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RESEARCH ARTICLE

NRF-2 and KEAP-1 Expression in Pancreatic Cancer Patients: A Clinicopathological Study

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ABSTRACT

Background: Pancreatic cancer is one of the main causes of death in many nations. A delayed diagnosis would be detrimental because it has a bad prognosis and has poor specific symptoms and indicators. Additionally, it demonstrates a significant level of chemotherapeutic treatment resistance. Oxidative stress has a key role in the aetiology of pancreatic cancer. The transcription factor NRF2 controls how the body responds to oxidative stress since it interacts with Keap1, causing it to be targeted for ubiquitylation and proteasomal destruction. In cancer biology, Nrf2 has a double-edged sword depending on the stage of carcinogenesis. **Aim**: The study aimed to investigate the expression of Keap1 and Nrf2 in pancreatic cancer cells and to compare the findings to other clinicopathological parameters. **Materials and Methods**: A retrospective study was done on 45 pancreatic carcinoma patients. Immunohistochemical staining for Nrf2 and Keap1 was done and correlated with clinicopathological parameters. **Results**: Significant statistical associations were found between NRF2 expression with duodenal and pancreatic infiltration. A significant relation was seen between Keap1 expression and duodenal infiltration as well as between NRF2 expression and Keap1 expression in tumor cells. **Conclusion**: Nrf2 and Keap1 may play important roles in pancreatic carcinogenesis.

Keywords: Delayed diagnosis Nrf2, Keap1, Pancreatic cancer, poor outcome

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INTRODUCTION

Pancreatic cancer is one of the main causes of death in many nations. A delayed diagnosis would be detrimental because it has a bad prognosis and has poor specific symptoms and indicators. Additionally, it demonstrates a significant level of chemotherapeutic treatment resistance (Lister et al., 2011; Capasso et al., 2018). Pancreatic cancer has many risk factors and a multistep carcinogenesis process like colorectal cancer (Capasso et al., 2018). Oxidative stress plays a key role in the etiology of pancreatic cancer (Cykowiak and Krajka-Ku´zniak, 2022). Regulation of oxidative stress levels is important in both tumor development and anti-cancer therapies. Reactive oxygen species (ROS) levels rise in cancer cells, but they adapt to oxidative stress by developing antioxidant defense mechanisms (Canning et al., 2015).

The transcription factor known as nuclear factor E2-related factor 2 (NRF2) controls how the body responds to oxidative stress. It plays an important role in maintaining homeostasis by inducing the expression of many genes that control antioxidant defense (Purohit et al., 2020). The E3 ubiquitin ligase Keap1 (Kelch ECH-associating protein 1) normally keeps NRF2 low. The transcription factor Nrf2 interacts with Keap1, causing it to be targeted for ubiquitylation and proteasomal destruction (Jung et al., 2018). This provides a regulated antioxidant response, detoxification, and cancer prevention (Hartikainen et al., 2012). However, excessive stress causes the dissociation of Nrf2 from Keap1, causing constitutive activation of Nrf2 and increasing the expression of genes required for the growth of cancer cells, ferroptosis, autophagy, angiogenesis, drug resistance, and metastasis (Cykowiak and Krajka-Ku´zniak, 2022).

So, in cancer biology, Nrf2 has a double-edged sword depending on the stage of carcinogenesis. In the early stages, Nrf2 activation prevents carcinogenesis at late stages, it causes cancer cell progression (Cykowiak and Krajka-Ku´zniak, 2022).

Numerous cancerous cells, including ductal adenocarcinomas and pancreatic cancer cell lines, express Nrf2 at an increased level. This explains the capacity of these cells to respond to stress signals, resist chemotherapeutic interventions, and suffer poor patient survival (Lister et al., 2011). So, the study aimed to investigate the expression of Keap1 and Nrf2 in pancreatic cancer cells and to compare the findings to other clinicopathological parameters.

MATERIAL AND METHODS

Patients and specimens

A retrospective study was done on 45 patients who were pathologically diagnosed with pancreatic carcinoma and treated at Mansoura University Oncology Center from January 2014 to December 2021.Clinical data were collected from patients' records, including patients age and gender. Pathological data including, tumor grade, nodal metastasis, peri-neural invasion, lympho- vascular invasion, tumor extension and pancreatic safety margins and TNM staging were assessed by three pathologists. Distant metastasis was approved clinically, radiologically and pathologically. The median follow-up duration was 14 (1-99) months.

