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Zinc and Probiotic Supplementation: Impact on Health Outcomes in Hospitalized Malnourished Children Under Five with Diarrhea

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Abstract

A randomized control study was conducted to evaluate the effect of zinc and probiotic supplementation on health outcomes of hospitalized malnourished children under five with diarrhea. A total of 40 subjects were enrolled for this study and divided into four groups consisting 10 subjects in each. The study was conducted in nutrition rehabilitation unit (NRU) established at pediatric ward govt. Lady Reading Hospital (LRH) Peshawar Pakistan. The biochemical parameters including red blood cells (RBC), white blood cells (WBC), platelets, hemoglobin, sodium, potassium, calcium, chloride and anthropometric measurement including weight, height, OFC and z-score values were analyzed at different intervals. Repeated measure analysis was used followed by post hoc bonferroni test for multiple comparisons at 5% level of significance. The group-1 and group-3 children received zinc syrup Osiris (zinc sulphate), dosage 50 mg/day along with F-75 and F-100 therapeutic milk. Similarly, the group-2 and group-3 children received probiotics enterogermina oral suspension (1 impulse/day). Significant improvement has been observed in RBC's mean level ($p<0.05$) in children consumed zinc and probiotic supplements compared to control group. Similar increase was also observed in the platelets and sodium mean level significantly ($p<0.05$) compared to mixed group respectively. However, weight gain of malnourished children was also increased significantly ($p<0.05$). The mean age of the children was 22.6000±15.88990 and 63.4% of the families were living in joint family system, 85.4% of mothers were illiterate, the mean income per month was PKR: 12000. Medical history include diarrhea, consistency of watery stool, semi watery, blood in stool, mucus in stool, vomiting and dehydration were 100%, 85.4%, 12.2%, 12.2%. 14.6% 9.8% and 75.6% respectively. Similarly, the means weight, height/length, Z-score and occipital frontal circumference (OFC) of the children were 6.09±1.23, 71.30±6.23, 3.50±0.52 and 43.30±2.16 respectively. The bottle-feeding and improper dilution were found a strong predictor for causing diarrhea, with the increase in episodes of diarrhea and more chances of infections were exposed. The finding on breastfeeding showed a positive relation for the occurrence of diarrhea within the families. It was concluded that Zinc and probiotics therapy in recovery of admitted malnourished children play significant role in their health promotion.

Keywords

Zinc, Probiotics Supplementation, Diarrhea, Hospitalized Malnourished Children

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1. Introduction

Malnutrition is the broad term for protein caloric malnutrition, when the human body requirements for energy, powers protein or in combine can't be fulfil by the eating routine. Protein caloric malnutrition might be primary, when nutrient intake is not adequate as per the body necessities, or secondary, when there is some sickness that coordinates too little consumption of food supplements [1]. For the most part, Child under/over nutrition rates all over Pakistan remains high. As in the latest survey the

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hunger proportion in Khyber Pakhtunkhwa are above from the normal which shows the genuine general medical issue; underweight (23.1%), stunted (40%) and wasting (15%) [2]. Nutrients assume a critical part to improve tolerant wellbeing results and reduce subsequent term of recovery; decreasing length of stays in hospital and also likely to decreases costs. Around 60% of all deaths among under-5 children in developing world could be form poor health. It has been assessed that almost 50.6 million under-5 children are malnourished having 90% of children from developing countries. All around, 35% of deaths among children under 5 are identified with lack of healthy food intake [3]. In addition to growth failure and micronutrient deficiencies, under nutrition can cause cognitive and behavioral impairments even with long-term nutritional rehabilitation [4-5]. Zinc is a micronutrient which assumes an essential part in a well-functioning of invulnerable framework. A dietary and physical insufficiency in zinc adds to the dismalness and death from compelling afflictions [6]. Malnourished mothers having children is zinc lacking impacting the mass along with reliability of the baby and, afterward, the neonate [7]. Breastfeeding to infant children is a basic consider survivability in making healthy nation, there is truths that the malnourished mothers having breastfed as a rule needs sufficient levels of zinc to boost up the necessities of children, keeping up the distinguishable sustaining, insufficiency of children in both substantial scale and micronutrients including zinc, is the effect of little delivery zinc levels, and feedings of breast milk contain stumpy level of zinc trailed by complementary feeding [8].

