Introduction

Pancreatic cancer (PC) is a highly aggressive malignant tumor that ranked as the fourth leading cause of cancer-related deaths in the Europe and United States **(Hu et al., 2021).** Considering all stages combined, PC has the poorest survival rates among all types of cancer, with only an 11% survival rate. It is projected to become the second leading cause of death by the year 2030 **(Siegal et al., 2022).** In Egypt, pancreatic cancer caused 2,906 deaths in 2020, accounting for 0.54% of total deaths, and with a mortality rate that varies by province, with northern districts having approximately 2.85 times higher rates than southern districts. This variation may be attributed to exposure to different environmental factors, as the northern regions have the highest rates of soil and water pollution in the country **(Baum et al., 2020).** Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, accounting for nearly all cases of pancreatic malignancies **(Sarantis et al., 2020).** PDAC is defined as a tumor microenvironment that is highly stromal and exhibits weak immunogenicity. This microenvironment facilitates tumor evolution and plays a role in the development of resistance to therapy **(Mizrahi et al., 2020).**

Surgical resection is the most commonly used strategy for PDAC treatment followed by adjuvant therapy but with long-term limited effectiveness **(Wood et al., 2022).**

Hence, it is crucial to investigate novel therapeutic approaches and supportive treatments that can improve the quality of patients' lives (Kim et al., 2021). Recently, there has been a growing focus on exploring the association between pancreatic cancer and different aspects of the gut microbiota **(Pushalkar et al., 2020; Sammallahti et al., 2021).** Consequently, the manipulation of the gut microbiota and the restoration of its diversity and balance might have a substantial impact on the management of this disease **(Mendez et al., 2021).**

According to the consensus statement by the International Scientific Association for Probiotics and Prebiotics (ISAPP), probiotics are defined as living microorganisms that confer health benefits when consumed in adequate quantities **(Varela-Trinidad et al., 2022).** Within the field of cancer research, probiotics have demonstrated the ability to bolster the immune response by attracting diverse immune cells, regulating inflammation, enhancing the integrity of the gut barrier, and exerting direct antitumor effects. These effects are achieved through mechanisms such as the production of antimicrobial peptides and the inhibition of cancer cell proliferation **(Śliżewska et al., 2020; Azad et al., 2018)**. The majority of research investigating the potential benefits of probiotic consumption on pancreatic cancer has been conducted using animal models. However, further investigation is necessary to fully comprehend its inhibitory role in the progression of pancreatic cancer. This requires additional exploration through preclinical and clinical studies **(Chen et al., 2020).**

Prebiotics are a substrate that is selectively utilized by host microorganisms, conferring a health benefit, such as mannan oligosaccharides, conjugated linoleic acids, polyunsaturated fatty acids, oligosaccharides such as fructooligosaccharides, inulin, galactooligosaccharides **(Gibson et al., 2017).** Prebiotics have also been shown to play a beneficial role in reducing the risk of inflammation and exhibiting antitumor effects **(Mahdavi et al., 2021).** On the other hand, Synbiotics encompass the combination of probiotics and prebiotics, providing a potential synergistic effect in modulating the gut microbiota and promoting overall health **(Markowiak and Śliżewska, 2017).** In the context of cancer therapy, synbiotics may exert their effects through mechanisms such as immunomodulation, augmentation of chemotherapeutic effectiveness, and mitigation of treatment-related side effects **(Singh et al., 2023).**

Recent studies and a meta-analysis have suggested that the inclusion of probiotic/synbiotic supplements is associated with a significant reduction in the risk of postoperative infectious complications in individuals who have undergone surgery **(Veziant *et al*., 2022).** However, further research is needed to validate these findings, as there may be publication bias and limitations in the quality of evidence that could influence the results **(Chen *et al*., 2022).**

The objective of this study was to assess the synergistic effect of synbiotics (combination of probiotics and prebiotics) compared to probiotics alone in providing a significant anti-tumor immunomodulation effect in PDAC patients, as well as their impact on postoperative complications and outcomes.

Methods

Ethical approval

The protocol of this study was approved by the Institutional Review Board (IRB) of Theodor Bilharz Research Institute (PT.652, 2021). The human subjects study were enrolled according to REC-TBRI's ethical standards and the 1964 Helsinki Declaration. Written informed consent forms were obtained from all participants.

Study design and patients

This single-blind randomized control study was conducted on patients with PDAC who underwent tumor resection at the General Surgery Department of Theodor Bilharz Research Institute Hospital from 2021 to 2023. The data of all patients, including laboratory and pathological data, were collected from the electronic

medical records of the TBRI hospital. All cases underwent Ct pancreatic protocol with EUS (endoscopic ultrasound) to exclude local vascular invasion before surgery. The inclusion criteria included patients with primary PDAC, with complete pathological and follow-up data, without long-distance metastasis, without chronic diseases, and without any treatments before the surgery. The exclusion criteria included the patients who suffered from other tumors or other chronic diseases or died from accidental death or other diseases, lack of pathological and follow-up data, and long-distance metastasis before the surgery. Once the informed consent was obtained, the included patients were randomized, using randomization software, into placebo, probiotic, and synbiotic receiving groups. All included patients were blinded for the intervention.

Probiotic and synbiotics treatment program

The probiotic and prebiotic medications utilized in this investigation are commercially available FDA-approved forms and have a well-established safety record. Throughout the administration process, both the patient and the nursing staff were obligated to report any possible side effects or unfavorable incidents. The study was halted if a patient withdrew their consent or if any significant adverse events related to the medication administration occurred. The probiotic agent 25 Billion CFU (Nowfoods, USA), composed of ten strains of bacteria (*Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus planta Lactobacillus paracasei, Bifidobacterium breve, Streotococcus thermophiles, Lactobacillus salivarius, and Bifidobacterium longum),* was taken in a dosage of two capsules once daily, two weeks (oral route) before the surgery and continued postoperatively for one month (first day post-operative by diluting the capsules in 50ml sterile water and given through feeding jejunostomy tube). For the synbiotic group, the same probiotic drug

was used in addition to taking Inulin 1000mg (Herbamama USA) two capsules once daily. For the placebo group, the same regimen was applied using a placebo drug.

