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The effect of probiotics on fecal calprotectin in critically Ill children

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Abstract

Gastrointestinal complications are very common in the critically ill children due to the disruption of normal gut microbiota thus facilitating the colonization and overgrowth of the harmful bacteria across the intestinal mucosal barrier which precipitates to GIT inflammation reflected by fecal calprotectin as well as sepsis, hence comes the importance of probiotics in maintaining the equilibrium between the pathogenic and gut microflora. In this study, we evaluated the relation between fecal calprotectin and intestinal complications in critically ill children and the efficacy of enteral probiotics (Lactobacillus) in reducing gastrointestinal inflammation in critically ill children. This prospective single blinded randomized control trial (RCT) study included 45 critically ill children who were admitted to PICUs, Faculty of Medicine, Cairo University, over a duration of 9 months recruitment, from March 2021 to October 2021.Probiotics had a positive effect on lowering fecal calprotectin, total leucocytic count and CRP but showed no effect on reducing the incidence of gastrointestinal complications in PICU.

Keywords: Critically ill child, Probiotics, Fecal Calprotectin, GIT complications, Sepsis.

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1. Introduction

Gastrointestinal complications are very common in the critically ill children but unfortunately, they do not get the importance they deserve and are not included in any of the scoring systems used to assess multi-organ system failure (MOSF). These complications may be due to the hostile environment in the intensive care units (ICUs) causing the disruption of normal gut microbiota, secretion of stress hormones, the use of broad-spectrum antibiotics and corticosteroids, cessation of oral intake, mechanical ventilation and other invasive procedures [1]. Most gastrointestinal complications in the ICUs are transient and overlooked by the physicians such as constipation, diarrhea and feeding intolerance but other complications are very noxious and need more attention such as stress ulcers and gastrointestinal bleeding [2]. Gut microbiota act as a barrier to the pathogenic organisms across the gut mucosa. Critical illness facilitates the colonization and overgrowth of the harmful bacteria across the intestinal mucosal barrier which precipitates to systemic inflammatory response syndrome and sepsis, hence comes the importance of probiotics in maintaining the equilibrium between the pathogenic and gut microflora [3].

Commonly used probiotics are derived from gut microbiota so help in restoring them and providing their same effect in the prevention of gastrointestinal colonization and subsequent sepsis, as proved by Alfaleh et al, 2014 that enteral probiotics help in the prevention of necrotizing enterocolitis, late onset sepsis and candidiasis in critically ill neonates, antibiotic associated diarrhea in neonatal intensive care units (NICUs) and in the pediatric intensive care units (PICUs). Probiotics proved also to reduce candiduria by 50% as well as candidemia and decrease gastrointestinal candida colonization, On the other hand; probiotics should be used cautiously with immunocompromised ill children as they may predispose to fungemia, bacteremia and sepsis [4].

The beneficial effects of probiotics are due to their role in the enhancement of gut flora hence the improvement of the immune response. Probiotic strains activate mucosal immunity and stimulate cytokine production,

Immunoglobulin A (IgA) secretion, phagocytosis, and production of substances (such as organic acids and hydrogen peroxide) that are inhibitory to pathogens. They also compete for nutrients with pathogenic bacteria and inhibit pathogen attachment and action of microbial toxin. Probiotics also have a trophic effect on intestinal mucosa (by stimulating the proliferation of normal epithelium that maintains mucosal barrier defences), modulate innate and adaptive immune defence mechanisms via the normalization of altered gut flora, and the prevention of bacterial translocation [5]. Fecal calprotectin (FC) measurement is a test used to measure the protein calprotectin in the stool and its elevation indicates neutrophil migration to the intestine denoting underlying gastrointestinal inflammation, Calprotectin is isolated from almost all the body fluids, its elevation is a marker of inflammation and many studies have correlated between its elevation and certain gastrointestinal inflammatory diseases such as inflammatory bowel disease (IBD), cow milk protein allergy, necrotizing Enterocolitis (NEC), celiac disease and bacterial gastrointestinal infection by Salmonella or Campylobacter Jejeuni [6].

