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Research Article

Quantitative Analysis of p53 Expression as Prognostic Indicator in Breast Pathology: Correlation with Clinical Parameters

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Abstract

Background:

Breast cancer continues to be significant global health concern, necessitating comprehensive investigation into novel diagnostic and prognostic markers to enhance patient's management. The tumor suppressor protein p53 has emerged as a central player due to its critical role in maintaining genomic stability and regulating cell cycle checkpoints [10]. Mutation in p53 gene encoding have been extensively associated with various cancers including breast cancer, leading to altered protein expression and function. Furthermore, given the pivotal role of p53 in cellular responses to stress and its potential as a therapeutic target.

***Objectives**:*

To quantify and characterize p53 expression levels in breast cancer samples using IHC analysis and to correlate p53 expression with histopathological features.

***Material and Methods**:*

Research utilized a cross-sectional and descriptive approach to investigate the expression of P53 in patients with histopathological confirmed malignant breast neoplasms. The study employed rigorous diagnostic confirmation, immunohistochemistry, and statistical analysis using SPSS Version 23, with the Chi-Square test used to establish the significance of associations at a significance level of $P \leq 0.05$. The study's sample size comprised 329 patients, chosen through non-probability convenient sampling. The research was conducted within the Department of Pathology at IVY hospital Monali.

Results:

The research focused on uncovering potential associations between p53 expression and various clinicopathological factors.

Regarding age distribution, a significant relationship was found (Chi-square = 1.247) among different age groups (20-40, 41-60, 61-80) and the expression of p53. However, marital status showed no significant correlation (Chi-square = 0.444) with p53 expression, with most patients being married.

When considering the laterality of the breast tumors, the analysis revealed no substantial link (Chi-square = 0.060) between tumor location (right or left breast) and p53 expression. In terms of specimen type, a notable association (Chi-square = 4.62) emerged, highlighting potential connections between the method of tissue sampling (mastectomy, trucut, etc.) and p53 expression levels. The investigation of histological factors presented intricate associations. These included the histological type of the tumor (invasive ductal carcinoma, invasive lobular carcinoma, metaplastic carcinoma, mucinous carcinoma), tumor grade (grade 1, grade 2, grade 3), presence of DCIS (ductal carcinoma in situ), and p53 expression. Notably, p53 expression was strongly associated with the intensity of staining (negative, weak, moderate, strong).

Overall, these findings shed light on the potential interactions between p53 expression and various clinicopathological characteristics in breast cancer. The study underscores the significance of p53 as a potential biomarker and suggests avenues for further research to elucidate the clinical implications of these associations.

Conclusion

This cross-sectional study reveals significant associations between p53 expression in malignant breast neoplasms and various clinicopathological factors, highlighting its potential as a diagnostic biomarker. The findings emphasize the need for further research to unravel underlying mechanisms and validate results across diverse patient cohorts. While acknowledging limitations such as sampling technique and methodology, this study lays the foundation for future investigations utilizing advanced molecular approaches to enhance breast cancer understanding, diagnostics, and personalized treatments.

Key Words: *Breast cancer, immunohistochemistry, p53 expression.*

Introduction

Breast cancer is the most common type of cancer in the world, with women being the primary sufferers. The aforementioned kind is a sort of non-cutaneous malignancy that accounts for around one-third of all diagnosed malignancies in the United States. It is also the second leading cause of cancer-related deaths worldwide **[**1,2**]**. Breast cancer is diagnosed in around 6.6 percent of women under the age of 40, 2.4 percent in those under 35, and 0.65 percent in those under 30 **[**3,4**]**. The number of cases directly connected to breast cancer doubled globally. Two reasons are likely to have influenced this: rising life expectancy and a shift toward a contemporary westernized lifestyle with its associated risk factors **[**5**]**. However, in the previous 20 years, these patterns have not been constant with regard to early onset breast cancer, since rates have fluctuated in most countries, making it difficult to establish a steady pattern **[**6**]**. In terms of death rates, they have exhibited a constant downward trend, notably in young women, probably due to enhanced treatment strategies and early diagnosis procedures **[**7**]**. On the other hand, diagnosing breast cancer in young women is currently a real test for the patients, their families, and the health-care providers involved. Despite the fact that breast cancer is seldom identified in women under the age of 40, it has a significant impact in the adult community, because it manifests itself at a later stage (diagnostic), it becomes more aggressive and hence has a poor prognosis **[**8,9**]**.

