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Association of Immunohistochemical Expression of P53 in Pancreatic Ductal Adenocarcinoma

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ABSTRACT

Objective: To assess the immunohistochemical expression of p53 in pancreatic ductal adenocarcinoma and chronic pancreatitis.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan from Jul 2020 to Jun 2022.

Methodology: Our study inducted 116 individuals in total (56 with cancer and 60 with pancreatitis). Following the recovery of the cases, each block was divided into sections and stained with hematoxylin-eosin and p53 antibodies. Microsoft Excel 2010 was used for the statistical analysis.

Results: Out of the 116 cases studied, 56 cases were found to be adenocarcinoma. Among these cases, 42(75%) showed p53 positivity according to the Sophia scoring system, with +3 and +4 staining considered positive. Of these 56 cases, 3 cases were scored 1+, 11 cases were scored 2+, 21 cases were scored 3+, and 21 cases were scored 4+. 8 out of 60 cases (13.33%) of pancreatitis showed p53 positivity.

Conclusion: The majority of pancreatic adenocarcinomas exhibit immunohistochemical overexpression of p53, which can be used in conjunction with other immunohistochemical markers to aid in the diagnosis of carcinoma and differentiate it from benign mimickers.

Keywords: Adenocarcinoma pancreas, Chronic pancreatitis. P53 immunohistochemistry

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INTRODUCTION

Pancreatic cancer is the seventh foremost cause of mortality in the world's industrialized countries.[1] After colorectal cancer, it is the second most common digestive tract cancer, with a predicted 56,000 cases and 45,000 deaths in the USA in 2019.[2] It is one of the top 10 cancers that cause death globally. According to data from the USA, people with pancreatic cancer had a median survival rate of 6 to 12 months and an overall 5-year survival rate of 4.8%.[3] In the USA, it ranks as the fourth-leading cause of cancer fatalities.[4] In contrast to 2012, pancreatic cancer incidence has doubled in 2020, with a 97.4% fatality rate. According to a Globocan analysis, the incidence of pancreatic cancer is predicted to quadruple by the year 2040, with a high fatality rate.[5,6] In Pakistan, pancreas cancer fatalities have reached 1069, or 0.07% of all deaths, according to WHO data that was released in 2020.[7] Statistically, 97.8% of pancreatic cancer cases in Pakistan result in early death. Although the situation is concerning, there has been little research conducted in this area.[8] Overall, pancreatic cancer has a higher incidence and fatality rate in older people and affects women more frequently than males.[1] There are two primary subtypes of pancreatic adenocarcinoma. 90% of instances are pancreatic ductal adenocarcinomas, which develop from the pancreatic exocrine glands, and 5% are pancreatic neuroendocrine tumors (PanNETs), which develop from the organ's endocrine tissue.[9]

A variety of etiological factors contribute to the development of pancreatic adenocarcinoma. It is associated with genetic mutations, obesity, inflammation, chronic pancreatitis, autophagy dysfunction, and metabolic syndromes.[10] Genetic modifications play the most significant role. It includes oncogene activation mutations, inactivation of tumor suppressor genes such as TP53, dysregulation of the Hedgehog pathway, deregulation of EGFR signaling, and activation of the Akt and NF-κB signaling pathways. p53, also known as tumor protein 53 (TP53), is a transcription factor that controls the cell cycle and thus serves as a tumor suppressor gene. It is also referred to as "the guardian genome" due to its significance in averting genomic destabilization by inhibiting the growth of mutated cells. p53 mutation is seen in a wide range of

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malignancies, including ovarian, breast, urinary bladder, colon, lung, and melanoma. Multiple cancers co-occurring and a worse clinical outcome are both linked to the TP53 mutation. The study was carried out to assess the immunohistochemical expression of p53 in pancreatic ductal adenocarcinoma and chronic pancreatitis

METHODOLOGY

At the Armed Forces Institute of Pathology, Department of Histopathology, the comparative cross-sectional study was conducted from July 2020 to June 2022, following approval by the Ethical Review Committee (Certificate No. HSP19-1/READ-1RB/20/470). The sample size was calculated using the "WHO Sample Size Calculator," where incident rates of adenocarcinoma and pancreatitis were taken as 12.1% and 18.6%, respectively.[11] In all, 116 individuals were included in our research, including 60 with pancreatitis and 56 with adenocarcinoma.

