disability, psychological structures and coping strategies (10).

The PBPI questionnaire has been translated into several languages with different target populations (6, 11-15). Although the Persian version of this questionnaire is available, due to the different nature of chronic cancer pain and chronic musculoskeletal pain, the psychometric characteristics of the Persian version have not been investigated among people with chronic

low back pain. Therefore, the aim of the present study is to investigate the validity and reliability of the Persian version of PBPI among this group of patients. Based on the COSMIN checklist, the following hypotheses were considered to express the correlation between the PBPI questionnaire and other scales (16).

1. There is a positive and significant correlation between the subscales of the PBPI questionnaire and the constructs of pain catastrophizing, Roland Morris disability questionnaire, coping mechanisms, pain-related anxiety symptoms and pain intensity of people dealing with chronic back pain.

2. There is a negative and significant correlation between the subscales of the PBPI questionnaire and the quality of life of people with chronic back pain.

Methods

This study of localization, validity and reliability of PBPI scales is a methodological one. 118 people suffering from chronic back pain who visited the physical therapy centers of Tehran in the summer and fall of 2017 and 2018 participated in this study (15). The criteria for entering the study include: suffering from back pain for more than three months, the ability to speak Farsi (Persian language), and being in the age range of 18 to 55 (17). People with cognitive disorders, known pathologies (such as discopathy, spinal canal stenosis, fractures in the spine and osteoporosis), and spondylolisthesis as well as those who were pregnant were excluded from the study (17). Eventually, 118 people were eligible to participate in the study and all of them signed the participation consent form. This study was approved by the Ethics Committee of The University of Social Welfare and Rehabilitation Sciences (No:IR.USWR.REC.1396.205).

Pain Beliefs Perception Inventory (PBPI)

The questionnaire was designed by Williams and colleagues in 1989 to assess people with chronic non-cancer pain. The original version of this questionnaire has 16 items and three subscales including mystery, time, and self-blame. Patients rate their pain beliefs on a four-point Likert scale from -2 (completely disagree) to +2 (completely agree). The scoring of 3, 9, 12 and 15th items are calculated in reverse (7). After the factorial structure of the PBPI was examined, four factors (mystery, permanence, constancy, and self-blame) were ultimately identified (9). Asghari et al. localized this questionnaire in Persian language in 2005, which resulted in 15 items with four similar subscales (10). The factor of belief in pain permanence in the future is obtained through summation of the scores achieved from statements Nos. 4, 8, 11 and 14. Summing up the scores of statements Nos. 6, 10 and 12 presents us with the factor of belief in self-blame. Moreover, the score from statements Nos. 5, 3, 9 and 15, states the factor of belief in the constancy of pain in the present time. The factor of belief in the mystery of pain is obtained from the sum of the scores related to statements Nos. 2, 1, 7 and 13.

Coping Strategies Questionnaire (CSQ-8)

The CSQ questionnaire was designed by Rosenstiel and Keefe (1983) in people with chronic back pain. This tool had 50 items, 7 diverse cognitive and behavioral strategies. The six mentioned cognitive strategies include diverting attention, catastrophizing, ignoring pain sensations, reinterpretation, coping self-statements, and praying. It is considered a behavioral coping strategy to increase the level of activity. Behavioral and cognitive coping strategy scales of each item have seven options (0 = never use, 3 = sometimes use, 6 = always use) (18). Each scale is scored between 0 and 36. The Persian version of this scale is available, which, similar to the original version, has Cronbach's alpha coefficient of above 0.70 for subscales (19).

Roland Morris Disability Questionnaire (RMDQ)

This questionnaire is used to measure the disability caused by chronic back pain. It contains 24 questions with yes and no answers. Its score is from 0 to 24, where 0 indicates no disability and 24 indicates severe disability. This scale is widely used in various researches and has favorable internal consistency and construct validity (20).

Visual Analog Scale (VAS)

Visual analog scale is used to measure pain intensity. This scale includes a straight horizontal line of 100 mm, with one end being "no pain" and the other being "the most severe pain possible". The patient marks the pain intensity on the 100 mm continuum of this straight line (21).

Pain Catastrophizing Scale (PCS)

The scale of pain catastrophizing was designed by Sullivan (1995) with the aim of evaluating the level of catastrophic thoughts and behaviors of a person (22). In this questionnaire, subjects are asked to reflect on past painful experiences. Then, rate the degree they experience the thirteen mentioned thoughts and feelings during these events on a 6-point scale. The scale ranges from 0, "not at all or at all" to 4, "always or always" (23).

Beck Depression Inventory-II (BDI-II)

This questionnaire was first designed by Beck. Today, its 21-item version is used which includes specific symptoms of depression. The samples are selected with one of these items that indicates the severity of depression symptoms (24). Each item has a score between 0 and 3. The total score is between 0 and 63. This questionnaire can be used in people over 13 years old and it was localized by Ghasemzadeh in 2005. Its Cronbach's alpha was reported as 0.87 (25).

Pain Anxiety Symptom Scale (PASS-20)

Pain Anxiety Symptom Scale is a self-report tool designed by McCracken in 1992. It is deployed to assess anxiety and fear reactions caused by pain in people who suffer from chronic pain. The total score is between 0 and 100. A higher score indicates pain-related anxiety (26). Shanbezadeh et al (2017) scrutinized the validity and reliability of this tool among the chronic back pain group. Intraclass correlation coefficients for all subscales were higher than 0.70%. Also, Cronbach's alpha was more than 0.70% for all the subscales (27).

Short Form-36 (SF-36)

The quality-of-life scale, a shortened 36-itemed form, was designed by Ware (1992) to evaluate the quality of life and general health (28). This questionnaire was translated into Farsi in 2005 and its psychometric properties were examined (29).

Statistical Analysis

Ceiling and floor effects determine the number and percentage of people who got the lowest and highest score in each of the subscales. If more than 15% of patients have a minimum or maximum score, the questionnaire cannot differentiate between patients at the extremes of the scale (30) .

To evaluate the reliability, this scale was given to 54 patients with chronic back pain in two stages, with a time interval of one week. The purpose of retest assessments was to differentiate between actual score variance and temporary error, which arises from time-related variations in individuals' emotional states, physiological conditions, or cognitive processes (31). In order to measure relative and absolute reliability, Intraclass correlation coefficient (ICC), Standard error of measurement (SEM) and Minimal detectable change (MDC) were calculated between the two stages of measurement (32). By using absolute reliability indices, it is possible to distinguish clinical changes in the sample's condition from changes that may be due to measurement error. To calculate ICC in SPSS version 17, Two-Way Random-Effects Model or (1 and 2) was used.

ICC equal to or higher than 0.7 was considered as the acceptable limit of the reliability level. SEM was obtained using ICC and standard deviation, and MDC was obtained using SEM, with its calculation formula stated as below (33):

$$SD\sqrt{1-ICC}$$

$$1.96 \times \sqrt{2} \times SEM$$

Internal consistency reliability was assessed with Cronbach’s Alpha on the 4 subscales of the PBPI, which is used to evaluate the strength of the relationship between individual's questions within the scale. Mean scores, an alpha coefficient of more than 0.80 was considered as sufficient and acceptable (32).

The Bland-Altman analysis was used to assess how well subscales agree between tests and retests. The mean difference and limits of agreement with a 95% confidence interval served as the method's outcome measures (17).

To evaluate the construct validity of the Persian version of the PBPI scale, the correlation between the score of their subscales and the scores of the Persian version of RDMQ, PCS, CSQ, CSQ, PASS-20, SF-36 and pain intensity was calculated in people with non-specific chronic back pain.

In order to calculate the Item-Total correlation, Dimensionality on an item level, after individually removing the score of each item from the subscale score related to it, Spearman's correlation coefficient was measured for each item with its corresponding subscale score. Acceptable correlation coefficients are 0.4 or lower, and each item's correlation with each of the other subscales should be less than that of the relevant subscale (34).

Results

The background information of people was collected through a self-report questionnaire designed by the researcher. The average age of the subjects was 36.36 with a standard deviation of 10.51 years. The average pain intensity during the test was 30.9 mm based on the linear scale. 29.2% of the subjects in this research were men and 70.8% were women. 19.1% of subjects had education up to diploma, 48.4% had bachelor's degree and 32.5% had master's and doctorate education. The results of the Shapiro-Wilk statistical test showed that the distribution of data in all subscales of the PBPI questionnaire was not normal. Therefore, in the present study, non-parametric statistical methods were used to check the correlation of data.

Table 1 shows the floor and ceiling effect for the subscales' scores of the Persian version of PBPI. As can be seen in the table, less than 15% of people had the minimum or maximum scores of the subscales, except the self-blame subscale.

The obtained results from ICC, SEM, MDC and Bland-Altman agreement along with the mean and standard deviation of each subscale are also mentioned in Table 1. Munro's classification was used to describe the degree of relative reliability (17).

Reliability between zero and 0.25 was considered very low, 0.26 and 0.49 low, 0.50 and 0.69 medium, 0.7 and 0.89 high, and finally, 0.9 and 1 very high. For the majority of the subscales, ICC values between 0.70 and 0.78 were found, which is above the acceptable limit. However, for the subscale of belief in the mystery of pain, an average score of 0.58 was reported. According to Table 1, Cronbach's alpha values in this study for the subscales' scores ranged from 0.74 to 0.88.

Table 1. Flooring and ceil effects, Test-retest reliability, limitation of agreement of Persian version of PBPI (n=118).

SUBSCALE	Permanence	Self-blame Pain	Constancy	Mysteriousness
mean	-6.23	0.26	-2.85	-1.25
SD	5.58	3.13	3.2	3.02
Cronbach's alpha	0.82	0.83	0.88	0.74
ICC	0.70(0.53-0.81)	0.72(0.56-0.83)	0.78(0.65-0.9087)	0.58(0.38-0.73)
SEM	3.05	1.65	1.5	1.95
MMDC	8.47	4.59	4.16	5.42
flooring effect %	0.80%	3.40%	1.70%	3.40%
ceiling effect%	2.50%	28%	4.20%	1.70%
mean difference (95% CI)	-0.301 (-1.47-0.87)	0.37 (-0.635-0.71)	-0.339 (-0.93-0.25)	0.43 (-0.34-1.21)
LOA	-8.67-8.07	-4.74-4.82	-4.58-3.9	-5.08-5.95

SD: standard deviation, ICC: intraclass correlation coefficient, SEM: Standard Error of Measurement, MDC: minimal detectable change, LOA: limitation of agreement.

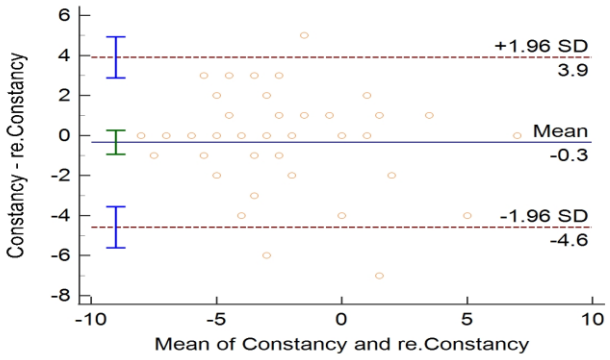

Figure 1. Bland-Altman Plot of constancy subscale of Persian version of PBPI in individual with non-specific Chronic Low Back pain.

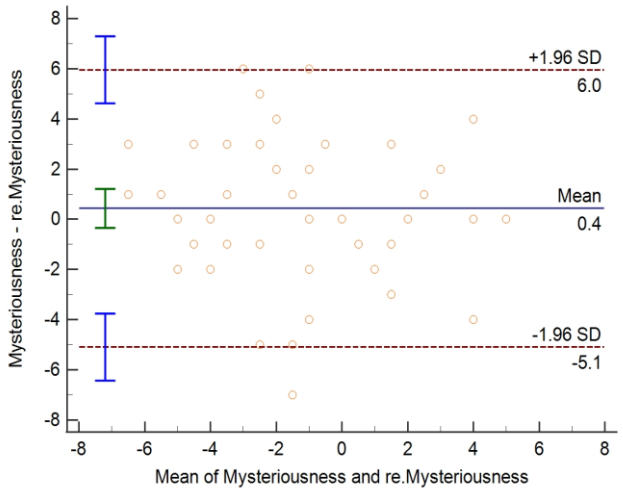

Figure 2. Bland-Altman Plot of Mysteriousness subscale of Persian version of PBPI in individual with non-specific Chronic Low Back pain.

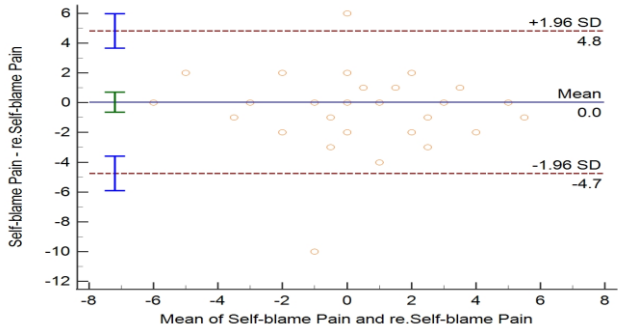

Figure 3. Bland-Altman Plot of self-blame subscale of Persian version of PBPI in individual with non-specific Chronic Low Back pain.