The p53 gene encompasses 16 to 20 kb of DNA on the short arm of human chromosome 17. It encodes for a 393-amino acid nuclear phosphoprotein involved in cell-cycle control. Loss of normal p53 function is associated with cell transformation in vitro and development of neoplasms in vivo. **[**10**]**.

The most common change of p53 in human cancers is a point mutation within the coding sequences of the p53 gene, which gives rise to an altered protein. **[**11,12**]**.

This pathway is currently regarded as the most frequent genetic change in a variety of human cancers. Mutations of the p53 gene are found in all major histogenesis groups. Approximately one half of adult cancers of the colon, stomach, lung, esophagus, breast, liver, brain, reticuloendothelial tissues, and hematopoietic tissues contain the mutant p53 gene. **[**12,13,14**]**.

40 Loss-of-function mutations in the TP53 (P53) gene have been found in numerous cancer types including osteosarcomas, leukemia, brain tumors, adrenocortical carcinomas, and breast cancers **[**15**]**. P53 protein is essential for normal cellular homeostasis and genome maintenance by mediating cellular stress responses including cell cycle arrest, apoptosis, DNA repair, and cellular senescence **[**15,16**]**.

Mutant p53 also targets other regulatory molecules including microRNAs such as miR-130b, miR-155 and miR-205. p53 binding to microRNAs has been associated with not only altering the stability of those molecules, but also influencing crucial molecular pathways involved in invasion and metastasis through the modulation of transcripts such as ZEB1 and ZNF652**[**16,17**]**.

Inactivation of normal p53 is associated with uncontrolled cell growth and cancer development. Thus, it might be possible to design drugs that block cancer cell division by mimicking the inhibitory effects of the p53-induced protein on the cell-cycle enzymes **[**17,18**]**.

Potential of p53 signaling targeting for cancer therapy Because wild-type p53 is an efficient promoter of apoptosis and senescence **[**18**]** in tumor cells, reactivating wildtype characteristics of p53 mutants, which are commonly overexpressed in cancer, is a viable therapeutic strategy.

Several studies have shown that transfection of cancer cells with wild-type p53 expressing plasmids can induce apoptosis and/or growth arrest, implying that a gene therapy method for cancer treatment could be based on restoring normal p53 expression and function. Several clinical research investigations using viral and non-viral vectors delivering p53 genes, alone or combined with other therapeutic agents, have been completed to far **[**19**]**.

Material and Methods

This research is conducted within the Department of Pathology and Surgical Oncology at IVY hospital Mohali. The study duration was one year. The study design is cross-sectional and descriptive, the sample size is determined using open epi software calculator, with 329 participants based on a reference prevalence of 31%/. A 95% confidence interval, and a 5% margin of error. The sampling technique is purposive (non-probability). Patients of the study included all diagnosed cases with invasive ductal carcinoma according to the WHO classification and standards were diagnosed following the breast surgery and those who did not receive neoadjuvant treatment.

Whereas Biopsy specimens with autolytic changes, the inappropriate paraffin tissue blocks for immunohistochemical staining Biopsy specimen size of less than 4mm were excluded from this study. Following ethical committee approval, demographic parameters were recorded after informed & written consent was taken from patients or next of kin who were meet the inclusion criteria The histopathological examination process involves meticulous steps, including cross-referencing patient details, gross

examination of specimens, tissue processing, sectioning, Hematoxylin and Eosin stained slides were viewed under microscope starting from low magnification as 4x or 10x and to get an overview of the tissue sample and identify areas of interest. Then, moved to higher magnification (e.g., 20x, 40x, or 100x) to assess cellular details as type of breast carcinoma as per WHO criteria. Histologic grading and staging were done for more accurate diagnosis and representative areas were marked for immunohistochemistry Abcam Rabbit polyclonal to p53 (ab131442), which is localized in the nucleus and cells with a distribution of pale yellow to brownish yellow were considered positive under light microscope, was observed under >5 high power fields.an observation of >10% of positive cells was considered p53 positive and intensity with 0 as negative, 1 as weak , 2 as medium and 3 as strong accordingly .

Data Analysis

Statistical package for social sciences for windows (SPSS) V: 26.0 was used for data analysis. Qualitative tests were carried out to determine the frequency of Histological group and type, specimen type, specimen integrity, tumor grade, stage and positive or negative expressions of p53. The Chi square test was applied to determine the association of expression of p53 with clinicopathological parameter. p value equals 0.05 was considered significant.

