# Effect of *Lactobacillus acidophilus* versus *Bifidobacterium bifidum* on clinical and immunological responses of children with acute diarrhea

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**Abstract: Aim:** To determine the value of Lactobacillus acidophilus versus Bifidobacterium bifidum in shortening the duration of acute diarrhea treatment in children. Also to measure the immune response to such treatment by measuring serum total IgA, IL-10 and TNF-α. **Methods:** Ninety Five children suffering from acute diarrhea were examined microbiologically to detect the causative organism, and then were divided into four groups: Group1; received L.acidophilus, Group 2; received B. bifidum, Group III; received both probiotics, and Group IV; (control group) received oral rehydration therapy. Only those patients suffering from bacterial, fungal or parasitic infections received antibacterial, antifungal or antiparasitic therapy respectively. Serum IgA, IL10 and TNF-α were measured in all patients using ELISA. **Results:** There was a significant decrease in the duration and frequency of diarrhea in groups I, II and III compared to group IV. Serum levels of IgA, TNF-α and IL-10 after treatment were significantly higher in groups I, II and III compared to group IV with no significant difference between group I and II, whereas group III showed higher levels of TNF-α and IL-10. However, in parasitic infections the levels of IgA, TNF-α and IL-10 were only increased in group III versus group IV. Moreover, fungal infections were not associated with change in IgA and TNF-α in any of the groups with probiotic treatment compared to the control group. A significant positive correlation between serums TNF-α and IL-10 was also observed in all the groups with probiotic treatment but not in group IV.
**Conclusions**: Both *L.acidophilus and B.bifidum* are exhibiting antimicrobial activities in pediatric viral and bacterial diarrhea and their combined use may be useful in parasitic infections. A great advantage of the selected probiotics is their ability to induce IgA and TNF-alpha with their anti-infectious effects and IL-10 which down regulates pro-inflammatory cytokines secretion, thereby maintaining the delicate balance between necessary and excessive defense mechanisms.

## 1. Introduction

Diarrhea is a frequent cause of morbidity and mortality during infancy and childhood, especially in developing countries. In the United States, 16.5 million children below the age of five years have at least one episode of diarrhea a year. In developing countries, 3.2 million children die every year due to diarrhea and it can account for as much as 25% of their national healthcare costs [1]. The WHO advises fluid and electrolytes replacement as the therapy of choice for treatment of dehydration [2]. However, although the mortality rate is decreased by rehydration [3], the duration and number of diarrhea episodes are not affected [4].

Probiotics are defined to be non-pathogenic organisms that are incorporated into the diet to modify gut microbial ecology, leading to beneficial structural and functional changes in the gut. Probiotics serve as a barrier for the colonization of pathogens to prevent disease and the enhancement of the immune system. In addition, they carry out metabolic functions such as helping the fermentation of nondigestible fibers, and

storing energy in the form of short-chain fatty acids [5]. There is evidence that administration of probiotics as an adjuvant is successful in the management of acute diarrhea [6]. However, the majority of studies have been conducted in industrialized countries where nutritional status, drug availability and diarrhea etiology are different from those in low-income countries [7]. Furthermore, each probiotic strain has a different level of efficacy as diarrhea-adjuvant treatment [8], and the type of preparation, killed or lived bacteria, may result in heterogeneous results [7,9]. *Lactobacilli* and *Bifidobacteria* are the two main types of bacteria that stay in the intestine that contain antimicrobial properties and play a role in both local and systemic immunity of the intestine. With these two residential bacteria in the intestine, pathogenic microbe will less likely be able to colonize because *Lactobacilli* and *Bifidobacteria*'s presence can compete with and inhibit the growth of these potentially pathogenic microbes. Thus, these two types of resident bacteria act as a gut defense barrier to

protect the intestine from the harboring of potential pathogens. Aside from providing a gut defense barrier, these two types of bacteria can secrete antimicrobial substances and mucins to inhibit the growth of pathogens and have been shown to improve the secretory immune function and the intestinal flora such as influencing secretory immunoglobulin A (sIgA) synthesis and sIgA precursors [10]. These bacteria also promote good digestion, boost the immune system, produce lactic and acetic acid that control intestinal pH and inhibit the growth of *Candida albicans*, *E. coli*, and other bacteria that have more pathogenic qualities [11].

