Original Article

Research in Oncology
2023; Vol. 19, No. 2: 31-38
DOI: 10.21608/resoncol.2024.239557.1202

Evaluation of Immunohistochemical Expression of Human Epidermal Growth Factor Receptor 2 (HER2) and its Association with Clinicopathological Variables in Patients with Pancreatic Duct Adenocarcinoma in Upper Egypt

Maisa H. Mohammed [1] ✉, Nagwa A. Ahmed [1], Maha M. A. Ahmed [2], Mohammed H. Sayed [3]

1 Department of Pathology, Faculty of Medicine, Sohag University, Sohag, Egypt; 2 Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Sohag University, Sohag, Egypt; 3 Department of Pathology, Faculty of Medicine, Al Azhar University, Assiut, Egypt

Abstract

Background: Pancreatic duct adenocarcinoma (PDAC) is an aggressive neoplasm. It shows progressive and cumulative genetic mutations, with subsequent development of therapy-resistant clones. Human epidermal growth factor receptor 2 (HER2) is a transmembrane glycoprotein that is normally involved in cellular proliferation and differentiation. Overexpression of HER2 was detected in several human malignant neoplasms. HER2 was successfully targeted in breast carcinoma and gastric carcinoma with improved prognosis in these neoplasms.
Aim: This study aimed to evaluate the immune expression of HER2 in PDAC, and to correlate HER2 expression with a variety of clinical and pathological parameters.
Methods: Formalin-fixed, paraffin-embedded tissue blocks from 37 patients with PDAC were studied. Two tissue sections were prepared from each tumor-laden tissue block; one section was stained by hematoxylin and eosin to confirm the diagnosis of PDAC, determine tumor grade, and detect perineural and/or vascular invasion. The other section was stained immunohistochemically by an anti-human HER2 antibody.
Results: Lympho-vascular invasion was detected in 89.2% of cases, it was associated with both advanced pathological tumor stages ($p = 0.023$) and regional lymph node involvement ($p = 0.028$). Circumferential perineural invasion was found in 75.7% of cases and correlated significantly with the advanced tumor stage ($p < 0.0001$), high tumor grade ($p= 0.014$), and regional lymph node involvement ($p = 0.002$). Positive expression of HER2 was detected in 51.4% of cases, HER2 positivity was significantly associated with grade III tumors (0.046), advanced pathological stages ($p = 0.002$), regional lymph node involvement ($p = 0.005$), perineural invasion ($p < 0.0001$) and lymphovascular invasion ($p = 0.046$).
Conclusion: HER2 protein is over-expressed in advanced stages of PDAC and is associated with poor prognosis pathologic features. Targeting HER2 in therapeutic protocols may improve the future prognosis of PDAC.

Keywords: HER2, Immunohistochemistry, Pancreatic duct adenocarcinoma, Perineural invasion
Corresponding author: Maisa Mohammed, MD; Department of Pathology, Faculty of Medicine, Sohag University, Sohag, Egypt; Email: maisaahashem@med.sohag.edu.eg
Received: 28-September-2023, **Accepted:** 13-January-2024, **Published online:** 27-January-2024

Introduction

Pancreatic carcinoma (PC) is a highly aggressive malignant tumor that has shown an obvious increase in its worldwide incidence in the last decade [1]. There are three distinct subtypes of pancreatic carcinoma: ductal, acinar, and neuroendocrine subtypes [2]. Pancreatic duct adenocarcinoma (PDAC) accounts for about 85% of all pancreatic malignant neoplasms. It is associated with a dismal prognosis; more than half of affected patients already have metastatic lesions at their

initial diagnosis and the 5-year survival doesn't exceed 7% [3].

Delayed diagnosis of PDAC is attributed to the non-specific symptoms associated with it. These vague symptoms include weight loss, malaise, and abdominal or back pain. Pancreatic duct adenocarcinomas that arise from the pancreatic head may obstruct the bile duct causing obstructive jaundice, or obstruct the pancreatic duct producing steatorrhoea, acute pancreatitis, or symptoms related to gastric outlet obstruction [4].