Inclusion Criteria: Patients of both genders and all ages were included in the study. Biopsy samples, both incisional and excisional, containing adequate pancreatic tissue, were included who had clinical and radiological suspicion of malignancy.

Exclusion Criteria: Patients who had received prior chemotherapy and radiotherapy for adenocarcinoma were excluded from the study. Cases in which microscopic morphology is not clear, tissue processing is inadequate, poor fixation, or there is excessive hemorrhage and necrosis were also excluded.

Blocks of tissue that had been paraffin-embedded were recovered from individuals' prior medical records. Two 5-µm-thick slices were cut, and these sections underwent H&E staining before being immunohistochemically stained with a p53 antibody. We employed the mouse monoclonal antibody p53 clone DO7 and isotype IgG2b/K. The Sophia scoring system was used to evaluate the nuclear staining pattern as positive.

The Sophia scoring system was used to evaluate the criterion for positive p53 staining, as shown in Table-I. At least 100 cells were assessed using a 40x objective.[12,13] Scores of 0,1+ and 2+ were considered negative, whereas score of 3+ and 4+ was considered positive.[14]

SPSS (Statistical Package for the Social Sciences) version 26 and Microsoft Excel 2010 were used for statistical analysis. Tables including frequencies, percentages, means, standard deviations, and standard errors of the mean were subjected to data analysis using the Chi-square test. When the *p*-value is equal to or less than 0.05, values are deemed statistically significant.

Table-I: Sophia Scoring System of p53 Immunohistochemical Expression

Score	Staining pattern
Score 0	<5% of the cells revealed positivity of marker
Score 1+	(≥5≤10%) positive tumor cells
Score 2+	11≤25% positive tumor cells
Score 3+	26≤50% positive tumor cells
Score 4+	>50% positive tumor cells

RESULTS

In the study we performed, 42 out of 56 pancreatic adenocarcinoma patients, or 84%, possessed statistically significant p53 expression (*p*-value 0.05). As shown in Table-II, 8 (16%) instances of non-neoplastic pancreatic tissue also exhibited overexpression of p53. Nuclear staining of p53 was taken as positive, while cytoplasmic staining was disregarded.

Table-II: Immunohistochemical Expression of p53 in Pancreatic Adenocarcinoma and Pancreatitis (n=116)

		p53		Total	*p*-value
		Positive	Negative		
Adenocarcinoma	Number	42	14	56(48.3%)	0.000
	Percentage within p53	84%	16%		
Pancreatitis	Number	8	52	60(51.7%)	
	Percentage within p53	21.2%	78.8%		
Total		50(43.11%)	66(56.89%)	116	

According to the Sophia Scoring System, the p53 score was interpreted, and as shown in Figure, the majority of cases were negative. Thirty-one cases (26.72%) were scored 2+, 23 cases (19.83%) were 1+, and 12 cases (10.34%) were scored 0, with the remainder of cases labeled positive and having scores of 3+ in 28 cases (24.14%) and 4+ in 22 cases (18.96%).

Among adenocarcinoma patients, the majority of cases stained positive for the p53 immunohistochemical marker, with p53 nuclear staining taken into account along with the percentage of cells. Forty-two cases (75%) were p53 positive, with

21 cases each at scores of 3+ and 4+, whereas 14 cases (25%) of adenocarcinoma patients were negative for the p53 immunomarker. In pancreatitis, the immunohistochemical expression of p53 was negative in a larger number of individuals. Fifty-two individuals (86.67%) had negative cases for p53 immunohistochemical expression. Eight cases (13.33%) were positive for p53 expression. Among these positive cases, 7(11.67%) and 1(1.67%) scored 3+ and 4+, respectively, as shown in Figure.