Modulation of host immunity is one of the most common benefits of the administration of probiotics [12]. The immunomodulatory studies demonstrated that the probiotic culture fractions are modulating the expression of the most important cytokines in the development of the anti-infectious immunity against enteric pathogens, expressed by the stimulation of TNF-alpha and INF- gamma pro-inflammatory cytokines and the inhibition of IL-6 and IL-8 cytokines, known to be implicated in the occurrence of lesion effects upon the infected host [13]. The probiotic effects may be mediated via control of the balance between pro- and anti-inflammatory cytokines [14]. Therefore, increase in the level of IL-10 may be necessary, since IL-10 prevents inflammatory reactions such as toxic colitis [15].

The aim of this work was to determine the value of *Lactobacillus acidophilus* versus *Bifidobacterium bifidum* in shortening the duration of acute diarrhea treatment in children. Also to measure the immune response to such treatment by measuring serum total IgA, IL-10 and TNF-α.

## 2. Patients, Material and Methods:
### 2.1. Inclusion criteria

This study is a case, control trial that was conducted between January and July 2011 on children admitted to the pediatric unit of AL Eman Hospital – Assiut Egypt, with a diagnosis of acute diarrhea. Decisions regarding admission and general management were made, respectively, by the emergency and attending physicians. Children who had passed abnormal watery and/or mucous stools more than three times within the previous 24 hours with duration of diarrhea for more than 72 hours were eligible for enrolment. Patients with evidence of systemic infections, neurological disturbances, and a history of convulsions or conditions such as chronic immunodeficient gastro-intestinal conditions or severe dehydration were excluded. Children who had received treatment with probiotics and medications which interfere with intestinal motility during the present illness were also excluded. Informed consent

was obtained and the study was approved by the ethics committee of the Faculty of Medicine of Assiut University.

### 2.2. Demographic analysis

After enrolment, demographic characteristics and clinical history were recorded. Weight to the nearest 100 g and length/height to the nearest mm were measured. Clinical evidence of dehydration was documented in accordance with WHO guidelines [16].

### 2.3. Laboratory investigations

Serum sodium, potassium, bicarbonate, blood urea nitrogen, creatinine and complete blood counts were measured. Urine analysis and microscopic faecal examination were routinely performed on admission.

### 2.4. Faecal examination for rotavirus by enzyme immune assay (EIA)

Detection of Rota virus in faecal samples was performed using Roodscreen II EIA (M480, Microgen Biproducts - Spain). Microtitration wells were coated with rabbit antibodies raised against pooled rotavirus isolates representing subgroups 1, 2 and 3. Serotype 4 was recognized by cross-reaction with the common group antigen. Diluted faecal samples were pipetted directly into the coated wells. The color developed with TMB substrate was read at 450 nm wavelengths.

### 2.5. Microbiological examination

Faecal samples were cultured on MacConkey's agar (Himedia – Cat. No. MM081), Mannitol salt agar (Himedia – Cat. No. M118 – 500G), ORSAB (Himedia – Cat. No. M1454 – 500G), Bismuth Sulphide agar (Himedia – Cat. No.MU027 – 500G), Salmonella agar, modified HiCrome RajHans Medium, Sabouraud's dextrose agar (HiMedia ™ M063) (all the media were provided from Himedia company – India). Confirmation of the isolated organisms was performed using biochemical reactions (coagulase test, TSI test, Urease test, sugar assimilation test (HiMedia ™ M139), germ tube test, IMVC test for identification of isolated organisms [17].

### 2.6. Experimental protocol

After obtaining written consent from care-givers, 95 patients were randomized by a computerized programme into one of four groups: Group I; 25 patients, received live *L. acidophilus* (minimum of $10^9$/sachet) (Rameda, ARE) and Group II; 25 patients, received *B. bifidum* (Cerelac, Nestla, ARE); Group III; 25 patients, received both types, and Group IV; 20 patients, received powdered oral rehydration solution (ORS) (Cid, ARE). Serum samples were taken from patients at time of admission (0 day) to measure serum total IgA and after recovery (at 5th day of treatment) to measure serum levels of IgA, IL-10 and TNFα after recovery. Both patients and attending physicians were blinded to which children were receiving which medication. Children in each group received the assigned medication three

times daily until the end of the diarrhea episode and up to a maximum of 5 days. Patients suffering from bacterial, fungal or parasitic infections received antibacterial, antifungal or antiparasitic therapy respectively. The end of a diarrhea episode was defined as the first of two consecutive semi-formed stools or the last stool followed by 12 hours without passing stool. Duration and frequency of diarrhea were the main clinical outcomes. Adverse events were recorded by interviewing parents or guardians.