Immunohistochemical staining

Tissue microarray was prepared from formalin-fixed paraffin embedded pancreatic cancer tissue samples. Sections of 4-µm thickness were mounted on glass slides, and then deparaffinized in graded alcohol. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 20 min. The slides were incubated overnight at 4°C with the primary antibody NRF2 (rabbit polyclonal antibody) and Keap1 (mouse monoclonal antibody). The primary antibody binding was detected using peroxidase labelled secondary antibody and chromogen, diaminobenzidine (DAB) DakoEnVision™ Detection Systems (Dako, Denmark) according to the manufacturer's recommendations. Tissue sections were counterstained with hematoxylin. Three pathologists examined immune stain for both NRF2 and Keap1. Results were scored for both nuclear and cytoplasmic NRF2 and cytoplasmic Keap1 expression in tumor cells as follows, Negative: 0%–5%; Mild: >5% to 25; Moderate: >25% to 75%; and High :>75% to 100%. The results are then divided into 2 groups: low expression (25%) and high expression (>25%) (Hartikainen et al., 2012).

Statistical Analysis

The Statistical Package of Social Science (SPSS) Program for Windows (Standard Version 21) was used to analyze the data. The Kolmogorov-Smirnov test was initially used to determine whether the data were normal. Numbers and percentages were used to describe qualitative data. The association between categorical variables was tested using the Chi-square test, while the Fischer exact test and Montecarlo test were used when the expected cell count was less than 5. For data that was regularly distributed, continuous variables were presented as the mean SD (standard deviation). Two markers were correlated using Spearman correlation. The Kaplan-Meier test was employed for the survival analysis, and the Log-Rank test was performed to establish the statistical significance of differences between curves. For all the above-mentioned statistical tests, the significance threshold is fixed at 5% (p-value). The results were considered significant when the $p \leq 0.05$. The smaller the p-value obtained, the more significant the results.

RESULTS

Descriptive data

The study was applied to 45 pancreatic cancer cases obtained from 18 female and 27 male patients. Thirty cases were more than or equal to 60 years old, the clinical criteria of these cases are presented in Tables 1 and 2. Forty-two cases (93.3%) were located in the pancreatic head and only 3 cases (6.7%) were seen in the body and uncinate process. Thirty-five cases (77.8%) were less than or equal to 2.5cm and 43 cases (95.6%) were of mild to moderate differentiation. Twenty-eight cases (62.2%) were seen with tumor infiltration to the pancreas and 27 cases (60.0%) were seen with positive nodal metastasis.