There are multiple risk factors for PDAC. Cigarette smoking and alcohol consumption, both predispose to PDAC through an ongoing chronic inflammation of the pancreatic tissue and subsequent DNA damage [5]. Chronic pancreatitis is another risk factor for PDAC as it evokes genomic stress and instability [6]. About 10% of PDAC cases have an inherited background or occur as a component of inherited syndromes such as familial breast carcinoma syndrome, Lynch syndrome, and Peutz-Jeghers syndrome [7].

Microscopically, PDAC is characterized by abundant desmoplastic stroma. This desmoplastic stroma is a challenging entity; it exceeds the neoplastic cells, so it hinders the biological study of pancreatic carcinoma. Furthermore, this desmoplastic stroma harbors fibroblastic cells which provide nutrition to the tumor cells and secrete growth factors that stimulate their proliferation [4]. It is believed that abundant desmoplasia reduces the number of vascular channels in the vicinity of the tumor which may in turn reduce the effect of chemotherapeutic agents on tumor cells [3].

Studying the genetic basis and biological nature of a tumor, to identify its altered genes is beneficial to develop more effective, tumor-specific therapeutic agents.

Human epidermal growth factor receptors are a family constituted of 4 members: HER1, HER2, HER3, and HER4. HER receptors are involved in cellular proliferation and differentiation. They have membranous location and intracellular tyrosine kinase catalytic activity [8].

HER2 is a 185 KDa transmembrane glycoprotein, encoded by *Neu* oncogene that is located on chromosome 17. HER2 is involved in cellular proliferation and differentiation. It is over-expressed in many human malignant neoplasms such as carcinoma of mammary, ovarian, gastrointestinal, and urothelial tissues, and its overexpression is associated with aggressive behavior with nodal and distant metastasis [9-13].

Because of its membranous location and its distribution in neoplastic tissues, several studies were conducted to target HER2 with novel therapeutic medications. HER2 has been successfully targeted in mammary and gastric carcinomas with subsequent improvement in the treatment outcome of these tumors [14].

This study aimed to detect the expression of HER2 in PDAC and to correlate HER2 expression with a variety of clinical and pathological features.

Methods

Clinical data and specimen collection

A retrospective, observational study was performed on archived formalin-fixed, paraffin-embedded tissue blocks that belonged to 37 patients who were admitted to Assiut and Sohag Oncology Centers and Sohag University Hospital from January 1st, 2017, to December 31st, 2020. Patients' presentations included obstructive jaundice, abdominal pain, and vomiting. Radiological assessment revealed heterogeneous suspicious pancreatic lesions from which tru-cut biopsies were obtained and examined by the standard staining method; hematoxylin and eosin (H&E). Cases diagnosed as pancreatic carcinoma based on true-cut biopsies underwent pancreaticoduodenectomy (Whipple's procedure) or distal pancreatectomy, depending on tumor location. Inclusion criteria included cases of PDAC who underwent radical operations, tissue blocks with sufficient material, and cases with available clinical data. Exclusion criteria included cases of pancreatic carcinoma other than PDAC, patients who received preoperative chemotherapy or radiotherapy, patients with PDAC who were diagnosed by tru-cut biopsies only and didn't undergo radical operations, cases with insufficient/destructed material or patients with poor clinical data.

Each tumor-laden tissue block was sectioned into 2 tissue sections; one was stained by H&E to confirm the diagnosis of PDAC, determine tumor grade, and detect perineural and/or vascular invasion. The other tissue section was stained by an anti-human HER2 antibody.

Immunohistochemical staining of HER2

Formalin-fixed, paraffin-embedded tissue blocks were cut into 4 µm- 4-thick sections and fastened on coated slides. Two steps of hot and cold xylene were

used to remove paraffin wax. Tissue rehydration was done by downward grades of ethyl alcohol. Endogenous peroxidase activity was impeded by hydrogen peroxide (H2O2) for 10 minutes. Exposure of surface antigens was achieved by heating sections in sodium citrate, PH 6, at 92°C for 4 cycles, 5 minutes each. Sections were incubated overnight with anti-h-Erb B2/HER2, a purified mouse monoclonal Ig G, in a dilution of 1:200, (clone: 191924, Catalog # MAB1129, Concentrated form, Bio-Teche company, Minneapolis, USA). Reaction results were detected by immersing the sections in diaminobenzidine (DAB) for 10 minutes at room temperature (ScyTek, P.O. Box 3286- Logan, Utah 84323, USA). Nuclear counterstaining was done by Harris' Hematoxylin. Sections were dehydrated and cleared by upward grades of ethyl alcohol and xylene, respectively. Sections obtained from oral squamous cell carcinoma were used as a positive control [15]. Negative control was prepared by the replacement of anti-h-Erb B2/HER2 by phosphate-buffered Saline (PBS).