Blood samples collection

For all studied groups, blood samples were collected at the baseline, 14 days pre-operative (Pre-14d), on the operation day (OP-0d), and 14 and 30 days post-operative (PO-14d and PO-30 respectively). Blood was collected into BD Vacutainer tubes and allowed to clot for 30 minutes. Afterward, it was centrifuged for 15 minutes at 1300 rpm. The separated serum was then stored in a -80°C freezer until analysis.

Histopathological preparation and technique

Following tumor resection, received pancreatic samples were submitted to the Pathology Department, TBRI for gross and microscopic examination. Pancreatic tissue was fixed for 24 hours in 10% neutral buffered formalin solution, then dehydrated in ascending grades of ethyl alcohol (70%- 90%- 100%), then processed in xylene and paraffinized in wax for preparation of formalin-fixed paraffin-embedded blocks. Sections of 4 um thick were cut and spread on positively charged glass slides for better adherence, then stained by Hematoxylin and Eosin (H&E) for evaluation of histopathological changes.

Immunohistochemical (IHC) technique

One paraffin-embedded block was selected from each case and was cut into 4 μm sections. unmasking for antigens was performed with 10 mM sodium citrate buffer, pH 6.0, at 90°C for 30 minutes, then incubated in 0.03% hydrogen peroxide for 10 minutes at room temperature to remove endogenous peroxidase activity, followed

by adding the blocking serum (0.04% BSA, A2153, Sigma-Aldrich, Shanghai, China, and 0.5% normal goat serum X0907, Dako Corporation, Carpinteria, CA, USA) for 30 minutes at room temperature. The sections were incubated with an anti-CD8 polyclonal antibody (YPA2235, Biospes, Chongqing, China), and anti- IFNγ polyclonal antibody (YPA2285, Biospes, Chongqing, China), at dilution of 1:100 for overnight at 4°C. Slides were incubated with ImmunoDetector DAB HBR detection system (BioSB, Santa Barbra, USA). Finally, staining was developed with 3,3'- diaminobenzidine (DAB) solution, and sections were counterstained with hematoxylin, dehydrated with graded ethanols, and mounted. Negative controls were carried out in which PBS was used instead of the primary antibody

Immunohistochemical interpretation and scoring

Scoring of CD8 and IFNγ immunostaining was performed blindly to the patients' clinicopathological data. CD8+T cells and IFNγ staining was interpreted as positive when >10% of cells showed distinct nuclear staining. The mean percentage of positive cells was calculated in 10 randomly selected fields at high magnification (x400). Interpretation of stained slides was done using a light microscope (Scope A1, Axio, Zeiss, Germany). Photomicrographs were taken using a microscope camera (AxioCam, MRc5, Zeiss, Germany).

Enzyme-linked immunosorbent assay (ELISA)

Serum concentrations of IL-10, IL-6, and IL-1B were determined using sandwich-based enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions (Sunlong Biotech, China). Briefly, 50 µL of diluted samples or standards were incubated in a micro ELISA strip plate that was pre-coated with antibodies specific to the tested cytokines. Horse radish peroxidase (HRP)-Conjugate was employed as a detection antibody. After incubation for 30

minutes at 37°C and three subsequent washes, TMP substrate was used to visualize the HRP-enzymatic reaction. The absorbance of the produced color was measured at 450 nm using an ELISA reader (Multiscan TMFC, Thermofisher, USA), and the cytokine concentration was then calculated from the standard curve.

Statistical analysis

Data were analyzed using statistical software package (IBM-SPSS) version 23 software. Kolmogorov–Smirnov test showed that the raw data were normally distributed. One ANOVA was applied to study the effect of treatment on the studied parameters. Two-way ANOVA was applied to study the effect of time and treatment on the studied parameters. The least significant difference (LSD) test was used to illustrate the statistical differences among the experimental groups. Duncan's test was used to illustrate the homogeneity among the different intervals. Data is displayed as mean ± standard error of the mean.

Results

Demographic characteristics of the studied patients

Between December 2021 and April 2023, and following the assessment of 115 primary eligible patients, 90 patients were randomly allocated into probiotics, synbiotics, and placebo groups (Fig. 1). Some patients were primarily excluded (n=10) because they denied consent (n=4), participating in another study (n=3) or were diagnosed by COVID 19 (n=3). Furthermore, during the follow-up period, we missed two patients in the placebo group due to discontinued follow-up, and we lost 4 patients in the probiotics group due to death (n=2) and discontinued follow-up (n=2), while in the synbiotics group, we missed three patients due to discontinued follow-up.

Baseline features homogeneity was displayed in the three groups (Table 1). There were no significant differences between the groups regarding demographic characteristics including sex, age, history of chronic disease, and body mass index.