## 2.7. Measurement of serum total IgA by EIA

Serum IgA was measured before start of treatment and after recovery using commercially available ELISA kits (Spinreact, SAU, Spain); antihuman IgA antibodies were mixed with samples containing IgA, to form insoluble complexes. These complexes caused an absorbance change, dependant upon the IgA concentration of the patient sample that has been quantified by comparison from a calibrator of known IgA concentration, at wavelength 600 nm. Values 70 – 400 mg/dl were considered positive, any values above this were considered elevated [18].

## 2.8. Measurement of serum Il-10 and TNF-α by EIA

Serum IL-10 and TNF-α were measured after recovery using commercially available ELISA kits (Koma Biotech – Korea); Standards and samples were added to precoated plates and after incubation for two hours at room temperature, they were washed for four times with PBST. Detection antibody (biotinylated rabbit antihuman IL-10 or TNF-α respectively) was added and incubated again for two hours at room temperature. After washing, Streptavidin – HRP was added and incubated for 30 minutes at room temperature. The color developed with TMB substrate was read at 450 nm wavelengths. Standard curves and concentrations were calculated using ELISA reader.

## 2.9. Statistical analysis:

Data were analyzed using SPSS version 16 program. Values were expressed as mean ± standard deviations (SD) and percentages. Comparisons between two groups were analyzed by unpaired t-test and Chi square. Comparisons between more than two groups were analyzed by ANOVA test. Comparisons between IgA before and after probiotic treatment were analysed by paired t-test and Chi square. Spearman's correlation test was applied to analyze correlations between different quantitative variables within each group. The changes in IgA concentrations over the first five days were expressed as a relative percentage of change from the admission concentrations (IgA day 5- IgA admission/ IgA admission %). $P$ value < 0.05 was considered significant.

## 3. Results:

## 3.1. Demographic and clinical data of different groups of patients

As shown in table (1), there was no significant difference in the age and sex of the different study groups ($P$=0.382 and 0.996, respectively). The duration of diarrhea and frequency/day were decreased in groups I, II and III compared to the control group IV ($P$=0.000) and <0.03, respectively).

## 3.2. Distribution of the causative agents among different groups of patients

The distribution of the causative agents of diarrhea detected in the different groups of patients is shown in table (2).

## 3.3 Serum IgA response to treatment among different groups of patients

As shown in table (3), the percentage of patients with high serum IgA levels increased significantly after treatment in all study groups. It increased from 8% to 40% in group I ($P$<0.001), from 4% to 48% in group II ($P$=0.000), from 8% to 44% in group III ($P$ =0.000) and from 20% to 55% in group IV ($P$ <0.02).

Table (4) shows the mean levels of serum IgA in different groups of patients before and after treatment. Before treatment, there was no significant difference between the levels of serum IgA in the different groups ($P$ = 0.316). Whereas after treatment, serum IgA levels were significantly higher in the three groups who received probiotic treatment; group I (712.45±128.6), group II (851.65±169.40) and group III (978.57±186.56) versus the control group IV (467.87±103.56) ($P$ <0.03).

## 3.4. Serum levels of TNF-α in different groups after treatment

As shown in Table (5), all study groups who received probiotic treatment showed higher levels of serum TNF-α compared to the control group ($P$ <0.01, $P$<0.001 and $P$=0.000 for groups I, II and III, respectively). There was no significant difference between the serum level of TNF-α in group I versus group II, while it was significantly higher in group III, who received combined probiotic treatment, compared to groups I and II ($P$ <0.01 and $P$ <0.03, respectively).

## 3.5. Serum levels of IL-10 in different groups after treatment

As shown in Table (5), all study groups who received probiotic treatment showed higher levels of serum IL-10 compared to the control group ($P$<0.02, $P$<0.001 and $P$=0.000 for groups I, II and III, respectively). There was no significant difference between the serum level of IL-10 in group I versus group II, while it was significantly higher in group III compared to groups I and II ($P$ <0.03 and $P$ <0.02, respectively).

