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Welcome to the Int J Cancer and Biomedical Research (IJCBR)**!**

It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

Mohamed L. Salem,

Editor in Chief

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

The Expression of Immune Checkpoint Inhibitors PDL-1 and CTLA-4 in Pancreatic Versus Non-Pancreatic Periampullary Adenocarcinoma: An Immunohistochemical Study

Noha M. El-Anwar[1,2], A. Kamal[3], Marwa Shabana[4] and Reham S.E. Esmail[5,2]

[1]Department of pathology, Faculty of Medicine, Tanta University, Egypt
[2]Department of Pathology, Armed Forces College of Medicine, Egypt
[3]Department of Surgical Oncology, National Cancer Institute.
[4]Department of Pathology, National Research Centre.
[5]Department of Pathology, Faculty of Medicine, Fayum University

ABSTRACT

Background: Periampullary cancers constitute about 5% of gastrointestinal malignancies. They are comprised of tumors of diverse origins and are generally subdivided into pancreatic and non-pancreatic carcinomas. Immune checkpoint regulators, cytotoxic T-lymphocyte antigen 4 (CTLA-4), and the programmed cell death ligand-1 (PDL-1) have emerged as promising new targets for cancer therapeutics. **Aim**: This study aims to determine the possible role of immune checkpoint inhibitors PDL-1 and CTLA-4 in periampullary carcinoma of pancreatic and non-pancreatic adenocarcinoma subtypes, in an attempt to investigate the possible introduction of their related immunotherapy in the management of these tumors. **Materials and Methods:** Expression of immune inhibitory molecules was examined by immunohistochemistry in 40 cases including (20) pancreatic adenocarcinoma and (20) non-pancreatic adenocarcinoma. The association between markers and clinicopathological parameters was evaluated. **Results:** Statistically significant differences in the immunoexpression of both CTLA-4 and PDL-1 in the two studied groups were noticed with higher expression in non-pancreatic adenocarcinoma in relation to pancreatic adenocarcinoma (P=0.004, P=0.008) respectively. PDL-1 expression was positive in 15% and 55% of pancreatic and non-pancreatic adenocarcinoma cases, respectively with a significant correlation with lymph nodes metastasis in non-pancreatic adenocarcinoma cases. CTLA-4 was positive in 20% of pancreatic carcinoma with a significant correlation with lymph node metastasis, perineural invasion and T stage. In non-pancreatic periampullary adenocarcinoma, CTLA-4 was positive in 65% of cases with a significant association with lymph nodes metastasis and T stage. **Conclusions:** Immunotherapy using anti-PDL-1 and CTLA-4 are proposed as a novel promising management tool in non-pancreatic periampullary adenocarcinoma not in pancreatic adenocarcinomas.

Keywords: CTLA-4, non-pancreatic periampullary adenocarcinoma, pancreatic adenocarcinoma, PDL-1

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Correspondence to:
Noha M. El -Anwar
Department of Pathology
Faculty of Medicine
Tanta University, Egypt
Armed Forces College of Medicine
Tel.: +201224368268
E-mail: nohaelanwar@gmail.com

INTRODUCTION

Periampullary cancers constitute about 5% of gastrointestinal malignancies and are classically defined as cancers arising within the distance of 2 cm of the papilla of Vater. They represent a complex disease entity with divergent heterogenous histogenesis, including pancreatic, ampullary, biliary and duodenal cancers (Bansal et al., 2017). They comprised tumors of diverse origins and generally subdivided into pancreatic (PC) and non-pancreatic carcinomas with the latter having a more favourable prognosis and better overall survival rates (Siegel et al., 2015; Sunil et al., 2017). Histologically, all periampullary cancers are predominantly adenocarcinomas and all are regarded as tumors with the worst prognosis worldwide, with spiky disease-related mortality (Saluja et al., 2019).

Despite the dynamic progress in both the diagnosis and management, including the newly introduced targeted therapies, surgical resection via pancreaticodoudenectomy (PD) with or without pylorus preservation is still the mainstay of management of periampullary carcinoma as a whole; the role of adjuvant chemotherapy and adjuvant chemoradiation treatment is still questionable and uncertain (Baghmar et al., 2019).

In the last years, the research has approached great achievements in the way to understand the intersection between immune surveillance and tumor initiation and progression, and the concept which has installed novel effective therapeutic tools in many cancer types (Jesus et al., 2018). The overall survival and prognosis of many solid tumors as melanoma, non-small lung carcinoma, and hematologic malignancies have been significantly improved after the introduction of such immunotherapeutic modalities (Kalbasi and Ribas, 2020).