Other researchers such as Campeotto et al, (2007) [7] and Yang et al, (2008) [8] were interested in studying the relationship between elevated fecal calprotectin and gastrointestinal distress, which was defined as gastrointestinal bleeding, diarrhea, and/or abdominal distension in sick children and proved its elevation in sick children in comparison to ‘’not sick’’ children [6].

2. Materials and Methods

This prospective single blinded randomized control trial (RCT) study included 45 critically ill children who were admitted to PICUs, Faculty of Medicine, Cairo University, after taking an informed consent, over a duration of 9 months recruitment, from March 2021 to October 2021. 45 critically ill children whose age ranged from 1 month to 14 years were enrolled and were divided into 2 groups. Group A (22 critically ill children) received one sachet of Lacteol fort® (Lactobacillus) once daily by enteral route for 5-7 days. Group B (23 critically ill children) received placebo. Patients with congenital anomalies, inborn errors of metabolism, immunodeficiency or conditions necessitating nothing per os were excluded from the study. Simple randomization was done using computerized sequence generation (www.randomizer.org). Blindness was done using aliquots covered with opaque plaster. After inclusion, patients' detailed history was taken to gather data related to their age, gender, cause of admission, chronic diseases and nutritional history and their clinical and laboratory evaluation was done. Fecal Calprotectin was withdrawn on admission & before discharge (day 7 of admission) from a fresh stool sample 1-5gm that was kept in the refrigerator for a period up to 4 days then was frozen at -20 degree Celsius until the time of essay then fecal calprotectin was measured by ELISA (enzyme-linked immunosorbent assay). Probiotics (Lactobacillus) was started when oral intake is started in a daily dose of one sachet by dissolving it in 5 ml distilled water and was given orally or by a Ryle daily for 7 days.

Statistical analysis was done using Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric while data with non-parametric distribution were presented as median with inter-quartile range (IQR). Also, qualitative variables were presented as number and percentages. The comparison between groups with qualitative data were done by using Chi-square test and Fisher exact test instead of the Chi-square only when the expected count in any cell found less than 5. The comparison between two groups with quantitative data and parametric distribution were done by using independent t-test while with non-parametric distribution were compared using Mann-Whitney test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: $P > 0.05$: Nonsignificant, $P < 0.05$: Significant, $P < 0.01$: Highly significant.

3. Results and discussion

Our study was conducted over 45 critically ill children, with a median age of 4 years, with male predominance (62.2%). The most common cause of admission was respiratory causes such as pneumonia (51%), followed by neurological causes such as convulsions (22%) or miscellaneous causes (11%) such as DKA and AKI, sepsis and septic shock (8.8%) and finally gastrointestinal causes (6.6%) such as chronic diarrhea and dehydration. Regarding anthropometric measures, 62.2% had normal weight versus 37.8% were underweight, 71.1% had normal height/length and 28.9% were of short stature. Vital signs assessment was as following: 64.4% were normothermic versus 35.6% were hyperthermic and needed antipyretics administration, 73.3% were normotensive, 17.8% were hypertensive, 8.9% were hypotensive, 93.3% had normal pulse versus 6.7% who were tachycardic, 93.3% had normal respiratory rate versus 26.7% who were tachypnic. Regarding laboratory evaluation including blood culture, initial (on admission) and follow up (on discharge) C-reactive protein (CRP), initial and follow up Total leucocytic count (TLC) as summarized in table 1.