Water and electrolyte imbalance is the principle concern, and children are at high risk to grown up of life weakening with lack of water in the body [9]. Looseness of the bowel is reliably found in children with lack of zinc. There are additionally expanded fecal misfortunes of zinc in continues diarrhea [10-11]. Zinc insufficiency is uncommon in children from developing world as a result of the absence of ingestion of nutrients, high dietary phytate that declines zinc assimilation, and lacking sustenance consumption. As an administrative, synergist, and auxiliary segment, zinc manages the movement of proteins, interpretation elements, and flagging atoms [12]. It balances the soundness of cell films by decreasing per oxidative harm and gives security against the disturbance of cells [13] by keeping up the structure and capacity of the layer boundary in a few tissues [14-16]. In zinc deficient states, animals and humans have publicized an increased vulnerability towards the structural and functional destruction of numerous organ systems, such as the epidermal, central nervous, and immune systems and the intestinal tract [17]. Clinical symptoms include anemia, loss of appetite, reduced growth, immune dysfunction and developmental problems [18]. Zinc deficiency often leads to diarrheal diseases and pathogenic shifts within the intestinal micro biota with regards to intestinal tract [19]. The strength of the intestinal micro biota has a marvelous impact on the non-specific resilience of an individual towards transmittable diseases. Disturbances in the balance of the micro biota can direct to an excessive proliferation of indigenous and exogenous pathogenic bacteria, one effect of which can be gastrointestinal disorders [20]. Furthermore, zinc depletion can reach to the disruption of the intestinal barrier integrity because the delocalization of compounds of the intestinal tight junction [21].

The term probiotic was presented in 1965 by Lilly and Stillwell [22]. In any case, the logical premise of this definition has as of late been addressed in light of the fact that individual examines recommend that a quantity of probiotic impacts be capable of accomplished by nonviable microscopic organisms and even by bacterial genetic material [23]. In this way, probiotics have all the more as of late been characterized as "microbial cell arrangements or segments of microbial cells with a useful impact on the wellbeing and prosperity of the host [24]. The technique for thinking for using probiotics in extreme compelling detachment of the inside relies on upon the doubt that they are showing in opposition to intestinal pathogens. Regardless, the portion through which probiotics work is unclear. The possible segments join the amalgamation of antimicrobial substance ion for supplements required for improvement of pathogens forceful restriction of grasp of pathogens [25-27]. Change of toxins or toxic substance receptors and instigation of nonspecific and specific safe responses to pathogens [28-29]. Keeping in perspective of above certainties, the present study is an endeavor for the successful utilization of zinc and probiotics to enhance the wellbeing results in less than 5 malnourished children.

Objectives

1. To investigate the effect of zinc and probiotics supplementation on physical and biochemical markers in malnourished diarrheal children under 5 years of age.
2. To determine the effect of zinc and probiotics supplementation on nutritional status of severely malnourished diarrheal children under 5 years of age.
3. To identify the best zinc and probiotics supplements for preventing diarrhea in malnourished under 5 children.

2. Material and Methods

2.1. Study Design And Study Location

A randomized control trial was conducted to explore the relation between zinc and probiotics supplementation on the health outcomes in under 5 malnourished hospitalized children. The study was carried out at nutrition rehabilitation unit (NRU) of lady reading hospital Peshawar. LRH is one of the establish hospital in the Khyber Pakhtunkhwa province. The administrative structure of LRH is well organized and pediatric specialists; nutritionists along with supportive staff serve their best regarding the medical and nutritional care of the patients. Total six beds are settled in nutrition rehabilitation ward.

2.2. Inclusion Criteria and Exclusion Criteria

The children whose Z score value becomes < -3sd or above were admitted and put on research protocol. The subject who have Z score value < -2sd or below and having edema are also a part of research study. The children who have some chronic illness including liver, kidney, heart and other metabolic diseases were not admitted in the research study. Defaulter subjects were also excluded from study.

2.3. Study Protocol, Sampling Technique And Patient's Identification

Total 40 Subjects were enrolled in the study. Four groups were made, and each group was containing 10 Subjects. One group was receiving only zinc supplement (ZS), another group were given probiotic supplement (PS), third group was receiving zinc and probiotic (ZPS) in combine form and the last group were kept as control. The patients were admitted in the pediatric ward of nutrition rehabilitation unit/ stabilization center either through emergency or outpatient department (OPD) or referred Patients from community basic health units. They were assessed by nutritionists in child assessment room, as malnourished children on the visible and noticeable clinical signs and symptom. After admission in nutrition rehabilitation unit the diarrheal malnourished children were find out. Every third child was randomized for study and putted on research protocol.