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is known to be the master of all the immune checkpoint inhibitors, as it prevents T-cell activation at very early initial stages (Buchbinder and Desai, 2016). On the other hand, the programmed cell death protein-1/programmed death-ligand 1 PDL-1 acts on the regulation of the previously activated T lymphocytes on later stages of the body immune mechanism (Akinleye and Rasool, 2019). Both CTLA-4 and PDL-1 are responsible for controlling T cell-mediated immune response by "switching off" T-cell effects on the self-cells. Therefore, their blockage will allow for proper stimulation and expression of the immune system against tumor cells (Rapoport et al., 2017).

Both anti-CTLA-4 and anti-PDL-1 have shown promising results in many gastrointestinal malignancies (Sharma and Allison, 2020), however, some studies have approximated the possible role of immunotherapy in periampullary cancers, with relatively conflicting results and unsteady conclusions (Jiang et al., 2019).

In the current study, we examined the possible role of immune checkpoint inhibitors PDL-1 and CTLA-4 in cases of periampullary carcinoma of pancreatic and non-pancreatic adenocarcinoma subtypes in the management of these tumors by investigating their immunohistochemical expression.

MATERIAL AND METHODS

Cases and study groups

This retrospective multi-center study included 40 cases; 20 cases of pancreatic head adenocarcinoma type and 20 cases of non-pancreatic periampullary adenocarcinoma.

Formalin-fixed paraffin-embedded blocks were collected from archived cases during the last 3 years from Air forces specialized hospital, Naser Institute for treatment and research and Tanta University hospital, according to the following inclusion criteria:

- Availability and integrity of paraffin blocks.
- Full clinicopathological data as illustrated in table 1 including (histopathological diagnosis of adenocarcinoma with no specific variant and precise anatomic location of the tumor).

Cases without a full clinicopathological sheet or bad quality of tissue blocks were excluded. This study was performed in accordance with the Declaration of Helsinki. The evaluation of archived pathology specimens described in the study was approved by the ethical committee of National Research Center (approval code:IRB:6413042020)

The slides were reviewed for:

- Confirmation of the diagnoses
- Reporting the histopathologic findings as perineural invasion and nodal metastasis.

Staging of the tumor was defined according to TNM American Joint Committee on Cancer Union International Center Cancer staging system (AJCC-UICC) (Amin et al., 2017)

Immunohistochemistry methods

Two unstained slides were cut from the tumor paraffin blocks at 3–5-micron thickness for further immunohistochemical staining for CTLA-4 & PDL-1 according to the following protocol through an automated immunostainer (Ventana BenchMark XT; USA) which runs the following basic steps: Section deparaffinization by xylene, rehydration by graded alcohol then saturation using 0.03% hydrogen peroxide. This was followed by antigen retrieval by Tris-

buffered 0.1% saline and Tween-20 at pH = 7.6. Incubation was done with the primary antibodies using PDL-1 antibody (M3653 clone 22C3, Dako, Glostrup, Denmark) and CTLA-4 (Mouse monoclonal antibody, Santa Cruz) at 1:400 dilution for 30 min at room temperature. The UltraView Universal DAB Detection Kit was used as the secondary antibody in the device. Finally, Samples were counterstained with hematoxylin and mounted in DPX. Sections incubated without the primary antibody were used as negative controls. Tonsil sections were used as a positive control for both PDL-1 and CTLA-4.

Immunohistochemical stains evaluation

PDL-1 expression was detected as brown membranous staining and CTLA-4 expression was detected as cytoplasmic staining. According to Schlober et al. (2016). In the expression of PDL-1 and CTLA-4 on tumor cells, samples with >10% stained tumor cells were considered positive.

Statistical analysis

Microsoft excel 2013 was used for data entry and the statistical package for social science (SPSS version 24) was used for data analysis. All collected data were revised for competencies and logical consistency. Simple descriptive statistics (arithmetic mean and standard deviation) were used for the summary of normal quantitative data and frequencies used for qualitative data. Bivariate relationship was displayed in cross-tabulations and comparison of proportions was performed using the chi-square and Fisher's exact tests where appropriate. The level of significance was set at a probability (P) value < 0.05.

RESULTS

The present study was performed on 40 biopsies; 20 cases of pancreatic adenocarcinoma and 20 cases were non-pancreatic periampullary adenocarcinoma. The studied cases included 23 males and 17 females in the two groups with a mean age of 56 for pancreatic carcinoma cases and 55 for non-pancreatic adenocarcinoma cases with mean standard deviations of 9.434 and 9.691 respectively. The clinicopathological features of the studied cases are summarized in Table 1.

Immunohistochemical of PDL-1 (Figure 1)

PDL-1 expression was detected as brown membranous staining.

Pancreatic adenocarcinoma cases: It was only positive in 3 (15%) of the studied cases. The relations between PDL-1 expression and the studied clinicopathological parameters are summarized in Table 2. No significant correlation was found with all clinicopathological parameters.