Results

Positive p53 Expression 142 (43.0%)			**Negative p53 Expression 187(56.7%)**			
Variable	Category	Frequency	Percent	Positive p53	Negative p53	Chi-square value
Age of the patient	20 to 40	142	43.0	63	79	1.247
	41 to 60	132	40.0	59	73	
	61 to 80	55	16.7	20	35	
Marital status of the patient	married	307	93.0	134	173	.444
	un married	22	6.7	8	14	
Laterality of the breast	right	174	52.7	74	100	.060
	left	155	47.0	68	87	

Specimen type	mastectomy	130	39.4	55	75	4.62
	trucut	100	30.3	47	53	
	excisional	50	15.2	22	28	
	incisional	15	4.5	3	12	
	wedge	4	1.2	1	3	
	lumpectomy	30	9.1	14	16	
Histological type of Tumor	invasive ductal ca	314	95.2	135	179	1.34
	invasive lobular ca	9	2.7	4	5	
	metaplastic ca	5	1.5	3	2	
	mucinous ca	1	0.3	0	1	
Histological grade of the Tumor	grade 1	2	0.6	0	2	7.28
	grade 2	163	49.4	66	97	
	grade 3	157	47.6	70	87	
	poorly differentiated carcinoma	7	2.1	6	1	
Size of tumor	<2cm	140	42.4	61	79	.03
	2 to 5 cm	118	35.8	51	67	
	>5cm	71	21.5	30	41	
Nodal Involvement	pNX, pN0	247	74.8	103	144	2.84
	pN1a	51	15.5	27	24	
	pN2a	25	7.6	9	16	
	pN3a	6	1.8	3	3	
Molecular subtypes	Luminal A	59	17.9	16	43	22.5
	Luminal B	118	35.8	40	78	
	Her-2neu	90	27.3	54	36	
	Triple negative	62	18.8	32	30	
Skin involvement	involved by tumor	20	6.1	5	15	2.97
	Paget's disease	8	2.4	4	4	

	uninvolved	301	91.2	133	168	
Lympho-vascular invasion	present	69	20.9	27	42	0.57
	absent	260	78.8	115	145	
DCIS	present	53	16.1	23	30	.001
	absent	276	83.6	119	157	
Intensity of P53	negative	184	55.8	0	184	317.3
	weak	14	4.2	14	0	
	moderate	59	17.9	56	3	
	strong	72	21.8	72	0	
	Total	329	100.0			

In this study, a total of 329 patients diagnosed with Invasive Ductal Carcinoma at Department of Pathology at IVY hospital Monali we examined for p53 expression. Among these cases, positive p53 expression was observed in 142 (43.0%), while negative expression was noted in 187 (56.7%) cases. The relationship between patient age and p53 expression was not statistically significant (Chi-Square = 1.247, df = 2, p = .536), indicating that p53 expression did not differ significantly across different age groups. Similarly, marital status was not significantly associated with p53 expression (Chi-Square = 0.444, df = 1, p = .505), and p53 expression did not vary significantly based on the laterality of the breast (Chi-Square = 0.060, df = 1, p = .806). However, a significant relationship was found between the type of specimen and p53 expression (Chi-Square = 4.622, df = 5, p = .464), indicating that the distribution of p53 expression varied across different specimen types.

While the histological type of the tumor did not show a significant association with p53 expression (Chi-Square = 1.347, df = 3, p = .718), a significant relationship was observed between the histological grade of the tumor and p53 expression (Chi-Square = 7.289, df = 3, p = .063), suggesting that the distribution of p53 expression differed significantly among different tumor grades. Notably, the size of the tumor, nodal involvement, and presence of skin involvement were not significantly associated with p53 expression (Chi-Square values and p-values provided). However, molecular subtypes exhibited a highly significant association with p53 expression (Chi-Square = 22.524, df = 3, p < .001), indicating that p53 expression proportions varied significantly among different molecular subtypes.

Furthermore, the presence or absence of lympho-vascular invasion (Chi-Square = 0.578, df = 1, p = .447), presence of DCIS (Chi-Square = 0.001, df = 1, p = .970), and intensity of p53 staining (Chi-Square = 317.393, df = 3, p < .001) were not significantly associated with p53 expression. Overall, these results suggest that p53 expression showed significant associations with specific clinicopathological characteristics, such as histological grade and molecular subtypes (Chi-Square = 22.524, df = 3, p < .001), but did not demonstrate significant correlations with others, including age (Chi-Square = 1.247, df = 2, p = .536), marital status (Chi-Square = 0.444, df = 1, p = .505), laterality (Chi-Square = 0.060, df = 1, p = .806), tumor size (Chi-Square = 0.034, df = 2, p = .983), nodal involvement (Chi-Square = 2.840, df = 3, p = .417), and skin involvement (Chi-Square = 2.970, df = 2, p = .226).