2.4. Supplements Used

Zinc = Admitted children were given zinc syrup Osiris (zinc sulphate) according to the following dosage 50 mg/day along with F-75 and F-100. Patient was kept on only zinc syrup after discharge from nutrition rehabilitation unit.

Probiotics = Patient were given probiotic in the form of enterogermina oral suspension (1 impule /day).

ResoMal = ResoMal were given to treat diarrhea.

3. Data Collection

3.1. Socio - Demographic Status

Information with respect to age, occupation, education of guardians, family size, family type and family monthly income of the selected children were gathered by interviewing the client guardian and the data was recorded in questionnaire. (appendix 1).

3.2. Anthropometric Measurements

Weight of selected children was taken by prescribed WHO standard systems [30]. Weight of the subjects was taken by using baby weight scale. The scale was collaborated to zero for taking actual data. The children mother was requested to expel shoes, wearing light garments and to step on the focal point of the scale. The estimation was recorded in kilograms (kg).

Length of children was determined by infantometer. The child was place on length board without shoes. Legs of child were straight. The movable foot board was brought to the legs and record the reading. Height of the subjects was determined by stadiometer height board. The children were without shoes (i.e., unshod or wearing flimsy socks), wear light fabrics, stand equally on both feet, mends together, arms were hanging by the sides, head and back were in contact with vertical board. The subject was then asked to breathe in and extend to their tallness. The estimation was recorded in centimeters (cm).

The OFC was measured by using a non-stretchable measuring tape, the mother of the subjects was asked to hold the child. The tape was determined to children shed that lie over the frontal bone of the skull over the eyebrows. The tape was then balance and locates the maximal edge. Estimation was taken to the nearest 0.1 cm.

The Z-score value was recorded by using Z-score outlines prescribed by world health organization.

3.3. Clinical and Biochemical Assessments

The doctors and nutritionists clinically inspected the patients and were then send for biochemical assessment. Sign and symptoms were also being overlooked. Edema was also checked as; Grade +: mildly edematous (both feet/lower legs are influenced). Grade ++: Moderately edematous (either feet, bring down legs, hands or lower arms are influenced). Grade +++: Severely edematous (Generalized reciprocal edema including legs, arms and face).

For biochemical assessment, the blood was collected from admitted children in ward and then putt in the EDTA tube marked with subject admission information. The blood was then sent to the research laboratory for biochemical assessment within 15 to 20 minutes. After centrifugation serum was analyzed for serum electrolytes sodium, potassium, chloride and calcium also complete blood count like RBC, WBC, HB and platelets tests were done by trained laboratory pathologist.

3.4. Dietary History/Information

Data regarding IYCF including complementary feeding, exclusive breast feeding, bottle feeding and partially breastfeeding were recorded in the questionnaire by interviewing the child mothers.

4. Statistical Analysis

Data regarding all parameters including anthropometry, biochemical was entered into excel sheet and was analyzed by SPSS. Repeated measure analysis was used followed by bonferroni (post hoc) test for multiple comparisons at 5% level of significance.

5. Results and Discussion

Zinc and probiotics supplements have been provided to the admitted malnourished children in nutrition rehabilitation unit (NRU), Lady Reading Hospital Peshawar. Malnutrition prevail at high level both in rural and urban communities. No treatment services exist in the community for the treatment of malnutrition in children except referred to the tertiary care hospital for treatment. The recommended intake of zinc and probiotic is one of the most common tools to overcome the severity of malnutrition. In our study zinc and probiotics supplements were orally given to the admitted hospitalized children.

5.1. General Socio-Demographic Characteristic of the Respondents

Table 1 shows the general socio-demographic characteristics of the study subjects. Results of the categorical parameters are presented as frequency and percentages while those of continuous variables are presented as mean ± standard deviation (SD). The mean age (months) of the enrolled children was 22.60±15.88 (n=40), the mean family size and total monthly income were 12.50±5.503; mean family size in our study is slightly above the 5th quintile and monthly income at the 3rd quintile of the general population living in the semi urban areas of Khyber Pakhtunkhwa [31]. It was found that 63.4% (n=25) of children were living in joint family system indicating that more than half of the families were of joint type. Results on family type are in accordance to the prevailing culture in Pakistan. In a cross-sectional study conducted by Aga Khan University stated that 56.5% of the families were living in joint families [32]. Results on mean parental education indicate that more than half of the parents were illiterate (mothers=85.4%, fathers=63.9%), which may be due to the reason that the sample was derived from semi-urban areas of District Peshawar. According to the Pakistan Social and Living Standard Measurement Survey (PSLM), the overall literacy rate (age 10 years and above) is 58% (47% in female and 70% in male) for 2011-2012. It shows that overall literacy rate is higher in male than in female in Pakistan [33].