Non-pancreatic adenocarcinoma cases: PDL-1 expression was positive in 11 (55%) of cases. It was found that PDL-1 expression was significantly correlated with lymph nodes metastasis (P=0.025) as shown in Table 3.

Immunohistochemical of CTLA-4 (Figure 2)

it was detected as cytoplasmic staining. The relation between CTLA-4 and clinicopathological parameters in pancreatic and non-pancreatic adenocarcinoma cases was illustrated in Tables 4 & 5.

Pancreatic adenocarcinoma cases: CTLA-4 was positive in only 4 (20%) of cases, bearing a significant correlation with lymph node metastasis, perineural invasion, and T stage (P=0.014, P=0.025 and P=0.009) respectively.

Non-pancreatic adenocarcinoma cases: CTLA-4 expression was positive in 13 (65%) of cases. It was found that CTLA-4 expression was significantly correlated with lymph nodes metastasis and T stage (P=0.019, P=0.023) respectively.

Correlation of expression of both CTLA-4 and PDL-1 expression in both groups

There was a significant correlation for higher expression of CTLA-4 and PDL-1 in non-pancreatic adenocarcinoma in relation to pancreatic adenocarcinoma (P=0.004, P=0.008) respectively as illustrated in Table 6.

DISCUSSION

Primary adenocarcinomas derived from the head of the pancreas, ampulla, duodenum, or distal bile duct are unitedly referred to as "periampullary" adenocarcinomas (Bakshi et al., 2019).

Table 1. Clinicopathological features in pancreatic adenocarcinoma and non-pancreatic periampullary adenocarcinoma

		Group			
		Pancreatic carcinoma		Non-pancreatic carcinoma	
		Count	%	Count	%
Gender	Male	13	65.0%	10	50.0%
	Female	7	35.0%	10	50.0%
	Total	20	100.0%	20	100.0%
Margin status	Negative	12	60.0%	18	90.0%
	Positive	8	40.0%	2	10.0%
	Total	20	100.0%	20	100.0%
LN metastasis	Negative	12	60.0%	10	50.0%
	Positive	8	40.0%	10	50.0%
	Total	20	100.0%	20	100.0%
Differentiation	Poorly	4	20.0%	4	20.0%
	Moderately	9	45.0%	11	55.0%
	Well	7	35.0%	5	25.0%
	Total	20	100.0%	20	100.0%
Perineural invasion	Negative	10	50.0%	10	50.0%
	Positive	10	50.0%	10	50.0%
	Total	20	100.0%	20	100.0%
T stage	T1	10	50.0%	8	40.0%
	T2	6	30.0%	8	40.0%
	T3	2	10.0%	3	15.0%
	T4	2	10.0%	1	5.0%
	Total	20	100.0%	20	100.0%

Table 2. Relation between PDL-1 and clinicopathological parameters in pancreatic adenocarcinoma

		PDL-1				P value
		Negative		Positive		
		Count	N%	Count	N%	
Margin status	Positive	7	41.2%	1	33.3%	1.000
	Negative	10	58.8%	2	66.7%	
	Total	17	100.0%	3	100.0%	
LN metastasis	Negative	11	64.7%	1	33.3%	0.537
	Positive	6	35.3%	2	66.7%	
	Total	17	100.0%	3	100.0%	
Differentiation	Poorly	3	17.6%	1	33.3%	0.383
	Moderately	7	41.2%	2	66.7%	
	Well	7	41.2%	0	0.0%	
	Total	17	100.0%	3	100.0%	
Perineural invasion	Negative	10	58.8%	0	0.0%	0.060
	Positive	7	41.2%	3	100.0%	
	Total	17	100.0%	3	100.0%	
T stage	T1	9	52.9%	1	33.3%	0.478
	T2	5	29.4%	1	33.3%	
	T3	1	5.9%	1	33.3%	
	T4	2	11.8%	0	0.0%	
	Total	17	100.0%	3	100.0%	

Table 3. Relation between PDL-1 and clinicopathological parameters in non-pancreatic periampullary adenocarcinoma cases

		PDL-1				P value
		Negative		Positive		
		Count	N%	Count	N%	
Margins status	Negative	8	88.9%	10	90.9%	0.067
	Positive	1	11.1%	1	9.1%	
	Total	9	100.0%	11	100.0%	
LN metastasis	Negative	7	77.8%	3	27.3%	0.025
	Positive	2	22.2%	8	72.7%	
	Total	9	100.0%	11	100.0%	
Differentiation	Poorly	0	0.0%	4	36.4%	0.056
	Moderately	5	55.6%	6	54.5%	
	Well	4	44.4%	1	9.1%	
	Total	9	100.0%	11	100.0%	
Perineural invasion	Negative	6	66.7%	4	36.4%	0.178
	Positive	3	33.3%	7	63.6%	
	Total	9	100.0%	11	100.0%	
T stage	T1	6	66.7%	2	18.2%	0.095
	T2	3	33.3%	5	45.5%	
	T3	0	0.0%	3	27.3%	
	T4	0	0.0%	1	9.1%	
	Total	9	100.0%	11	100.0%	