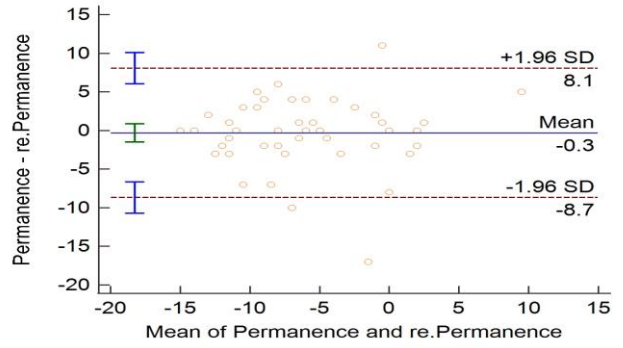

Figure 4. Bland-Altman Plot of Permanence subscale of Persian version of PBPI in individual with non-specific Chronic Low Back pain.

The correlation coefficients between the subscales' scores of the PBPI questionnaire with the scores of the RMDQ, CSQ, PCS, PASS-20, SF-36 and pain intensity are summarized in table 2.

Table 2. Correlation coefficients between PBPI questionnaire scores with RMDQ, CSQ, BDI-II, PCS, PASS-20, SF-36 questionnaire scores and pain intensity (n=118).

Scales/ subscales	Permanence	Self-blame Pain	Constancy	Mysteriousness
PCS	0.424**	0.139	.361** 0	0.332**
PASS.20	0.353**	0.110	00.266**	0.230*
BDI-II	0.416**	0.073	.367** 0	0.266**
SF36.PH.T	-0.511**	-0.021	-0.500**	-0.306**
SF36.MH.T	-0.323**	-0.069	-0.237*	-0.269**
SF36.T	-0.455**	-0.050	-0.401**	-0.315**
Diverting attention	-0.027	0.076	0.031	-0.102
Reinterpretation	0.025	.192*	0.054	-0.006
Catastrophizing	.500**	0.150	0.398**	0.300**
Ignoring pain	-0.198*	0.137	-0.118	-0.061
Praying-hope	0.110	0.075	0.154	-0.007
self-statement	-0.074	0.141	0.050	-0.167
Increasing activity levels	0.023	0.182	0.093	0.088
VAS.	0.212*	0.147	0.194	0.062
RMDQ	0.462**	0.172	0.482**	0.190*

** Correlation coefficients significant at P<0.000, *Correlation coefficients significant at P<0.05. PCS; Pain Catastrophizing Scale, VAS; Visual Analogue Scale, RMDQ; Roland Morris Disability Questionnaire; BDI-II; Back Inventory Index, SF-36; Short Form, MH; Mental Health, PH; Physical Health, PASS; Pain Anxiety Symptom Scale, Pain Intensity.

The results of Table 3 shows that the Spearman correlation between each item and its corresponding subscale was between 0.360 and 0.689, whereas the correlation with other subscales was between 0.089 and 0.589. This means that the correlation of each item with its own subscale was more than the correlation between the score of that item with other subscales. A significant value for the correlation between all items and subscales was reported to be less than 0.001.

Table 3. Item-total correlation of Persian version of PBPI (n=118).

Item	Permanence	Self-blame Pain	Constancy	Mysteriousness
I4	0.689**	0.259**	0.616**	0.399**
I8	0.399**	0.019	0.311**	0.143**
I11	0.454**	0.002	0.326**	0.377**
I14	0.461**	0.072	0.333*	0.243*
I6	0.12	0.684**	0.123	0.038
I10	0.057	0.658**	0.027	-0.009
I12	0.213*	0.36**	0.123	0.116
I3	0.608**	0.037	0.578**	0.207*
I5	0.654**	0.035	0.584**	0.256**
I9	0.701**	0.015	0.602**	0.248**
I15	0.667**	0.059	0.478**	0.357**
I1	0.037	0.197*	0.801**	0.551**
I2	0.061	0.333**	0.654**	0.636**
I7	0.081	0.289**	0.744**	0.512**
I13	-0.131	0.186	0.677**	0.486**

Discussion

In the current study, less than 15% of the participants met the minimum and maximum scores in the subscales, with the exception of self-blame, which had a floor impact of 0.28%. This can show the power of the Persian version of the PBPI scale in differentiating the various beliefs and pain perception in patients with back pain. Findings from the current study corroborated results from a research by Monticone et al. (2014) and Azevedo et al. (2017), where more than 15% of individuals had at least a minimal score on the self-blame subscale. (6, 15).

All subscales' ICC values fell between 0.7 and 0.78, with the exception of the mystery of pain subscale, which had a score of 0.58. This result validates the average of the mystery of pain subscale and the other three subscales' strong reliability. It also shows that in both tests, the order of people with respect to the entire test group has stayed appropriate. The results of another study including individuals with chronic pain fell within a same range (0.88-0.79) (15). The results of the other research, which included participants with chronic back pain, were similar (6). Cronbach's alpha coefficient of the subscales of mystery of pain was reported to be in the range of 0.74 to 0.88, which is in line with the results of other studies that had been done previously (6, 10, 15).

The minimum MDC for the subscales of belief in pain permanence, self-blame, pain constancy, and mysteriousness were 89.47,4.59, 4.16, and 5.42, respectively. With the aid of the MDC results, therapists and researchers are able to ascertain the true changes and validity of the subscales' scores (27). The agreement between the mean difference and the results indicates that each subscale fell within the predetermined limitations. Failure to calculate MDC and SEM and agreement in previous studies has limited the possibility of comparing their results.

The PBPI subscales' construct validity results suggested that, all subscales, except self-blame exhibited a positive and significant association with disability, pain-related anxiety symptoms, depression, and catastrophizing. Also, a significant negative relationship was observed between the quality of life and the subscales of pain permanence, pain constancy, and pain mystery. Among the coping strategies, only catastrophizing showed a positive and significant relationship with three subscales of the PBPI questionnaire, except self-blame. A positive and significant relationship was reported between pain intensity and the subscales of pain

among 122 people with chronic pain. A negative relationship was observed between the level of quality of life and the subscales of pain mystery, pain constancy, and pain permanence. Similar to the present study, they did not report a significant relationship between this questionnaire and the subscale of self-blame (15).

A notable positive correlation was observed between the permanence subscales and pain intensity, while no such association was identified for the remaining subscales. The permanence subscales of the PBPI concentrate on the daily life encounters of pain, suggesting a potentially more robust connection with the factual experience of pain intensity as assessed through the VAS. Contrary findings were reported by

Blanch et al., who evidenced a strong correlation between all PBPI subscales and pain intensity. Discrepancies in results may be attributed to variations in sample sizes; notably, the study by Blanch et al. predominantly involved participants afflicted with fibromyalgia (8).

According to the Cognitive-Behavioral Theory and the Biopsychosocial model, there is a significant correlation between disability, pain catastrophizing, and predictable coping strategies (8). This statement confirms the results of previous studies as well as the present one. The lack of correlation between self-blame and other scales was also found in previous studies. This could be due to the lack of a structure related to self-blame, which calls for more attention in future studies (1, 13, 35, 36). The construct validity results confirmed the hypotheses considered at the beginning of the present research.

The strong correlation between the items of the Persian version of PBPI with their corresponding subscale indicates the appropriate structure of this version. In addition, it shows that each subscale consists of appropriate items (6, 15).

Limitation

This study's limited number of participants may compromise its external validity and generalizability. Moreover, lack of implementation of content validity and exploratory factor analysis is another limitation that can be addressed in future studies.

Conclusion

The psychometric properties of the Persian version of the Pain Beliefs and Perceptions Inventory (PBPI) were examined among individuals suffering from chronic back pain, demonstrating commendable levels of validity and reliability. This instrument can be effectively employed by physical Therapists and researchers to assess patients' beliefs and perceptions regarding pain, contributing to enhanced treatment outcomes.

Competing interests

The authors declare that they have no competing interests.

Statement of the Institutional Review Board Approval

Informed consent form approved by the Ethics Committee at University of Social Welfare & Rehabilitation Sciences (No: IR.USWR.REC.1396.205).

Authors contributions

BA, **STM** and **AS** contributed to the concept and design of the study and collected the data. **SKGA** drafted the manuscript and prepared the final version, read and revised the manuscript critically for important intellectual content. Finally, all authors approved the final version of the manuscript for publication.

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Case report Free Access

A case of mucous gland adenoma of lung: a benign mimicker of malignancy

Veeksha Venugopal Gowda [1], Divya Vijayanarasimha [1], Rekha Vijay Kumar [1], Sanjeev Kulkarni [2]

[1] Department of Oncopathology, Sri Shankara Cancer Hospital and Research Centre, Bangalore, Karnataka, India

[2] Department of Surgical Oncology, Sri Shankara Cancer Hospital and Research Centre, Bangalore, Karnataka, India

Abstract

Introduction: Mucous gland adenoma of the lung (MGA) is an uncommon benign tumor. MGA of lung is extremely rare with less than 70 cases documented in the literature to the best of our knowledge. At present, according to the World Health Organization's classification of thoracic tumors, MGA is categorized as epithelial tumor and subclassified under adenomas. This is characterized by endobronchial growth of mucous cells with no atypia. We report a case of MGA of lung which was clinico-radiologically suspected to be a malignant tumor and discuss the diagnostic approach, differential diagnosis, treatment and need for close follow-up, with a thorough review of the literature.

Case presentation: A 57-year-old lady presented with pain in the left chest wall and arm for a duration of 3 months. After clinical examination, an x-ray showed collapse, and consolidation on ipsilateral lung. The subsequent CT scan of the thorax showed an 14 x 12 x 11 mm lesion in the proximal left main bronchus. Clinico-radiologically, carcinoma of lung was suspected. The patient underwent endoscopy and the endobronchial biopsy from the lesion showed features of a papillary glandular neoplasm. There was no immunostaining of the lesional cells for TTF1, synaptophysin, chromogranin, and p40, with a low Ki67 index of <5%. Although the possibility of malignancy was deemed unlikely, resection was suggested for confirmation. The patient then underwent pneumonectomy on which a histological diagnosis of mucous gland adenoma was made. The patient is well and on follow-up for 12 months.

Discussion: Due to its rarity and clinical presentation mimicking malignancy, MCA presents challenges in diagnosis. Malignant entities like invasive mucinous adenocarcinoma, low-grade mucoepidermoid carcinoma, and endobronchial metastasis from extraneous sites need to be considered in the differential diagnosis.

Conclusion: Mucous gland adenoma of lung is a rare tumour; this case report highlights the challenges faced while reporting small biopsy samples of lung and the need to be aware of the benign mimickers of malignancy. For the accurate diagnosis of this rare entity, a multimodality approach that includes histological examination, immunohistochemical analysis and radiological findings is key.

Keywords: Mucous gland adenoma, Lung, Endobronchial biopsy

Corresponding Authors: Veeksha Venugopal Gowda

✉ Email: veekshavenugopal @gmail.com

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Introduction

Mucous gland adenoma of the lung is an uncommon benign tumor (1) first reported in 1882 by Muller. The majority of these cases arise within the main, lobar, or segmental bronchi .

Patients typically present with symptoms of obstruction, cough, haemoptysis, dyspnoea, and recurrent pneumonia. Mucous gland adenoma is extremely rare. It has no sex predilection and has a wide age range (25–67years). The exact etiology and pathogenesis are unknown. The differentials include invasive mucinous adenocarcinoma, low-grade mucoepidermoid carcinoma, endobronchial glandular bronchial papilloma, bronchiolar adenoma and other adenomas (2). The purpose of this report is to spread awareness regarding this rare entity, highlight the diagnostic challenges on small biopsy samples and differentiation from its histological mimics.

CASE STUDY

A 57-year-old lady presented with pain in the left chest wall and arm since 3months; the pain was intermittent. There were no symptoms of fever, weight loss or dyspnoea. She underwent CECT scan of the thorax which showed a 14x12x11mm lesion in the distal main bronchus (Figure 1).

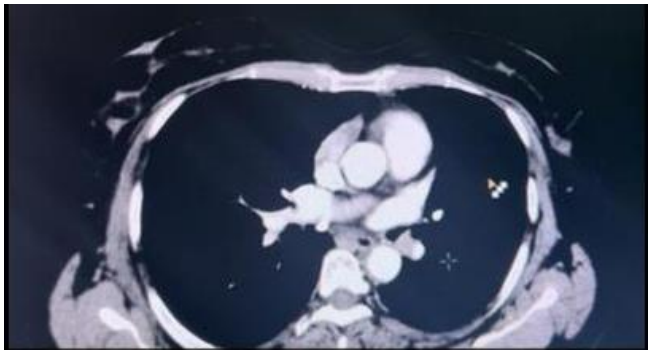

Figure 1. CECT thorax showing a well-defined enhancing lesion near the left perihilar region.