**3.6. Correlation between serum levels of TNF-α and IL-10 in different groups after treatment**

There was a significant positive correlation between the serum levels of TNF-α and IL-10 in the groups of patients who received probiotic treatment (groups I, II and III). Whereas in group IV, no significant positive correlation was observed as shown in table (6).

**3.7 Percentage of increase in serum levels of IgA in different groups classified according to the causative agents of diarrhea (Figure 1)**

In Rota virus infections, the percentage of increase in serum IgA levels was significantly higher in groups I, II and III compared to the control group IV ($P$ =0.000 for each), but there was no significant difference between the three groups I, II and III together ($P$ =0.352). In bacterial infections, the percentage of increase in serum IgA was also significantly higher in each of three groups compared to group IV ($P < 0.02$, $P<0.04$, $P<0.001$ for groups I, II and III, respectively), with no significant difference between the three groups together ($P$ =0.643). However, in fungal infections, there was no significant difference between the percentage of increase in each of the three groups I, II and III versus the control group IV ($P$ =0,271, 0.574 and 0.756, respectively) nor between the three groups together ($P$ =0.462). In parasitic infections, only group III who received combined probiotic treatment, showed significantly higher percentage of increase in serum IgA levels compared to the control group IV ($P$=0.000) and also compared to groups I and II ($P<0.01$).

**3.8 Serum levels of TNF-α in different groups classified according to the causative agents Figure (2)**

In Rota virus infections, serum levels of TNF-α were significantly higher in the three groups with probiotic treatment compared to the control group IV ($P <0.001$ for groups I and II, and $P$ =0.000 for group III). Comparing between the three groups together, the serum TNF-α level was significantly

higher in group III than in the two other groups I and II ($P <0.04$). In bacterial infections, only groups I and III showed higher TNF-α levels compared to the control group IV ($P <0.03$ and $P$ =0.000, respectively). Its levels were significantly higher in group III than in the two other groups I and II ($P <0.001$). In fungal infections, there was no significant difference between the serum TNF-α levels in the three groups with probiotics compared to the control group IV ($P$ =0.276 for group I, $P$ =0.361 for group II and $P$ =0.261 for group III, respectively). In parasitic infections, only groups I and III showed higher TNF-α levels compared to the control group IV ($P$ =0.000 for each) and its level was significantly higher in group III than in groups I and II ($P <0.001$).

**3.9 Serum levels of IL-10 in different groups classified according to the causative agents Figure (3)**

In Rota virus infections, serum levels of IL-10 were significantly higher in group III with combined probiotic treatment compared to the control group IV ($P <0.001$). Comparing between the three groups with probiotics together, serum IL-10 levels were significantly higher in group III than in the two other groups I and II ($P <0.04$). In bacterial infections, all the three with probiotics (groups I, II and III) had significantly higher levels of IL-10 compared to the control group ($P <0.02$, $P <0.001$ and $P$ =0.000, respectively). However, there was no significant difference between the levels in the three groups together ($P$ =0.354). Also in fungal infections, all the three groups with probiotic treatment had significantly higher levels of IL-10 compared to the control group ($P <0.01$, $P<0.001$ and $P<0.03$, respectively) but there was no significant difference between the levels in the three groups together ($P$ =0.476). In parasitic infections, only group III showed higher IL-10 levels compared to the control group IV ($P$ =0.000). Its levels were also significantly higher in group III than in groups I and II ($P$ =0.000).

**Table (1): demographic and clinical data of different groups of patients.**

| Item | Group I (n=25) | Group II (n=25) | Group III (n=25) | Group IV (n=20) | $P$ - value |
|---|---|---|---|---|---|
| Age ( yrs) | 4.61±0.89 | 3.28±0.62 | 3.85±0.24 | 3.45±0.59 | 0.382 |
| Sex: Male | 13 (52%) | 13 (52%) | 13 (52%) | 11 (55%) | 0.996 |
| Female | 12 (48%) | 12 (48%) | 12 (48%) | 9 (45%) | |
| Duration (days) | 1.88±0.72 | 2.32±1.06 | 1.76±0.72 | 4.4±0.94 | 0.000 |
| Frequency /day | 4.72±1.17 | 4.68±1.51 | 3.68±1.07 | 5.1±1.97 | <0.03 |

Values are expressed as mean ±SD.