Table 4. Relation between CTLA-4 and clinicopathological parameters in pancreatic adenocarcinoma cases

		CTLA-4				P value
		Negative		Positive		
		Count	N%	Count	N%	
Positive margins	Positive	8	50.0%	0	0.0%	0.117
	Negative	8	50.0%	4	100.0%	
	Total	16	100.0%	4	100.0%	
LN metastasis	Negative	12	75.0%	0	0.0%	0.014
	Positive	4	25.0%	4	100.0%	
	Total	16	100.0%	4	100.0%	
differentiation	Poorly	2	12.5%	2	50.0%	0.133
	Moderately	7	43.8%	2	50.0%	
	Well	7	43.8%	0	0.0%	
	Total	16	100.0%	4	100.0%	
Perineural invasion	Negative	10	62.5%	0	0.0%	0.025
	Positive	6	37.5%	4	100.0%	
	Total	16	100.0%	4	100.0%	
T stage	T1	10	62.5%	0	0.0%	0.009
	T2	4	25.0%	2	50.0%	
	T3	0	0.0%	2	50.0%	
	T4	2	12.5%	0	0.0%	
	Total	16	100.0%	4	100.0%	

Table 5. Relation between CTLA-4 and clinicopathological parameters in non-pancreatic periampullary adenocarcinoma cases

		CTLA-4				P value
		Negative		Positive		
		Count	N%	Count	N%	
Positive margins	Negative	6	85.7%	12	92.3%	0.059
	Positive	1	14.3%	1	7.7%	
	Total	7	100.0%	13	100.0%	
LN metastasis	Negative	6	85.7%	4	30.8%	0.019
	Positive	1	14.3%	9	69.2%	
	Total	7	100.0%	13	100.0%	
differentiation	Poorly	0	0.0%	4	30.8%	0.171
	Moderately	4	57.1%	7	53.8%	
	Well	3	42.9%	2	15.4%	
	Total	7	100.0%	13	100.0%	
Perineural invasion	Negative	5	71.4%	5	38.5%	0.350
	Positive	2	28.6%	8	61.5%	
	Total	7	100.0%	13	100.0%	
T stage	T1	6	85.7%	2	15.4%	0.023
	T2	1	14.3%	7	53.8%	
	T3	0	0.0%	3	23.1%	
	T4	0	0.0%	1	7.7%	
	Total	7	100.0%	13	100.0%	

The subtyping of periampullary adenocarcinoma into pancreatic and non-pancreatic subtypes appeared to be an important prognostic determination; as the non-pancreatic type has shown to possess better prognosis and overall survival in many studies (Schiergens et al., 2015).

Immune checkpoint regulators, (CTLA-4) and (PDL-1), have recently emerged as promising novel targets for cancer therapeutics, with inspiring results in many solid tumors and leukaemia (Kassardjian et al., 2018). The critical goal of the immune checkpoint therapeutic antibodies is inactivating the immune checkpoint proteins shifting the balance from immune suppression to immune activation (McArthur and Page, 2016). The possible role of immune checkpoint inhibitors in periampullary carcinomas remains obscure.

According to our results, PDL-1 expression was detected in only (15%) of PC cases as opposed to statistically significantly higher expression in the non-pancreatic group; which reached up to 55% of cases (P=0.008). In contrast to our results, Sideras and coworkers showed that many immune inhibitory molecules especially PDL-1 are expressed by pancreatic and ampullary cancer cells and these molecules can become valuable targets for immunotherapy (Sideras et al, 2017). Their study concluded that cancers arising from the ampulla aren`t biologically different from cancers arising from the pancreas. However, their findings couldn`t be generalized to a broader definition of peri-ampullary tumors, which include distal cholangiocarcinomas and duodenal adenocarcinoma, which were excluded from their studied cases.

In our cases, the expression of PDL-1 in PC cases showed no significant correlation with any of the studied prognostic clinicopathological parameters. Whilst in non-pancreatic periampullary adenocarcinoma cases, the expression was significantly correlated with positive lymph nodes metastasis (poor prognostic parameter) (P=0.025).

Conspicuous variation in the results of PDL-1 expression in periampullary carcinoma as well as other cancers was observed; the reported expression of PDL-1 in tumor cells of PC in different studies ranged from 12 up to 90% (Soares et al., 2015; Lu et al., 2017).