Table 1. Relation between NRF2 expression and clinico-pathological parameters

	Total	NRF2		χ^2 (p value)
		Low expression (n=20, 44.4%)	High expression (n=25, 55.6%)	
Age/ years				χ^2 =0.18
• <60 y	15 (33.3%)	6 (40.0%)	9 (60.0%)	P=0.67
• ≥60 y	30 (66.7%)	14 (46.7%)	16 (53.3%)	
Gender				χ^2 =1.5
• Male	27 (60.0%)	10 (37.0%)	17 (63.0%)	P=0.22
• Female	18 (40.0%)	10 (55.6%)	8 (44.4%)	
Tumor site				FET
• Head	42 (93.3%)	18 (42.9%)	24 (57.1%)	P=0.58
• Body\ uncinate process	3 (6.7%)	2 (66.7%)	1 (33.3%)	
Tumor size				χ^2 =0.16
• ≤2.5	35 (77.8%)	15 (42.9%)	20 (57.1%)	P=0.69
• >2.5	10 (22.2%)	5 (50.0%)	5 (50.0%)	
Grade				FET
• Poorly differentiated	2 (4.4%)	1 (50.0%)	1 (50.0%)	P=1.0
• Mild\moderately differentiated	43 (95.6%)	19 (44.2%)	24 (55.8%)	
Duodenal infiltration				χ^2 =7.95
• No	17 (37.8%)	3 (17.6%)	14 (82.4%)	P=0.005*
• Yes	28 (62.2%)	17 (60.7%)	11 (39.3%)	
LN				χ^2 =0
• Positive	27 (60.0%)	12 (44.4%)	15 (55.6%)	P=1.0
• Negative	18 (40.0%)	8 (44.4%)	10 (55.6%)	
TNM				MC
• IA	4 (8.9%)	1 (25.0%)	3 (75.0%)	P=0.75
• IB	9 (20.0%)	5 (55.6%)	4 (44.4%)	
• IIA	4 (8.9%)	1 (25.0%)	3 (75.0%)	
• IIB	23 (51.1%)	10 (43.5%)	13 (56.5%)	
• III	5 (11.1%)	3 (60.0%)	2 (40.0%)	
TNM.GP				χ^2 =0.12
• IIA\IA	17 (37.8%)	7 (41.2%)	10 (58.8%)	P=0.73
• IB\IIB\III	28 (62.2%)	13 (46.4%)	15 (53.6%)	
Lymph vascular emboli				χ^2 =0.21
• Positive	33 (73.3%)	14 (42.4%)	19 (57.6%)	P=0.65
• Negative	12 (26.7%)	6 (50.0%)	6 (50.0%)	
Survival				χ^2 =1.40
• Survived	16 (35.6%)	9 (56.2%)	7 (43.8%)	P=0.24
• Died	29 (64.4%)	11 (37.9%)	18 (62.1%)	
Metastasis				χ^2 =3.37
• Positive	27 (60.0%)	15 (55.6%)	12 (44.4%)	P=0.06
• Negative	18 (40.0%)	5 (27.8%)	13 (72.2%)	
Perineural invasion				χ^2 =0.29
• Positive	25 (55.6%)	12 (48.0%)	13 (52.0%)	P=0.59
• Negative	20 (44.4%)	8 (40.0%)	12 (60.0%)	
Pancreatic safety margin				FET
• Free	39 (86.7%)	17 (43.6%)	22 (56.4%)	P=1.0
• Infiltrated	6 (13.3%)	3 (50.0%)	3 (50.0%)	

χ2: Chi square test, FET: Fisher exact test, MC: Monte Carlo test, *significant p≤0.05

Table 2. Relation between Keap1 expression and clinico-pathological parameters

	Total	Keap1		χ^2 (p value)
		Low expression (n=19, 42.2%)	High expression (n=26, 57.8%)	
Age/ years • <60 y • ≥60 y	 15 (33.3%) 30 (66.7%)	 6 (40.0%) 13 (43.3%)	 9 (60.0%) 17 (56.7%)	χ^2 =0.046 P=0.83
Gender • Male • Female	 27 (60.0%) 18 (40.0%)	 10 (37.0%) 9 (50.0%)	 17 (63.0%) 9 (50.0%)	χ^2 =0.74 P=0.39
Tumor site • Head • Body\ uncinate process	 42 (93.3%) 3 (6.7%)	 17 (40.5%) 2 (66.7%)	 25 (59.5%) 1 (33.3%)	FET P=0.56
Tumor size • ≤2.5 • >2.5	 35 (77.8%) 10 (22.2%)	 14 (40.0%) 5 (50.0%)	 21 (60.0%) 5 (50.0%)	χ^2 =0.32 P=0.57
Grade • Poorly differentiated • Mild\moderately differentiated	 2 (4.4%) 43 (95.6%)	 1 (50.0%) 18 (41.9%)	 1 (50.0%) 25 (58.1%)	FET P=1.0
Duodenal infiltration • No • Yes	 17 (37.8%) 28 (62.2%)	 3 (17.6%) 16 (57.1%)	 14 (82.4%) 12 (42.9%)	χ^2 =6.76 P=0.009*
LN • Positive • Negative	 27 (60.0%) 18 (40.0%)	 11 (40.7%) 8 (44.4%)	 16 (59.3%) 10 (55.6%)	χ^2 =0.06 P=0.81
TNM • IA • IB • IIA • IIB • III	 4 (8.9%) 9 (20.0%) 4 (8.9%) 23 (51.1%) 5 (11.1%)	 1 (25.0%) 5 (55.6%) 1 (25.0%) 9(39.1%) 3 (60.0%)	 3 (75.0%) 4 (44.4%) 3 (75.0%) 14(60.9%) 2 (40.0%)	MC P=0.703
TNM.GP • IIA\IA • IB\IIB\III	 17 (37.8%) 28 (62.2%)	 7 (41.2%) 12 (42.9%)	 10 (58.8%) 16 (57.1%)	χ^2 =0.012 P=0.912
Lymph vascular emboli • Positive • Negative	 33 (73.3%) 12 (26.7%)	 14 (42.4%) 5 (41.7%)	 19 (57.6%) 7 (58.3%)	χ^2 =0.002 P=0.964
Survival • Survived • Died	 16 (35.6%) 29 (64.4%)	 8 (50.0%) 11 (37.9%)	 8 (50.0%) 18 (62.1%)	χ^2 =0.62 P=0.43
Metastasis • Positive • Negative	 27 (60.0%) 18 (40.0%)	 14 (51.9%) 5 (27.8%)	 13 (48.1%) 13 (72.2%)	χ^2 =2.57 P=0.11
Perineural invasion • Positive • Negative	 25 (55.6%) 20 (44.4%)	 11 (44.0%) 8 (40.0%)	 14 (56.0%) 12 (60.0%)	χ^2 =0.07 P=0.78
Pancreatic safety margin • Free • Infiltrated	 39 (86.7%) 6 (13.3%)	 16 (41.0%) 3 (50.0%)	 23 (59.0%) 3 (50.0%)	FET P=1.0