And for the clinical evaluation of sepsis guided by temperature instability, hyperventilation, tachycardia and confusion evaluated by the pediatric risk of mortality score (PRISM III) it ranged from 9 to 42 and 46.7% of our patients had clinical signs of sepsis versus 53.3% didn't show any signs of sepsis. Table 10. Finally, 22% of our patients needed triple or more antibiotics versus 77.7% who only needed single or double antibiotics. 33.3% of our patients suffered from GIT complications where diarrhea was the most common complication by 13.3%, 6.7% had feeding intolerance, vomiting or regurgitation and finally 4.4% suffered from aspiration. And 66.7% of our patients didn't have any GIT complications. 42% of our patients had elevated initial FC, 10 patients from probiotics group versus 9 patients from placebo group. And the follow up FC was elevated in 26.6 % of our patients, 7 patients from probiotics group versus 5 from placebo group. The median length of stay (LOS) of our patients was 10 days (7-20 days) with 6.7% mortality. Our study found statistically significant decreased fecal calprotectin after administration of probiotics in critically ill children. In agreement with Fallahi et al. in 2013 [9] study which was conducted on 47 patients with cystic fibrosis and found statistically significant lower fecal calprotectin concentrations in probiotic group compared with placebo with p-value <0.001.

Another study by Jung et al. (2022) [10] which evaluated the clinical and laboratory effects of lactobacillus plantarum in functional diarrhea patients with elevated levels

of fecal calprotectin for 2 months and found statistically significant decreased fecal calprotectin in probiotic group with p-value=0.046 with no significant change in fecal calprotectin in placebo group with p-value=0.141. Similarly, another study by Ananthan et al. (2016) [11] which was conducted on 275 children with cystic fibrosis to evaluate the effect of probiotic use on gut inflammation and revealed statistically significant decreased level of calprotectin after probiotic use with p-value=0.002 with significant improvement of gastrointestinal symptoms. Another Egyptian study by El Fragy & Hassan (2014) [12] was conducted to evaluate the effect of probiotic supplementation on fecal calprotectin level which was tested as an early marker of necrotizing enterocolitis and found statistically significant decreased level of fecal calprotectin level in probiotic group with pvalue =0.024 in the first week and p-value=0.019 after 2weeks. Similarly, Mohan et al. (2008) [13] found that bifidobacterial supplementation (probiotics) was associated with a significant decrease in calprotectin level and NEC occurrence in neonates. Our current study revealed statistically significant difference between probiotics and placebo as regards effect on infection determined by CRP and WBCs but statistically non-significant difference regarding blood culture, clinical signs of sepsis, PRISM score, mechanical ventilation, length of hospital stay and the need for more than 2 antibiotics. Our current study found statistically significant reduction of WBCs in probiotics group compared to placebo group with p-value=0.014. In agreement with Enayet et al. (2013) [14] study which aimed to evaluate the effect of probiotics to decrease risk of post-surgical infection in under 5 children following gastrointestinal surgery and revealed statistically significant decreased risk of postsurgical infection in probiotic group determined by WBCs and CRP with p-value <0.05. Similarly, another study by Cavalcante et al. (2020) [15] which was conducted to evaluate the effect of probiotics to improve immunity and revealed statistically significant decreased WBCs count in probiotic group compared to control group with p-value=0.004. This can be explained by the effect of probiotics in modulation of immune system and inhibiting growth of pathogenic bacteria [16]. The current study found statistically significant decrease of CRP in probiotic group compared to placebo with p-value=0.04. This goes in line with Agrawal et al. (2018) [17] which evaluated the role of probiotics on CRP in preterm infants and found no statistically significant decrease of CRP. Asemi et al. demonstrated that consumption of probiotic supplements results in reduction in CRP compared to placebo. This can be explained by the effect of probiotics on serum CRP by the effect of short chain fatty acids that are produced from probiotics in the colon with subsequent decrease CRP synthesis from liver [18]. Angurana et al. (2018) [19] conducted a study to evaluate the effect of probiotics on cytokine levels denoting immune response in critically ill children and revealed statistically significant increase anti-inflammatory cytokines e.g., IL-10 and transforming growth factor B1 in probiotics compared with placebo with pvalue=0.001. Wang et al. (2014) [20] performed a randomized controlled study on 100 critically ill infants who were administered a probiotic mix (L.casei, L.acidophilus, Bacillus subtitlis, and Enterobacterococcus faecalis) three times daily for 8 days and that enhanced immune activity, decreased incidence of nosocomial pneumonia and MODS and reduced length of hospital stay. Banupriya et al. (2015) [21] conducted a randomized control trial on 150 children aged 12 years or younger who needed mechanical ventilation for more than 48 hours who were divided into 2 groups (intervention group received a probiotics mix of L.acidophilus, L.rhamnosus, Lactobacillus plantarum, L.casei, Lactobacillus bulgaricus, Bifidobacterium longum, B.infantis, for 7 days or until discharge, and control group who did not receive either probiotics or placebo) and revealed that probiotics resulted in significant decrease in incidence of VAP, hospital stay, and mechanical ventilation. Also, probiotic group had lower colonization rates with potentially pathogenic organisms (klebsiella and pseudomonas) (34.3% versus 51.4%, p-value=0.05), and reduction of VAP caused by Klebsiella (42.2% versus 19.4%, p-value=0.01) and pseudomonas (4.2% versus 16.7%, p-value=0.03). Srinivasan et al. (2006) [22] conducted a prospective study on children admitted to PICU (n=28) to establish clinical safety (invasive infection/colonization) of L.casei Shirota by bacteriologic surveillance in surface swabs and endotracheal aspirates (colonization) as well as blood, urine, and sterile body fluid cultures and found no evidence of either colonization or bacteremia with L.casei Shirota.