Table 1. General socio-demographic characteristic of the respondents.

Variables	Yes/No	N (%)
Father education	Yes	14 (34.1)
	No	26 (63.9)
Mother education	Yes	5 (12.2)
	No	35 (85.4)
Father occupation	Yes	8 (19.5)
	No	32 (78.0)
Family Type	Joint	25 (63.4)
	Separate	15 (36.6)
Socio-economic status	Satisfactory	25 (61.0)
	Non-satisfactory	15 (36.6)

Parameters	Mean ± SD			
	G-I	G-II	G-III	G-IV
Family Size	12.50 ± 5.503	12.80 ± 7.300	10.80 ± 5.789	10.50 ± 5.276
Age	22.60 ± 15.88	22.60 ± 15.88	22.60 ± 15.88	22.60 ± 15.88
Diarrhea days	5.70 ± 1.829	5.50 ± 2.173	4.10 ± 2.025	4.50 ± 0.707
# of Dependents	2.00 ± 0.667	2.50 ± 1.509	2.70 ± 1.337	2.50 ± 1.269

G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

5.2. General Health Characteristic of the Study Subjects

Table 2 shows the results on overall health statuses of malnourished children. Most of the children have watery diarrhea 85.4% (n= 35) and 12.2% (n=5) have semi-watery diarrhea. 12.2% n=5 among 40 children have blood in their stool while remaining 35 children have no blood in their stool. 6 children about 14.6% have mucous in their stool while the rest have no mucous in their stool. Most of the children were dehydrated 75.6% (n=31) while 22.0% (n=9) non-dehydrated. 9.8% of children have vomiting while the rest were non- vomited children. About 2 children among 40 were having temperature while 92.7% of children were non- temperate. Vaccinated children were 25 among 40 while the rest of children were non- vaccinated, or vaccination was in progress. 29.3% (n=12) children were on antibiotics while 68.3% (n=28) were non- taking antibiotics. About one third of the children were taking rehydration solution 75.6% (n=31) while the others were not taking any rehydration solution. 19.5% of child was on iv fluids while the other were not receiving iv fluids. 12.2% (n=5) children were breastfed children, 43.9% (n=18) was given noted formula milk feed children while 41.5% (n=17) was partially breastfed children. 70.7% (n=29) of the mothers were giving boiled water during diarrhea to their children while 26.8% (n=11) were not giving boiled water during diarrhea. 70.7% (n=29) mothers were boil the baby feeder before use while the others were not boil the feeder before use.

Table 2. General health characteristic of the study subjects.

Variables	Status	n (%)
Consistency of stool	Watery	35 (85.4)
	Semi-watery	5 (12.2)
Blood in stool	Yes	5 (12.2)
	No	35 (85.4)
Mucous in stool	Yes	6 (14.6)
	No	34 (82.9)
Hydration status	Dehydrated	31 (75.6)
	Non-dehydrated	9 (22.0)
Vomiting	Yes	4 (9.8)
	No	36 (87.8)
Temperature	Yes	2 (4.9)
	No	38 (92.7)
Vaccination	Yes	25 (61.0)
	No	20 (19.5)
Antibiotics	Yes	12 (29.3)
	No	28 (68.3)
Rehydration solution	Yes	31 (75.6)
	No	9 (22.0)
IV fluids given	Yes	8 (19.5)
	No	32 (78.0)
Kind of milk feeding	Breast	5 (12.2)
	Bottle	18 (43.9)
	Partial milk	17 (41.5)
When complementary feed started	>6 months	6 (14.6)
	<6 months	34 (82.9)
Offer boiled water during diarrhea	Yes	29 (70.7)
	No	11 (26.8)
Boil the feed before use	Yes	29 (70.7)
	No	11 (26.8)