**Table (2): Distribution of the causative agents among different groups.**

| Causative agent | Group I (n=25) | Group II (n=25) | Group III (n=25) | Group IV (n=20) |
|---|---|---|---|---|
| Rota virus | 10 (40%) | 12 (48%) | 11 (44%) | 11 (55%) |
| Bacterial: | 18 (72%) | 14 (56%) | 7 (28%) | 7 (35%) |
| E. coli | 10 (40%) | 9 (36%) | 4 (16%) | 3 (15%) |
| S. aureus | 4 (16%) | 2 (8%) | 0 (0%) | 3 (15%) |
| Klebsiella spp. | 3 (12%) | 2 (8%) | 0 (0%) | 0 (0%) |
| Proteus spp | 1 (4%) | 1 (4%) | 3 (12%) | 1 (5%) |
| Parasitic | 17 (68%) | 16 (64%) | 16 (64%) | 17 (68%) |
| Fungal | 2 (8%) | 0 (0%) | 1 (4 %) | 4 (20%) |

**Table (3): IgA response to treatment among different groups.**

| Serum IgA | Group I (n=25) | Group II (n=25) | Group III (n=25) | Group IV (n=20) |
|---|---|---|---|---|
| **Before:** | | | | |
| Normal | 23 (92%) | 24 (96%) | 23 (92%) | 16 (80%) |
| Increased | 2 (8%) | 1 (4%) | 2 (8%) | 4 (20%) |
| **After:** | | | | |
| Normal | 15 (60%) | 13 (52%) | 14 (56%) | 9 (45%) |
| Increased | 10 (40%) | 12 (48%) | 11 (44%) | 11 (55%) |
| P value | <0.001 | 0.000 | 0.000 | <0.02 |

Normal serum level of IgA: 70-400 mg/dl.

**Table (4): IgA serum levels in different groups before and after treatment**

| Groups | GI | GII | GIII | GIV | $P$-value |
|---|---|---|---|---|---|
| Before | 51.23±7.86 | 56.56±8.43 | 70.14±12.34 | 59.65±15.56 | 0.316 |
| After | 712.45±128.6 | 851.65±169.40 | 978.57±186.56 | 467.87±103.56 | < 0.03 |
| $P$-value | 0.000 | 0.000 | 0.000 | 0.000 | |

Values are expressed as mean ±SD (mg/dl).

**Table (5): Serum levels of TNF-α and IL-10 in different groups**

| Groups | GI | GII | GIII | GIV | $P$-values | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1vs II | 1vs III | II vs III | I vs IV | II vs IV | IIIvsIV |
| IL-10 | 520.65 ±56.64 | 650.21 ±59.63 | 827.34 ±87.56 | 315.67 ±78.56 | 0.364 | <0.03 | <0.02 | <0.02 | <0.001 | 0.000 |
| TNFα | 462.56 ±48.35 | 436.63 ±39.76 | 600.67 ±78.79 | 210.56 ±30.54 | 0.629 | <0.01 | <0.03 | <0.01 | <0.001 | 0.000 |

Values are expressed as Mean ±SD (Pg/ml).

**Table (6): Correlation between Serum levels of IL-10 and TNF-α in different groups**

| Groups | GI | GII | GIII | GIV |
|---|---|---|---|---|
| IL-10 | 520.65±56.64 | 650.21±59.63 | 827.34±87.56 | 315.67±78.56 |
| TNF-α | 462.56±48.35 | 436.63±39.76 | 600.67±78.79 | 210.56±30.54 |
| Correlation | r= 0.675 $P$< 0.04 | r= 0.693 $P$ < 0.03 | r= 0.487 $P$ <0.02 | r= 0.487 $P$ = 0.583 |