Evaluation of HER2 immunostaining

Immunohistochemical expression of HER2 in examined tumor tissue sections was detected as a brownish granular membranous and cytoplasmic stain.

HER2 was scored based on the percentage of HER2 expression in tumor cells and the intensity of its staining into score 0 (negative) if there is no HER2 expression by tumor cells, 1+ (faint) if there is incomplete membranous staining, 2+ (equivocal) if > 10% of the tumor cells showed moderate staining or 10-30% showed complete intense staining, and 3+ (positive) if > 30% of tumor cells showed intense complete staining [16]. The morphometric assessment was done with a binocular Olympus microscope CX40 RF200 (Olympus Optical Co., LTD).

Statistical analysis

Quantitative data was represented as mean and standard deviation. Categorical data was described as numbers and percentages. Shapiro-Wilk test was used to determine if quantitative data was normally distributed or not. The Chi-square test was used to compare the distribution of categorical data between groups and the *t*-test to compare the means between two groups. A p value <0.05 was considered significant. Statistical analysis was performed with the IBM SPSS Statistics for Windows, Version 26.0. (Armonk, NY: IBM Corp).

Results

Patients' characteristics

The current study enrolled 37 patients with PDAC, and their characteristics are summarized in Table 1. The male to female ratio was 1.3:1 and the age of patients ranged from 40 to 72 years.

The majority (86.4%) of tumors were in the head of the pancreas. Histopathological evaluation of H&E-stained tumor tissue sections (Figure 1) revealed that most of the cases (75.7%) were graded as grade II. The pathological staging (pT stage) of the studied cases revealed a pT1 in 7 cases, pT2 in 16 cases, pT3 in 10, and pT4 in 4. Evaluation of H&E-stained tissue sections prepared from the resected regional lymph nodes revealed neoplastic deposits in 21 cases.

Table 1: Clinical and pathological characteristics of 37 patients with pancreatic duct adenocarcinoma

Variable	Description
	Mean (SD)
Age (years)	57.2 (8.8)
	***n* (%)**
Sex	
Men	21 (56.8
Women	16 (43.2)
Tumor location	
Pancreatic head	32 (86.4)
Pancreatic body	5 (13.6)
Tumor Grade	
Grade I	2 (5.4)
Grade II	28 (75.7)
Grade III	7 (18.9)
pT stage	
pT1	7 (18.9)
pT2	16 (43.2)
pT3	10 (27.1)
pT4	4 (10.8)
Nodal status	
Positive	21 (56.8)
Negative	16 (43.2)
Lymphovascular invasion	
Present	33 (89.2)
Absent	4 (10.8)
Perineural invasion	
Present	28 (75.7)
Absent	9 (24.3)