Table 6. Correlation between PDL-1 and CTLA-4 immunohistochemical expression in both groups

		Group				P value
		Pancreatic Carcinoma		Non-pancreatic carcinoma		
		Count	%	Count	%	
PDL1	Negative	17	85.0%	9	45.0%	0.008
	Positive	3	15.0%	11	55.0%	
	Total	20	100.0%	20	100.0%	
CTLA4	Negative	16	80.0%	7	35.0%	0.004
	Positive	4	20.0%	13	65.0%	
	Total	20	100.0%	20	100.0%	

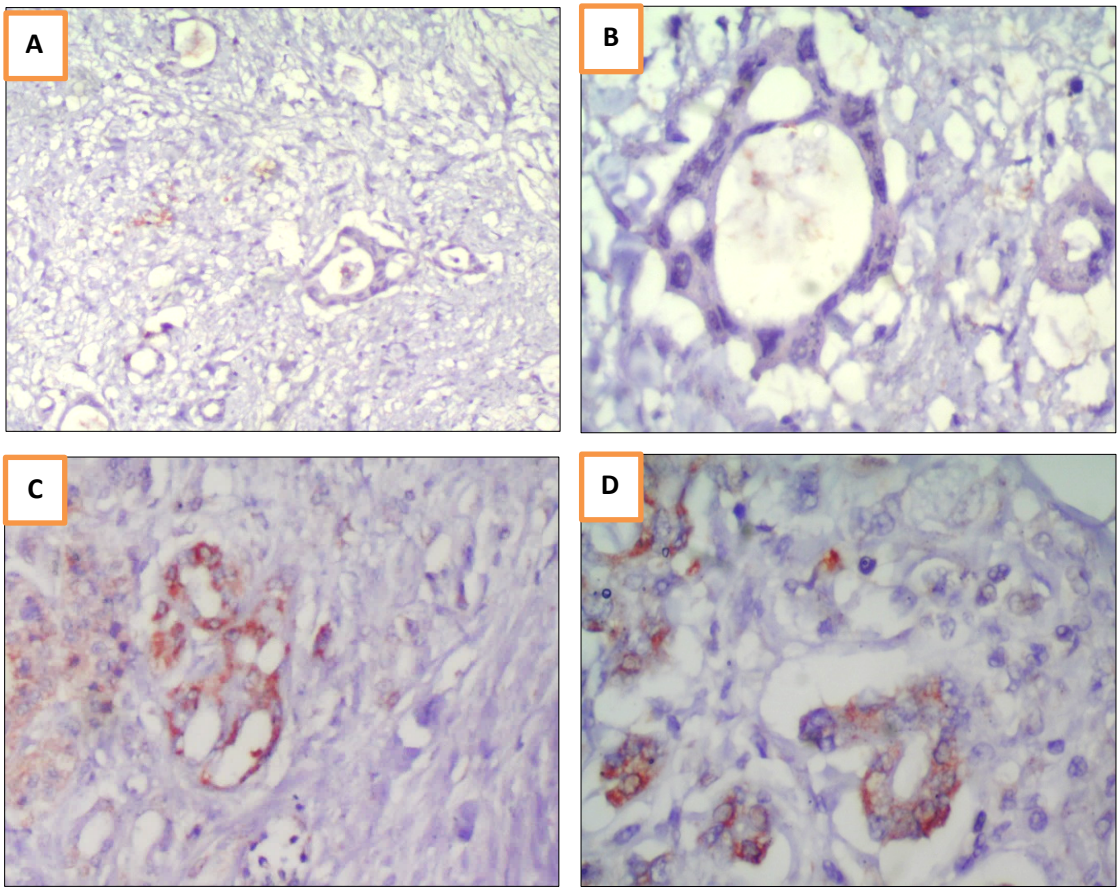

Figure 1. PDL-1 immunohistochemical expression in the cases: A & B - Pancreatic adenocarcinoma with a negative expression for Pdl1 Low and high power (x200, x400). C & D - Non-pancreatic periampullary adenocarcinoma showed positive membranous expression of PDL-1 low and high power (x200, x400).

The possible most accepted explanation for this variation is the probable use of different antibodies of different PDL-1 clones, as well as the use of divergent positivity scoring systems in interpreting the obtained results. Moreover, some studies focused on PDL-1 expression in tumor cells only, while others calculated its expression in immune cells or stromal cells as part of their scoring parameters (Wang et al. 2010). In agreement with our results, Birnbaum et al. (2016) examined PDL-1 mRNA expression in PC and noticed that only 19% of their PC cases expressed PDL-1 and that the expression was related to worse survival rates. Nomi et al. (2007) got higher PDL-1 expression reaching 39.2% of their studied PC cases, however, they stated as well, that the expression was associated with poor prognostic parameters. They, therefore, concluded that PDL1 may be introduced as a novel prognostic marker for human pancreatic cancer and that targeting PDL-1/ PD-1, especially in combination with standard chemotherapy, may exhibit significant therapeutic repercussion.