Twenty-eight cases (62.6%) were seen in stage IB\IIB or III. Lympho-vascular invasion was seen in 33 cases (73.3%), 27 cases (60.0%) were seen with distant metastasis, 25 cases (55.6%) with peri-neural invasion and 39 cases (86.7%) with free pancreatic safety margins. Out of 45 cases, 29 died (64.4%) and 16 survived (35.6%).

NRF2 expression and clinicopathological features

Among the 45 cases, NRF2 expression in tumour cells was high 25 cases (55.6%) and 20 cases (44.4%) showed low expression (Figure 1). The relationship between NRF2 expression and the clinicopathological parameters is summarized in Table 1. Significant statistical associations were found between NRF2 expression and duodenal infiltration (P=0.005), as (82.4%) of high expression was seen in tumors with no pancreatic infiltration whereas (60.7%) of low expression cases were seen in tumors with pancreatic infiltration.

Keap1 expression and clinic-pathological features

Out of 45 cases, twenty-six cases (57.8%) showed high Keap1 expression in tumor cells while 19 (42.2%) showed low expression. The relationship between Keap1 expression and the clinicopathological parameters is summarized in Table 2. A significant relation was seen between Keap1 expression and duodenal infiltration (P=0.009), as (82.4%) of high expression was seen in tumors with no duodenal infiltration where (57.1%) of low expression cases was seen in duodenal infiltration. A significant correlation between NRF2 expression and Keap1 expression in tumor cells (≤0.001) was seen in Table 3.

Overall survival and disease-free survival

As shown in Table 4, there was a significant association between overall survival and peri-neural invasion. Negative peri-neural invasion showed better overall survival than cases with positive invasion (P=0.039) (Figure 2). No significant associations were found between overall survival and NRF2 and Keap1 expressions in tumor cells (p=0.356, P=0.434, respectively). Table 5 showed significant association between free survival, perineural invasion and pancreatic safety margin.