Rao et al. (2016) [23] conducted a study to evaluate the effect of probiotics on late onset sepsis in preterm and revealed statistically significant decreased risk of late onset in probiotics group with p-value=0.007. In contrast to Honeycutt et al. (2007) [24] who conducted a study on 61 critically ill children to evaluate efficacy of probiotics in reducing rate of nosocomial infection, where the children received either a probiotic (one capsule of L.rhamnosus strain GG daily) or placebo (one capsule of inulin) until discharge from the hospital and observed a statistically non-significant trend toward an increased rate of infection with probiotic strain (P-value=0.31). The current study found no statistically significant difference between the two studied groups as regards the need of more than two antibiotics. In contrast to King et al. (2019) [25] who performed a systemic meta-analysis study which included 17 RCT to evaluate the role of probiotics and revealed statistically significant decrease the need for antibiotics in probiotics group with p-value =0.02. This can be explained by the effect of probiotics to reduce the risk of common illnesses in PICU (Hao et al., 2015). Five RCTs by Hatakka et al. (2001)[26], Hatakkaa et al. (2007) [27], Kumpu et al. (2012) [28], Rautava et al. (2009) [29], and Taipale et al. (2011) [30] described the total number and percentage of patients treated at least once with antibiotics in the probiotic and placebo groups and found that in two studies Hatakka et al. (2001) [26] and Kumpu et al. (2012) [28], there was no difference between groups, in two other studies Hatakka et al. (2007) [27] and Rautava et al. (2009)[29] observed that antibiotics were prescribed in placebo group more than probiotic group. Our current study found no statistically significant difference as regards the need for using antifungal between probiotic group and placebo group with p-value >0.05. Another study by Kumar et al. (2013) [31] was conducted on 150 children on broad spectrum antibiotics for at least 48 h to compare the effect of probiotics vs placebo on colonization of GIT and found significant increase in patients colonized by candida species and significant decrease of candida in urine and using of probiotics was associated with significant reduction in gastrointestinal candida colonization.

Table 1: Laboratory and clinical indicators of sepsis in our studied patients

Blood culture	No	18 (40.0%)
	Negative	20 (44.4%)
	Positive	7 (15.6%)
Initial TLC	Median (IQR)	12 (8.6 – 17)
	Range	2.1 – 34
Follow up TLC	Median (IQR)	10 (8.2 – 13)
	Range	3.2 – 26
Initial CRP	Median (IQR)	36 (11 – 104)
	Range	0.2 – 315
Follow up CRP	Median (IQR)	34 (6 – 58)
	Range	1 – 159
PRISM score	Mean±SD	21 ± 7.20
	Range	9 – 42
Signs of sepsis	No	24 (53.3%)
	Yes	21 (46.7%)
Need for > 2 antibiotics	Yes	10 (22%)
	No	35 (77.7%)