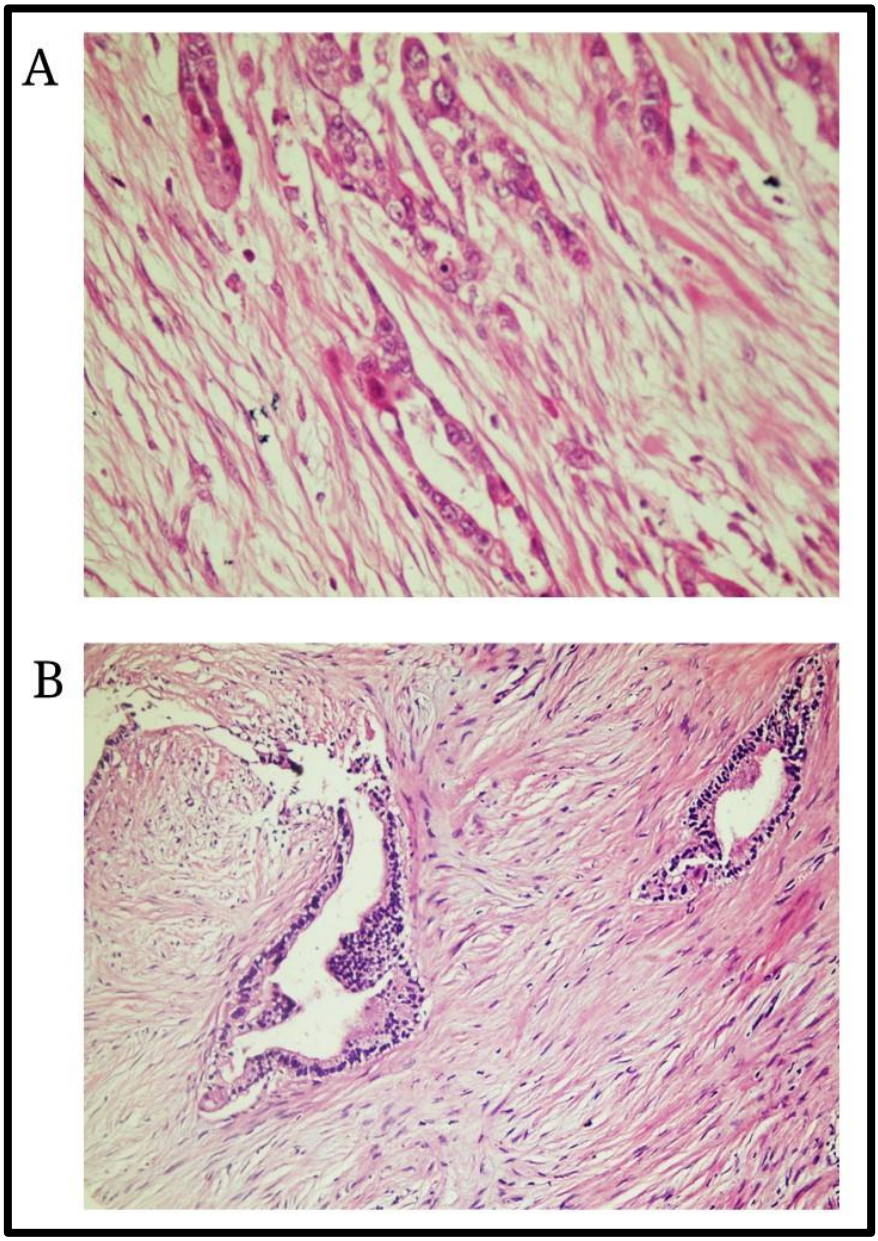

Figure 1: A- Pancreatic duct adenocarcinoma (X200), **B-** Abundant desmoplastic stroma (X200)

Table 2: Lymphovascular invasion and perineural invasion in relation to tumor grade and stage and lymph node status

Variable		Lymphovascular invasion			Perineural invasion		
		Positive (*n*=33)	Negative (*n*=4)	*p* value	Positive (*n*=28)	Negative (*n*=9)	*p* value
		n (%)			*n* (%)		
Tumor grade	G I	1 (3)	1 (25)	0.133	0	2 (22.2)	**0.014**
	G II	25 (75.8)	3 (75)		21 (75)	7 (77.8)	
	G III	7 (21.2)	0		7 (25)	0	
pT stage	pT 1	4 (12.1)	3 (75)	**0.023**	0	7 (77.8)	**<0.0001**
	pT 2	15 (45.5)	1 (25)		14 (50)	2 (22.2)	
	pT 3	10 (30.3)	0		10 (35.7)	0	
	pT 4	4 (12.1)	0		4 (14.3)	0	
Lymph node status	Positive	21 (63.6)	0	**0.028**	20 (71.4)	1 (11.1)	**0.002**
	Negative	12 (36.4)	4 (100)		8 (28.6)	8 (88.9)	