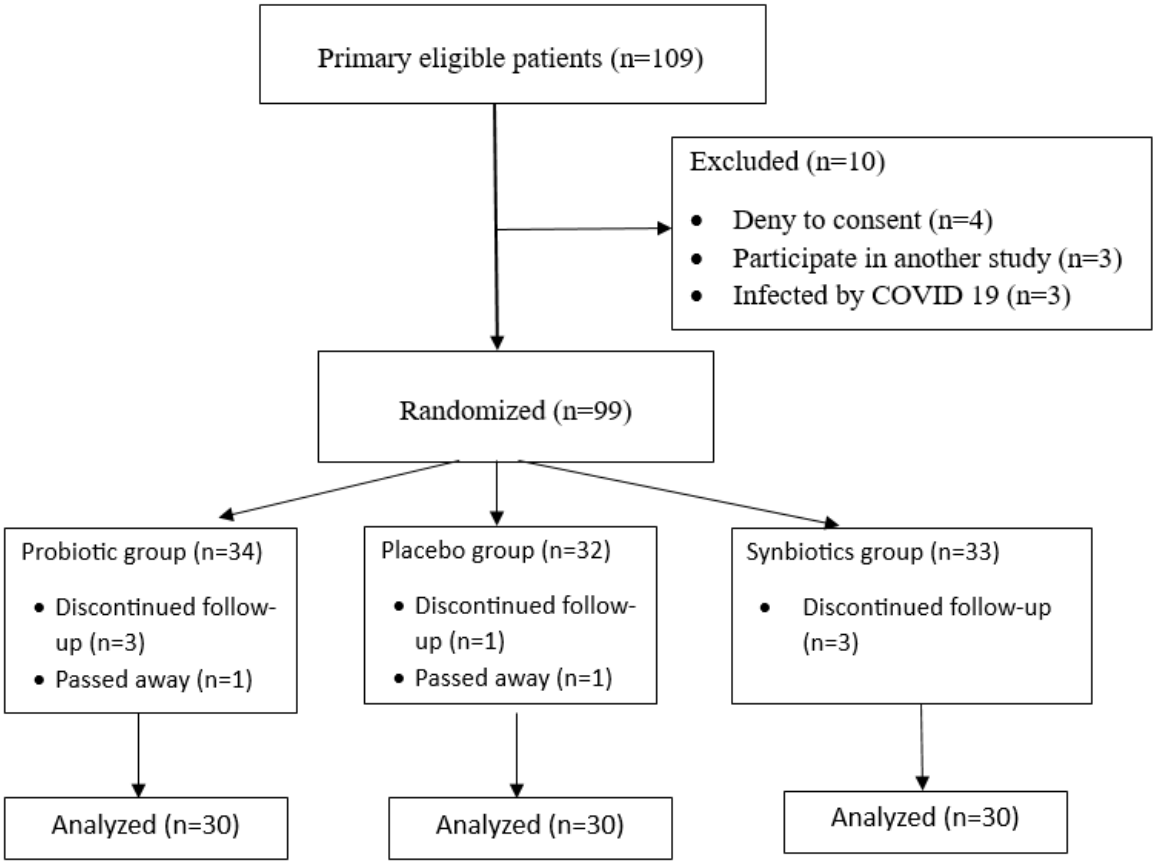


Figure 1: Consort diagram of study design

Table 1: Demographic characteristics of participants

Variable		Placebo N (%)	Probiotics N (%)	Synbiotics N (%)	P-value
Age category (year)	< 50	17(56.7)	12 (40)	16 (53.3)	0.393
	> 50	13 (43.3)	18 (60)	14 (46.7)	
Gender	Male	21 (70)	17 (56.7)	24 (80)	0.147
	Female	9 (30)	13 (43.3)	6 (20)	
Disease history	None	25 (83)	27 (90)	26 (86)	0.210
	Diabetes	2 (7)	1 (3)	2 (7)	
	Hypertension	3 (10)	2 (7)	2 (7)	
BMI (Mean ± SE)		22.40 ± 1.85	23.00 ± 1.27	22.00 ± 1.96	0.919

P>0.05: represents an insignificant effect.

BMI: body mass index

Tissue infiltration of CD8+ T cells and IFN-γ expression

Tumor tissue infiltration of CD8+T cells as well as the expression of IFN γ in tissue samples from 90 patients with PDAC were assessed by IHC (Figures 2(a)–(f)). It was observed that patients who received synbiotics and probiotics prior to surgery exhibited a significant increase in both the proportion of CD8+T cells as well as the expression of IFN-γ compared to those in the placebo group (p=0.000). Moreover, the expression of IFN-γ as well as CD8+ T cells infiltration was notably higher in the synbiotics-treated group in comparison to the probiotics-treated group (p=0.013, p=0.049 respectively) (Table 2)

Table 2: Tissue expression of CD8+cells and INF-γ in subjected groups

Variable	Group	Mean ± SE	P-values
INF-γ	Placebo	15.83 ± 2.28	---
	Probiotics	52.50 ± 5.66 *	P_1=0.002
	Synbiotics	60.50 ± 6.03*	P_1=0.000, P_2=0.013
CD8	Placebo	15.83 ± 2.63	---
	Probiotics	54.83 ± 8.32*	P_1=0.005
	Synbiotics	61.50 ± 6.62*	P_1=0.002, P_2=0.049

*: represents significant differences (P_1< 0.05), as compared to the placebo group

#: represents significant differences (P2< 0.05), as compared to probiotics group