Carcinoma was suspected clinico-radiologically. Endobronchial biopsy from the lesion showed features of a papillary glandular neoplasm, with the epithelial cells showing minimal atypia (Figure 2a and 2b).

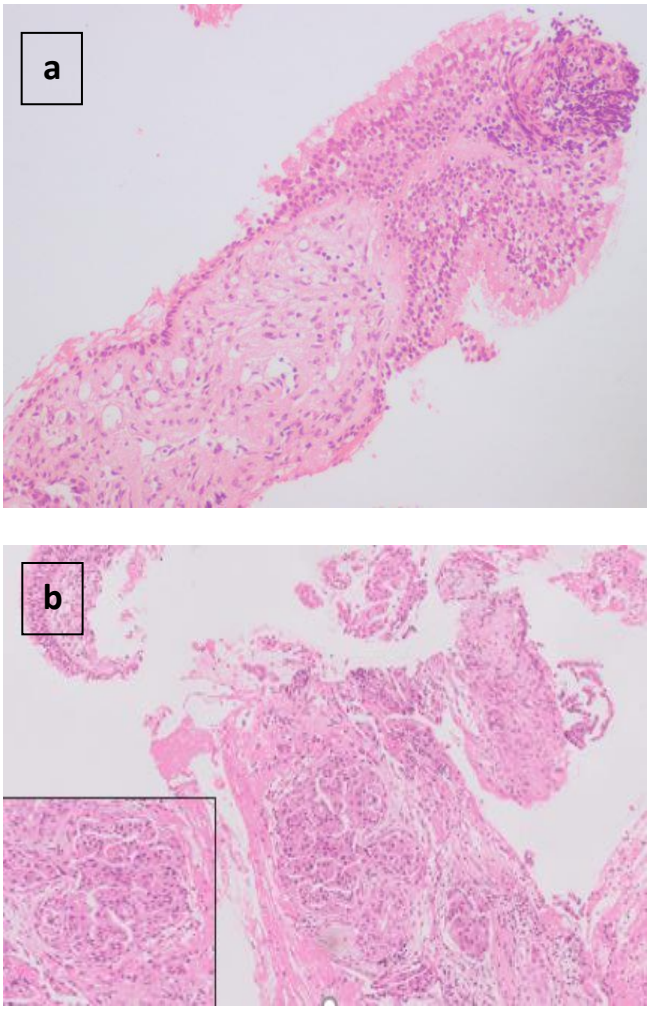

Figure 2. a. H&E stain, 10x. shows normal bronchial epithelial lining, **b.** H& E stain,20x Papillary glandular neoplasm, the atypical cells are arranged in papillary architecture lined by cuboidal to columnar cells with minimal atypia, Inset shows higher power of the neoplasm (H&E at x40).

The neoplastic cells showed no immunostaining for TTF1(Fig 3a), synaptophysin, chromogranin, and showed a Ki 67 proliferation index of <5%. P40 stained the continuous basal cell layer of bronchial epithelium, however no staining in the neoplastic cells. An additional immunohistochemical (IHC) marker NKX3.1 showed weak to moderate nuclear staining in occasional neoplastic cells (Figure 3b).

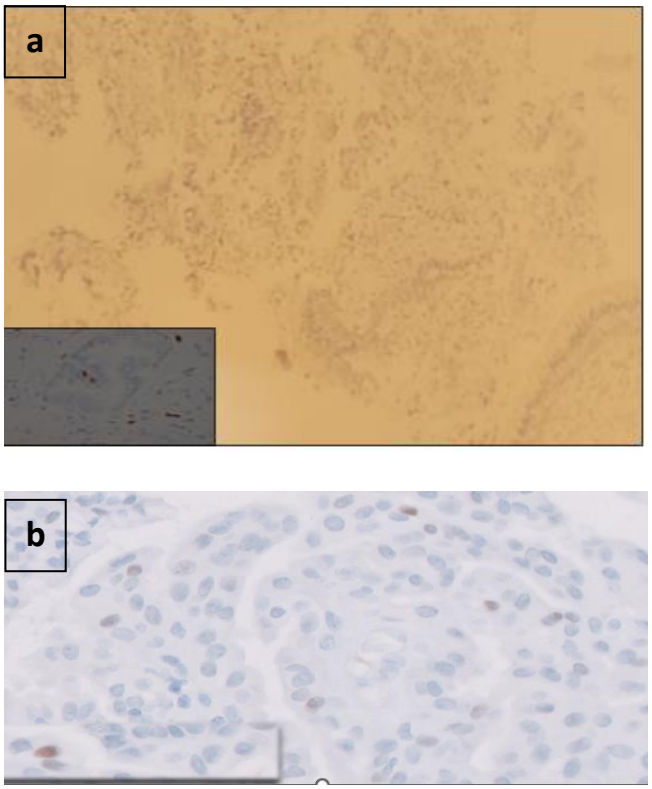

Figure 3. a. IHC stain for TTF1, x20 No staining of the neoplastic cells for TTF1; Inset shows Ki67 <5% (IHC at 20X); **b.** IHC stain for NKX3.1, 40X, weak to moderate nuclear staining of few cells, inset shows focal strong staining of occasional cells.

Although the possibility of malignancy was unlikely, resection was suggested for confirmation in view of the limited tissue examined. The patient then underwent pneumonectomy. Intraoperatively, the surgeons noticed a grey-white tumour in the bronchus (Figure 4), not involving the lung parenchyma.

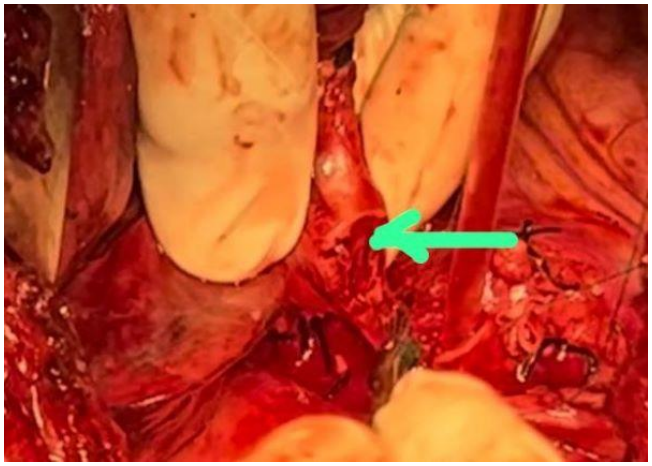

Figure 4. Intraoperative image shows grey-white tumour in the bronchus.

Histopathological examination of the specimen showed a grey-white papillary tumor in the bronchus that measured 1.5x1.1x1.3cm which did not involve the lung parenchyma. Histologically, a circumscribed neoplasm was identified in the bronchial wall, composed of papillae and numerous cystically dilated mucous–filled glandular structures lined by flattened cuboidal epithelium with oncocytic change and none to minimal atypia. Mitoses were rare. (Figure 5 a,b,c).

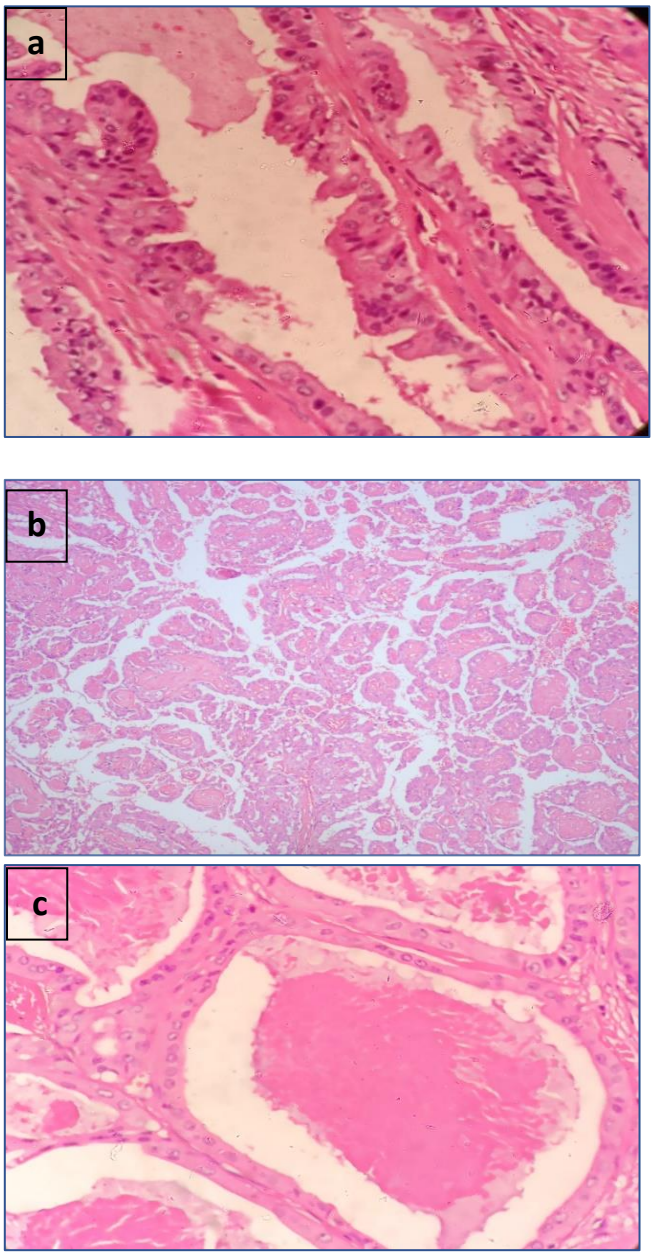

Figure 5. a and **b** H&E stain 40x and 10x respectively shows papillary architecture lined by cuboidal to columnar cells with moderate amounts of cytoplasm and none to minimal atypia; **c.** H&E at 40x Cystically

dilated mucous–filled glandular structures lined by bland flattened cuboidal epithelial cells.

Hence, a final diagnosis of mucous gland adenoma was made. The patient is on follow-up for 12 months postoperatively, remains asymptomatic. Clinically and radiographically disease-free

Discussion

Mucous gland adenoma was considered a salivary-type of tumor and presumed to arise from the submucosal sero-mucous glands of the bronchus. Fewer than 70 cases have been documented in the literature to the best of our knowledge (1,3,4).

Our patient presented with left chest wall and arm pain which is an unusual finding; the common clinical manifestations of this tumor include hemoptysis, cough, dyspnoea and wheezing, often complicated with pneumonia (5,6,7,8).

These tumors can also present as chronic obstructive pulmonary disease as they obstruct the airway lumen (9). Therefore, it usually takes longer to come to the correct diagnosis. One case report highlights that this entity can be misdiagnosed as tuberculosis for up to 2 years (10). The lesion was located peripherally in our patient, which may explain why she had no obvious symptoms.

The majority of the tumors present as intraluminal exophytic masses in the proximal airways. Our case showed an endobronchial mass with no parenchymal involvement, unlike the cases reported by Zhang XT et al. and M. A. Weinberger et al. which showed parenchymal involvement (4,6).

The diagnosis of papillary glandular neoplasm on a small biopsy sample is considered appropriate because adenocarcinoma cannot be conclusively excluded without thorough sampling.

Macroscopically, mucus gland adenoma appears as a grey-white smooth mass, with a solid-cystic cut surface, with cystic change often being the predominant feature (4). Our case differed in being predominantly solid on cut surface.

Our histology findings were in accordance with those described in the reports by Zhang XT, England et al, and Sasaki E et al; showing a well-circumscribed lesion with proliferation of mucosal glands in the form of an exophytic nodule composed mainly of variably dilated, cystic glands filled with mucus. Tubules and papillae were present focally. The lining cells were mucous-secreting columnar, cuboidal or flattened cells with stratification or papillary luminal folds. The stroma was composed of hyaline connective tissue. There was no invasion of the cartilage or bronchial wall. The unusual histological finding in our case was the presence of papillary architecture which is rare or absent in other case reports. Zhang et al. noted that several normal dilated bronchi can be distributed among neoplastic glands. This characteristic finding may be helpful in differential diagnosis (4,5). However, this was not a finding in our case.

The immunohistochemical findings in our case were in concordance with the findings of Badyal RK et al (1) whose case showed positivity for CK, CK7, 34βE12 and EMA; focal positivity for CEA and consistent negativity for TTF-1, SPA, Napsin A, ALK (D5F3), CDX2, CK20, p53, vimentin and synaptophysin; p63 and S100 staining highlighted the myoepithelial cells scattered at the periphery of the glands. In our case, the epithelial cells were TTF1, p40 synaptophysin and chromogranin negative. The Ki-67 index was less than 5%. NKX3.1 positivity was seen in two cases of MGA in the study by Sasaki et al and it was concluded that NKX3.1 immunohistochemistry could be sensitive and specific ancillary marker that distinguish MGA from other histologic mimics (5). However, it is likely that staining patterns in more tumours is required to know its specificity for MGA.