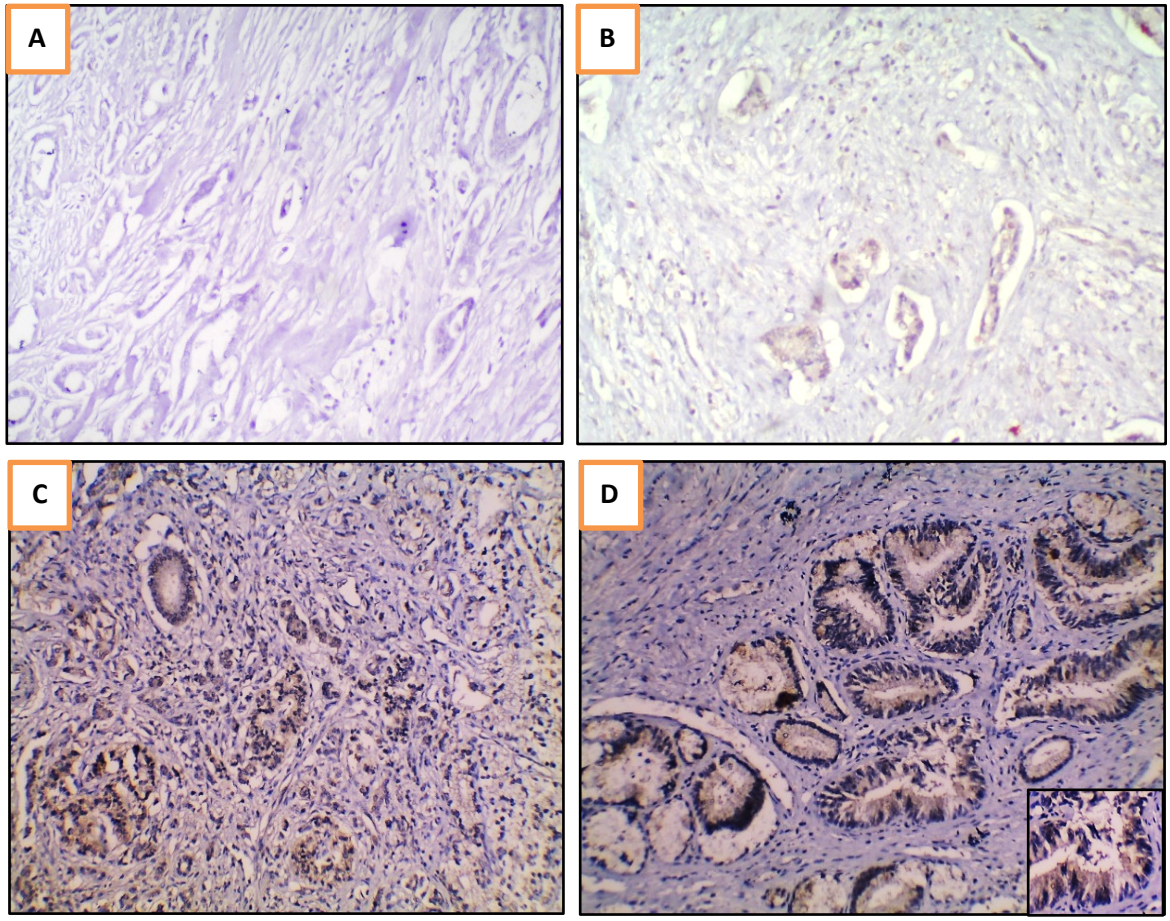

Figure 2. CTLA-4 immunohistochemical expression in the cases: A & B - Pancreatic adenocarcinoma with a negative expression for CTLA-4 (x200). C - Positive cytoplasmic expression of CTLA-4 in a case of non-pancreatic periampullary adenocarcinoma (x100). D - Another case of non-pancreatic periampullary adenocarcinoma with positive expression of CTLA-4, with inset picture showing cytoplasmic staining (x200).

On the other hand, Sideras et al. (2017) studied PDL-1 expression on PC and ampullary adenocarcinomas and related its expression with better cancer-specific survival. Compared with colorectal carcinoma, other studies reported that high PDL-1 expression in tumor cells of colorectal carcinoma is significantly associated with nodal and distant metastases (Zhu et al., 2015) as well as with poor differentiation, infiltrating growth pattern and increased lymphovascular invasion (Kim et al., 2016). In our cases, CTLA-4 expression was detected in only 20% of PC cases contrasted with 65% in non-pancreatic periampullary adenocarcinoma cases, and the difference was statistically significant (P = 0.004). CTL4 expression in PC demonstrated a significant correlation with poor prognostic factors as positive lymph node metastasis (P= 0.014), presence of perineural invasion (P= 0.025), and advanced tumor stage (P=0.009).