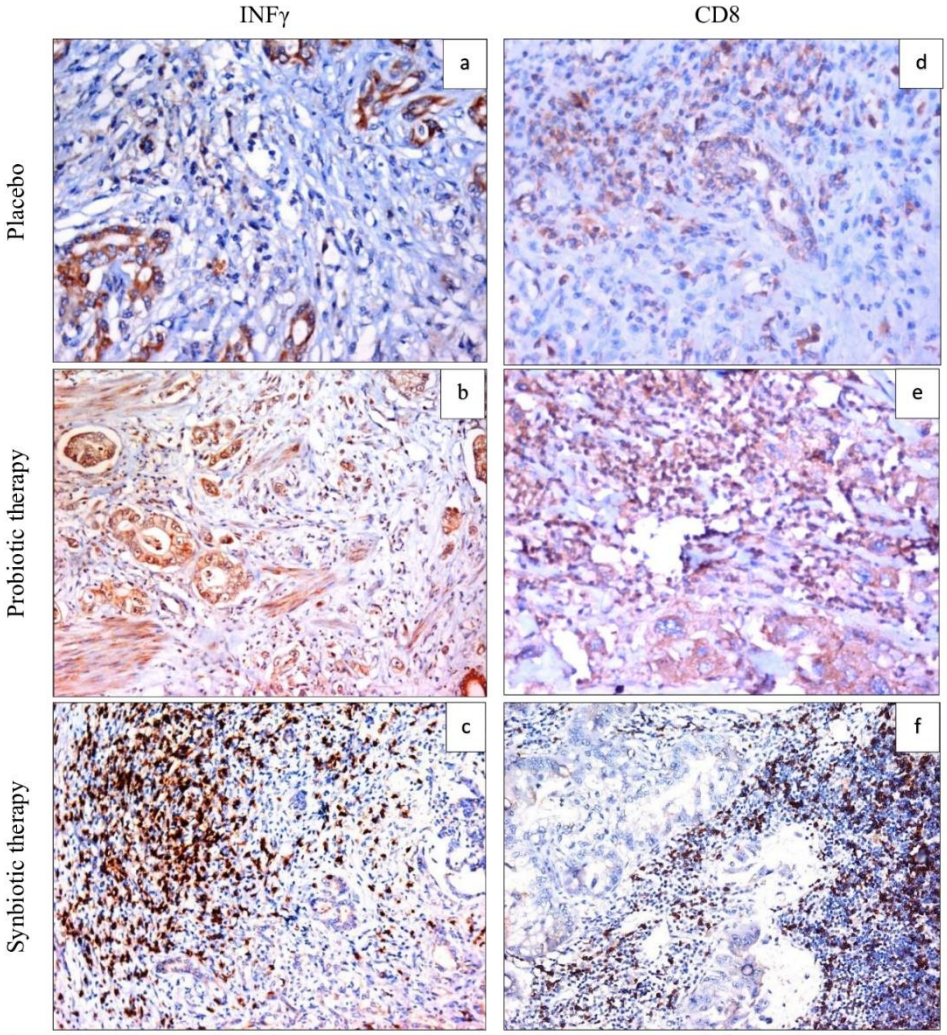


Figure 2: IHC expression of INFγ and CD8 in PDAC (a) PDAC, not receiving probiotic/synbiotic therapy, showing positive expression of INFγ in malignant glands and in <10% of associated inflammatory cells, (b) PDAC, receiving preoperative probiotic therapy, showing positive expression of INFγ in malignant glands and in >50% of associated inflammatory cells, (c) PDAC, receiving preoperative synbiotic therapy, showing positive expression of INFγ in >60% of associated inflammatory cells (IHC x200, (d) PDAC, not receiving probiotic/synbiotic therapy, showing positive expression of CD8 in <10% of associated inflammatory cells, (e) PDAC, receiving preoperative probiotic therapy, showing positive expression of CD8 in >50% of associated inflammatory cells, (f) PDAC, receiving preoperative synbiotic therapy, showing positive expression of CD8 in >60% of associated inflammatory cells (IHC x200).
IHC; immunohistochemistry, PDAC; pancreatic ductal adenocarcinoma

Association between clinicopathological parameters and tissue expression of INF-γ and CD8+T cells

On studying the association between the expression of INFγ and CD8 infiltration in the tumor tissues, and the clinicopathological parameters in the preoperative studied groups, we observed that pancreatic tissue exhibited a significant upregulation in the expression of INF-γ and CD8+T cells infiltration in both synbiotics and prebiotics treated groups compared to the placebo group. Moreover, as compared to the probiotic group, significant increases in the expression of INFγ and CD8 in the synbiotic group were obtained. In probiotics treated group, a significant elevation in the expression of INFγ was observed in female patients (p=0.011) younger than 50 years old compared to males, while in probiotics and synbiotics groups there were significant elevations in the expression of INF-γ with the pN-stage (P=0.002, P=0.025) as well as TNM stage (P=0.000, P=0.025), respectively. In the Synbiotic group, the significantly (P=0.036) higher pN-stage as well as TNM stage is proportional to the greater expression of CD8 (Table 3).

Table 3: The effect of clinicopathological parameters on the expression of INFγ and CD8+T cells in the tumor tissues of all groups

Clinicopathological parameters		INFγ			CD8		
		Placebo	Probiotic	Synbiotic	Placebo	Probiotic	Synbiotic
Age category	< 50	13.19 ± 0.74	27.50 ± 2.26*	35.89 ± 1.76*#	23.88 ± 0.77	32.25 ± 1.56*	42.78 ± 2.40*#
	p value	---	P2=0.000	P2=0.000, P3=0.001	---	P2=0.000	P2=0.000, P3=0.000
	> 50	13.65 ± 0.90	26.47 ± 2.01*	36.75 ± 1.34*#	26.15 ± 0.96	31.40 ± 1.30*	46.50 ± 1.50*#
	p value	---	P2=0.000	P2=0.000, P3=0.000	---	P2=0.005	P2=0.000, P3=0.000
	p value	P1=0.703	P1=0.735	P1=0.714	P1=0.083	P1=0.677	P1=0.188
Gender	Male	12.43 ± 0.55	23.43 ± 1.97*	36.60 ± 1.56*#	23.48 ± 0.76	32.29 ± 1.34*	46.60 ± 2.06*#
	p value	---	P2=0.000	P2=0.000, P3=0.000	---	P2=0.000	P2=0.000, P3=0.000
	Female	14.87 ± 1.11	30.69 ± 1.74*¶	36.42 ± 1.42*#	27.47 ± 0.85¶	31.23 ± 1.49	44.68 ± 1.67*#
	p value	---	P2=0.000	P2=0.000, P3=0.004	---	P2=0.064	P2=0.000, P3=0.000
	p value	P1=0.063	P1=0.011	P1=0.939	P1=0.001	P1=0.602	P1=0.491
pT-stage	pT2	12.50 ± 0.61	27.50 ± 1.88*	35.92 ± 1.75*#	23.00 ± 0.70	33.57 ± 1.02*	46.77 ± 1.82*#
	p value	---	P2=0.000	P2=0.000, P3=0.000	---	P2=0.000	P2=0.000, P3=0.000
	pT3	14.05 ± 0.87	26.31 ± 2.37*	36.94 ± 1.33*#	26.50 ± 0.86	29.85 ± 1.60	44.19 ± 1.82*#
	p value	---	P2=0.000	P2=0.000, P3=0.000	---	P2=0.069	P2=0.000, P3=0.000
	p value	P1=0.206	P1=0.695	P1=0.642	P1=0.007	P1=0.057	P1=0.329
pN-stage	pN1	13.78 ± 0.75	21.73 ± 1.91*	34.07 ± 1.11*#	24.89 ± 0.90	33.45 ± 1.72*	42.57 ± 1.60*#
	p value	---	P2=0.000	P2=0.000, P3=0.000	---	P2=0.000	P2=0.000, P3=0.000
	pN2	13.11 ± 0.92	30.50 ± 1.62*¶	38.73 ± 1.58*#¶	25.39 ± 0.97	30.63 ± 1.12*	47.93 ± 1.80*#¶
	p value	---	P2=0.000	P2=0.000, P3=0.000	---	P2=0.004	P2=0.000, P3=0.000
	p value	P1=0.58	P1=0.002	P1=0.025	P1=0.707	P1=0.162	P1=0.036
TNM stage	Stage II	13.78 ± 0.75	20.60 ± 1.71*	34.07 ± 1.11*#	24.89 ± 0.90	32.90 ± 1.80*	42.57 ± 1.60*#
	p value	---	P2=0.001	P2=0.000, P3=0.000	---	P2=0.000	P2=0.000, P3=0.000
	Stage III	13.11 ± 0.92	30.65 ± 1.53*¶	38.73 ± 1.58*#¶	25.39 ± 0.97	31.12 ± 1.16*	47.93 ± 1.80*#¶
	p value	---	P2=0.000	P2=0.000, P3=0.000	---	P2=0.002	P2=0.000, P3=0.000
	p value	P1=0.58	P1=0.000	P1=0.025	P1=0.707	P1=0.393	P1=0.036