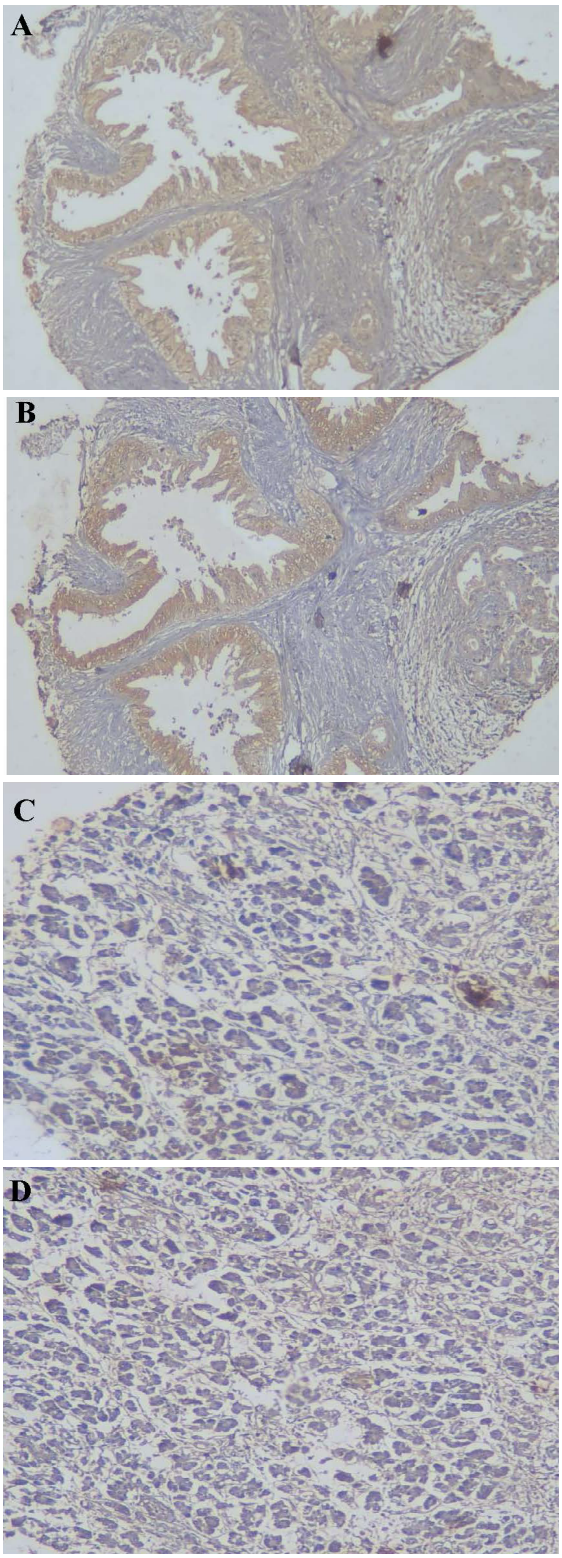

Figure 1. (A) Photomicrograph showing high and cytoplasmic Expression (>25%) of NRF2 in well-differentiated tumor (100×). (B) Previous case that shows High cytoplasmic keap1 expression(>25%).C) Photomicrograph showing low expression (<25%) of NRF2 in poorly differentiated tumor D) Previous case that shows low expression (<25%) of keap1.

Cases with perineural invasion showed shorter disease-free survival (P=0.003) (Figure 2). Cases with infiltrated pancreatic safety margin showed shorter disease-free survival (P=0.003). Also, there was no significant association between NRF2 and Keap1 expression and disease-free survival (P=0.301, P=0.393, respectively).