In non-pancreatic periampullary adenocarcinoma cases, significant correlation was shown with positive lymph nodes metastasis (P= 0.019) and advanced tumor stage (P=0.023) as well.

To the best of our knowledge, until this time, no other studies examined CTLA-4 expression in pancreatic versus non-pancreatic periampullary adenocarcinoma. Various studies showed that CTLA-4 expression in many tumors was associated with poor prognostic histopathological parameters. Karpathiou et al. (2020) showed that high CTLA-4 tumor cell expression in carcinoma of the uterine cervix was also associated with advanced tumor stage and positive lymph nodes metastasis.

Contrastingly, a study carried out by Schlober et al. (2016) on gastric adenocarcinoma revealed that CTLA-4 was expressed in 86% of cases with no significant association with lymph nodes metastasis.

Thus, our study pointed out that the expression of both immune checkpoint inhibitors CTLA-4 and PDL-1 was ultimately significantly higher in non-pancreatic periampullary adenocarcinoma cases in relation to pancreatic adenocarcinoma (P=0.004, P=0.008) respectively, the fact that may be attributed to the confessed nature of PC stroma which is known to be dense, highly desmoplastic, with poor microenvironment and scanty immune cells niche, which make the intra-tumoral stroma generally immunosuppressive (Mahmood et al., 2018).

The above results give new evidence supporting the established concept of the better overall prognosis in non-pancreatic periampullary carcinoma compared to PC, therefore, the segregation between the two tumor types is becoming essential.

While our study provides information on the immunologic profile of pancreatic adenocarcinoma and non-pancreatic periampullary carcinoma, further work is needed to immunologically characterize all different subtypes of periampullary cancer, to illustrate any possible differences within this group. We speculate that the immunological features in periampullary carcinoma may help to guide immunotherapeutic strategies in the future.

CONCLUSIONS

The study concluded that high expression of PDL-1 and CTLA-4 in non-pancreatic periampullary adenocarcinoma could provide a novel promising management tool of immunotherapy in these tumors,not in the pancreatic adenocarcinomas . Therefore, the differentiation between the two groups is recommended with further large-scale studies to immunologically characterize all different subtypes of periampullary cancer. Besides, our results figured out that the expression of both PDL-1 and CTLA- 4 in non-pancreatic periampullary carcinomas; maybe a poor prognostic parameter.

CONFLICTS OF INTEREST

All authors declared no conflicts of interest.

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REFERENCES

Akinleye A, Rasool Z (2019). Immune checkpoint inhibitors of PD-L1 as cancer therapeutics. Journal of Hematology & Oncology, 12: 92-105.

Amin, MB, Greene FL., Edge SB, Compton CC, Gershenwald JE, Brookland RK and Winchester DP (2017). The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more “personalized” approach to cancer staging. CA: a cancer journal for clinicians, 67: 93-99.

Baghmar S, Agrawal N, Kumar G, Bihari C, Patidar Y, Kumar S, Chattopadhyay T , Panda D , Arora A , Pamecha V (2019). Prognostic Factors and the Role of Adjuvant Treatment in Periampullary Carcinoma: a Single-Centre Experience of 95 Patients. Journal of Gastrointestinal Cancer. 50:361-369.

Bakshi N, Dhawan S, Nundy S, Rao S, Chopra P, Bhalla S (2019). Role of Immunohistochemistry in the Subtyping of Periampullary Adenocarcinoma. International journal of surgical pathology, 27: 598-608.

Bansal A, Dalal V, Kaur M, Siraj F (2017). Periampullary Carcinoma: Unusual Sites of Metastasis. Ochsner Journal, 17 (4) 426-429.

Birnbaum, DJ, Finetti P, Lopresti A, Gilaber, M, Poizat F, Turrini O, Mamessier E. (2016). Prognostic value of PDL1 expression in pancreatic cancer. Oncotarget, 7: 71198- 71210..

Buchbinder E and Desai A (2016). CTLA-4 and PD-1 pathways similarities, differences, and implications of their inhibition. American Journal of Clinical Oncology, 39: 98–106.

Jesus VHFD, Felismino TC, Barros e Silva MJD, Souza e Silva VD, Riechelmann RP (2018). Current approaches to immunotherapy in noncolorectal gastrointestinal malignancies. Clinics, 73 (Suppl. 1), e510s.

Jiang P, Zhou H, Ni C, Hu X, Mou Y, Huang D, Yang L (2019). Immunotherapy in pancreatic cancer: New hope or mission impossible?. Cancer Letters 445, 31: 57-64.