pT-stage; pathological tumor stage, pN-stage; pathological lymph node stage, TNM: tumor lymph nodes metastasis stage. Data are displayed as mean ± standard error. *, #: significant differences as compared to the placebo (P2<0.05) and probiotic (P3<0.05) groups. In each group, ¶: significant differences (P1<0.05), as compared to values at age <50 or Males or pT2 or pN1 or Stage II.

Serum concentrations of IL-1 β, IL 6, and IL 10

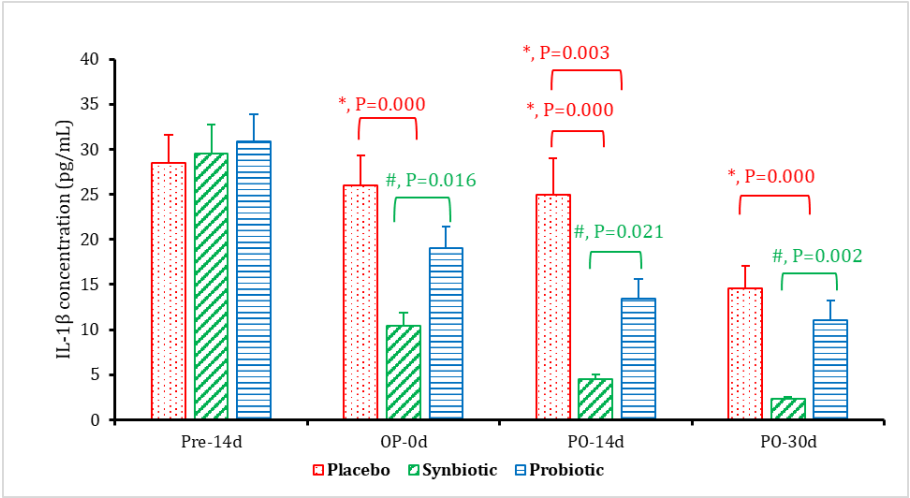
Cytokine concentrations at different time points pre and post-tumor resection were evaluated for all subjected groups, 14 days before surgery (Pre-14D), at the operation day (OP-0d), 14 and 30 days post-operative (PO-14d and PO-30d respectively). In the placebo group, there was no significant difference in the IL-1β concentrations at different time points but at PO-30d, where a significant decline was recorded, as compared to the baseline. In addition, as compared to the placebo group, a significant decline was observed at OP-0d, PO-14, and PO-30d (P=0.000) in the synbiotics-treated group and at PO-14 and PO-30d in the probiotics-treated group (P=0.000). However, the IL-1 β concentrations showed a significant decline in the synbiotics group compared with the probiotics group at the OP-0d (P=0.016), PO-14d (P=0.021), and P)-30 (P=0.002) (Table 4, Figure 3A). For IL-10 assessment, in the placebo group, there was no significant difference in the IL-10 concentrations among all the time intervals. In the synbiotics group and probiotics group, a gradual reduction in the IL-10 concentrations was observed from the OP-0d to PO-30d, as compared to the baseline (p= 0.002, p=0.000, p=0.000 respectively). Moreover, as compared to the placebo group, on the PO-14d and PO-30d, the IL-10 concentrations significantly declined (P=0.000). However, as compared to the probiotics group, the IL-10 concentrations showed a significant decline at OP-0d (P=0.04) (Table 4, Figure 3B). Regarding IL-6 concentration, there was no significant difference among all time intervals. In the probiotic and synbiotics group, a gradual reduction was detected from the OP-0d to PO-30d, as compared to the baseline. In addition, as compared to the placebo, on the OP-0d, PO-14d, and PO-30d, the IL-6 concentrations significantly declined (P=0.000) in both groups (p=0.000). However, as compared to the probiotic group, the IL-6 concentrations showed a significant decline at PO-14d (P=0.039) (Table4, Figure 3C)

Table 4. The concentrations of IL-1β, IL-10 and IL-6 in all the studied groups. Data is displayed as mean ± standard error.