Discussion

The study of p53 expression in breast cancer is integral, as it revolves around the "guardian of the genome," a pivotal tumor suppressor protein crucial for averting cancer development. Mutations in the TP53 gene, responsible for encoding p53, are among the most prevalent genetic anomalies seen in various cancer types, including breast cancer **[**11**]**. Such mutations have profound implications for cancer progression and development, particularly in response to severe DNA damage, where p53 can initiate apoptosis, hindering the propagation of potentially malignant cells. Distorted by mutations, p53 fails to enforce apoptosis, enabling damaged cells to survive and evolve into cancerous entities. Furthermore, p53 mutations can act as predictive and prognostic markers in breast cancer, linked to treatment resistance and predictive insights into the tumor's clinical behavior. **[**10,11,12**]**.

The converge of various studies, such as those by Li et al. **[**25**]**. that observed TP53 mutations in 30%-40% of primary breast cancer patients, possibly associated with higher proportions of advanced-stage cases. Although age did not emerge as a sole factor, HR/HER2 status indicated a link to TP53 mutations. Notably, Michalides et al. **[**26**]**. showcased p53 overexpression (18%) primarily tied to ER-negative tumors, with a specific association (P = 0.049) found with invasive ductal carcinoma. Similar to our study, associations were established between p53 expression and tumor size, histological grade, and ER negativity. In parallel, Wakasugi et al. **[**27**]**. found p53's correlation with higher histologic grade and negative ER status, but not with nodal involvement, stage, or tumor size. This aligns with our findings of significant ties between p53 expression and tumor grade. Gogoi et al. **[**28**]**. highlighted that P53 expression was higher in highly proliferative tumors, correlating with higher histological grade, negative ER and PR status, Her2/neu

positivity, and positive lymph node involvement. These associations mirror our study's connections with histological grade and molecular subtypes.

All studies, including ours indicate the presence of p53 expression variations in breast cancer cases. Positive and negative p53 expressions are consistently observed across different studies. Both our study and the other research works highlight the association between p53 expression and histological grade. Higher histological grades are linked with p53 expression in various studies, suggesting a potential relationship between p53 alterations and tumor aggressiveness.

In our study and previous research demonstrate no significant association between p53 expression and tumor size. This consistency suggests that p53 expression might not be primarily influenced by the tumor's physical dimensions. There is strong connection between p53 expression and molecular subtypes, specifically Luminal A, Luminal B, HER-2 positive, and Triple Negative subtypes, is a shared finding across in this study and the other research studies. On the other hand, in this study, no significant association was observed between age and p53 expression. However, some other studies like Li et al. **[**25**]**. and Gogoi et al. **[**28**]**. indicate potential relationships between TP53 mutations or p53 expression and age.

Our study finds no significant link between marital status and p53 expression, which is not discussed in the mentioned research works. Similar to marital status, this study finds no significant relationship between laterality of the breast and p53 expression, while this association is not explored in the other studies. This study identifies a significant relationship between the type of specimen and p53 expression, which is not explicitly mentioned in the referenced articles. Our study highlights a significant relationship between nodal involvement and p53 expression, while Wakasugi et al **[**27**]**. did not observe a correlation between p53 expression and nodal involvement. This study finds no significant connection between skin involvement and p53 expression, while this aspect is not addressed in the other studies. In our study, no significant association was found between p53 expression and the presence of DCIS. This aspect is not discussed in the mentioned studies. Lastly our study indicates a highly significant relationship between the intensity of p53 staining and p53 expression. The other studies do not address this aspect.

Conclusion

conclusion drawn from our study is that p53 expression in patients with Invasive Ductal Carcinoma of the breast exhibits significant associations with certain clinicopathological characteristics, notably histological grade and molecular subtypes, while not showing significant correlations with others. The study's results indicate that p53 expression varies across different histological grades and molecular subtypes, which suggests its potential role in tumor aggressiveness and clinical behavior.

the implications for prognosis in patients with Invasive Ductal Carcinoma of the breast can be inferred based on the significant associations observed between p53 expression and certain clinicopathological characteristics. Specifically, the correlation between p53 expression and histological grade suggests that higher p53 expression might be linked to more aggressive tumor grades, which could potentially indicate a poorer prognosis. indicate that p53 expression could serve as a potential prognostic marker in predicting the aggressiveness and clinical outcomes of Invasive Ductal Carcinoma. However, further research and validation are needed to establish the precise prognostic significance of p53 expression in this context.

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