Expression of p53 in Pancreatic Ductal Adenocarcinoma

Estabraq Ali Abdul Ameer , Kifah Hamdan Abdul Ghafour

ABSTRACT:

BACKGROUND:

Pancreatic adenocarcinoma is one of the major causes of cancer death in the world**.** Alterations in the p53 tumor suppressor gene stand out as the most common alteration in many cancers including 75% of pancreas cancer. Pancreatic adenocarcinoma in which p53 is mutated carry a poor prognosis, because of late-stage detection, the presence of vascular invasion, metastases, and ineffective treatment options.

OBJECTIVE:

To evaluate the expression of P53 in pancreatic ductal adenocarcinoma immunohistochemically and clinicopathologic correlation with grade and stage.

MATERIALS AND METHODS:

This is a retrospective study of 40 pancreatic biopsies (formalin fixed, paraffin embedded) were collected from archived materials from GIT and Hepatology teaching hospital in Baghdad medical city (from December 2018 to October 2019). Study group include 40 cases of Pancreatic adenocarcinoma, 20 were EUS guided core needle biopsy, and the other 20 were pancreatic tissue from patients sustained whipple surgery.

Two sections of 5 µm thickness were taken from each block**,** the first was stained with H&E, the second was stained Immunohistochemical for P53.

RESULTS:

Forty cases are studied and show +ve staining for P53 (100%), 24(60.0%) were males and 16(40.0%) were females, The age of patients ranged from 35 to 70 years with mean ± SD of 56.28±10.2 years. Seventeen were score 4+, thirteen were score 3+, six were score 2+ and four were score 1+.

Tumors with low grade were 20 (50.0%) cases, moderate grade were 15 (37.5%) cases, and high grade were 5 (12.5%) cases.

20 cases were staged from patients underwent whipple surgery.

Tumors with stage T1 N0 Mx were 2(10%), stage T2 N0 Mx were 5(25%), stage T2 N1 Mx were 4(20%), stage T3 N0 Mx were 8(40%), and stage T3 N1 Mx were 1(5%)

CONCLUSION:

There was statistical correlation between P53 expression and tumor grade but not with stage.

KEYWORDS: P53 expression, Pancreatic adenocarcinoma.

INTRODUCTION:

Pancreatic cancer is an intractable malignancy and is the seventh leading cause of global cancer deaths in industrialized countries [(1)], and the third most common in the USA. [(2)]

In Iraq it ranks as the 15th most common cancer in 2018. [(1)]

In USA it is overall relative 5-year survival rate of only 4.8%, with the median survival time ranging from 6 to 12 months. [(3)]

Worldwide incidence and mortality of pancreatic cancer correlate with increasing age and is slightly more common in men than in women. [(1)]

Pancreatic cancer is mainly divided into two types: pancreatic ductal adenocarcinoma (PDAC), which is the most common (90% of cases) arising in exocrine glands of the pancreas, and pancreatic neuroendocrine tumor (Pan NET), which is less common (5%) and occurs in the endocrine tissue of the pancreas. [(4)]

Abnormalities of p53 protein accumulation in human cancers can be studied with Immunohistochemistry (IHC) methods, which has been of key importance in revealing the clear association between increased p53 protein stability and mutation. [(5)]

MATERIALS AND METHODS:

This is a retrospective study of formalin fixed, paraffin embedded tissue blocks were collected from archived materials from GIT and Hepatology teaching hospital in Baghdad medical city (from December 2018 to October 2019).

Department of Pathology, College of Medicine/ University of Baghdad., Baghdad, Iraq.

Study group included 40 cases of Pancreatic adenocarcinoma, 20 were EUS guided core needle biopsy, and the other 20 were pancreatic tissue from patients sustained whipple surgery.
*Control group include 10 cases half of them from EUS guided core needle biopsy that show normal pancreatic tissue and the other half from patients sustained whipple surgery for another malignancy.
Two sections of 5 µm thickness were taken from each block, the first was stained with H&E , The 2nd was stained immunohistochemically for P53.

Scoring system:

The criterion for positive immunoreaction is dark brown precipitate in the nucleus for p53. The percent scored according to Sophia scoring system, A minimum of 100 tumor cells were scored, Scoring was done at X40 objective. [6] as shown in table below.

Table 1 : Sophia Scoring system of P53 expression.

Score	Staining pattern
Score 0	<5% of the cells revealed positivity for the marker.
Score 1+	(5-10%) positive of tumor cells.
Score 2+	less than 25% of tumor cells are stained positive.
Score 3+	(25-50%) of tumor cells are stained positive.
Score 4+	over 50% of tumor cells are stained positive.