The lack of significant atypia, mitotic activity and parenchymal infiltration helped in differentiating this neoplasm from the relatively more common diagnosis of invasive mucinous adenocarcinoma. Lack of intermediate and epidermoid cells excluded the diagnosis of low-grade mucoepidermoid carcinoma. Endobronchial glandular bronchial papilloma, bronchiolar adenoma and other adenomas are close differential diagnoses, but their cellular composition and location are different from mucous gland adenoma. The rare possibility of an endobronchial metastasis should be excluded by correlating with the clinical history, histomorphology and immunohistochemistry (2).

Surgical airway resection, sparing lung parenchyma, is the treatment of choice for centrally located adenomas. Lobectomy is preferred in patients with bronchial obstruction or parenchymal involvement. If the patient has comorbidities like chronic obstructive pulmonary disease and the tumor is polypoid and attached to the bronchial wall, bronchoscopic resection is the surgery of choice. In our case, pneumonectomy was performed in view of the broad stalk of the mass in the main bronchus and diagnostic ambiguity (8,11).Since mucous gland adenomas are benign tumours, they are cured by resection (2).

Most of the patient do well without recurrences; however, due to its rarity a longer follow up may be advisable. Our patient is at 12 months of follow up and is recurrence-free.

Conclusion

This case report highlights the challenges faced while reporting small biopsy samples of rare lung neoplasms and the need for heightened awareness of the various benign histological mimics of adenocarcinoma.

Competing interests

The authors declare that they have no competing interests.

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We sincerely thank the technical staff, our pathology and clinical collegues.

Consent

Consent of the patient is taken.

Authors contributions

VG collection of case details , image capturing, data acquisition, review of literature, article writing and corresponding author, **DV** collection of case details, article writing, revision of article, **RVK** revision and improvement of the article, **SK** procuring operative, radiological images and revision of the article. All the authors contributed to the article and approved the submitted version.

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Review Free Access

The effect of green tea consumption on depression

Mohammad Esmaeilpour Bandboni [1] *, Arefeh Zabeti Touchaei [2], Peyman Rabiei [3], Zinat Seyedpour [4]

[1] Zeynab (P.B.U.H) School of Nursing And Midwifery, Guilan University of Medical Sciences, Rasht, Iran

[2] Department of Chemistry, Lahijan Branch, Islamic Azad University, Lahijan, Iran

[3] Babol-Branch, Islamic Azad University, Babol, Iran

[4] Guilan University of Medical Sciences, Rasht, Iran

Abstract

Introduction: Depression has become an epidemic disorder in the world, which according to the World Health Organization is the first debilitating factor in the world. There are different approaches to its treatment, including the use of complementary medicine and herbal medicines. In recent years, there have been reports about the protective role of tea consumption in reducing the risk of depression, especially in the elderly. This study aimed to determine the effect of tea consumption on depression by reviewing quality published studies in this regard.

Methods: To conduct a comprehensive review on the topic of tea and depression between 2005 and 2024, we used various search engines such as Persian language scientific resources SID and Magiran, as well as Google Scholar, Web of Science, PubMed, and Scopus. The search was conducted using the keywords "tea" and "depression" in combination.

Results: Out of 215 articles that were reviewed, the final analysis was conducted on 22 of them. Among these, 20 articles validated the correlation between tea consumption and a decrease in symptoms of depression. In most of these studies, it was highlighted that consuming at least three cups of tea daily had a positive impact.

Conclusion: The results of this study have verified the advantages of drinking over 3 cups of green tea per day. It is recommended to consume green tea in various groups, especially in populations with a high incidence of depression, such as the elderly living in boarding centers.

Keywords: Herbal therapy, Tea, Depression, Green tea

Corresponding Authors: Mohammad Esmaeilpour Bandboni

✉ Email: esmaeilmmm@yahoo.com

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Introduction

The world is facing a challenge as the population ages, particularly in developing countries where this issue is expected to be more significant in the years to come. The percentage of elderly people aged 60 and above has increased globally from 9% in 1994 to 12% in 2014 (1). This growth is predicted to be about 3.5 times by 2025, while the total population will also increase. The growth of the total population reaches (2). According to the general population and housing census of Iran in 2015, about 9.3% of the country's population is made up of elderly people over 60 years old (3). It is predicted that the percentage of Iran's elderly population will reach more than 12% in 2025 (4). Aging is a natural process and one of the stages of human growth and development. During this process, changes occur in the physiological, psychological and social aspects of people. This process is gradual and progressive and diet, environment, personal habits and genetic factors affect its severity and extent (5). These changes in homeostasis that occur during old age can lead to various diseases, including stroke, dementia, mental illness, and cardiovascular diseases (6).

At present, depression is one of the most common mental illnesses that has become a general problem and is widespread throughout the world (7). Depression includes a set of mental disorders that affect people's activity, behavior and thoughts (8). In depressed patients, the feeling of sadness and the decrease in the feeling of pleasure and interest leads to a decrease in personal and social functioning, which is accompanied by changes in sleep, nutrition, energy level and motivation (9). According to the report of the World Health Organization, depression is the most important debilitating factor worldwide (8). Depression is usually associated with other diseases. Such diseases may precede depression and cause depression or be its consequence and result. The priority of diagnosing and treating depression should remain consistent despite differences in people and conditions (10). Age is an independent and important variable that can affect the appearance of depression, its symptoms and its natural course. Generally, depression is a major cause of death worldwide, which is associated with a reduction in social, occupational, and interpersonal roles. As we age, this dreaded mental state is often exacerbated by environmental and physical factors. In general, demographic characteristics (age, gender, place of residence, level of education, occupation, type of family, cohabitation with spouse, economic dependence), lifestyle (inactivity, disability in the areas of mobility and displacement, duties Home and family and social participation, nutritional status, having fun activities, cultural variables, religion, alcohol consumption, smoking) psychological variables (social acceptability and extroversion, duty orientation, dissatisfaction with personal income, loneliness, sadness) and sadness, perception of general health), physiological variables (average red blood cells, hemoglobin level or hematocrit values, degree of anemia, low weight, chronic diseases, sleep disorders, use of sleeping pills) are predictive factors. They cause depression in the elderly (11). These issues have caused mental problems to be observed in old age. About 15 to 25 percent of elderly people have important mental problems, which have a potential effect on their physical diseases (12). The feeling of depression is the most common problem that threatens mental health among the elderly, which is included in the category of mood disorders (13). Depression is associated with significant prevalence and mortality in the elderly (14). The prevalence of obvious clinical symptoms of depression among the elderly in the community is 8-15% and in the elderly living in nursing homes, it is about 30%. According to research findings, about 15% of the elderly suffer from depression (12).

Antidepressants, psychosocial interventions, and in severe cases, shock therapy are used to treat depression (9). Although many drugs are available for the treatment of psychiatric disorders, including depression, researchers and psychiatrists believe that a large number of patients have not recovered after taking these drugs, and some are also able to tolerate the side effects of these drugs (15). Today, non-drug treatments and treatments with fewer side effects have received much attention in the control of some psychiatric syndromes, one of which is the use of medicinal plants (16), which has led researchers and psychiatrists to It has led to use of non-invasive and low-complication methods such as the use of medicinal plants (17). Throughout history, medicinal plants have established their place in human life as medicinal and

therapeutic agents and are used in various forms such as tablets, capsules, and ointments (18).

Tea (Camellia Sinensis L) is one of the oldest drinks in the world, which was first discovered by the ancient Chinese, and then other countries also learned how to produce it (19). Tea is one of the oldest drinks in the world, which was first discovered in ancient China. This plant is cultivated well in tropical and semi-tropical areas that have sufficient annual rainfall, proper drainage and acidic soil (20). After water, tea is the most popular and consumed drink in the world (21). Green tea is one of the plants that has received a lot of attention, and it is generally considered a common tea drink all over the world (18). Green tea strengthens and stimulates the activity of brain cells, especially in the parts related to memory. Also, by drinking green tea, the body becomes more resistant and calms the nerves and relieves nervous tension. Since many diseases are directly related to stress and the nervous system, drinking this tea can be considered beneficial for many diseases (22). Various studies have been conducted on the relationship between tea consumption and depression. However, the findings in this field are often contradictory. Therefore, we decided to conduct a comprehensive review to summarize the various findings. Table 1 provides an overview of key information related to tea, specifically focusing on its historical significance, cultivation requirements, popularity, the importance of green tea, its potential benefits for brain function and stress relief, and the connection between tea consumption and depression.

Table 1. The essential details about tea, include its origins, cultivation conditions, global popularity, the significance of green tea, potential cognitive and stress-relief benefits, and the research on tea's association with depression.

Topic	Information
Tea (Camellia Sinensis L)	- One of the oldest drinks in the world. - First discovered by ancient Chinese
Tea Cultivation	- Thrives in tropical and semi-tropical areas. - Requires sufficient annual rainfall, proper drainage, and acidic soil
Popularity	- Second most popular and consumed drink in the world after water
Benefits of Green Tea	- Strengthens and stimulates brain cell activity, particularly related to memory. - Increases resistance and promotes relaxation, relieving nervous tension. - Potentially beneficial for various diseases related to stress and the nervous system.

Methods

The current review study was conducted to investigate the effect of tea consumption on depression in the elderly. To find articles and studies related to the topic, search engines of Persian language scientific resources SID, Magiran and English language Google Scholar, Web of Science, PubMed, and Scopus were used. The keywords tea and depression were used in combination. The study period was between 2005 and 2024. The inclusion criteria for this study were all original Persian and English articles and reviews whose full text was available. Initially, two researchers conducted a search using selected keywords across all relevant databases. The articles found were then saved. In the second step, both researchers reviewed the saved articles and agreed to exclude any that were not related to the subject of the study. In the next step, duplicate and similar articles were removed. The steps of selecting the articles are shown in Figure 1.

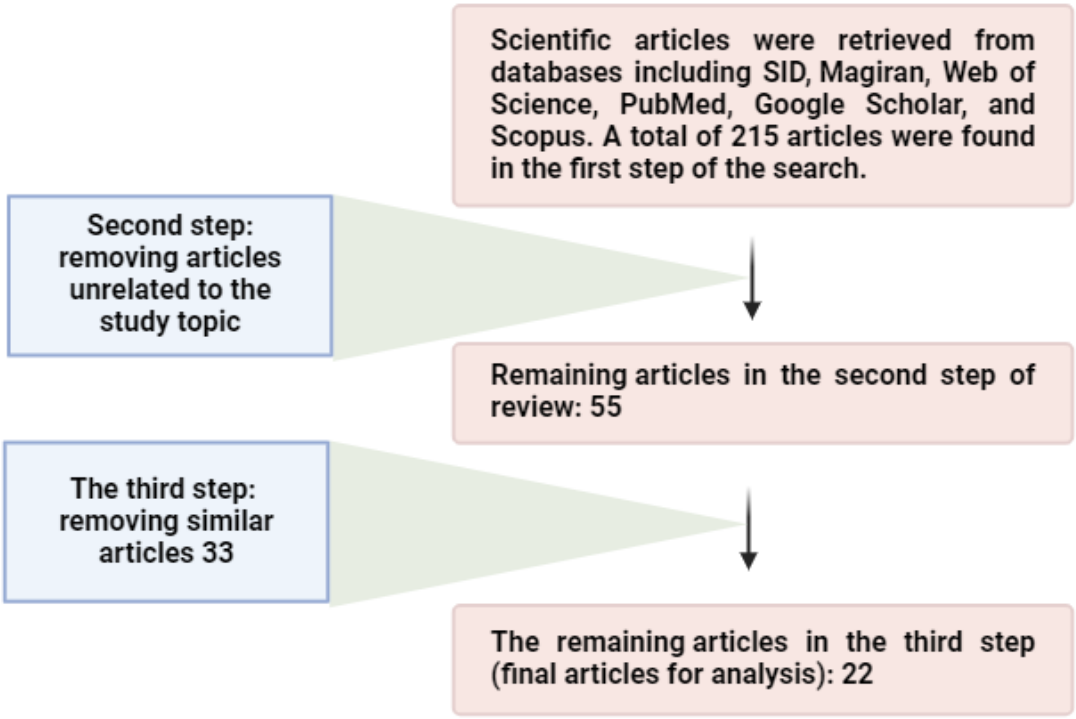

Figure 1. The steps and description of selecting the articles.