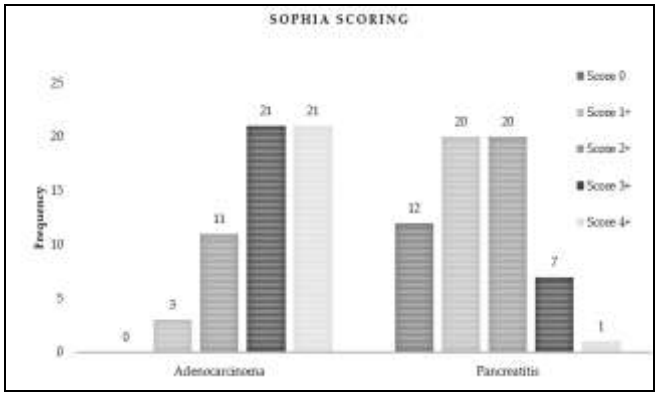

Figure: The p53 Immunohistochemical Sophia Score in Pancreatic Adenocarcinoma and Pancreatitis (n= 116)

DISCUSSION

One of the most common causes of death from epithelial gastrointestinal malignancies is pancreatic adenocarcinoma. Several known risk factors contribute to its etiology. Among non-modifiable (hereditary) risk factors include hereditary breast and ovarian syndrome, hereditary non-polyposic colorectal cancer or Lynch syndrome, Familial adenomatous polyposis, Peutz-Jeghers syndrome, and cystic fibrosis. Modifiable risk factors for the development of pancreatic adenocarcinoma include smoking, alcohol consumption, chronic pancreatitis, obesity, diabetes mellitus, fatty diet, and biliary obstructive diseases. However, one of the most important etiological causes is genetic mutation.[15-16]

KRAS is the most frequent genetic mutation in pancreatic adenocarcinomas, followed by TP53, CDKN2A, SMAD4, and BRCA1 and BRCA2.[17] 20-76% of pancreatic adenocarcinomas have the TP53 mutation.[18,19] In one study, its prevalence rises to 50-75% of the cases of pancreatic adenocarcinoma after initiating activation mutation of the KRAS gene, and it is thought to be one of the major oncogenic driving mutations in pancreatic adenocarcinoma. These frequently lead to the accumulation of mutant p53 proteins, which may have dominant-negative or gain-of-function characteristics.[20]

According to our research, only 8 out of 60 pancreatitis patients (16%) displayed p53 expression, compared to 42 out of 56 instances of adenocarcinoma (84%) that showed p53 immunohistochemical expression. There are statistically significant cases of p53 immunohistochemical expression in adenocarcinomas (p-value <0.005).

Zhao *et al*., conducted research in 2016 involving 260 patients, comprising 186 cases of pancreatic adenocarcinoma, 23 cases of benign pancreatic lesions, and 51 cases of normal pancreatic tissue. In 3(13%) cases of normal pancreatic tissue, 1(1.96%) case of a benign pancreatic lesion, and 44(23.67%) cases of pancreatic adenocarcinoma, immunohistochemical expression of p53 was observed. This study supported the findings of our study. The p-value was <0.005, which was statistically significant.[21] Similar research was conducted by Apple *et al*., which comprised 15 cases of pancreatic ductal adenocarcinoma. Thirteen (86.67%) of these cases exhibited positive immunohistochemical p53 expression. Immunohistochemical staining for p53 was also performed on normal pancreatic tissue from 30 patients, which only exhibited a very insignificant positivity in 1(3%) of the individuals.[22]

There were variable results when compared to the study conducted by DiGiuseppe *et al*., They studied 48 cases of adenocarcinomas, of which 19 (or 40%) showed nuclear p53 staining and 29 (or 60%) did not. Forty-nine individuals were included in the study by Slebos *et al*., of whom 25(51%) had nuclear p53 staining, whereas 24(49%) did not. There was no significant association between p53 mutation and pancreatic adenocarcinoma among these studies.[23,24]

Similar to this, a study by Blanck *et al*., found that pancreatic adenocarcinoma expressed p53 immunohistochemically in 45% of cases, with no statistically significant correlation.[25] Because p53 does not operate synonymously in normal cells and because p53 deletions, frame-shift mutations , and MDM2 overexpression may not display p53 staining, there are differences in the immunohistochemistry expression of p53.