Statistical Analysis:

Statistical analysis was performed with **SPSS** v18.88 (Statistical package for social sciences) and also Excel **2010** programs. Data analysis was done using t-test, chi-square test for tables with frequencies, percentages, ranges, means standard deviation and standard errors of mean. Values were considered Statistically significant when p-value is equal or less than 0.05.

RESULTS:

Half of patients were EUS guided core biopsies, and the other half were biopsies from whipple surgery.
In this study, 100% of Pancreatic adenocarcinoma cases were showed positive P53 expression with a significant association (P= 0.001) (<0.05) Non neoplastic pancreatic tissue was negative (score 0) for P53 expression.

Table 2: Association between neoplastic and non_neoplstic biopsies with P53 expression.

	P53 Expression		Total (%) n= 50	P - Value
	Positive (%) n= 40	Negative (%) n= 10		
CA	40 (100%)	0	40 (80%)	0.001
Non neoplastic pancreatic tissue	0	10(100%)	10 (20%)	

Forty patients were studied, 24(60.0%) males and 16(40.0%) females.
The age of patients ranged from 35 to 70 years with mean ± SD of 56.28±10.2 years, 3 cases (7.5%) aged from 31-40years, 10 cases (25.0%) aged from 41-50 years, 12 (30.0%) aged from 51-60 years, and 15 (37.5%) aged from 61-70 years.

Score of P53:-

Score of P53 according to Sophia scoring system, which show the majority were score 4+ (42.5%) or 3+(32.5%) and the remaining were score 2+(15.0%) and score 1+(10.0%).

Grading Of study group:

Tumors with low grade were 20 (50.0%) cases, moderate grade were 15 (37.5%) cases, and high grade were 5 (12.5%) cases.
Regarding grade of tumor relation with P53 expression the p-value was 0.005642 (<0.05) and the result was significant so there is relation between P53 expression score and tumor grade.

Table 3: Association between tumor grade and P53 expression.

Tumor Grade	+1	+2	+3	+4	Total	P - Value
Well	1(5%)	2(10%)	4(20%)	13(65%)	20(50%)	
Moderate	3 (20%)	1(6.7%)	8(53,3%)	3(20%)	15 (37.5%)	0.005642
Poor	0 (0 %)	3(60%)	1 (20%)	1(20%)	5 (12.5%)	

Staging of study group:

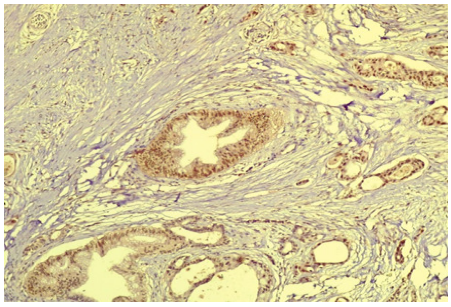
20 cases were staged from patients underwent whipple surgery.

Tumors with stage T1 N0 Mx were 2(10%), stage T2 N0 Mx were 5(25%) , stage T2 N1 Mx were 4(20%), stage T3 N0 Mx were 8(40%), and stage T3 N1 Mx were 1(5%).

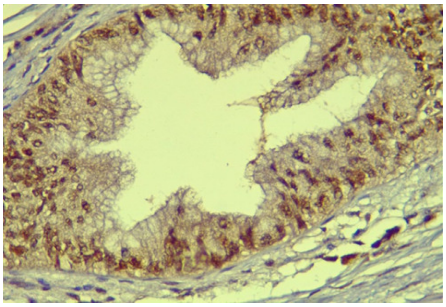
Relation of P53 score with the stage was insignificant with p-value 0. 331 (>0.05).

Table 4 : shows the association between tumor stage and P53 expression.

Stage	Score			Total	P value
	2+	3+	4+		
T1 N0 Mx	0 (0.0)	0 (0.0)	2 (20.0)	2 (10.0)	0.331
T2 N0 Mx	1 (25.0)	1 (16.7)	3(30.0)	5 (25.0)	
T2 N1 Mx	0 (0.0)	1 (16.7)	3(30.0)	4 (20.0)	
T3 N0 Mx	3 (75.0)	4 (66.7)	1(10.0)	8 (40.0)	
T3 N1 Mx	0 (0.0)	0 (0.0)	1 (10.0)	1 (5.0)	
Total	4 (100.0)	6 (100.0)	10 (100.0)	20 (100.0)	



(IHC /X10)



(IHC /X40)

Figure 1: Well differentiated pancreatic ductal adenocarcinoma/IHC. Biopsy from whipple surgery, +ve P35 nuclear staining score +3 .