Results

A significant portion of this research was published in 2021. In the first step of the present study, 215 articles with the desired keywords were found, after which 160 were removed, and then the similar articles were left out so that the final analysis was done with 22 articles. One article was published in Farsi and 21 studies were published in English. The year of publication of the articles was between 2005 and 2024. The target population of these studies includes; They were elderly, middle-aged, diabetic and cancer patients. studies in terms of research type; four articles, it was a systematic review and meta-analysis, and the other articles were cross-sectional and cohort analysis, and only one article was a control case. The findings of 19 articles provide evidence that regular tea consumption (green and black) reduces the risk of depression symptoms, but 5 articles did not report a significant relationship between tea consumption and depression risk reduction. The results of two articles out of three review and meta-analysis studies show that tea consumption reduces the risk of depression. In this regard, Dong et al. found that consuming 3 or more cups of tea per day reduces the risk of depression (23). Farajzadeh et al. found that those who drank tea had a 35% lower risk of depression symptoms than those who did not (24). On the other hand, Grosso et al. reported that the relationship between green tea consumption and the risk of depression is insignificant (25). In general, 19 articles confirmed the relationship between tea consumption and reducing the risk of depression, and in most of these studies, daily consumption of at least three cups was emphasized. Some studied green tea, some black tea, and some studied both types of tea. Of the 22 articles that investigated the effects of tea, 3 articles (13%) did not report a significant relationship between tea consumption and the risk of depression, but another 19 articles (86%) confirmed the positive and significant effect of tea on reducing the risk of depression. Of the articles that specifically investigated the effects of green tea, 2 articles reported that green tea consumption does not affect reducing depression. These results are detailed in Table 2.

Table 2. Information on selected articles on the relationship between green tea consumption and depression

Author(s)	Year	Title	Target Group	Study Type	Intervention/Independent Variable	Key Findings	Final Conclusion
Farajzadeh & et al	2017	Does tea consumption reduce the chances of depression in the elderly? Case-control study	Iranian elderly	Case-control	Daily tea consumption	Elderly individuals who consume more than 3 cups of tea per day have a 66% lower chance of depression compared to those who consume less (p=0.001).	Tea consumption reduces the risk of depression in the elderly
Pham & et al	2014	Green tea and coffee consumption is inversely associated with depressive symptoms in a Japanese working population	Japanese men and women (68-20 years old)	Cross-sectional, analytical	Daily green tea and coffee consumption, estimated caffeine intake	Individuals who consume 4 or more cups of green tea per day have a 51% lower prevalence of depressive symptoms compared to those who consume less than 1 cup (p=0.01).	Coffee and green tea consumption may have a protective role against the development of depressive symptoms
Niu & et al	2009	Green tea consumption is associated with depressive symptoms in the elderly	Japanese elderly	Cross-sectional, analytical	Green tea consumption status through a self-administered questionnaire	Elderly individuals who consume 4 or more cups of green tea per day have a 56% lower prevalence of depressive symptoms compared to those who consume a maximum of 1 cup (p=0.001).	Increased consumption of green tea leads to a decrease in depressive symptoms
Grosso & et al	2016	Coffee, tea, caffeine and risk of depression: A systematic review and dose-response meta-analysis of observational studies	Published articles from January to June 2015	Meta-analysis	Investigation of the relationship between tea consumption and the occurrence of depressive symptoms	A weak association between tea consumption and the risk of depression was observed.	The results of this meta-analysis indicate an insignificant relationship between tea consumption and the risk of depression.
Ruusunen & et al	2010	Coffee, tea and caffeine intake and the risk of severe depression in middle-aged Finnish men: the Kuopio Ischemic Heart Disease Risk Factor Study	Middle-aged Finnish men	Cohort study	Categorization of individuals based on daily tea and coffee consumption into 4 groups	No association was found between tea consumption and the risk of developing depressive symptoms.	Coffee consumption may reduce the risk of depression, while no association was found for tea and caffeine consumption.
Chen & et al	2010	Exercise, Tea Consumption, and Depression Among Breast Cancer Survivors	Chinese women with breast cancer (stage 0-3)	Cohort (April 2002 to December 2006)	Assessment of tea consumption in the past 18 months through interviews	Regular tea consumption (100 grams of dried tea leaves per month) leads to a reduction in the occurrence of depression.	Regular tea consumption reduces the risk of developing depression.
Park & Moon	2015	Coffee and depression in Korea: the fifth Korean National Health and Nutrition Examination Survey	South Korean population (97-20 years old)	Cross-sectional, analytical	Assessment of daily coffee and tea consumption through a questionnaire	A weak association was found between green tea consumption and the risk of depression.	Green tea consumption has no significant effect on the occurrence of depression.

Author(s)	Year	Title	Target Group	Study Type	Intervention/Independent Variable	Key Findings	Final Conclusion
Dong & et al	2015	Tea consumption and the risk of depression: A meta-analysis of observational studies	Published articles from the beginning to August 2014	Meta-analysis	Investigation of the relationship between tea consumption and the occurrence of depressive symptoms	Consuming 3 or more cups of tea per day reduces the risk of depression.	Tea consumption leads to a decreased risk of developing depressive symptoms.
Guo & et al	2014	Sweetened Beverages, Coffee, and Tea and Depression Risk among Older US Adults	US adults aged 50 to 71 years	Prospective cohort study	Tea consumption assessed through the National Health and Nutrition Examination Survey	No association was found between tea consumption and the risk of depression.	Tea consumption does not influence the occurrence of depression.
Omagari & et al	2014	Coffee consumption is inversely associated with depressive status in Japanese patients with type 2 diabetes	Japanese patients with type 2 diabetes	Cross-sectional, analytical	Green tea, black tea, and coffee consumption assessed through a questionnaire	Coffee consumption was associated with a reduction in depression, but green tea and black tea consumption did not affect depression in patients with type 2 diabetes.	Green tea consumption did not have an effect on reducing depression.
Feng & et al	2013	Tea Consumption and Depressive Symptoms in Older People in Rural China	Older people in rural China	Cross-sectional, analytical	Tea consumption assessed through interviews	After adjusting for cardiovascular diseases, regular tea consumption was associated with a reduction in depressive symptoms in older people in rural areas.	Tea consumption reduces the risk of depression in older people.
Hintikka & et al	2005	Daily tea drinking is associated with a low level of depressive symptoms in the Finnish general population	Finnish general population	Cohort study	Daily tea consumption assessed through a questionnaire	Regular daily tea consumption is associated with a lower occurrence of depressive symptoms.	Tea consumption reduces the occurrence of depression.
Akinori Yaegashi & et al	2022	Green Tea Consumption and Risk of Depression Symptoms: A Systematic Review and Meta-Analysis of Observational Studies	-	Meta-analysis	Association between green tea consumption and the risk of depression symptoms	High consumption of green tea is inversely associated with depression symptoms.	Green tea consumption reduces the risk of depression symptoms.
Ratnani & Malik	2022	Therapeutic Properties of Green Tea: A Review	-	Review	Therapeutic properties and various compounds in green tea	Strong evidence suggests that daily consumption of green tea, due to its antioxidant properties, may be used as a preventive measure for various types of cancer and other diseases.	Green tea consumption reduces the risk of depression and various types of cancer.

Author(s)	Year	Title	Target Group	Study Type	Intervention/Independent Variable	Key Findings	Final Conclusion
Min Luo & et al	2021	Effects and Mechanisms of Tea on Parkinson's Disease, Alzheimer's Disease and Depression	Narrative review	-	Tea consumption and its effects on Parkinson's disease, Alzheimer's disease, and depression	Tea consumption had protective effects against Parkinson's disease, Alzheimer's disease, and depression.	Tea can be used for the prevention and treatment of Alzheimer's disease and depression.
Xinrong Dong & et al	2022	Green tea consumption and risk of depressive symptoms: Results from the TCLSIH Cohort Study	Participants aged 25 to 90 years	Cohort study	Green tea consumption and its association with depressive symptoms	During a follow-up period of 14,661 person-years (with an average follow-up of 2.0 years), 1,064 cases of depressive symptoms occurred.	The prospective study showed that regular green tea consumption is associated with a reduced risk of depressive symptoms in the general population of China.
Zhenyu Wan & et al	2023	Long-Term Consumption of Green Tea Can Reduce the Degree of Depression in Postmenopausal Women by Increasing Estradiol	Postmenopausal women	Case-control study	Long-term green tea consumption and its effects on inflammation, endocrine glands, and depression	Significant differences in insomnia, degree of depression, BMI, SII, and estradiol levels were found between the intervention and control groups.	Long-term green tea consumption can reduce the risk of depression in postmenopausal women by reducing inflammation and increasing estradiol levels.
Ke Shen et al	2019	Association between tea consumption and depressive symptoms among Chinese older adults	Chinese adults aged over 65 years	Longitudinal study	The impact of tea consumption on depressive symptoms	Regular and continuous tea consumption has an effect on depressive symptoms among older adults, with a greater effect reported in very old male adults compared to younger female adults.	Regular and continuous green tea consumption is an effective and cost-effective method for reducing depressive symptoms in older adults.
Yao Yao et al	2021	Type of tea consumption and depressive symptoms in Chinese older adults	Chinese adults aged 65 to over 100 years	Case-control study	The influence of different types of tea on depressive symptoms in older adults	Consuming 1 to 2 cups of green tea per day reduces the chance of developing depressive symptoms by 50%. Age, gender, and type of tea have an impact on depressive symptoms in older adults, and further clinical trials are needed.	-

Discussion

Depression has become a global pandemic in recent years. The elderly are more vulnerable to depression due to several reasons such as suffering from chronic diseases like diabetes, living alone, losing their loved ones or spouse, losing their independence in carrying out daily tasks, reduced social participation, and living in nursing homes. As a result, the prevalence of clinical symptoms of depression among the elderly in the community ranges from 8-15%, while it's about 30% for those living in nursing homes (12).

In order to control this pandemic, there are different ways of changing lifestyles, reducing environmental factors, identifying patients in the early stages and treating them using chemical drugs, psychological techniques and complementary medicine. Complementary medicine and the use of herbal medicines as part of it have a special place in modern nursing. Currently, nurses play a more supportive role than before and make double efforts to promote self-care behaviors. Complementary medicine can be one of the appropriate tools to play the supportive role of nurses due to its popularity among people and its non-invasive nature. The treatment of depression, as a chronic disease, certainly needs to improve the patient's self-care behaviors, and a comprehensive nurse should try in this direction by playing a supportive role. Tea is one of the popular drinks among different strata of people, which has a high consumption in Iran as well. It is cultivated as a strategic agricultural product in the north of the country and is available to everyone at a cheap price. There are reports on its favorable effects in reducing depression symptoms, which shows its protective role against depression. In the present study, the available evidence in this field was reviewed. And finally, 22 completely related articles that met the inclusion criteria were analyzed in this connection.

These articles included review, meta-analysis, cohort, cross-sectional, and case-control studies with different target groups. The terms tea and green tea were used in the title of this article. The findings of the studies are mainly in favor of the positive effect of drinking tea on reducing the risk of depression, so 67% of the 15 articles (10 articles) have confirmed the positive effect of tea on reducing the risk of depression. In this regard, the chance of depression in the elderly who consume more than 3 cups of tea per day is 66% lower than those who consume less than 3 cups of tea per day (P=0.001) (26). Esmaeilpour-Bandboni et al. also emphasized that regular consumption of green tea significantly reduces depression in the elderly (27). Li et al reported that drinking black tea reduces depression, but green tea has no effect (14). Farajzadeh et al found that people who drank tea had a 35% lower risk of depressive symptoms compared to those who did not drink tea (24). Chen et al also reported that regular consumption of tea (at the rate of 100 grams of dry tea leaves per month) reduces the incidence of depression (28). In this regard, the study of Dong et al showed that consuming 3 or more cups of tea per day reduces the risk of depression (23). The findings of the study by Feng et al in China also revealed that after adjusting for the effects of cardiovascular diseases, it was found that regular consumption of tea reduces depression in the elderly living in rural areas (29). Hintikka et al, one of the oldest studies in this field, confirmed the effects of regular daily consumption of tea on reducing the incidence of depression symptoms (30).

A research was conducted to investigate the potential of green tea in improving mood similar to depression, using an animal model in an experimental setting. The study's findings revealed that the stress-reducing effects of green tea were influenced by the ratio of caffeine to epigallocatechin gallate (CE/TA). When mice were administered green tea components with CE/TA ratios ranging from 2 to 8, it resulted in the suppression of depression-like behavior, adrenal hypertrophy, and brain inflammation. Furthermore, mice with a CE/TA ratio of 4 exhibited sustained expression of Npas4, a protein associated with reduced stress response in anxiety and depression. In clinical trials, green tea with CE/TA ratios of 3.9 and 4.7 demonstrated a decrease in susceptibility to subjective depression (31).

Research carried out in Japan examined the potential link between consuming green tea and experiencing depressive symptoms. The study involved 1987 employed individuals, of which 916 initially did not exhibit any depressive symptoms. To assess green tea

consumption, participants completed a reliable diet history questionnaire, while depression symptoms were evaluated using the CES-D scale. Multiple logistic regression was utilized to estimate the odds ratio of depressive symptoms based on the intake of green tea. The findings indicate that, contrary to previous cross-sectional and prospective studies suggesting an inverse association, there is no evidence of a connection between consuming green tea and experiencing depressive symptoms among the Japanese population (32).