	Group	Sampling time				Effect of Time
		Pre-14d	**OP-0d**	**PO-14d**	**PO-30d**	
IL-1B	**Placebo**	28.43 ± 3.15^{b}	25.97 ± 3.31^{b}	24.91 ± 4.08^{b}	14.60 ± 2.51^{a}	$F_{3,116}$=3.39, P=0.02
	Synbiotic	29.49 ± 3.24^{c}	10.44 ±1.44^{b*}	4.47 ± 0.59^{a*}	2.27 ± 0.23^{a*}	$F_{3,116}$=47.22, P=0.000
		P1=0.813	P1=0.000	P1=0.000	P1=0.000	
	Probiotic	30.88 ± 3.03^{c}	19.06 ± 2.36$^{b\#}$	13.42 ± 2.16$^{a*\#}$	11.05 ± 2.21$^{a\#}$	$F_{3,116}$=12.89, P=0.000
		P1=0.583, P2=0.754	P1=0.053, P2=0.016	P1=0.003, P2=0.021	P1=0.198, P2=0.002	
	Treatment	$F_{2,87}$=0.153, P=0.858	$F_{2,87}$=3.75, P=0.000	$F_{2,87}$=14.55, P=0.000	$F_{2,87}$=10.76, P=0.000	
IL-10	**Placebo**	21.42 ± 2.56^{a}	20.36 ± 2.44^{a}	22.48 ± 3.22^{a}	24.79 ± 2.36^{a}	$F_{3,116}$=0.504, P1=0.681
	Synbiotic	19.34 ± 1.98^{c}	11.31 ± 0.94^{b*}	7.69 ± 0.49^{a*}	5.85 ± 0.29^{a*}	$F_{3,116}$=27.96, P1=0.000
		P1=0.524	P1=0.002	P1=0.000	P1=0.000	
	Probiotic	20.31 ± 2.32^{c}	17.19 ± 2.26$^{b\#}$	12.81 ± 1.50^{a*}	8.89 ± 0.77^{a*}	$F_{3,116}$=7.48, P1=0.000
		P1=0.734, P2=0.765	P1=0.265, P2=0.04	P1=0.001, P2=0.084	P1=0.000, P2=0.14	
	Treatment	$F_{2,87}$=0.205, P2=0.815	$F_{2,87}$=5.29, P2=0.007	$F_{2,87}$=13.14, P2=0.000	$F_{2,87}$=49.69, P2=0.000	
IL-6	**Placebo**	18.93 ± 1.74^{a}	18.85 ± 1.59^{a}	23.42 ± 1.95^{a}	22.27 ± 1.56^{a}	$F_{3,116}$=1.84, P1=0.143
	Synbiotic	18.98 ± 1.59^{c}	11.38 ± 1.05^{b*}	6.91 ± 0.40^{a*}	4.79 ± 0.24^{a*}	$F_{3,116}$=40.97, P1=0.000
		P1=0.984	P1=0.000	P1=0.000	P1=0.000	
	Probiotic	18.14 ± 1.56^{c}	12.42 ± 1.22^{b*}	9.07 ± 0.75^{a*}	7.67 ± 0.58$^{a*\#}$	$F_{3,116}$=18.02, P1=0.000
		P1=0.733, P2=0.718	P1=0.001, P2=0.578	P1=0.000, P2=0.218	P1=0.000, P2=0.039	
	Treatment	$F_{2,87}$=0.083, P2=0.92	$F_{2,87}$=9.58, P2=0.000	$F_{2,87}$=53.45, P2=0.000	$F_{2,87}$=93.06, P2=0.000	

*: a significant difference (P1<0.05), as compared to the control group.
#: a significant difference (P2<0.05), as compared to the symbiotic group.

A


IL-1β concentration (pg/mL)
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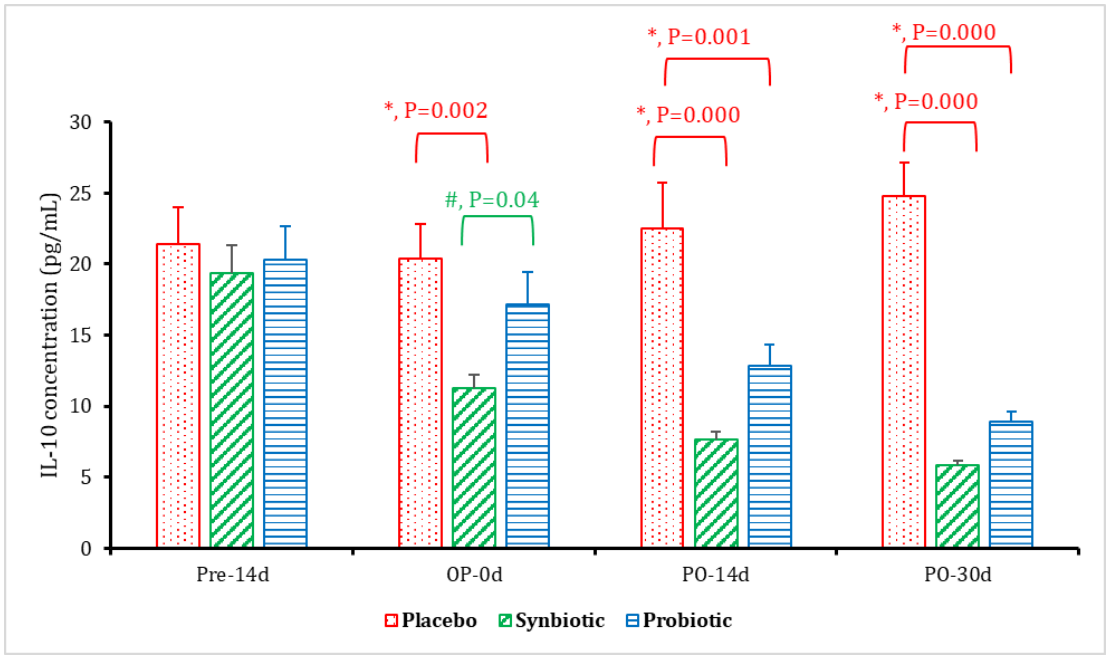
Pre-14d
OP-0d
PO-14d
PO-30d
Placebo
Synbiotic
Probiotic

B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IL-10 concentration (pg/mL)
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PO-14d
PO-30d
Placebo
Synbiotic
Probiotic