DISCUSSION:

-P53 expression:

This study show that p53 gene mutations and/or abnormal p53 protein accumulation are present in 100% of pancreatic adenocarcinomas and low or undetectable P53 immunoreactivity in normal pancreatic tissue with significant P value (0.001) ,this finding is nearly agree with study of **H Zhao et al 2016**, which also found the same p value in comparing cases of normal pancreatic tissue and pancreatic ca with staining for P53 [7] and of the 15 cases studied by **Sophia K Apple MD et al 1999** , all normal epithelial ducts from infiltrating adenocarcinomas of the pancreas specimens, showed no positivity for p53 staining and of infiltrating adenocarcinoma cells, 13 had positive p53 staining . [6]

But differ from studies of , **Joseph A. Digiuseppe et al 1994** found in study of 48 patients that nuclear staining for p53 was seen in 26 (54%) of the 48 infiltrating carcinomas examined , and **Robbert J. C. Slebos et al 2000** study 49 patients and 25 of them were +ve for P53 . [8,9] The reasons for these contradictory results may be that the absence of p53 expression is not always synonymous with normal function of p53 gene and it is still possible that p53 deletion mutations, frame shift mutations,

nonsense mutations, or MDM2 overexpression do not show p53 expression.

- Sex:

- Regarding the sex 24 (60%) patients were male and 16 (40%) were female, and the ratio is 1.5:1 . This study sex groups totally fit the result of **Asim Qureshi et.al 2011** [10], and **KH Abdulghafour et al 2013** [11] with a male to female ratio of (2:1), but it differ from **Robbert J. C. Slebos et al 2000** [9] with nearly equal male and female number.

-Age:

- Considering age of study group the age ranged from 35 to 70 years with mean ± SD of 56.28±10.2 years. Which explained by the occurrence of pancreatic adenocarcinoma in older age group, and the study group nearly agreed with study age group of **Akeil Hussien at al 2014** [12] and **Jeong J. et al 2005**. [13]

But is lower than results of **Young Choon Kim et al 2009** [14] with a mean ± SD of 66.0± 10.2 years, and higher than study age group of **KH Abdulghafour et al 2013** .[11] with the Mean age (45+2.02)and range (28-65) year .

-Tumor grade:

In this study there is 20 cases with low grade (50.0%) , moderate grade were 15 (37.5%) cases , and high grade were 5 (12.5%) cases.

Most cases were well differentiated, this nearly agree with the study of **Robbert J. C. Slebos et al 2000** [9] with study group of 49 patients 19 of them were well differentiated.

But against other studies that showed higher percent for moderate differentiation as **Asim Qureshi et al 2011** [10] and **Young Choon Kim et al 2009** [14] . And in study of **Linder S et al 1997**, showed higher percent (30 of 53) of poorly differentiated carcinoma. [15]

Tumor grade shows significant statistical correlation with expression of P53 with p value (0.005642) (<0.05). And this also found in previous studies: **Robbert J. C. Slebos et al 2000**.in his study the P value is 0.004 [9] ,**Talar-Wojnarowska R et al 2006** , found a significant correlation between p53 mutation and tumor differentiation ($p < 0.01$) [16]

and **Joon Jeong et al 2005** studied 41 cases of ductal adenocarcinoma and the p value with P53 was significant (0.038) [13]

This explained by strong evidence for a relationship between p53 accumulation and nuclear abnormalities, consistent with a role of p53 in the maintenance of genomic integrity.

But disagree with study of **M Dong et al 2007** [17], that found insignificant correlation with P value (0.477),and **H Zhao et al 2016** [7] with P value (0.737).

-Tumor stage:

In this study only 20 cases staged according to (AJCC Cancer Staging Manual Eighth Edition) due to limited cases found that underwent whipple surgery. Most cases are stage T2 or T3 with stage T2 N0 Mx were 5(25%), stage T2 N1 Mx were 4(20%) , stage T3 N0 Mx were 8(40%) and stage T3 N1 Mx were 1(5%).

The least is stage T1 which are 2(10%).

This results nearly agree with study of **Young Choon Kim et al 2009** [14] ,and **Asim Qureshi et al 2011**[10] .**Robbert J. C. Slebos et al 2000** [9] and **Jeong J et al 2005** [13] with most cases are stage III.

In study of **S Linder1 et al 1997** on 53 patients diagnosed as pancreatic adenocarcinoma found most patients are stage I which differs from our study. [15] Because of presence of facilities for early diagnosis.

There is no significant correlation in this study between stage and P53 expression with P value 0.343(>0.05), also the same result found in previous studies:

M Dong et al 2007 [17] that study 59 cases of pancreatic adenocarcinoma and the P value was (0.174) between stage and P53 expression, and **Domenico Coppola et al 1998** [18] with the P value (0.45).