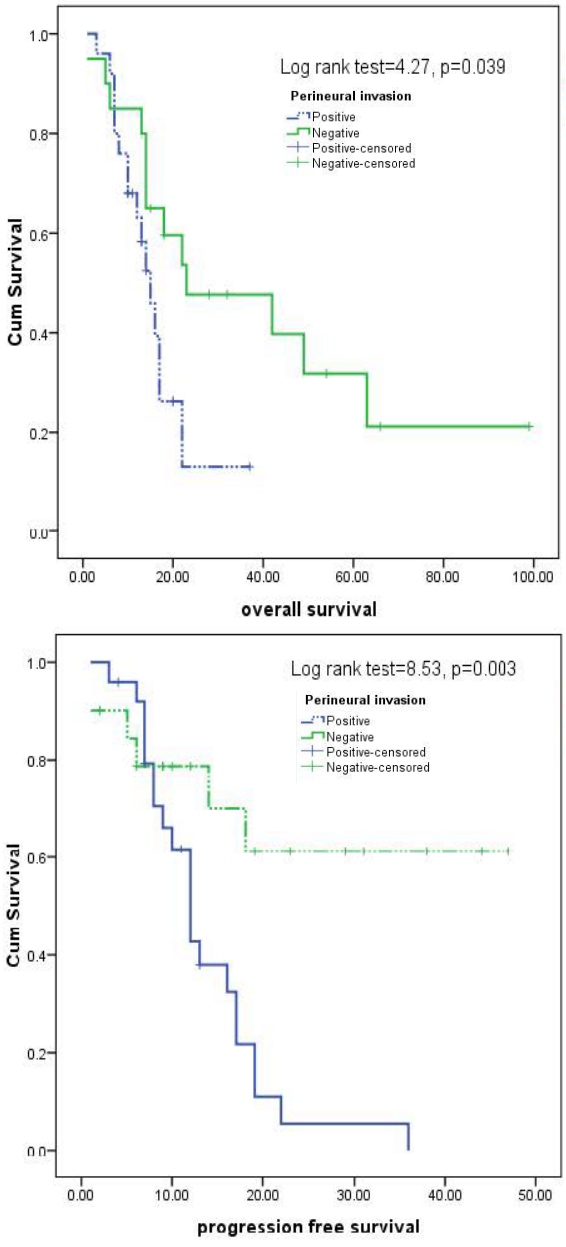

Figure 2. (A) Kaplan-Meier survival curve for overall survival among patients with and without perineural invasion (B) Kaplan-Meier survival curve for progression-free survival among patients with and without perineural invasion.

DISCUSSION

Pancreatic cancer is one of the leading causes of death in many countries. It has a poor prognosis, which is related to poor specific symptoms and signs and delayed diagnosis (Lister et al., 2011; Capasso et al., 2018). Pancreatic cancer has a multistep carcinogenesis process like colorectal cancer, in which oxidative stress plays an important role (Capasso et al., 2018). The transcription factor nuclear factor E2-related factor 2 (NRF2) controls the oxidative stress response. It contributes significantly to homeostasis maintenance by promoting the expression of several genes that regulate antioxidant defense (Purohit et al., 2020). The E3 ubiquitin ligase Keap1 (Kelch ECH-associating protein 1) normally keeps NRF2 low. The transcription factor Nrf2 interacts with Keap1, causing it to be targeted for ubiquitylation and proteasomal destruction (Jung et al., 2018).

However, excessive stress causes the dissociation of Nrf2 from Keap1, causing constitutive activation of Nrf2 and increasing the expression of genes necessary for cancer cell proliferation, ferroptosis, autophagy, angiogenesis, drug resistance, and metastasis. So, in cancer biology, Nrf2 has a double-edged sword depending on the stage of carcinogenesis. In the early stages, Nrf2 activation prevents carcinogenesis at late stages, it causes cancer cell progression (Cykowiak and Krajka-Ku´zniak, 2022).

Numerous cancerous cells, including ductal adenocarcinomas and pancreatic cancer cell lines, express Nrf2 at an increased level. This explains why these cells can react to stress signals, resist chemotherapy treatments, and have low patient survival rates (Lister et al., 2011).

In this work, 45 cases of pancreatic cancer were stained by NRF2 and Keap1 antibodies to assess their expression in pancreatic cancer cells and correlate these results with clinicopathological parameters and patient survival. There was a high nuclear expression of NRF2 staining in 55.6%, and 44.4% showed low expression. This expression was reported in different studies on different organs (Ondodera et al., 2014).