Table 2: Correlation between FC and GIT complications in our patients

		Initial FC	**P-value**	**Follow up FC**	**P-value**	**FC difference**	**P-value**
		Median (IQR)		**Median (IQR)**		**Median (IQR)**	
GIT complications	No	60.2 (13.6 – 593)	0.665	37.8 (8.45 – 163.05)	0.286	-18.95 (-354.05 – 1.25)	0.251
	Yes	76.2 (9.7 – 1504)		103.75 (13.2 – 290.3)		-1.75 (-493.7 – 38.2)	
Feeding intolerance	No	74.6 (13.4 – 821.2)	0.439	51.2 (11.5 – 181.9)	0.435	-11.7 (-448.4 – 2)	0.449
	Yes	12.9 (3.2 – 755)		24.7 (2.3 – 111.7)		21.5 (-752.7 – 98.8)	
Diarrhea	No	46.9 (11.3 – 593)	0.023	34.8 (8.7 – 111.7)	0.005	-9.3 (-259.7 – 2)	0.861
	Yes	1222 (577.1 – 1855.7)		512 (290.3 – 1893)		-428 (-493.7 – 37.3)	
Vomiting	No	74.6 (13.4 – 821.2)	0.237	40.95 (10.1 – 181.7)	0.679	-10.5 (-438.2 – 2.05)	0.637
	Yes	9.7 (1.5 – 577.1)		92.95 (83.4 – 102.5)		-196.35 (-493.7 – 101)	
Regurgitation	No	116.9 (12.9 – 821.2)	0.122	67.9 (12.2 – 181.9)	0.079	-16.8 (-493.7 – 21.5)	0.420
	Yes	13.4 (3.9 – 16.3)		11.5 (2.3 – 13.2)		-1.9 (-3.1 – -1.6)	
Aspiration	No	47.4 (11.3 – 755)	0.226	40.95 (10.1 – 181.7)	0.376	-10.5 (-438.2 – 2.05)	0.839
	Yes	1065.1 (76.2 – 2054)		109.7 (105 – 114.4)		-955.4 (-1949 – 38.2)	

Table 3: Correlation between FC with age, length, weight/height, PRISM score, length of stay (LOS) and mortality in our studied patients

		Initial FC		Follow up FC		FC difference	
		r	P-value	r	P-value	r	P-value
Age (months)		-0.202	0.184	-0.183	0.246	-0.073	0.648
Length (cm)		-0.217	0.152	-0.125	0.430	0.065	0.681
Weight (kg)		-0.172	0.260	-0.106	0.504	-0.031	0.84
PRISM		-0.281	0.062	-0.375*	0.014	0.302	0.052
Length of stay (days)		-0.086	0.587	-0.005	0.973	0.324*	0.036
Mortality	Yes	116.9 (13.6 – 821.2)	0.025	46.15 (11.5 – 181.5)		10.5 (-448.4 – 2.1	
	NO	4.7 (1.8 – 9.7)		------		-----	

Table 4: Correlation between FC and TLC and CRP in all our studied patients

	Initial FC		Follow up FC		FC difference	
	r	P-value	r	P-value	R	P-value
Initial TLC	-0.054	0.722	0.099	0.535	-0.110	0.488
Follow up TLC	-0.013	0.932	0.123	0.439	0.123	0.440
TLC difference	-0.077	0.614	-0.158	0.317	0.247	0.115
Initial CRP	-0.195	0.198	0.037	0.814	0.395**	0.010
Follow up CRP	-0.259	0.086	-0.113	0.475	0.350*	0.023
CRP difference	-0.102	0.506	-0.299	0.055	-0.298	0.055