C

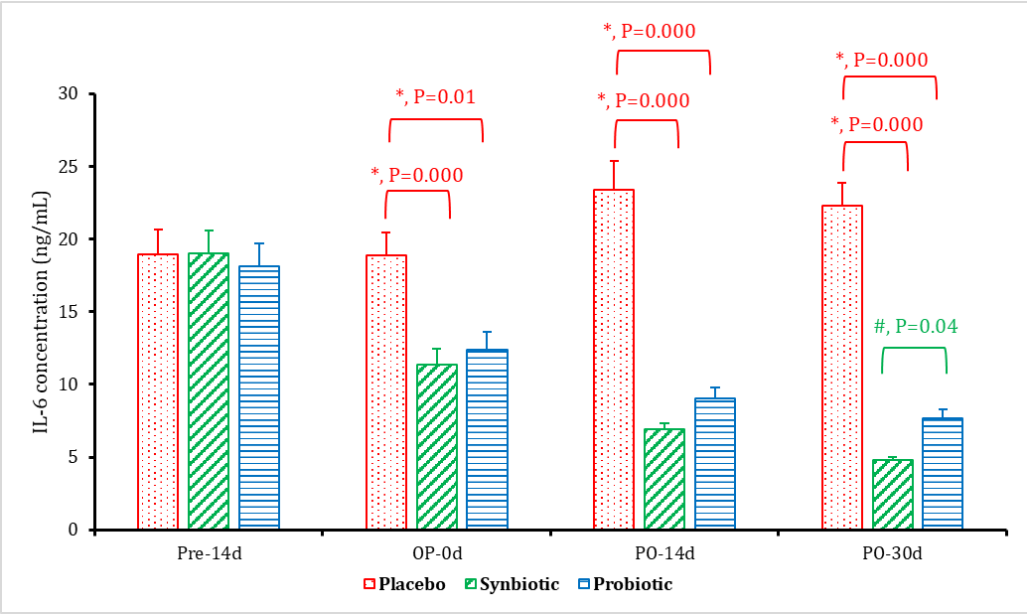

Fig 3. The concentrations of IL-1B (A), IL-10 (B) and IL-6 (C) in all the studied groups. Data is displayed as mean ± standard error. *: a significant difference (P1<0.05), as compared to the control group. #: a significant difference (P2<0.05), as compared to the symbiotic group.

Post-operative short-term outcome

Post-operative short-term outcome conditions were evaluated in all groups included in the study (Table 5). Both probiotics and symbiotics groups demonstrated a significant reduction in the average number of days until the first bowel movement, in comparison to the placebo group (P=0.000). There were no significant differences in the postoperative hospital stay period as well as the days till the return to normal activity. Incidence of infectious complications showed significant improvement in bacteremia in probiotics and synbiotics groups (0.047) with a remarkable decrease in the synbiotics group as well as the incidence of pneumonia. Non- infectious complications including anastomotic leakage, diarrhea, and abdominal distension, showed significant improvement in synbiotics and probiotics receiving- groups compared to the placebo group (p=0.032, p=0.044, and p= 0.42 respectively). Moreover, diarrhea and abdominal distension were particularly noteworthy decreases in the synbiotics group.

Table 5. Postoperative short-term outcomes

	Placebo cases (n=30)	**Probiotic cases (n=30)**	**Synbiotic cases (n=30)**	***P-Value***
Bleeding (%)	1/30 (3.3%)	1/30 (3.3%)	1/30 (3.3%)	1.000
Mean Day to First Stool (day)	5.00 ± 0.13	4.00 ± 0.09*	4.00 ± 0.14*	*0.000*
Post-operative stay (day)	10.00 ± 0.13	10.00 ± 0.14	10.00 ± 0.14	1.000
Return to usual activity (day)	14.00 ± 0.13	14.00 ± 0.17	14.00 ± 0.08	1.000
Incidence of infectious complications (%)				
Bacteremia	15/30 (50.0%)	7/30 (23.3%)	4/30 (13.3%)*	0.047
Wound infection	2/30 (6.7%)	1/30 (3.3%)	1/30 (3.3%)	0.770
Pneumonia	4/30 (13.3%)	2/30 (6.7%)	2/30 (6.7%)	0.578
Urinary tract infection	5/30 (16.7%)	5/30 (16.7%)	4/30 (13.3%)	0.919
Incidence of non-infectious complications (%)				
Anastomotic Leakage	3/30 (10.0%)	0/30 (0 %)*	0/30 (0 %)*	0.032
Diarrhea	17/30 (56.7%)	10/30 (33.3%)*	8/30 (26.7%)*	*0.044*
Abdominal distension	16/30 (53.3%)	10/30 (33.3%)*	7/30 (23.3%)*	0.042
Mortality (n)	N/P (0 %)	N/P (0%)	N/P (0%)	0.364

Data are presented as mean ± standard error and number (percentages). P<0.05: Significant differences according to Chi-squared and One-way ANOVA.

Discussion

In contrast to the numerous studies conducted on colorectal cancer (CRC) and gastric cancer (GC), there is a lack of research exploring the role of synbiotics in the prevention and treatment of other gastrointestinal (GI) cancers, such as pancreatic and liver cancer. Only a limited number of published reports have investigated the potential link between probiotics and prebiotics in pancreatic ductal adenocarcinoma (PDAC), focusing primarily on the suppression of tumorigenesis and the impact on post-surgical complications **(Abdul Rahman et al., 2023; Rad et al., 2021; Tang et al., 2021).** To the best of our knowledge, this is the first randomized clinical study to evaluate the immunomodulatory effect and post-surgical complications of oral synbiotics (probiotics and inulin) compared to probiotics alone in PDAC patients. Our results indicated that consuming synbiotics and probiotics supplements for six weeks (two weeks pre-operative and four weeks post-operative), significantly improved the immune response in the treated groups compared to the placebo group. Immunohistochemical results showed that the proportion of CD8+ T cells and the expression of IFN-γ in tumor tissue were significantly higher in patients treated with synbiotics or probiotics compared to those treated with a placebo. Additionally, the synbiotics group showed a greater elevation in CD8+ T cells and IFN-γ expression compared to the probiotics group. These findings are consistent with the results obtained by Mao et al. (2020), who suggested that probiotics could enhance the antitumor immune response of CD8+ T cells and IFN-γ+ T cells in the tumor microenvironment in patients with colorectal carcinoma **(Mao et al., 2020)**. In our study, we used a ten-strain cocktail of probiotics including *Bifidobacterium, Lactobacillus, and Streptococcus* bacteria strains in addition to inulin fibers prebiotics. Several studies have demonstrated the efficient immunomodulatory effects of such strains. For example, Yoon et al. (2021) found that supplementation with *B. breve* strains reduced tumor growth in mice with colon carcinoma by augmenting lymphocyte-mediated anti-cancer immunity **(Yoon et al., 2021)**. Similarly, Lee et al. (2015) concluded that *Lactobacillus acidophilus* strains increased serum IFN-γ, CD4+, and CD8+ cells in mice with induced colon cancer **(Lee et al., 2015).** Other studies have also shown the modulation of the anti-

tumor immune response by using a combination of *Bifidobacterium* or *Lactobacillus acidophilus* bacteria, leading to the improvement of dendritic cells function, infiltration of CD8+ T cells, and reduction of inflammatory cytokines **(Sivan et al., 2015; Gui et al., 2015).**