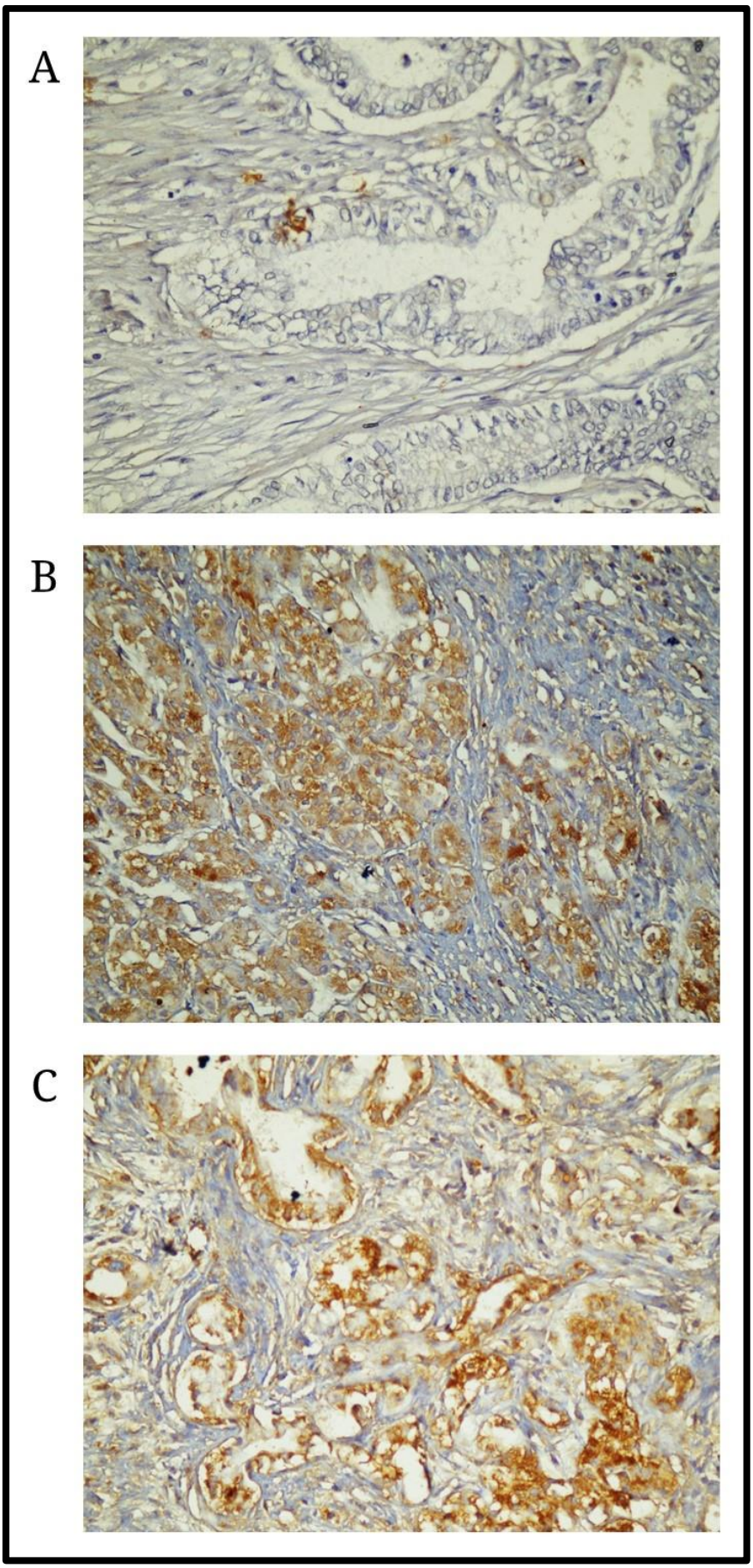

Figure 2: Pancreatic duct adenocarcinoma: A- Negative expressions of HER2 (X400), **B & C -** Positive expression of HER2 (X200).

Lymphovascular invasion (LVI) and Perineural invasion (PNI) were detected in 33 (89.2%) and 28 (75.7%) cases, respectively. Their relationship to the tumor grade, pT stage, and status of regional lymph nodes is illustrated in Table 2.

As regards the significance of LVI, all cases of PDAC that showed more advanced tumor stages (pT3 and pT4), showed angio-invasive emboli in their H&E-stained tissue sections (p = 0.023). Similarly, lympho-vascular emboli were detected in all cases of PDAC that showed neoplastic deposits in their regional lymph nodes (p = 0.028).