Table 5: Comparison between probiotics and placebo groups regarding TLC and CRP

TLC		Initial	Follow up	Difference	Test value≠	P- value	Sig.
Placebo group	Median (IQR)	12 (8.3 – 16.7)	11 (7.2 – 14.4)	0 (-5 – 1.4)	-1.218	0.223	NS
	Range	5 – 32	5.9 – 26	-12.8 – 10			
Probiotic group	Median (IQR)	12.5 (10 – 18)	10 (8.9 – 12)	-2 (-6 – 0)	-2.465	0.014	S
	Range	2.1 – 34	3.2 – 19	-27.1 – 5.8			
CRP		Initial	Follow up	Difference	Test value≠	P- value	Sig.
Placebo group	Median (IQR)	43 (21 – 113)	35 (10 – 80)	-8 (-38 – 0.2)	-2.138	0.033	S
	Range	1.8 – 194	1 – 159	-169 – 48			
Probiotic group	Median (IQR)	14 (8 – 100)	14 (5 – 50)	-2.5 (-20 – 0.1)	-2.280	0.023	S
	Range	0.2 – 315	1 – 143	-265 – 22			

Table 6: Comparison between placebo and probiotics groups regarding the length of stay (LOS) and mortality

				Test value	P-value	Sig.
		Placebo	Probiotics			
		No. = 23	No. = 22			
LOS	Median IQR	14 (9-20)	7 (7-14)	0.865	0.387	NS
	Range	5-30	5-25			
Mortality	Yes	2 (8.7%)	1 (4.5%)	0.311	0.577	NS
	No	21 (91.3%)	21 (95.5%)			

Table 7: Comparison between placebo and probiotics groups regarding GIT complications

			Test value	P-value	Sig.
	Placebo	Probioitcs			
	No. = 23	No. = 22			
No	14 (60.9%)	16 (72.7%)	0.711*	0.399	NS
Yes	9 (39.1%)	6 (27.3%)			
Feeding intolerance	2 (8.7%)	1 (4.5%)	0.311*	0.577	NS
Diarrhea	3 (13.0%)	3 (13.6%)	0.003*	0.953	NS
Vomiting	2 (8.7%)	1 (4.5%)	0.311*	0.577	NS
Regurgitation	2 (8.7%)	1 (4.5%)	0.311*	0.577	NS
Aspiration	1 (4.3%)	1 (4.5%)	0.001*	0.974	NS

Table 8: Comparison between placebo and probiotics groups regarding initial and follow up fecal calprotectin (FC)

Fecal calprotectin		Initial	Follow up	Difference	Test value≠	P- value	Sig.
Placebo group	Median (IQR)	21 (4.9 – 821.2)	102.5 (24.7 – 189.2)	2 (-6.3 – 37.3)	-0.921≠	0.357	NS
	Range	1.5 – 5012.4	3.9 – 5116	-1102.3 – 103.6			
Probiotic group	Median (IQR)	244 (21.3 – 755)	14.4 (4.5 – 103.8)	-244.8 (-659.4 – -21.1)	-3.945≠	0.000	HS
	Range	1 – 2054	1.9 – 512	-1949 – 2.1			

No reported side effects to probiotics like abdominal distension, signs of allergy or treatment intolerance in 100% of the observed patients.

Our study found no statistically significant decrease in invasive procedures, length of stay, mortality and need for mechanical ventilation with p-value>0.05. In contrast to, Siempos et al. (2010) [32] study which conducted a meta-analysis and revealed that the administration of probiotics, compared with control, was beneficial in terms of the incidence of VAP, length of ICU stays, and colonization of the respiratory tract with pseudomonas aeruognosa, but there was no difference in ICU mortality, in hospital mortality, duration of mechanical ventilation, and diarrhea. Compared to another study by Chawan and Rajan (2020) [33] which revealed statistically significant decreased ICU stay, duration of hospital stays and duration of MV with p-value=0.001 in probiotic group compared to placebo [33]. Our current study found no statistically significant difference between probiotic group and placebo group as regards mortality with p-value=0.577. This goes in run with Angurana et al. (2018) [34] who evaluated the effect of probiotics on inflammatory cytokines in children with severe sepsis and found that mortality was similar in the two studied groups. Our current study found no statistically significant difference between the two studied groups regarding GIT complications in PICU. In contrast to Hojsak et al. (2010) [35] who conducted a double-blind randomized placebo-controlled trial in hospitalized children receiving Lactobacillus GG (n=376) and placebo (the same post-pasteurized milk, deprived of Lactobacillus GG, placebo group (n=366), and found a significantly reduced risk for respiratory tract and GIT infections in