In our study, the significant increase in the expression of CD8+ T cells and IFN-γ in the synbiotics group compared to the probiotics group may be attributed to the beneficial effect of consuming inulin prebiotics along with these probiotic strains. These results are consistent with various pre-clinical studies that have suggested the anti-tumor immune system effect of inulin, particularly when combined with different probiotic regimens. For instance, Kassayová et al. (2016) found that consuming *Lactobacillus plantarum* along with inulin prebiotics reduced carcinogenesis by inducing apoptosis and diminishing pro-inflammatory cytokines in a colon cancer model **(Kassayová et al., 2016).** Also, Li et al. (2020) used inulin and mucin prebiotics in their study on colon cancer in mice and observed that dietary consumption of inulin, but not mucin, reduced tumor growth through the induction of CD8+ T and CD4+ T cells mediated anti-tumor immune response **(Li et al ., 2020**). Boucher et al. (2023) also demonstrated that an inulin-enriched diet along with probiotic strains triggered an enhanced CD4+ and CD8+ αβ T cell-mediated anti-tumor response and attenuated tumor growth in preclinical tumor-bearing mouse models **(Boucher et al., 2023).**

Elevated levels of inflammatory-mediated cytokines such as IL-6 and IL-10 have been associated with poor prognosis in patients with pancreatic cancer **(Feng et al., 2018; Lippitz et al ., 2016; Tanaka et al., 2014).** In our study, we evaluated the concentration of circulating inflammatory-mediated cytokines (IL-10, IL-6, and IL-1ß) in all groups at different time intervals before and after tumor resection. Our results showed a significant decrease in all tested cytokines in both the probiotics and synbiotics groups compared to the placebo group. The synbiotics group exhibited a more pronounced decline in IL-1ß levels on the day of the operation, 14 days, and 30 days after surgery. These findings are consistent with Das et al. (2020), who discussed the association between poor prognosis of pancreatic cancer and elevated concentrations of IL-1ß. They suggested that neutralizing IL-1ß could promote intratumoral CD8+ T cell infiltration and function and sensitize pancreatic ductal adenocarcinoma to checkpoint immunotherapy **(Das et al., 2020).** Additionally, we found that IL-6 and IL-10 levels gradually decreased on the day of the operation and 30 days post-operative compared to the baseline. The synbiotics group showed a significant reduction in IL-6 levels 14 days post-operative and in

IL-10 levels on the day of the operation compared to the probiotics group. This reduction in cytokine levels can be attributed to the synergistic anti-inflammatory effect of synbiotics, particularly due to the inclusion of inulin fibers as a prebiotic. These results align with other studies that have discussed the anti-inflammatory and immunomodulatory effects of inulin prebiotics **(Farabegoli et al., 2023; Abreu et al., 2022).**

Considering that surgery is the primary determinant of prognosis in PDAC, and holds curative potential, our study aimed to investigate the short-term post-operative outcomes when probiotics or synbiotics were administered. Limited research has been conducted on the supplementation of probiotics or synbiotics in patients undergoing pancreaticoduodenectomy. Between 2007 and 2021, only six studies with conflicting results were carried out, involving a total of 294 participants (147 in the control group and 147 in the intervention group). Three studies utilized probiotics with limited strains, Folwarski et al. (2021), Diepenhorst et al. (2011), and **Nomura et al. (2007),** and three studies used synbiotics, including Yokoyama et al. (2016), **Sommacal et al. (2015),** and Rayes et al. (2007). In our study, we observed a significant reduction in the average number of days until the first bowel movement and the incidence of diarrhea in both the synbiotics and probiotics groups compared to the placebo group. The reduction was particularly notable in the synbiotics group. These findings are consistent with the results obtained by **Folwarski et al. (2021) and Diepenhorst et al. (2011**), who observed that the consumption of *Lactobacillus* strains improved bacterial translocation and postoperative outcomes after pancreatoduodenectomy. Furthermore, our results showed a decrease in infectious complications in both the probiotics and synbiotics groups compared to the placebo group, with a more pronounced improvement observed in the synbiotics group. These findings are in line with the research conducted **by Rayes et al. (2007).** In their prospective randomized trial involving 80 patients who underwent pylorus-preserving pancreatoduodenectomy (PPPD) and received lactobacillus and fibers for 30 days, they observed a significant reduction in bacterial infection rates following PPPD **(Rayes et al., 2007).** Sommacal et al. (2015) also concluded that perioperative synbiotics supplementation decreases postoperative complications in periampullary neoplasms. Their study included 23 patients who received a cocktail of Lactobacillus strains and fructooligosaccharides twice daily for 14 days, resulting in improved hospital stay length and a decreased incidence of infection. Regarding postoperative hospital stay and the time required for normal activities, there were no significant differences observed among the

groups. We believe that this is directly related to our protocol in TBRI, which includes routine feeding jejunostomy in all cases and the use of a new technique for anastomosis, specifically end-to-side duct to mucosa with an internal stent and two-layered pancreaticojejunostomies with omental wrapping.

Conclusion

This study provided evidence of the significant immunomodulatory impact of synbiotics supplementation and its contribution to improving post-operative complications in PDAC patients. However, further large-scale studies are necessary to explore more pertinent clinical evidence in this field. These studies should involve diverse combinations and regimens of probiotics and prebiotics administered over extended periods. Additionally, investigating the potential synergies between synbiotics and immunotherapeutic drugs is crucial. By conducting such research, we can gain a deeper understanding of the topic and obtain more valuable clinical insights.