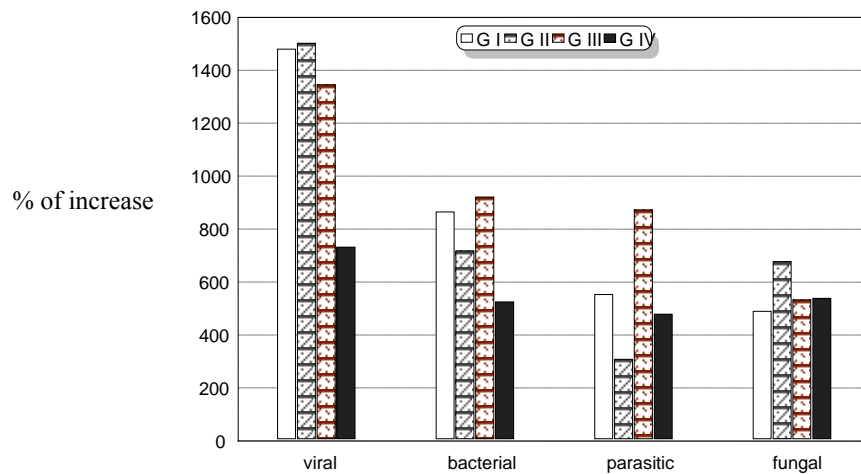Values are expressed as Mean ±SD (Pg/ml).

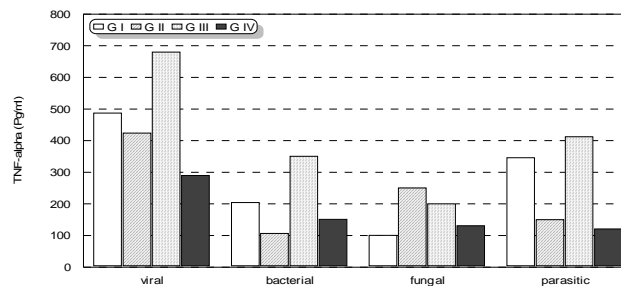**Figure 1. IgA serum levels in different groups classified according to the cause of diarrhea.**



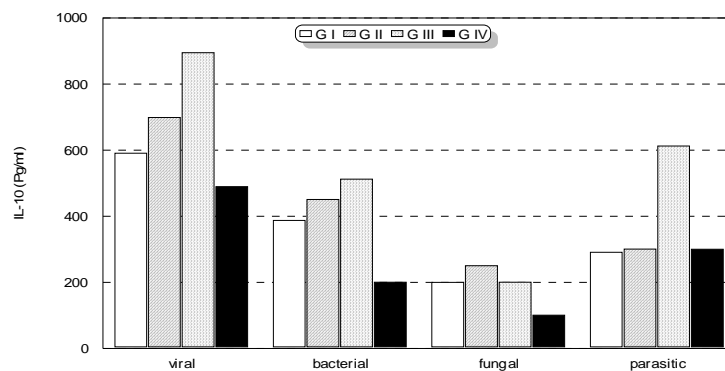**Figure 2. Serum levels of TNF-α in different groups classified according to the cause of diarrhea.**



**Figure 3. Serum levels of IL-10 in different groups classified according to the cause of diarrhea.**

## 4. Discussion:

Members of the genera Lactobacillus and Bifidobacterium are amongst the most common microorganisms in the human gastrointestinal tract. Because of their beneficial properties Lactobacillus and Bifidobacterium strains are commonly used in pharmaceutical probiotic preparations [19]. In this study, we investigated the value of usage of *L.*

*acidophilus* and / or *B. bifidum* in shortening the duration and frequency / day of acute diarrhea episodes in children. There was a significant reduction by more than two days in the duration ($P = 0.000$) and frequency ($P = <0.03$) of diarrhea in groups I, II and III who received *L.acidophilus*, *B.bifidum* and both probiotics, respectively compared to the control group IV who received ORS only. This is concomitant with

meta-analyses [20-22] that showed an overall reduction of one day in the duration of diarrhea in mild-to-moderate episodes but no demonstrable benefit in the more severe cases. Also Szajewska and Mrukowicz [23] reported that in infants and children, probiotics significantly reduce the duration of diarrhea by more than 3 days. The major benefits were achieved when there was infection with rotavirus. The beneficial effects of probiotics however are strain dependent, dose dependent, greater for doses of more than $10^{10}$ cfu/ml, significant in people with viral gastroenteritis and more evident when treatment with probiotics is initiated early in the course of the disease [24].