A study was conducted to examine the impact of consuming green tea over an extended period on levels of inflammation, endocrine function, and depression among postmenopausal women. The research involved 386 postmenopausal women residing in a village known for tea production. The findings revealed a notable distinction between the two groups in relation to insomnia, depression, BMI (body mass index), SII (systemic immune-inflammation index), and estradiol levels. The consumption of green tea demonstrated the potential to decrease the risk of depression by mitigating inflammation and increasing estradiol levels. As a result, this study suggests that promoting green tea consumption as a healthy lifestyle habit is beneficial for postmenopausal women (33).

On the other hand, in the study of Grosso et al, a weak relationship between tea consumption and the risk of depression was observed (25). Ruusunen et al also did not observe a relationship between tea consumption and the risk of depression symptoms in their study (34). The findings of the study by Guo et al also did not show a relationship between tea consumption and the incidence of depression (35).

Concerning green tea consumption, Pham et al reported that people who drank 4 cups or more of green tea per day had 51% less depressive symptoms than those who drank less than 1 cup of green tea per day (P=0.01) (36). The study by Niu et al also revealed that the elderly who drink 4 or more cups of green tea per day show 56% fewer symptoms of depression than those who drink up to 1 cup of green tea per day. P = 0.001) (37). Kim et al also found that those who drink at least 3 cups of green tea per week had 21% less depressive symptoms than those who did not drink green tea at all (38). Another study observed a weak relationship between green tea consumption and the risk of depression. The findings of the study of Omagari et al also showed that coffee consumption is related to reducing depression, but green tea and black tea consumption does not affect depression in patients with type 2 diabetes (39). Today, along with human studies, veterinarians recommend green tea consumption to reduce depression symptoms in animals. In an animal model of depression that was created by administering lipopolysaccharides (LPS) in mice, it was observed that daily consumption of green tea products reduced the intensity of depression symptoms in the animal and gradually cured the depression. Some studies have examined the combined and comparative consumption of black tea, coffee, and green tea in the elderly and middle-aged population, the result of which was the positive and significant effect of green tea consumption in reducing depression symptoms, especially in the elderly population. The little contradiction that was observed in the results of some studies can be related to the type of tea preparation, amount, time, and volume consumed as well as the number of cups per day, so in one study the researchers believed that the method of tea preparation in the actualization of its properties is effective (26). The type of study, the target group, and the amount of tea consumed during the day have also been reported as reasons for the difference in the findings of some studies.

Conclusion

The results of this study confirm that drinking three or more cups of tea per day has beneficial effects, particularly in populations with high rates of depression, such as the elderly residing in boarding centers. It is recommended to prepare green tea using water at 80 degrees Celsius and to drink it half an hour after each meal, including breakfast, lunch, and dinner.

Conflict of interest

The authors declare no conflicts of interest for this research.

Authors contributions

MEB performed the analysis and wrote the paper, and **AZT**, **PR**, and **ZS** contributed to some parts of the manuscript and collected data.

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A histopathological study on breast carcinoma with special reference to cyclin-D1 and estrogen receptor

Payel Hazari [1], Monoj Kumar Deka [1], Bandana Kanoo [1]

[1] Department of Pathology, Silchar Medical College and Hospital, India

Abstract

Introduction: Breast cancer is the most frequent cause of cancer-related death in women in developing nations. Breast cancer diagnoses have increased as a result of rising awareness among women. The expression of Estrogen receptors (ER) plays a crucial role in determining the responsiveness to specific treatments. Cyclin D1 being a marker for cell proliferation was used in this study. The primary objectives of the current investigation were to investigate the expression of Cyclin-D1 and Estrogen receptor (ER) in breast carcinoma and to establish a relationship between the expression patterns of Cyclin-D1 and ER with the histopathological features of the tumor in breast carcinoma.

Materials and methods: The study was conducted in the Department of Pathology, Silchar Medical College and Hospital, Silchar, India, from June 2021 to May 2022. A total of 59 cases of primary breast carcinoma MRM(Modified radical mastectomy) specimens were included in the study.

Results: The mean age of the patients was 52.12 ± 12.47 years, and the majority of the patients were in the post-menopausal phase. Lymph node metastasis was observed in 47.5% of the cases, and the majority of the cases were in grade II. The study demonstrated a trend towards increased Cyclin-D1 and ER-positive with aging. Cyclin-D1 positivity decreases and Cyclin-D1 negativity increases as the tumor growth increases. The study showed a statistically significant association (P=0.001) between ER and Cyclin-D1. The majority of post-menopausal patients had ER-positive.

Conclusion: The present study provides the incidence of different parameters associated with breast carcinoma and their statistical correlation with CyclinD1 and ER that will provide improved and crucial treatment guidance.

Keywords: Breast carcinoma, Histopathological grades, Lymph node metastasis, Estrogen receptor (ER), Cyclin-D1, Menopausal status

Corresponding Authors: Payel Hazari

✉ Email: hazari.payel2@gmail.com

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Introduction

Breast carcinoma accounts for 1 in 4 cancer diagnoses among women worldwide. Breast cancer, which accounts for an anticipated 2,261,419 cases (11.7% of all cancer sites) each year (2020) and 684,996 fatalities (6% of all cancer-related deaths), is the most common worldwide (2020). Breast cancer is the most frequent cause of cancer-related death in women in developing nations. In India, 178 361 (26.3%) new cases were found among the female population in 2020 (1). Breast cancer diagnoses have increased as a result of rising awareness among women.

Breast cancer has a high rate of survival when detected early and when there is access to effective therapy. Unfortunately, 50–80% of these illnesses are found at an advanced stage in the majority of low- and middle-income countries (2). A more sensitive assessment of a palpable breast lump has recently been employed with the help of the Triple Test approach, which consists of a clinical examination, mammography, and fine needle aspiration cytology (3). Early diagnoses of aggressive tumors (ER-ve, PR-ve, HER2/neu +ve, or triple-negative tumors) result from increased awareness campaigns (4, 5, 6).

As per the latest treatment guidelines for breast cancer (7), the expression of estrogen receptors (ER) plays a crucial role in determining the responsiveness to specific treatments. The ER expressions are critical in determining how well hormonal therapy will function (8).

Histopathologists commonly assess tumor proliferation activity, which provides data on the clinical behavior, diagnosis, and treatment of tumors (9). Cyclins bind to and activate Cyclin-Dependent Kinases (CDK), regulating the rate at which cells transition between different cell cycle phases. In this study, cyclin D1 was used as a marker for cell proliferation.

Cyclin D1 activates steroid hormone receptor-mediated transcription in the absence of estrogen hormone and enhances transcription in its presence. The anti-estrogens did not inhibit the activation of estrogen receptors by Cyclin D1.There is an increase in binding of the receptor to estrogen response element sequence that upregulates ER-mediated transcription owing to the direct binding of Cyclin-D1 to the hormone binding domain of ER. These results highlight a unique role for Cyclin D1 as a CDK-independent matter of the ER (10).

This study aims to investigate various parameters like age, laterality, menopausal status, tumour size, lymph node and the expression of ER and Cyclin-D1 in breast cancer and to establish a relationship between the expression patterns of Cyclin-D1 and ER with the histopathological features of the tumor in breast carcinoma. This will provide improved and crucial treatment guidance for breast cancer patients.

Materials and Methods

The present study was undertaken to study the clinic-pathological findings in breast carcinoma and to assess the expression of Cyclin-D1 and ER in them.

Place of study

The present study was undertaken in the Department of Pathology, Silchar Medical College and Hospital, Silchar. The study was approved by the Institute's Ethics Committee (No. SMC/15,222) dated 20/10/2022. According to the Helsinki Declaration's ethical guidelines, the study is compliant.

Study period

1 year: From June 2021 to May 2022.

Type of Study

Hospital-based prospective cross-sectional study.

Source of data and sample size

59 cases of primary breast carcinoma MRM specimens submitted to the Department of Pathology, Silchar Medical College and Hospital, Silchar, for histopathological examination (Figure 1). Immunohistochemistry with CyclinD1 and ER antibody was done on these specimens as per IHC protocol.

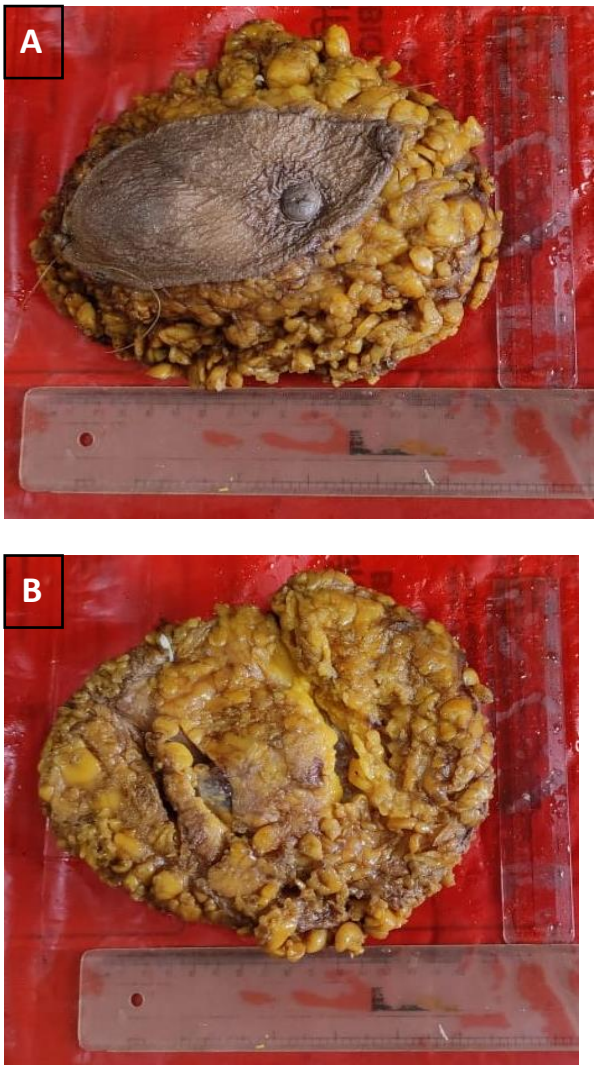


Figure 1. Gross pictures an MRM specimen (A is anterior view; B is posterior view).

Inclusion criteria

In the study, patients with invasive duct carcinoma, no special type (IDC, NST) as histopathological diagnoses were included.

Exclusion criteria

- All metastatic carcinoma of breasts.

- Male breast carcinomas

Parameters studies

I. Detailed clinical history is taken and all routine investigations are done after obtaining consent from the patients.

II. Hospital records of the patients.

III. Microscopic examination of the tissues.

IV. Immunohistochemistry on paraffin embedded tissue of histopathologically diagnosed cases.

The current study was conducted prospectively at a hospital in Silchar, India, in the Department of Pathology during a year, from 2021 to 2022. 59 biopsy/resection specimens for primary breast carcinoma were submitted in total. All regular investigations are carried out after obtaining the patients' agreement and a thorough clinical history is gathered. These specimens were first stained with H&E before being subjected to immunohistochemistry using CyclinD1 and ER antibodies by the IHC methodology.

Preparation of slides: Paraffin sections were cut and mounted on saline coated slides. The slides were heated at 65℃ to remove the paraffin and then immerse in xylene. After rehydration of the tissues, the slides were cleaned with distilled water. Subsequently, the slides were washed with Tris buffer and submerged in a 3% peroxide solution for three minutes to remove endogenous peroxidase activity.

Antigen detection and antigen retrieval: Heat retrieval was performed using a decloaking chamber with citrate buffer at 95℃ for 40 minutes. The slides were then transferred to Tris-Saline buffer to cool to room temperature. To prevent non-specific immunostaining , the tissue sections were treated with 1% mouse serum. Primary antibodies, including Rabbit monoclonal antibody QR022 for CyclinD1 and Rabbit monoclonal antibody QR013 for ER were applied to the sections approximately one hour before removal.

Secondary detection of the primary antibody: After 10 minutes of incubation with biotinylated mouse anti-species antibody, sections were washed in Tris buffer. The slides were then treated with a solution of chromogen 3,3'- diaminobenzidine (DAB) at a concentration of 1mg/mL in Tris buffer containing 0.016% fresh H2O2. Tap water was used to clean the DAB from the slides.

Counterstaining: Slides were immersed in a solution of hematoxylin diluted 1:1 with distilled water for

counterstaining. After counterstaining, the slides were cleaned in distilled water and dehydrated by dipping them in ethanol. Then a coverslip was used to view and report after cleaning in xylene (Figure 2).