5.3. Effect on RBCs Level

Table 3 indicates that at the start of intervention day 0, the mean values for RBC of doses and placebo groups were 3.63±0.80, 3.90±0.66, 4.60±3.86 and 3.42±1.79 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean RBC 3.99±0.68 was observed. However, after 30th and 60th day consumption, the mean RBC was 4.56±0.52 and 4.82±0.49 respectively. There was a significant (*p<0.05*) increase in mean value of RBC when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a significant increase in the mean RBC 4.52±0.42, 4.91±0.44 and 5.27±0.91 was observed at day 14th, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a significant increase in the mean RBC 4.86±2.01, 4.40±1.26 and 5.04±0.73 was observed at day 14th, 30th and 60th respectively. The present study demonstrated that zinc, probiotics and mixture significantly increased, while placebo was non-significantly increased the mean values of RBC.

Table 3. Effect of Zinc and Probiotic Supplementation on RBCs Level of Malnourished Children.

Doses	Duration			
	Mean ± SD (10.6/µl)			
	0 day	14th day	30th day	60th day
G-I	3.63±0.80^{d}	3.99±0.68^{cd}	4.56±0.52^{abc}	4.82±0.49^{abc}
G-II	3.90±0.66^{cd}	4.52±0.42^{abc}	4.91±0.44^{ab}	5.27±0.91^{a}
G-III	3.60±3.86^{cd}	4.86±2.01^{bcd}	4.40±1.26^{abc}	5.04±0.73^{ab}
G-IV	3.42±1.79^{abc}	3.75±1.78^{abc}	3.85±1.63^{ab}	4.46±1.57^{ab}

G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

5.4. Effect on WBCs Leve

Table 4 indicates that at the start of the intervention day 0, the mean values for WBC of doses and placebo groups were 8.62±6.54, 8.19±5.30, 8.60±3.86 and 8.42±1.79 respectively. In G-I, after consumption of zinc syrup for 14th day, an increase in the mean WBC 7.87±5.10 was observed. However, after 30th and 60th day consumption, the mean WBC was 7.48±3.62 and 7.38±2.35 respectively. There was no significant ($p<0.05$) increase in the mean value of WBC when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean WBC 7.70±3.57, 8.13±3.05 and 8.27±2.45 was observed at day 14th, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean WBC 7.86±2.01, 8.40±1.26, and 9.04±0.73 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was non-significantly increased the mean values of WBC. The immune related functions of zinc have been reviewed in the last few years [34]. They concluded that zinc is essential for highly proliferating cells, especially in the immune system and influences both innate and acquired immune functions.

Table 4. Effect of zinc and probiotic supplementation on WBCs level of malnourished children.

Doses	Duration			
	Mean ± SD (10. e3/µl)			
	0 day	14th day	30th day	60th day
G-I	8.62±6.54[a]	7.87±5.10[a]	7.48±3.62[a]	7.38±2.35[a]
G-II	8.19±5.30[a]	7.70±3.57[a]	8.13±3.05[a]	8.27±2.45[a]
G-III	8.60±3.68[a]	7.86±2.01[a]	8.40±1.26[a]	9.04±0.73[a]
G-IV	8.42±1.79[a]	7.75±1.78[a]	7.85±1.63[a]	8.46±1.57[a]

G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

5.5. Effect on HB Level

Table 5 indicates that at the start of intervention day 0, the mean values for HB of doses and placebo groups were 8.06±1.49, 8.00±1.38, 8.01±1.08 and 8.97±1.20 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean HB 8.40±1.29 was observed. However, after 30th and 60th day consumption, the mean HB was 9.10±1.04 and 10.04±1.08 respectively. There was a non-significant ($p<0.05$) increase in mean value of HB when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean HB 9.07±1.63, 10.14±1.44 and 11.38±1.15 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean HB 8.72±1.11, 17.83±24.67, and 11.21±0.61 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was non-significantly increased the mean values of HB. (Zulia et al., 2016) concluded that the combined zinc and probiotics over 4 weeks improve the immune system of the patient with severely malnourished measured by lymphocyte and monocytes.