Kalbasi, A and Ribas A (2020). Tumour-intrinsic resistance to immune checkpoint blockade. NatureReviews Immunology 20: 25–39.

Karpathiou G, Chauleur C, Mobarki M, Peoc'h M. (2020). The immune checkpoints CTLA-4 and PD-L1 in carcinomas of the uterine cervix. Pathology-Research and Practice, 216: 152782.

Kassardjian A, Shintaku PI, Moatamed NA (2018). Expression of immune checkpoint regulators, cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death-ligand 1 (PD-L1), in female breast carcinomas. PloS one, 13: e0195958.

Kim JH, Park HE, Cho NY, Lee HS and Kang GH (2016).

Characterisation of PD-L1-positive subsets of microsatellite-unstable colorectal cancers. British journal of cancer, 115: 490-496.

Lu C, Paschall AV, Shi H, Savage N, Waller JL, Sabbatini ME, Liu K. (2017). The MLL1-H3K4me3 axis-mediated PD-L1 expression and pancreatic cancer immune evasion. JNCI: journal of the National Cancer Institute, 109 (6): 283-295.

Mahmood J, Shukla HD, Soman S, Samanta S, Singh P, Kamlapurkar S & Vujaskovic Z (2018). Immunotherapy, radiotherapy, and hyperthermia: A combined therapeutic approach in pancreatic cancer treatment. Cancers, 10: 469-484.

McArthur HL and Page DB (2016). Immunotherapy for the treatment of breast cancer: checkpoint blockade, cancer vaccines, and future directions in combination immunotherapy. Clinical Advances in Hematology & Oncology, 14:922-933.

Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H and Nakajima Y (2007). Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clinical cancer research, 13: 2151-2157.

Rapoport BL, Van Eeden R, Sibaud V, Epstein JB, Klastersky J, Aapro M, Moodley D (2017). Supportive care for patients undergoing immunotherapy. Support Care Cancer 25, 3017–3030.

Saluja SS, Kiran S, Mishra PK, Ramaswamy D, Varshney VK, Godhi S, Sisodia K (2019). Long-Term Functional Outcome After Pancreatoduodenectomy for Periampullary Carcinoma with Morphological Correlation. Pancreas, 48:1182-1187

Schiergens TS, Reu S, Neumann J, Renz BW, Niess H, Boeck S and Kleespies A (2015). Histomorphologic and molecular phenotypes predict gemcitabine response and overall survival in adenocarcinoma of the ampulla of Vater. Surgery, 158:151-161.

Schlößer HA, Drebber U, Kloth M, Thelen M, Rothschild SI, Haase S, Alakus H. (2016). Immune checkpoints programmed death 1 ligand 1 and cytotoxic T lymphocyte associated molecule 4 in gastric adenocarcinoma. Oncoimmunology, 5: e1100789.

Sharma P and Allison JP (2020). Dissecting the mechanisms of immune checkpoint therapy. Nat Rev Immunol, 20: 75–76.

Sideras, K, Biermann K, Yap K, Mancham S, Boor PP, Hansen BE, Kwekkeboom J (2017). Tumor cell expression of immune inhibitory molecules and tumor-infiltrating lymphocyte count predict cancer-specific survival in pancreatic and ampullary cancer. International journal of cancer, 141: 572-582.

Siegel RL, Miller KD, Jemal A (2015). Cancer statistics. CA: Cancer Journal for Clinicians, 65: 5-29

Soares KC, Rucki AA, Wu AA, Olino K, Xiao Q, Chai Y, Yao S (2015). PD-1/PD-L1 blockade together with vaccine therapy facilitates effector T cell infiltration into pancreatic tumors. Journal of immunotherapy (Hagerstown, Md.: 1997), 38: 1-11.

Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT, Gajewski TF. (2013). Up-regulation of PD-L1, IDO, and Tregs in the melanoma tumor microenvironment is driven by CD8+ T cells. Science translational medicine, 5: 200ra116-200ra116.

Sunil B J, Seshadri R A, Gouthaman S, Ranganathan R (2017). Long-Term Outcomes and Prognostic Factorsin Periampullary Carcinoma. Journal of Gastrointestinal Cancer, 48:13–19.

Wang L, Ma Q, Chen X, Guo K, Li J, Zhang M (2010). Clinical significance of B7-H1 and B7-1 expressions in pancreatic carcinoma. World journal of surgery, 34: 1059-1065.

Zhu H, Qin H, Huang Z, Li S, Zhu X, He J, Yang J, Yu X, Yi X (2015). Clinical significance of programmed death ligand-1 (PD-L1) in colorectal serrated adenocarcinoma. International journal of clinical and experimental pathology, 8: 9351–9359.

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