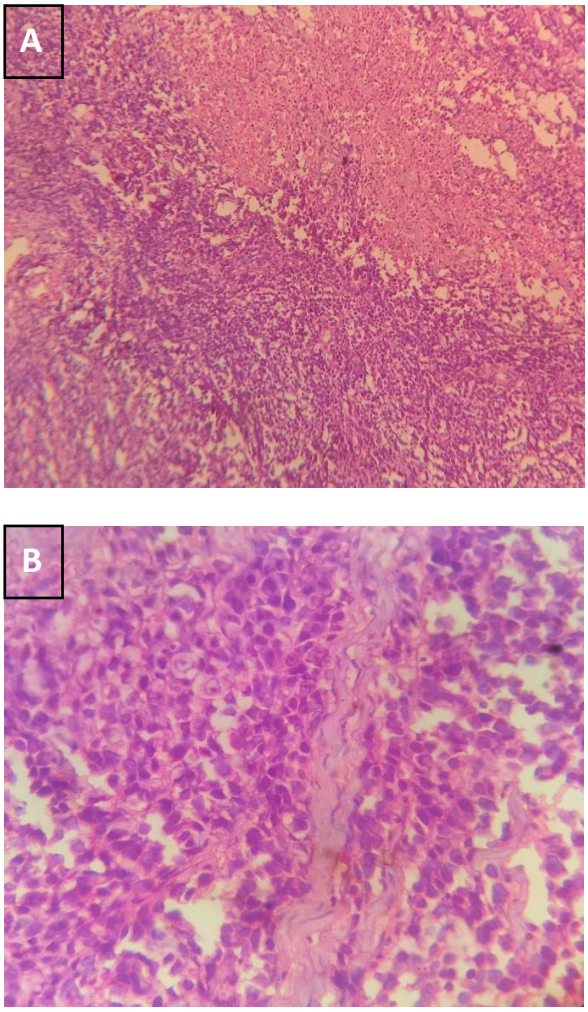


Figure 2. H&E pictures of IDC, NST (A: 10X and B: 40X).

Reporting of CyclinD1 immuno-histochemical study

A semi-quantitative scoring is used by the Allred score method for the nuclear staining (11) as

- 0: negative (no staining of any nuclei even at high magnification)
- 1: weak (only visible at high magnification)
- 2:moderate (readily visible at low magnification)
- 3: strong (strikingly positive even at low power magnification).

Additionally noted was the percentage of tumor nuclei that stained positively as:

0- none, **1**- <1/100, **2**- 1/100 to 1/10, **3**- 1/10 to 1/3, **4**- 1/3 to 2/3 and

5- >2/3.

After that, the intensity scores and proportion were combined to get a final score that varied from 0 to 8 (11).

Tumors were then categorized as:

- Negative/weak expression (total scores 0–2)
- Intermediate expression (total scores 3–5)
- Strong expression (total scores 6–8)

In this study, Intermediate and Strong positives were considered together as positive.

Reporting of ER immunohistochemical study

Strong brown to black nuclear staining was considered when assessing immune positivity for ER. Positive nuclei were expressed as the percentage of total nuclei counted.

Criteria for evaluating ER (12)

- **Negative for ER**: If, 1% or 0% of tumor cell nuclei are immunoreactive.
- **ER Low Positive**: If 1%-10% of tumor cell nuclei are immunoreactive.
- **Positive for ER**: 1%-100% of tumor nuclei are immunoreactive.

Statistical analysis

IBM SPSS software version 21.0 was used for data analysis. Qualitative data was presented as frequency and percentage, while quantitative data was presented as mean (±SD). The chi-square test was used to identify significant associations. A p-value of <0.05 was regarded as statistically significant.

Results

In our study, various clinicopathological parameters are analyzed and are presented as under.

The mean age of the patients having breast carcinoma was 52.12 ± 12.47 years and the majority of the patients belonged to 41 to 50 years of age (32.2%). This was followed by 28.8% and 18.6% cases belonging to the age range of 51 to 60 years and ≤40 years of age respectively (Table 1).

Table 1. Distribution according to age.

Age (in years)	Frequency (n = 59)	Percentage (%)
≤40	11	18.6
41 – 50	19	32.2
51 – 60	17	28.8
61 – 70	07	11.9
>70	05	8.5
Mean	52.12 ± 12.47	

In the present study, right-side predominance was observed for breast carcinoma. 52.5% of patients had carcinoma breast on the right breast while 47.5 % were over the left breast. (Table 2).

Table 2. Distribution according to laterality of breast carcinoma.

Laterality	Frequency (n = 59)	Percentage (%)
Left	28	47.5
Right	31	52.5

In our study, 33.9% of patients were in a pre-menopausal state and a majority of the cases 66.1% were in the post-menopausal phase. We considered menopause where no menstruation was reported over the last 12 months. (Table 3).

Table 3. Distribution according to menopausal status.

Menopausal status	Frequency (n=59)	Percentage (%)
Pre-menopausal	20	33.9
Post –menopausal	39	66.1

Most commonly affected (40.7%) cases of breast carcinoma patients had tumour of size 2-5cm. This was followed by ≤2cm tumour size in 30.5% and >5cm in 28.8% cases respectively (Table 4).

Table 4. Distribution according to tumour size.

Size of tumour	Frequency (n = 59)	Percentage (%)
≤2cm	18	30.5
2-5cm	24	40.7
>5cm	17	28.8

In our study, lymph node metastasis was observed in 47.5% of cases whereas, in 52.5% of cases, no lymph node metastasis was documented (Table 5) (Figure 3).

Table 5. Distribution according to lymph node metastasis of Breast carcinoma.

Metastasis	Frequency (n = 59)	Percentage (%)
Present	28	47.5
Absent	31	52.5

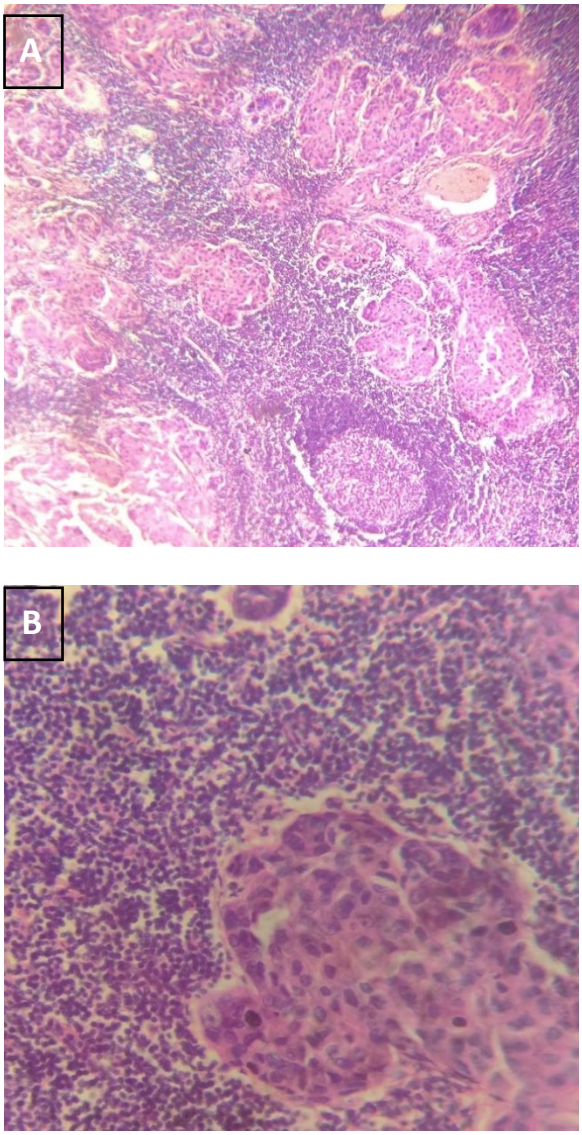


Figure 3. H&E picture of metastatic lymph node (A: 10X and B: 40X).

The Nottingham (Elston-Ellis) modification of the Scarff-Bloom-Richardson grading system also called as the Nottingham Grading System is applied for the above grading. The majority of cases 61% were found to be in grade II, this was followed by 30.5% and 8.5% in grade III and I respectively (Table 6).

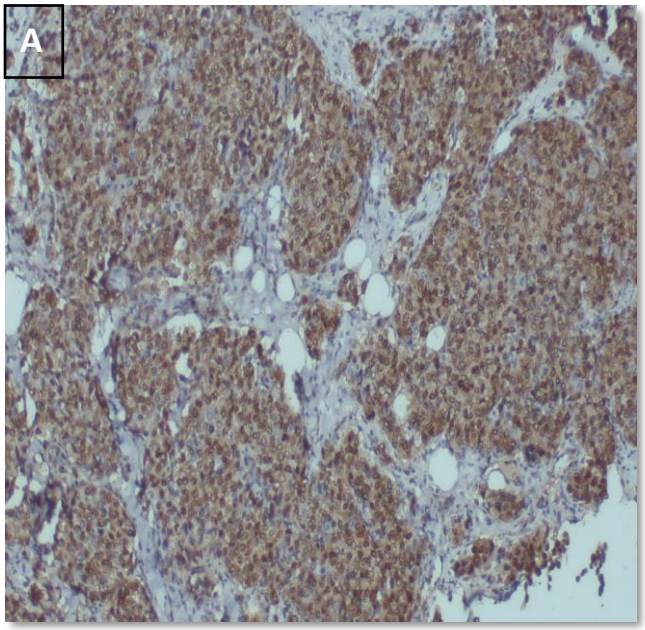
Table 6. Distribution according to the histological grades of the tumours.

Tumour grade	Frequency (n=59)	(%)
I	5	8.5
II	36	61
III	18	30.5

Tumour cells with >10% nuclear staining were regarded as positive and <10% or weak staining as negative. In this study, we found 36 out of 59 cases (61%) showed CyclinD1 positive expression whereas 23 cases (39%) cases had negative CyclinD1 expression (Table 7) (Figure 4).

Table 7. Distribution according to the expression of CyclinD1 in breast carcinoma.

CyclinD1 expression	Frequency (n = 59)	Percentage (%)
Positive	36	61
Negative	23	39



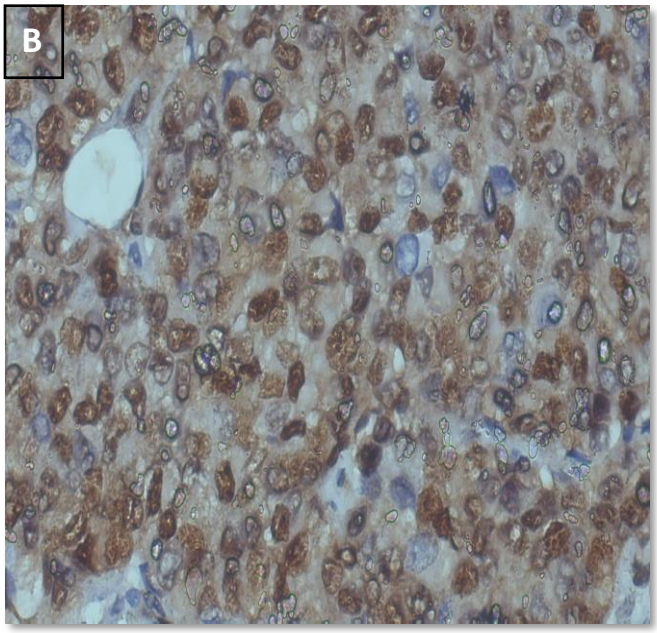


Figure 4. CyclinD1 positive in a case of IDC, NST (A: 10X, B: 40X).

For calculating the statistical significance, we grouped 59 cases into 2 categories based on their age as ≤40 years and >40 years age. The majority of patients, 48 out of 59(81.4%) are over 40 years of age and 11 (18.6%) are ≤40 years of age. 94.4% of patients >40 years show positive CyclinD1 expression and 60.9% of cases are negative for CyclinD1. However, 5.6% of cases of ≤40 years of age show positive CyclinD1 expression. The test of significance (chi-square test) showed a statistically significant association between age and CyclinD1 expression in the present study ($\chi 2$ = 8.334, P-value=0.0039) (Table 8).

Table 8. Association between age and CyclinD1 expression.

Age (in years)	Total cases (n=59)	CyclinD1 positive (n=36)	CyclinD1 negative (n=23)	P-value
≤40	11 (18.6%)	2(5.6%)	9(39.1%)	
41-50	19 (32.3%)	14 (38.9%)	5 (21.7%)	0.0039
51-60	17 (28.8%)	10(27.8%)	7 (30.4%)	
61-70	07 (11.9%)	06(16.6%)	01(4.4%)	
>70	05 (8.5%)	04(11.1%)	01 (4.4%)	

For calculating the p-value, we grouped the tumour size into 2 categories: ≤2 (18 cases) and >2cm (41 cases). CyclinD1 expression was seen in 47.2% of tumours with ≤2cm tumour size and 52.8%tumours with size >2cm. The difference was statistically significant ($\chi 2$ = 10.230, P-value=0.014). Also, the majority (65.2%) of CyclinD1 negative tumours have a size >5cm, followed by 2-5cm and ≤2cm with 30.4% and 4.4% respectively. This shows that with an increase in tumour size there is an increase in Cyclin-D1 negativity (Table 9).

Table 9. Association between tumour size and CyclinD1 expression.