Pancreatic intraepithelial neoplasia was detected in 75.7% of enrolled cases of PDAC. PNI was significantly associated with higher tumor grades (p = 0.014), advanced tumor stages (p < 0.0001), and the presence of regional lymph node tumor deposits (p = 0.002).

Immunohistochemical study of HER2

HER2 was detected in 34 out of 37 studied cases (91.9%). Faint expression of HER2 was detected in 6 (16.2%) cases, equivocal expression in 9 (24.3%), and positive HER2 expression in 19 (51.4%) (Figure 2).

The relationship between HER2 expression and the studied patient and tumor characteristics is shown in Table 3.

There was a statistically meaningful association between tumor grade and HER2 expression by tumor cells. Eighty-sex percent of grade III were HER2 positive compared to 43% of grade I-II (p = 0.0463). Similarly, on correlating pathological tumor stages with levels of HER2 expression, we found that 80% of pT3 and 100% of pT4 showed positive HER2 expression (p = 0.002). HER2 expression correlated significantly as well with the status of regional lymph nodes. Two-thirds of PDAC cases that revealed negative and/or non-specific HER2 expression in their immune-stained tumor tissue sections, didn't show metastatic deposits in their regional lymph nodes. On the other hand, 78.95% of cases that were scored as positive for HER2 showed regional lymph node metastases (p = 0.005). A significant relationship was detected between HER2 expression and both PNI (p < 0.0001) and LVI (p = 0.046). All cases that scored positive for HER2 revealed perineural and lympho-vascular invasion.

Age, gender, and tumor location did not correlate significantly with HER2 expression.

Table 3: Correlation between HER2 expression and the clinical and pathological criteria of patients with pancreatic duct adenocarcinoma

Variable	HER2 Expression		*p* value
	Negative / Faint / Equivocal (*n*=18)	Positive (*n*=19)	
	Mean (SD)		
Age (years)	59.8 (7.3)	54.8 (9.6)	0.08
	n (%)		
Gender			
Male	9 (50)	12 (63.2)	0.42
Female	9 (50)	7 (36.8)	
Tumor location			
Pancreatic head	16 (88.9)	16 (84.2)	1
Pancreatic body	2 (11.1)	3 (15.8)	
Tumor Grade			
Grade I-II	17 (94.4)	13 (68.4)	**0.0463**
Grade III	1 (5.6)	6 (31.6)	
pT stage			
pT 1	7 (38.9)	0	**0.002**
pT2	9 (50)	7 (36.8)	
pT3	2 (11.1)	8 (42.1)	
pT4	0	4 (21.1)	
Nodal status			
Negative	12 (66.7)	4 (21.1)	**0.005**
Positive	6 (33.3)	15 (79)	
Lymphovascular invasion			
Absent	4 (22.2)	0	**0.046**
Present	14 (77.8)	19 (100)	
Perineural invasion			
Absent	9 (50)	0	**< 0.0001**
Present	9 (50)	19	

Discussion

Despite the currently available surgical approaches and therapeutic medications, the prognosis of PDAC remains dismal [4]. The aggressive behavior of PDAC is related to cumulative genetic mutations which are translated histologically and

clinically in the form of LVI and PNI, involvement of tumor resection margins, extension to peri-pancreatic structures, and involvement of regional lymph nodes. All these features are responsible for the frequently encountered postoperative local recurrence and distant metastasis [1].

Lymphovascular invasion in malignant tumors is a threatening issue. It is associated with increased liability for nodal and distant metastasis with subsequent short disease-free survival and poor prognosis. In the current study, most of the cases of PDAC were associated with LVI. This angio-invasion was associated with advanced tumor stages and the presence of nodal metastasis. This is in keeping with what was observed by Epstein et al [17] and Takahashi et al [18]. Takahashi and co-workers found that the development of LVI in PDAC didn't occur because of overexpression of angiogenesis or lymphogenesis-promoting genes. Furthermore, there was no relationship between the occurrence of LVI in PDAC and the production of extracellular matrix-degrading products. Instead, they found that LVI was associated with augmented cell proliferation and overproduction of cell cycle-promoting genes [18].