Table 3. Correlation between NRF2 and Keap1

	NRF2	
	r	**P value**
Keap1	0.956	≤0.001

Table 4. Kaplan-Meier overall survival

	Overall Survival				
	Median Survival Time	**Std. Error**	**95% CI**	**Log Rank test**	**P - value**
Age/ years					
• <60 y	46.527	12.912	21.22-71.83		
• ≥60 y	22.099	4.113	14.03-30.16	3.76	0.052
Gender					
• Male	34.409	8.420	17.90-50.91		
• Female	26.832	5.438	16.17-37.49	0.012	0.912
Tumor site					
• Head	33.743	6.152	21.68-45.8		
• Body\ uncinate process	16.667	6.158	4.59-28.73	0.339	0.560
Tumor size					
• ≤2.5	38.672	7.439	24.09-53.25		
• >2.5	16.800	4.326	8.32-25.27	2.96	0.085
Grade					
• Mild\mod differentiated	33.780	6.110	21.8-45.7		
• Poorly differentiated	9.500	4.500	0.68-18.3	3.61	0.057
Duodenal infiltration					
• No	19.376	4.267	11.01-27.73		
• Yes	38.266	7.807	22.96-53.56	2.53	0.112
LN					
• Positive	30.331	5.181	20.17-40.48		
• Negative	28.186	7.879	12.74-43.62	0.973	0.324
5TNM.GP					
• IIA\IA	27.441	7.727	12.29-42.58		
• IB\IIB\III	30.637	5.188	20.46-40.80	1.29	0.255
Lymph vascular emboli					
• Positive	32.312	6.321	19.92-44.7		
• Negative	22.117	4.092	14.09-30.13	0.009	0.925
Metastasis					
• Positive	17.822	2.739	12.45-23.19		
• Negative	40.149	8.210	24.05-56.24	2.62	0.105
Perineural invasion					
• Positive	16.350	2.383	11.67-21.02		
• Negative	41.882	8.540	25.14-58.62	4.27	0.039*
Pancreatic safety margin					
• Free	32.310	6.869	18.84-45.8		
• Infiltrated	32.667	8.569	15.87-49.5	0.118	0.73
NRF2					
• Low expression	31.281	6.303	18.92-43.63		
• High expression	31.313	7.659	16.30-46.32	0.852	0.356
Keap1					
• Low expression	30.820	6.286	18.50-43.14		
• High expression	31.837	7.744	16.65-47.01	0.613	0.434
Overall OS	32.582	5.869	21.07-44.08		

Log Rank (Mantel-Cox) was used, CI: confidence interval

Table 5. Kaplan-Meier disease free survival

	Disease free Survival				
	Median Survival Time	**Std. Error**	**95% CI**	**Log Rank test**	**P - value**
Age/ years					
• <60 y	26.200	4.690	17.00-35.39		
• ≥60 y	16.394	2.837	10.83-21.95	3.03	0.082
Gender					
• Male	17.813	2.496	12.92-22.70		
• Female	22.765	4.768	13.42-32.11	0.1	0.752
Tumor site					
• Head	21.014	2.864	15.39-26.6		
• Body\ uncinate process	12.333	4.910	2.70-21.95	1.03	0.311
Tumor size					
• ≤2.5	20.457	3.067	14.44-26.5		
• >2.5	16.857	3.201	10.58-23.13	0.011	0.917
Grade					
• Mild\mod differentiated	20.447	2.704	15.14-25.7		
• Poorly differentiated	7.000	1.414	4.22-9.77	0.823	0.364
Duodenal infiltration					
• No	16.918	3.318	10.41-23.4		
• Yes	22.010	3.559	15.03-28.9	0.657	0.418
LN					
• Positive	22.456	3.558	15.48-29.4		
• Negative	15.303	2.936	9.54-21.1	1.22	0.269
TNM.GP					
• IIA\IA	15.607	3.139	9.45-21.7		
• IB\IIB\III	21.888	3.432	15.16-28.6	0.86	0.354
Lymph vascular emboli					
• Positive	23.181	3.640	16.04-30.3		
• Negative	16.037	2.616	10.91-21.16	0.813	0.367
Perineural invasion					
• Positive	13.600	1.615	10.43-16.7		
• Negative	32.306	4.857	22.78-41.8	8.53	0.003*
Pancreatic safety margin					
• Infiltrated	16.430	2.047	12.41-20.4		
• Free	39.250	6.712	26.09-52.4	4.54	0.033*
NRF2					
• Low expression	16.749	3.069	10.73-22.76		
• High expression	23.527	4.094	15.50-31.55	1.06	0.301
Keap1					
• Low expression	17.062	3.237	10.71-23.4		
• High expression	22.740	3.933	15.03-30.4	0.73	0.393
DFS	20.212	2.667	14.98-25.44		