Our study has certain limitations since specific cancerous and normal pancreatic cells can overexpress p53, and there is a possibility of observer-based errors. Retrospective sampling was also employed, which can introduce bias into the sample. Additionally,

immunohistochemistry data is semi-quantitative, which justifies us running additional tests to verify our findings.

LIMITATIONS OF STUDY

A small sample size limits the scope of our study. A large-scale, multi-institutional study should be conducted to obtain more generalized results. Future molecular genetic studies will be conducted to gain a precise understanding of the pathogenesis of pancreatic adenocarcinoma.

CONCLUSION

Our study demonstrates that the majority of pancreatic adenocarcinomas have reliably overexpressed p53, making it a valuable ancillary method in cancer diagnosis and differentiating it from benign mimickers, thus preventing a very complicated surgical procedure and reducing morbidity and mortality.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

HT & AA: Data acquisition, critical review, approval of the final version to be published.

WAK & SRAN: Conception, study design, drafting the manuscript, approval of the final version to be published.

SR & SH: Data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68(6): 394-424. https://doi.org/10.3322/caac.21492
2. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 2016; 66(4): 271-289. https://doi.org/10.3322/caac.21349
3. Pourhoseingholi MA, Ashtari S, Hajizadeh N, Fazeli Z, Zali MR. Systematic review of pancreatic cancer epidemiology in Asia-Pacific Region: major patterns in GLOBACON 2012. Gastroenterol Hepatol Bed Bench 2017; 10(4): 245-257.
4. Neoptolemos JP, Springfeld C, Hackert T. A Review of Pancreatic Cancer. JAMA 2021; 326(23): 2436. https://doi.org/10.1001/jama.2021.20065
5. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chin Med J 2021; 134(7): 783-791. https://doi.org/10.1097/CM9.0000000000001474
6. Abbas G, Shah S, Hanif M, Asghar A, Shafique M, Ashraf K et al. Cancer prevalence, incidence and mortality rates in Pakistan in 2018. Bull Cancer 2020; 107(4): 517-518. https://doi.org/10.1016/j.bulcan.2019.12.011
7. Ali SM, Adnan Y, Ali SA. Pancreatic Cancers: A Review of Studies from Pakistan and Comparison with Global Trends. Pak J Public Health 2021; 11(2): 120-127.
8. Ahmad Z, Din NU, Minhas K, Moeen S, Ahmed A. Epidemiologic Data, Tumor Size, Histologic Tumor Type and Grade, Pathologic Staging and Follow Up in Cancers of the Ampullary Region and Head of Pancreas in 311 Whipple Resection Specimens of Pakistani Patients. Asian Pac J Cancer Prev 2015; 16(17): 7541-7546. https://doi.org/10.7314/apjcp.2015.16.17.7541
9. Hidalgo M, Cascinu S, Kleeff J, Labianca R, Löhr JM, Neoptolemos J, et al. Addressing the challenges of pancreatic cancer: future directions for improving outcomes. Pancreatology 2015; 15(1): 8-18. https://doi.org/10.1016/j.pan.2014.10.001
10. Voutsadakis IA. Mutations of p53 associated with pancreatic cancer and therapeutic implications. Ann Hepatobiliary Pancreat Surg 2021; 25(3): 315-327. https://doi.org/10.14701/ahbps.2021.25.3.315
11. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6(269): pl1. https://doi.org/10.1126/scisignal.2004088
12. Ameer EA, Ghafour KH. Expression of p53 in Pancreatic Ductal Adenocarcinoma. Iraqi Postgrad Med J 2022; 21(2).
13. Qin LX, Tang ZY, Ma ZC, Wu ZQ, Zhou XD, Ye QH, et al. P53 immunohistochemical scoring: an independent prognostic marker for patients after hepatocellular carcinoma resection. World J Gastroenterol 2002; 8(3): 459-463. https://doi.org/10.3748/wjg.v8.i3.459
14. Cruz I, Snijders PJ, Van Houten V, Vosjan M, Van der Waal I, Meijer CJ. Specific p53 immunostaining patterns are associated with smoking habits in patients with oral squamous cell carcinomas. J Clin Pathol 2002; 55(11): 834-40. https://doi.org/10.1136/jcp.55.11.834
15. Cho J, Petrov MS. Pancreatitis, Pancreatic Cancer, and Their Metabolic Sequelae: Projected Burden to 2050. Clin Transl Gastroenterol 2020; 11(11): e00251. https://doi.org/10.14309/ctg.0000000000000251
16. Capasso M, Franceschi M, Rodriguez-Castro KI, Crafa P, Cambiè G, Miraglia C, et al. Epidemiology and risk factors of pancreatic cancer. Acta Biomed 2018; 89(9-S): 141-146. https://doi.org/10.23750/abm.v89i9-S.7923
17. Cicenas J, Kvederaviciute K, Meskinyte I, Meskinyte-Kausiliene E, Skeberdyte A, Cicenas J et al. KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 Mutations in Pancreatic Cancer. Cancers 2017; 9(5): 42. https://doi.org/10.3390/cancers9050042
18. Kern SE, Pietenpol JA, Thiagalingam S, Seymour A, Kinzler KW, Vogelstein B et al. Oncogenic forms of p53 inhibit p53-regulated gene expression. Science 1992; 256(5058): 827-830. https://doi.org/10.1126/science.1589764
19. Hwang RF, Gordon EM, Anderson WF, Parekh D. Gene therapy for primary and metastatic pancreatic cancer with intraperitoneal retroviral vector bearing the wild-type p53 gene. Surgery 1998; 124(2): 143-150.