Probiotic bacteria may exert beneficial effects and modulate the immune response to potentially harmful antigens via B lymphocytes and antibody production. In order to study the value of *L.acidophilus* and *B.bifidum* in boostering the immune response, we measured the serum levels of total IgA before and after treatment with probiotics. Before treatment, there was no significant difference between serum IgA levels in different groups ($P = 0.316$). Whereas after treatment, serum IgA levels were significantly higher in the three groups who received probiotic treatment compared to the control group IV ($P < 0.03$). In accordance to our results, Macpherson *et al.* [25] reported that B cells are stimulated in response to certain probiotic species to increase the IgA production and secretion. Salminen *et al.* [12] also demonstrated that probiotics especially bifidobacteria improve immunological response by increasing IgA synthesis. Galdeano *et al.* [26] indicated that probiotic signals are also able to interact with the gut epithelial cells to signal an increase in the number of IgA producing cells. An increase in IgA levels in the gut environment helps to promote a more effective gut immunological barrier [27]. The overall modification of secretory IgA production in the gut is important for mucosal immunity by aiding in blocking some pathogens and viruses from inhabiting the intestine [28].

Olas *et al.* [29] concluded that IgA has both a pro-inflammatory and an anti-inflammatory capability, and this dual function might contribute to the feedback mechanisms maintaining a balance between pro-inflammatory and anti-inflammatory activities.

To investigate the ability of *L.acidophilus* and *B.bifidum* to affect the production of pro-inflammatory and anti-inflammatory cytokines, serum levels of TNF-α and IL-10 were measured at 5th day of treatment in all study groups. Groups I, II and III who received probiotic treatment showed significantly higher levels of serum TNF-α and IL-10 compared to the control group, but there were no significant difference between the levels in group I versus group II. Whereas the serum levels were significantly higher

in group III who received combined probiotic treatment compared to groups I and II (Table 5). There was a significant positive correlation between the serum levels of TNF-α and IL-10 in groups I, II and III, whereas in group IV, such correlation was not observed (Table 6). This indicates that *L. acidophilus* and *B. bifidum* can achieve a balance between pro- and anti-inflammatory cytokines thereby maintaining the delicate balance between necessary and excessive defense mechanisms. This is concomitant with Ebaid and Hassanein [30] who found that bifidobacteria significantly increased the levels of pro-inflammatory and anti-inflammatory cytokines in patients receiving probiotics compared to the control. This is also concomitant with Pelinescu *et al.* [13] who studied the effect of *L. acidophilus* on inflammatory cytokines in patients receiving probiotics and demonstrated that the probiotic culture fractions are modulating the expression of the most important cytokines in the development of the anti-infectious immunity against enteric pathogens, expressed by the stimulation of TNF-alpha and INF-gamma pro-inflammatory cytokines and the inhibition of IL-6 and IL-8 cytokines.

In agreement with our results, concerning the role of IL-10 in the control of enteric inflammatory responses, Lammers *et al.* [19] found that exogenous bifidobacteria provided a stimulus for production of IL-10. Neirs *et al.* [31] have also shown that live lactic acid bacteria can modulate immune responses by inducing IL-10 in PBMCs, and these effects appeared to be strain specific. Our results contrast with the previous studies where IL-10 was not found to be induced by lactic acid bacteria [32, 33]. The use of different strains might explain this discrepancy, since Christensen *et al.* [34] reported great differences between six lactobacilli strains to induce production of key cytokines such as IL-12 and IL-10.

The efficacy of probiotics in ameliorating the course of diarrhea depends on the cause of diarrhea. Our results reveal that in Rota virus and bacterial infections, the percentage of increase in IgA levels was significantly higher in each of three groups who received probiotic treatment compared to the control group IV, with no significant difference between the three groups. However, the combined use of both probiotics in group III was associated with significantly higher levels of TNF-α and IL-10.