But disagree with study of **Robbert J. C. Slebos et al 2000** in which the P value was (0.01) [9]

This variation may be related to diverse inclusion criteria and selection bias of the studies which may be attributed to the limited cases we got.

CONCLUSION:

1. The present study showed that P53 activation is an important event in human pancreatic tumorigenesis as it is highly expressed in pancreatic cancer.
2. Tumor grade showed significant correlation with P53 expression.
3. Tumor stage showed insignificant correlation with P53 expression.

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: a cancer journal for clinicians. 2018;68:394-424.
2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer. 2018.

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. Gastroenterology and hepatology from bed to bench. 2017;10:245.
4. Hidalgo M, Cascinu S, Kleeff J, Labianca R, Löhr JM, Neoptolemos J, Real FX, Van Laethem JL, Heinemann V. Addressing the challenges of pancreatic cancer: future directions for improving outcomes. Pancreatology. 2015;15:8-18.
5. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal.. 2013;6:l1.
6. 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. Human pathology. 1999;30:123-29.
7. Zhao H, Wang Q, Wang X, Zhu H, Zhang S, Wang W, Wang Z, Huang J. Correlation between RAB27B and p53 expression and overall survival in pancreatic cancer. Pancreas. 2016;45:204.
8. DiGiuseppe JA, Hruban RH, Goodman SN, Polak M, Van Den Berg FM, Allison DC, Cameron JL, Johan A. Offerhaus G. Overexpression of p53 protein in adenocarcinoma of the pancreas. American journal of clinical pathology. 1994 Jun 1;101:684-88.
9. Slebos RJ, Hoppin JA, Tolbert PE, Holly EA, Brock JW, Zhang RH, Bracci PM, Foley J, Stockton P, McGregor LM, Flake GP. K-ras and p53 in pancreatic cancer: association with medical history, histopathology, and environmental exposures in a population-based study. Cancer Epidemiology and Prevention Biomarkers. 2000;9:1223-32.
10. Qureshi A, Hassan U, Azam M. Morphology, TNM staging and survival with pancreatico-duodenectomy specimens received at Shaukat Khanum Memorial Cancer Hospital and Research Centre, Pakistan. Asian Pac J Cancer Prev. 2011;12:953-56.
11. Abdulghafour KH, Khalf SA. CA19-9 and CK immunohistochemical expression in pancreatic and ampulla of vater carcinomas (A clinicopathological study. Journal of the Faculty of Medicine. 2014;56:308-12.
12. Eissa AH. Carcinoma of The Pancreas A Retrospective Study of Pancreatic Cancer of 320 Case from 1976 to 2011. Medical Journal of Babylon. 2014;11:971-83.
13. Jeong J, Park YN, Park JS, Yoon DS, Chi HS, Kim BR. Clinical significance of p16 protein expression loss and aberrant p53 protein expression in pancreatic cancer. Yonsei medical journal. 2005 Aug 31;46:519-25.
14. Kim YC, Kim HJ, Park JH, Park DI, Cho YK, Sohn CI, Jeon WK, Kim BI, Shin JH. Can preoperative CA19-9 and CEA levels predict the resectability of patients with pancreatic adenocarcinoma?. Journal of gastroenterology and hepatology. 2009 ;24:1869-75.
15. Linder S, Parrado C, Falkmer UG, Blåsjö M, Sundelin P, Von Rosen A. Prognostic significance of Ki-67 antigen and p53 protein expression in pancreatic duct carcinoma: a study of the monoclonal antibodies MIB-1 and DO-7 in formalin-fixed paraffin-embedded tumour material. British journal of cancer. 1997;76:54.
16. Talar-Wojnarowska R, Gasiorowska A, Smolarz B, Romanowicz-Makowskal H, Strzelczyk J, Janiak A, Malecka-Panas E. Comparative evaluation of p53 mutation in pancreatic adenocarcinoma and chronic pancreatitis. Hepato-gastroenterology. 2006;53:608-12.
17. Dong M, Dong Q, Zhang H, Zhou J, Tian Y, Dong Y. Expression of Gadd45a and p53 proteins in human pancreatic cancer: potential effects on clinical outcomes. Journal of surgical oncology. 2007;95:332-36.
18. Coppola D, Lu L, Fruehauf JP, Kyshtoobayeva A, Karl RC, Nicosia SV, Yeatman TJ. Analysis of p53, p21WAF1, and TGF-β1 in human ductal adenocarcinoma of the pancreas: TGF-β1 protein expression predicts longer survival. American journal of clinical pathology. 1998;110:16-23.