Lactobacillus GG group, compared with placebo group. Also, Szajewska et al. (2016) [36] reported five RCT studies with total 445 children for incidence of ICU acquired diarrhea episodes decreased in children treated with Lactobacillus rhamnosus GG from 23% to 9.6% and from 20.9% to 8.8% in 1653 children treated by S.boulardii. Another study by Allen et al. (2010) [37] on probiotics for acute infectious diarrhea who collected the results from 36 randomized control trials that compromised 8,014 participants from various geographical areas and revealed statistically shorter duration and reduced in stool frequency in acute infectious diarrhea in the probiotics recipients. Khodadad et al. (2013) [38] conducted a study on 66 H. pylori positive children to compare effect of probiotics on minimizing gastrointestinal side effects prevalence and improve eradication rate and revealed statistically significant eradication of H. pylori with decreased frequency of antibiotic induced side effects with p-value=0.04. Our study found a negative correlation between GIT complications which were detected in 33.3% of our patients and FC levels but a positive correlation between initial FC and diarrhea which was the most common GIT complication as shown in table (2). Similarly to Skokri et al. (2021) [39], who conducted a study over 89 individuals where 70 patients were confirmed Covid-19 patients and 19 individuals were healthy, they found a negative correlation between FC and GIT symptoms in Covid-19 patients where diarrhea was the most common symptom and explained this finding by the non-inflammatory and non-invasive mechanism in Covid diarrhea proved by the absence of fecal leucocytes in their diarrheal patients but they found a positive correlation between FC and Covid severity and prognosis. Also, Britton et al. (2020) [40], who collected stool samples from 44 confirmed covid patients and measured the level of eight cytokines and FC in these samples to correlate between them with GIT symptoms caused by Covid as well as Covid severity, they found that fecal cytokines and calprotectin levels were not correlated with gastrointestinal symptoms or with the level of virus detected,but found a significantly higher levels of IL23 in severe Covid, as well as higher levels of IL10 and lower levels of IL8 in covid patients versus normal individuals. In contrast to Ojetti et al. (2020) [41], who also conducted a study over 65 Covid patients and found that in the patients with elevated FC (>50 ug/g), GIT symptoms were more frequent in addition to finding a significant correlation between Covid-19 pneumonia and high levels of FC.

And Giuffrè et al. (2021) [43] who was interested in writing a brief report about FC and Covid-19, concluded that some Covid patients may develop severe gastro-intestinal complications and fecal calprotectin can be used to monitor intestinal disease activity levels. But in another study including 25 patients, Giuffrè et al. (2020) [42] found that FC was elevated in 21 Covid patients without GIT symptoms but there was a positive correlation between elevated FC and D-dimer. In our study, we found a statistically significant positive correlation between FC level and LOS, follow up FC and PRISM score and initial FC and mortality as shown in table (3) & (4). This goes in agreement with Attia et al. (2016) [44], who conducted a study over hospitalized severely malnourished children by observing the relation between severe acute malnutrition (SAM) with intestinal and systemic inflammation and found a positive correlation between high FC and high mortality rates in these children. Also, Kim et al. (2017) [45], Dróżdż et al. (2019) [46] and Voicu et al. (2021) [47] who were interested in studying the role of FC in hospitalized patients with clostridium difficile infection (CDI), found a positive correlation between FC levels and CDI severity and prognosis thus recommending the use of FC as a non-invasive predictor tool for CDI severity.

4. Conclusions

Probiotics are able to restore the imbalance of intestinal microbiota and function in critically ill children measured by fecal calprotectin which significantly decreased using probiotics. Also, probiotics had several important effects in critically ill children as improving inflammatory conditions determined by decreased inflammatory markers as WBCs and CRP, but still further larger studies are needed to evaluate its efficacy in decreasing the occurrence of GIT complications.

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