Studies of the immunomodulating effects of probiotics in children with acute rotavirus diarrhea revealed that human isolates of Lactobacilli have been extensively used in Finland to promote recovery from acute rotavirus diarrhea in children [35]. Lactobacilli were effective in shortening the course of acute diarrhea in children with rotavirus diarrhea since the duration of watery diarrhea was 1.5 days in the treated

infants *versus* 2.5 days in the matched control infants [36]. One of the beneficial effects of Lactobacill*i* during the time course of rotavirus diarrhea was to reinforce the local immune defenses through specific IgA response to rotavirus [37]. Moreover, the administration of viable Lactobacilli was more efficient in promoting rotavirus specific IgA in serum than inactivated bacteria [38]. Lactobacilli isolates significantly increased the number of rotavirus-specific IgA-secreting cells and serum IgA level in the convalescent stage [39].

The data concerning a protective role of lactic acid bacteria in bacterial diarrhea are scanty in humans, and contradictory results are often reported in animals. An *in vivo* study using mice orally infected with *Salmonella typhimurium* has shown that the human isolates *L. acidophilus* had an antibacterial activity [40]. In human volunteers ingesting an attenuated Salmonella typhi strain to mimic an enteropathogenic infection, the specific serum IgA titer was four-fold higher after three weeks in those who were supplemented with fermented milk (*L. acidophilus* and *bifidobacteria*) than in those in the control group [41]. Chatterjee *et al.*[42] and Johnson-Henry *et al.* (2004) [43] demonstrated that co-incubation of a *Lactobacillus* mixture with *H. pylori* decreases colonization of the pathogenic bacteria, decreases overall inflammation of the infection, and decrease the growing capabilities of the bacteria. Probiotics have been shown to not only to limit binding sites and exclude the infection, but also inducing mucin mRNA levels and increasing the mucus layer to trap the bacteria to be flushed out of the gastro-intestinal tract [44, 45] .

In the present study, the effects of probiotic treatment on the immune response to parasitic infections were different than those in viral and bacterial infections. The levels of IgA, TNF-α and IL-10 were only increased in group III who received combined probiotic treatment compared to the control group IV. In terms of intestinal parasitic infections, there have been fewer studies regarding probiotic therapy. Hawrelak[46] reported that T cells, neutrophiles, macrophages as well as IgM, IgG, and IgA antibodies are major players of the immune response necessary for resolution of giardiasis. T-cell cytokines may also induce production and release of antigiardial defensins. An important step towards the comprehension of the probiotic activity was the discovery that the culture supernatant of Lactobacilli was capable of controlling *G.lamblia* growth *in vitro* [47]. Benyacoub *et al.*[48] demonstrated that treatment of *Giardia* with different strains of probiotics has shown promising outcomes. Protection was associated with an enhancement of the immune response since a production of specific anti-*Giardia* intestinal IgA and

IgG was noticed in treated mice. Shukla and Sidhu [49] also indicated that treatment with Lactobacilli reduced both the severity and the duration of giardiasis in malnourished mice. Taking together all results, probiotics can be helpful in the prevention and treatment of parasitic infections, especially when combined with specific treatment.

*Candida* species have been often considered but infrequently documented as a credible cause of diarrhea and the mechanisms by which *Candida* species may induce diarrhea remain undefined [50]. Our study shows that *Candida* –associated diarrhea was not associated with change in IgA and TNF-α in any of the three groups who received probiotic treatment compared to the control group. In contrast to our results, Chaitow *et al.*[51] found that there is evidence that the probiotics *L. acidophilus* and *Bifidobacteria* inhibit the growth of *Candida albicans*. Williams *et al.*[52] have also indicated that *Lactobacillus* may be helpful in the prevention of Candidiasis in women with HIV. Manzoni *et al.*[53] also demontrated that orally administered *L. casei* significantly reduced the incidence and the intensity of enteric colonization by *Candida* species among very low birth weight neonates.

## 5. Conclusion

Our *in vivo* study demonstrates that both *L.acidophilus* and *B.bifidum* are exhibiting antimicrobial activities in pediatric viral and bacterial diarrhea, supporting their potential use in the treatment of pediatric gastro-intestinal disorders, as an alternative or in association with antibiotics. In addition, the combined use of both probiotics may be useful in parasitic infections. A great advantage of the selected probiotic strains is their ability to induce a beneficial immune and cytokine response expressed by the stimulation of IgA and TNF-alpha with their anti-infectious effects, and IL-10 which down regulates the secretion of pro-inflammatory cytokines, known to be implicated in the occurrence of lesional effects upon the infected host, thereby maintaining the delicate balance between necessary and excessive defense mechanisms.

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