Perineural invasion is a characteristic feature of PDAC to the extent that PNI must be reported in pathological reports of PDAC according to the seventh edition of TNM classification [19]. In the current study, PNI was associated with less differentiation of PDAC, advanced stage, and the presence of nodal involvement. This is in line with what was reported by Felsenstein et al that the presence of PNI is associated with positive surgical resection margins and shorter disease-free survival [20].

Another challenging issue in PDAC is that it is characterized by abundant desmoplastic stroma which quantitatively exceeds the tumor cells. This interferes with the genetic study of the tumor cells and provides a mechanical barrier against chemotherapeutic agents [3]. These challenges require the development of more targeted biological medications that can influence the tumor despite these hindrances.

HER2 is a transmembrane glycoprotein, encoded by a gene located on chromosome 17. HER2 is structurally and functionally like human epidermal growth factor receptors, hence it acquired its name. HER2 is involved in cellular proliferation and differentiation [21]. It is aberrantly expressed in several human carcinomas of mammary, ovarian, gastrointestinal, and urothelial origins; and such deregulated expression is associated with aggressive behavior and nodal and distant metastases [9 - 13].

In the current study, we evaluated the immunohistochemical expression of HER2 in PDAC and correlated HER2 expression to clinical and pathological parameters. We found that HER2 overexpression is associated with advanced pathological tumor stage and the presence of nodal metastasis; both findings are associated with unfavorable prognosis. These observations were in keeping with a previous study that was done to investigate the HER2 genetic profile and its protein status in 55 patients with PDAC. The study found that HER2 genetic amplification and/or protein overexpression were associated with a worse prognosis and shorter survival [22]. These observations could be explained on the basis that HER2 is involved in the activation of intracellular signaling pathways such as mitogen-activating protein kinase that stimulates cellular proliferation [13]. Furthermore, deregulated HER2 expression inhibits Cyclin D1 and p27 which control the cell cycle at the G1/S phase; thereby, deregulated expression of HER2 enhances uncontrolled cellular proliferation that acquires the tumor aggressive features [14].

The potential role of HER2 in facilitating LVI and PNI has been investigated in the current study. HER2 was found to be associated with the occurrence of LVI and PNI. We suppose that uncontrolled tumor cell proliferation by aberrant HER2 expression results in the evolution of highly aggressive tumor clones that can invade the vascular endothelial cells and the neuronal axons. We found also that HER2 is significantly overexpressed in high tumor grades which may be explained by its promoting effect on cell proliferation and inhibitory effect on cell cycle control agents. However, there was no detected relation between HER2 expression and tumor grade. This could be attributed to the small sample size.

Conclusion: HER2 expression is significantly associated with aggressive and metastatic PDAC. Targeting HER2 by biological therapeutic agents may represent a glimmer of hope in controlling PDAC.

Acknowledgments

The authors would like to thank all patients, physicians and medical assistants who participated in this study.

Authors' contribution

Conception & Design: Mohammed MH & Sayed MH; Acquisition, analysis, or interpretation of data: Ahmed NA, Ahmed MMA; Drafting / revising the manuscript: Mohammed MH, Ahmed NA & Sayed MH; Approval of the final version of

the manuscript: All authors; Agreement to be accountable for all aspects of the work: All authors.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

Data availability

Data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical considerations

The study was approved by the Ethical Committee of Sohag University (registration ID: Soh-Med-23-09-4PD).

Funding

Not applicable.

Study registration

ClinicalTrials.gov PRS (ID: NCT06055647).