In tumors without pancreatic infiltration, NRF2 expression was substantially connected with the presence of pancreatic infiltration (P = 0.005), whereas in tumors with pancreatic infiltration, NRF2 expression was significantly correlated with low expression (60.7%). This may contradict research (Shen et al., 2013) that shows NRF2 is involved in invasion and metastasis. They reported that Nrf2 inhibition suppressed the migration and invasion of ESCC cells under hypoxic conditions by promoting the expression of E-cadherin and suppressing the

expression of MMP-2. This study depends on detecting the relationship between the Nrf2 level and other genes that promote and induce invasion using different in vitro studies. However, depending on the stage of carcinogenesis reported by different studies, the dual sword theory can explain our results (Cykowiak and Krajka-Ku´zniak, 2022). In our work, there was no other significant correlation between Nrf2 expression and other clinicopathological parameters. These findings agree with those of Lister et al., who found no evidence of a significant relationship between the expression of Nrf2 in tumor cells and other clinicopathological variables. Also, Huang et al. found no significant correlation between Nrf2 expression in oral squamous cell carcinoma and tumor stage, lymph node status, or pathological grade. However, other studies showed significant correlations with some clinicopathological parameters, like Onodera et al. who found a significant association between Nrf2 expression in breast cancer cells and histological grade, p62 status, pathological tumor factor (pt.), lymph node metastasis, HER2 status, and Ki-67 status. This discrepancy can be explained by their larger sample size and their use of different in vitro studies to detect Nrf2 expression in cancer cells.

Keap1 has an important role in cancer prevention and progression. It is an adaptor for the ubiquitin ligase complex that regulates Nrf2 activity (Huang et al., 2013). Under physiological conditions, Keap1 binds to Nrf2, and through the ubiquitin-proteasome, it directs it to degradation and represses it (Ahtikoski et al., 2019). In oxidative stress, Nrf2 degradation ceases; it accumulates in nuclei and activates target genes for cytoprotecting (Taguchi et al., 2011). This may help cancer cells escape from oxidative stress induced by chemotherapeutic agents (Ahtikoski et al., 2019). In the present study, out of 45 cases, 57.8% showed high Keap1 expression in tumor cells, while 42.2% showed low expression. A significant relationship was seen between Keap1 expression and duodenal infiltration (P = 0.009), as 82.4% of cases of high expression were seen in tumors with no duodenal infiltration and 57.1% of low expression cases were seen in tumors with duodenal infiltration. These results may be explained by the study of Ohta et al., who concluded that weakened Keap1 function in lung cancer cells acquired multiple advantages for cancer cells to proliferate. Other clinicopathological characteristics have no meaningful correlation. Furthermore, Huang et al. discovered no link between Keap1 expression and tumor stage, grade, or lymph node status.

In the present study, there was a significant correlation between NRF2 expression and Keap1 expression in tumor cells (p=0.001). These results agree with Huang et al.'s results, which reported that Keap1 was statistically associated with nuclear Nrf2 (P = 0.000).

The Kaplan-Meier test was employed for survival analysis, and the log-rank test was performed to establish the statistical significance of differences between curves. The present study had a significant association between overall survival, disease-free survival, and perineural invasion. Negative peri-neural invasion showed better overall survival and disease-free survival than cases with positive invasion (P = 0.039, P = 0.003, respectively). This agrees with many studies that proved the importance of per-neural invasion as a prognostic factor in pancreatic cancer (Zhang et al., 2013).

Also, there was a significant association between disease-free survival and the pancreatic safety margin. Cases with an infiltrated pancreatic safety margin showed longer disease-free survival (P = 0.003). These results were opposite those of Chen et al., who posted that there was a significant difference in long-term survival with margins clear by 2mm and no significant difference if margins were less than 1mm. This discrepancy may be due to methods of margin assessment, as we didn’t estimate the distance statically (Chen et al., 2010).

No significant associations were found between overall survival and NRF2 and Keap1 expressions in tumor cells (p = 0.356 and 0.434, respectively). Also, there was no significant association between NRF2 and Keap1 expression and disease-free survival (P = 0.301 and P = 0.393, respectively). Huang et al. reported no significant association between

Nrf2 expression and Keap1 expression in oral squamous cell carcinoma and overall survival or patient outcome. This may be different from other studies that reported an association between Keap1 or Nrf2 expression and patient survival in different types of cancer cells (Ondodera et al., 2014, Isohookana et al., 2015, Ahtikoski et al., 2019). This may be explained by different sample sizes, the scoring system used in immune stain interpretation, and finally, other in vitro methods used for the detection of both Nrf2 and Keap1.

CONFLICT OF INTEREST

No conflict of interest.

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