20. Morton JP, Timpson P, Karim SA, Ridgway RA, Athineos D, Doyle B, et al. Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc Natl Acad Sci U S A 2010; 107(1): 246-251. https://doi.org/10.1073/pnas.0908428107
21. Zhao H, Wang Q, Wang X, Zhu H, Zhang S, Wang W, et al. Correlation Between RAB27B and p53 Expression and Overall Survival in Pancreatic Cancer. Pancreas 2016; 45(2): 204-210. https://doi.org/10.1097/MPA.0000000000000453
22. Apple SK, Hecht JR, Lewin DN, Jahromi SA, Grody WW, Nieberg RK. Immunohistochemical evaluation of K-ras, p53, and HER-2/neu expression in hyperplastic, dysplastic, and carcinomatous lesions of the pancreas: evidence for multistep carcinogenesis. Hum Pathol 1999; 30(2): 123-129. https://doi.org/10.1016/s0046-8177(99)90265-4
23. DiGiuseppe JA, Hruban RH, Goodman SN, Polak M, van den Berg FM, Allison DC et al. Overexpression of p53 protein in adenocarcinoma of the pancreas. Am J Clin Pathol 1994; 101(6): 684-688. https://doi.org/10.1093/ajcp/101.6.684
24. Slebos RJ, Hoppin JA, Tolbert PE, Holly EA, Brock JW, Zhang RH, et al. K-ras and p53 in pancreatic cancer: association with medical history, histopathology, and environmental exposures in a population-based study. Cancer Epidemiol Biomarkers Prev 2000; 9(11): 1223-1232.
25. Blanck HM, Tolbert PE, Hoppin JA. Patterns of genetic alterations in pancreatic cancer: a pooled analysis. Environ Mol Mutagen 1999; 33(2): 111-122. https://doi.org/10.1002/(sici)1098-2280