References

1. Taherian M, Wang H, Wang H. Pancreatic ductal adenocarcinoma: Molecular pathology and predictive biomarkers. *Cells*. 2022; 11(19): 3068.
2. Hackeng WM, Hruban RH, Offerhaus GJ, Brosens LA. Surgical and molecular pathology of pancreatic neoplasms. *Diagn Pathol*. 2016; 11(1): 47.
3. Cui Y, Wu J, Zong M, et al. Proteomic profiling in pancreatic carcinoma with and without lymph node metastasis. *Int J Carcinoma*. 2009;124(7): 1614-1621.
4. Wood LD, Canto MI, Jaffee EM, Simeone DM. Pancreatic carcinoma: pathogenesis, screening, diagnosis, and treatment. *Gastroenterology*. 2022; 163(2): 386-402.e1.
5. Li B, Nelson MS, Savari O, Loeffler AG, Eliceiri KW. Differentiation of pancreatic ductal adenocarcinoma and chronic pancreatitis using graph neural networks on histopathology and collagen fiber features. *J Pathol Inform*. 2022; 13: 100158.
6. Howes N, Neoptolemos JP. Risk of pancreatic ductal adenocarcinoma in chronic pancreatitis. *Gut*. 2002; 51(6): 765-766.
7. Esposito I, Konukiewitz B, Schlitter AM, Klöppel G. Pathology of pancreatic ductal adenocarcinoma: Facts, challenges and future developments. *World J Gastroenterol*. 2014; 20(38): 13833-13841.
8. Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in carcinoma: Overexpression and therapeutic implications. *Mol Biol Int*. 2014; 2014:852748.
9. Molina R, Escudero JM, Muñoz M, Augé JM, Filella X. Circulating levels of HER-2/neu oncoprotein in breast carcinoma. *Clin Chem Lab Med*. 2012; 50(1): 5-21.
10. Sidhanth C, Bindhya S, Krishnapriya S, et al. Phosphoproteome of signaling by ErbB2 in ovarian carcinoma cells. *Biochim Biophys Acta Proteins Proteom*. 2022; 1870(4): 140768.
11. Zhu Y, Zhu X, Wei X, Tang C, Zhang W. HER2-targeted therapies in gastric carcinoma. *Biochim Biophys Acta Rev Carcinoma*. 2021; 1876(1): 188549.
12. Han Y, Peng Y, Fu Y, et al. MLH1 Deficiency Induces Cetuximab Resistance in Colon Carcinoma via Her-2/PI3K/AKT Signaling. *Adv Sci (Weinh)*. 2020; 7(13): 2000112.
13. Albarrán V, Rosero DI, Chamorro J, et al. Her-2 targeted therapy in advanced urothelial carcinoma: From monoclonal antibodies to antibody-drug conjugates. *Int J Mol Sci*. 2022; 23(20): 12659.
14. Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human carcinoma pathogenesis. *Oncogene*. 2007; 26(45): 6469-6487.
15. Papavasileiou D, Tosios K, Christopoulos P, Goutas N, Vlachodimitropoulos D. Her-2 immunohistochemical expression in oral squamous cell carcinoma is associated with polysomy of chromosome 17, not Her-2 amplification. *Head Neck Pathol*. 2009; 3(4): 263-270.
16. Lehmann-Che J, Amira-Bouhidel F, Turpin E, et al. Immunohistochemical and molecular analyses of HER2 status in breast carcinoma are highly concordant and complementary approaches. *Br J Carcinoma*. 2011; 104(11): 1739-1746.
17. Epstein JD, Kozak G, Fong ZV, et al. Microscopic lymphovascular invasion is an independent predictor of survival in resected pancreatic ductal adenocarcinoma. *J Surg Oncol*. 2017; 116(6): 658-664.
18. Takahashi H, Katsuta E, Yan L, Tokumaru Y, Katz MHG, Takabe K. Transcriptomic profile of lymphovascular invasion, a known risk factor of pancreatic ductal adenocarcinoma metastasis. *Cancers (Basel)*. 2020; 12(8): 2033.
19. Sobin LH, Compton CC. TNM seventh edition: What's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer." *Cancer*. 2010; 116(22): 5336-5339.
20. Felsenstein M, Lindhammer F, Feist M, et al. Perineural invasion in pancreatic ductal adenocarcinoma (PDAC): A saboteur of curative intended therapies? *J Clin Med*. 2022; 11(9): 2367.
21. Rubin I, Yarden Y. The basic biology of HER2. *Ann Oncol*. 2001; 12(Suppl 1): S3-S8.
22. Han SH, Ryu KH, Kwon AY. The prognostic impact of HER2 genetic and protein expression in pancreatic carcinoma-HER2 protein and gene in pancreatic carcinoma. *Diagnostics (Basel)*. 2021; 11(4): 653.