Tumour size (in cm)	Total cases (n=59)	CyclinD1 positive (n= 36)	CyclinD1 negative (n=23)	P-value
≤2	18 (30.5%)	17 (47.2%)	01 (4.4%)	
2-5	24 (40.7%)	17 (47.2%)	07 (30.4%)	0.014
>5	17 (28.8%)	02 (5.6%)	15 (65.2%)	

In this study, from 36 overexpressed Cyclin-D1 cases, the majority of cases 80% are in grade I. This is followed by grade II and grade III with 69.4% and 38.9% cases respectively. For calculating the statistical significance (p-value) of this correlation, we grouped grade I and II as intermediate grade and grade III alone as high grade. This correlation was found to be statistically significant (p-value=0.0435). This implies Cyclin-D1 nuclear positivity is associated with lower tumour histological grade (Table 10).

In this study, from 40 ER-positive cases, the majority of cases 80% are in grade I. This is followed by grade II and grade III with 69.4% and 61.1% cases. For calculating the statistical significance (p-value) of this

correlation, we grouped grade I and II together as intermediate grade and grade III alone as high grade. This correlation was found to be statistically insignificant (p-value>0.05) (Table 10).

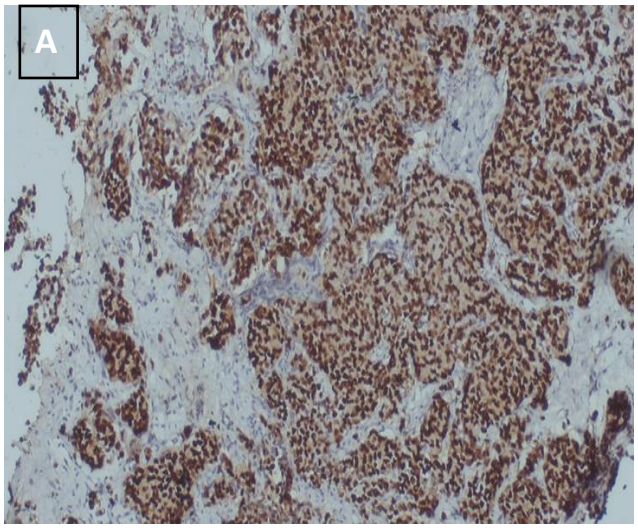
Table 10. Correlation between Cyclin-D1, ER expression and histological grade of tumors.

Grade	n	Cyclin-D1 positive	Cyclin-D1 negative	P-value	ER positive	ER negative	P-value
I	5	4(80%)	1 (20%)		4 (80%)	1(20%)	
II	36	25(69.4%)	11 (30.6%)	0.0435	25 (69.4%)	11(30.6%)	>0.05
III	18	7(38.9%)	11 (61.1%)		11 (61.1%)	7(38.9%)	

In the present study, the majority of breast carcinoma patients 40 out of 59 (67.8%) had positive ER expression whereas ER-negative expression was observed in 32.2% of cases (Table 11) (Figure 5).

Table 11. Distribution according to the expression of ER in breast carcinoma.

ER expression	Frequency (n=59)	Percentage (%)
Positive	40	67.8
Negative	19	32.2



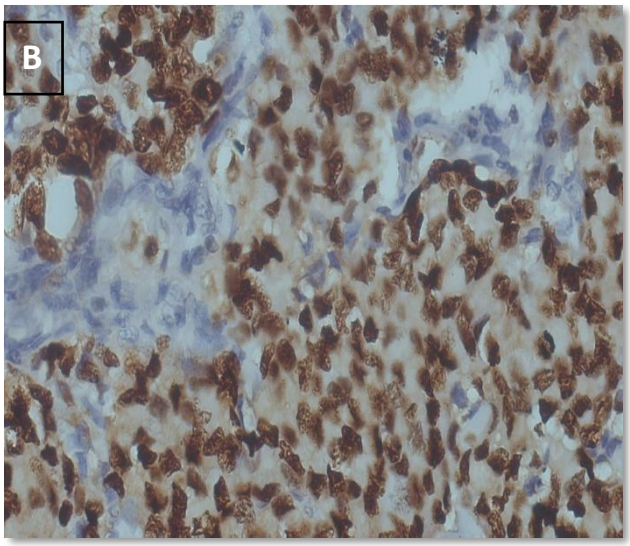


Figure 5. ER positive in a case of IDC, NST (A: 10X, B: 40x).

In this study, 40 ER-positive cases were found and expressed CyclinD1 in 100% of cases and 17.4% showed no expression for CyclinD1. Of 19 ER-negative cases, 82.6% were negative for CyclinD1. The association between CyclinD1 and ER expression was observed to be statistically significant ($\chi 2$ =40.163, P value= 0.001) (Table 12).

Table 12. Association between CyclinD1 and ER expression in breast carcinoma.

ER-expression	Frequency (n=59)	CyclinD1 positive (n=36)	CyclinD1 negative (n=23)	P-value
Positive	40(67.8%)	36 (100%)	04 (17.4%)	0.001
Negative	19(32.2%)	0(0%)	19 (82.6%)	

Among 20 pre-menopausal women, 15% expressed ER positivity while 73.7% of cases were ER-negative. Of 39 post-menopausal women, 85% showed ER positivity and 26.3% were ER negative. The difference was statistically significant (P-value< 0.05). This implies that there is increased ER expression in post-menopausal breast carcinoma patients (Table 13).

Table 13. Association between menopausal status and ER expression.

Menopausal status	Frequency (n=59)	ER-positive (n=40)	ER-negative (n=19)	P-value
Pre-menopausal	20(33.9%)	6(15%)	14(73.7%)	< 0.05
Post - menopausal	39(66.1%)	34(85%)	5(26.3%)	

Out of 11 cases of ≤40 years of age, ER expression was observed in 05% of cases. Of 48 cases in the>40 years age group, 95% showed positive ER expression. The difference was found to be statistically significant ($\chi 2$ = 4.477, P-value=0.0004). This implies that ER-positive expressions were more common in >40 years old patients with breast carcinoma (Table 14).

Table 14. Association between age and ER expression.

Age (in years)	Frequency (n=59)	ER-positive (n=40)	ER-negative (n=19)	P-value=
≤40	11 (18.6%)	2 (05%)	9 (47.4%)	0.0004
>40	48 (81.4%)	38 (95%)	10 (52.6%)	

Discussion

The present study includes 59 patients presenting to our institute with a breast lump that underwent biopsy and were diagnosed as intra-ductal carcinoma on histopathological examination from August 2021 to July 2022. The incidence was assessed with age, menopausal state, and laterality by carefully examining the patient profiles. A thorough investigation of Cyclin-D1 and ER expression was done using immunohistochemistry.

Among the 59 cases of breast carcinomas, the maximum cases (32.2%) were in the age group 41-50 years followed by 28.8%, and 18.6% cases from the age group 51-60 years and <40 years respectively. This is followed by the age group 61-70 years and >70 years respectively with each 11.9% and 8.5%. This is in concordance with Lengare PV et al. (2020) (13) where the maximum number of patients 38% lie in the age group 41-50 years.

Most of the patients in our study were in the 5th decade with a mean age of 52.12±12.47 years. This is in concordance with the study done by Servet K. et al (2019)(14), Lengare PV et al. (2020) (13) and Mohammadizadeh F et al. (2013) (15).

Among the 59 cases of breast carcinoma, 52.5% cases were reported from the right breast and 47.5% cases from the left breast.

In this study, majority of the cases (66.1%) were post-menopausal which is similar to studies conducted by Mazor M et al. (2018) (16), Singh R et al (2014) (17) and Roy et al (2010) (18) with 62.6%, 64% and 64% respectively.

This study showed that majority (40.7%) of tumour size were more than 2-5cm followed by ≤2cm tumour with 30.5% and >5cm tumour with 28.8%. This study was found in concordance with Servet K et al (2019)

(19), Ortiz AB et al (2017) (20), Bilalović N et al (2005) (21), Lee A et al (2007) (22) and Li Z et al(2016) (23).

As per this study, 47.5% of cases showed lymph node metastasis in breast carcinoma. Other significant studies where nodal metastasis were noted in breast carcinoma cases were Lengare PV et al (2020) (13) with 68% cases, Roy et al (2010) (18) with 51.5% cases, Ortiz AB et al (2017) (20) with 48% cases, Lee A et al (2007) (22) with 48.9% cases, Azizun-Nisa et al (2008) (24) with 71.3% cases, Peurala E et al (2013)(25) with 41.2% cases, Boström P et al(2009)(26) with 52.8% cases, Khabaz MN. (2014) (27) with 53.5% cases.

In the present study, Cyclin-D1 immune expression was positive in 36 out of 59 cases (61%) and negative in 23 cases (39%). Similar studies showing Cyclin-D1positivity are Mohammadizadeh F et al (2013) (15) with 78.6%, Reis-Filho JS et al (2006) (28) with 67.4%, Ortiz AB et al (2017)(20) with 52%, Lengare PV et al(2020) (13) with 64% , Peurala E et al. (2013) (25) with 60%, Siraj AK et al. (2021) (29) with 59.4%, Roy P. G et al. (2010) (18) with 63.4% and Lee A et al (2007) (22) with 63.9%.

In our study, it is found that with an increase in the age of patients, there is also an increase in the expression of CyclinD1 in breast carcinoma which is in concordance with Li Z et al (2016) (23) that showed 86.4% of CyclinD1 positive cases were ≥35 years and 13.6% were <35 years and Siraj AK et al (2021) (29) with 61.2% >50 years and 58.6% ≤50 years breast cancer patient expressing CyclinD1.

In the present study, out of 59 cases of breast carcinoma, ER was positive in 40 cases (67.8%) and negative in 19 cases (32.2%). This is in concordance with Roy et al (2010) (18) with ER positivity of 76.8%, Siraj AK et al (2021)(29) with 65.6%, Peurala E et al (2013)(25) with 76.5%, Mostafa M et al (2010) (30)with 69%, Singh R et al (2014) (17) with 44.6%, Bilalović N et al (2005) (21) with 79%, Lee A et al (2007)(22) with 64%, Mohammadizadeh F et al (2013)(15) with 60.7% and Lengare PV et al(2020) (13) with 56%.

In this study, 90% cases show ER-positivity in Cyclin-D1-positive breast carcinoma. Similar findings were found in studies conducted by Lee A et al (2007) (22) with 77.8% of Cyclin-D1 positive cases showing ER-positivity, Elsheikh, et al (2008) (31) with 54.6%, Lengare PV et al(2020) (13) with 54%, Li Z et al (2016) (23) with 81.2% , Roy et al (2010) (18) with 69.7%, Peurala E et al (2013) (25) with 96.6%, Reis-Filho JS et al (2006) (28) with 76.3% and Siraj AK et al. (2021) (29) with 72.6%.

85% of post-menopausal women show ER-positivity in breast carcinoma in our studies. This is in concordance with Singh R et al (2014) (17) with 48.4%, Md. Oliul Islam et al (2022) (32) with 57.1%.

In our study, ≤40 years women showed 05% ER positivity and >40 years patients showed 95% ER positivity which is statistically significant. This implies that there is a trend of higher ER positivity in older breast carcinoma patients (<40 years) than in younger (≤40 years).

This study is in concordance with Singh R et al (2014) (17) and Mostafa M et al (2010) (30) where among ER-positive cases, the maximum breast carcinoma cases were of the older age group (>50 years) with 50.9% and 69.1% respectively. Also, Aysha S. AlZaman et al (2016) (33) showed similar findings with ER-positive breast carcinoma having 72.6% of >40 years patients.

Conclusion

Regardless of regional variances, carcinoma of breast is the most frequent cancer in women. Among other things, the incidence of intra-ductal breast cancer is still very high.

Recent years have seen an increase in the early diagnosis of breast carcinoma due to increased public awareness of breast cancer and breast self-examination. This should be backed up by an immune-histochemical analysis of the numerous hormone receptors to pinpoint the cases that may respond well to hormonal therapy, extending the patients' disease-free survival.

In the current study, we found that the majority of breast cancer cases were seen in the fifth decade, then the sixth decade, and that the majority of patients were post-menopausal, with the right breast predominating.

In this study, the majority of cases were in grade II, followed by grade III and grade I.

The majority of the patients were positive for immune-staining for CyclinD1 and ER and were found to be associated with low histological grades.

This study demonstrated a trend towards increased CyclinD1 and ER-positive with aging.

We discovered that Cyclin-D1 positivity decreases and Cyclin-D1 negativity increases as the tumor growth increases.

The majority of post-menopausal patients had ER-positive, highlighting the fact that radiation and chemotherapy with anti-estrogens (such as Tamoxifen) may be beneficial in such instances.

Since CyclinD1 and ER-positive cases had better treatment outcomes than those negative tumors, they are now indicated as clinical prognostic markers for IDS, NST patients.

Limitations

The small number of cases due to SARS-COV-2 infections and the absence of post-operative information for the cases we analyzed.

Conflict of interests

The authors declare that they have no competing interests.

Authors contributions

All the authors have contributed equally and read and approved the final draft of the manuscript.

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