Table 5. Effect of Zinc and Probiotic Supplementation on HB Level of Malnourished Children

Doses	Duration			
	Mean±SD (g/dl)			
	0 day	14th day	30th day	60th day
G-I	8.06±1.49[a]	8.40±1.29[a]	9.10±1.04[a]	10.04±1.08[a]
G-II	8.00±1.38[a]	9.07±1.63[a]	10.14±1.44[a]	11.38±1.15[a]
G-III	8.01±1.08[a]	8.72±1.11[a]	17.83±24.67[a]	11.21±0.61[a]
G-IV	8.97±1.20[a]	8.29±1.23[a]	8.93±1.12[a]	9.74±1.10[a]

G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

5.6. Effect on Platelets Level

Table 6 indicates that at the start of intervention day 0, the mean values for platelets of doses and placebo groups were 222.30±48.93, 222.80±63.81, 222.30±43.79 and 222.50±34.38 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean platelets 223.10±34.94 was observed. However, after 30th and 60th day consumption, the mean platelet was246.50±34.69 and 288.50±39.61 respectively. There was a significant ($p<0.05$) increase in mean value of platelet when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a significant increase in the mean platelet 263.00±54.30, 291.00±62.82 and 308.30±39.90 was observed at day 14, 30th and 60th respectively In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean platelet 249.00±40.62, 297.20±32.26, and 319.90±45.32 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics and placebo significantly increased, while mixture was non-significantly increased the mean values of platelet. The findings indicated from the (Andrea et al., 2015) study that the platelets count decreased significantly in the experimental group (p=0.0014), with no correlation with serum zinc after oral supplementation. The aim of their study was to evaluate the effects of oral zinc supplementation on food intake, biochemical and hematological parameters in healthy children.

Table 6. Effect of Zinc and Probiotic Supplementation on Platelets Level of Malnourished Children

Doses	Duration			
	Mean ± SD (10. e3/µl)			
	0 day	14th day	30th day	60th day
G-I	222.30±48.93^{f}	223.10±34.94^{f}	246.50±34.69^{ef}	288.50±39.6^{def}
G-II	222.80±63.81^{cde}	263.00±54.30^{abcd}	291.00±62.82^{abc}	308.30±39.90^{ab}
G-III	222.30±43.79^{de}	249.00±40.62^{bcde}	297.20±32.26^{ab}	319.90±45.32^{a}
G-IV	222.50±34.38^{de}	253.60±29.25^{bcd}	293.40±27.661^{abc}	298.30±48.06^{ab}

G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

5.7. Effect on Sodium Level

Table 7 indicates that at the start of intervention day 0, the mean values for sodium doses and placebo groups were 131.90±4.22, 131.20±4.46, 131.60±4.62 and 131.30±1.56 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean sodium 133.50±2.46 was observed. However, after 30th and 60th day consumption, the mean sodium was 135.90±3.87 and 137.10±2.23 respectively. There was a significant (*p<0.05*) increase in mean value of sodium when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a significant increase in the mean sodium 133.10±2.96, 136.90±3.66 and 140.40±3.06 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean sodium 134.80±2.61, 138.30±1.41, and 138.90±1.44 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics and placebo significantly increased, while mixture was non-significantly increased the mean values of sodium. Sodium intake has been suggested to influence zinc excretion in normal subjects. In order to assess the effect of sodium on zinc excretion in subjects, urinary zinc excretion was measured in thirteen children and adolescents on both a high (140 mEq/day) and low (20 mEq/day) sodium intake. Urinary zinc excretion was elevated on both diets. The mean urinary zinc excretion on the high sodium diet 775 +/− 238 micrograms/24 h was significantly lower (P less than.005) than that on the low sodium diet 947 +/− 344 micrograms/24 h.

Table 7. Effect of Zinc and Probiotic Supplementation on Sodium Level of Malnourished Children

Doses	Duration			
	Mean ± SD (mmol/L)			
	0 day	14th day	30th day	60th day
G-I	131.90±4.22^{def}	133.50±2.46^{cde}	135.90±3.87^{abcd}	137.10±2.23^{abc}
G-II	131.20±4.46^{g}	133.10±2.96^{cdef}	136.90±3.66^{abc}	140.40±3.06^{a}
G-III	131.60±4.62^{f}	134.80±2.61^{bcde}	138.30±1.41^{ab}	138.90±1.44^{ab}
G-IV	131.30±1.56^{ef}	133.40±0.96^{cde}	136.50±1.50^{abc}	135.90±0.94^{abcd}

NA=sodium, G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

5.8. Effect on Potassium Level

Table 8 indicates that at the start of intervention day 0, the mean values for potassium doses and placebo groups were 3.42±0.89, 3.05±0.51, 3.43±0.31 and 3.71±0.20 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean potassium 3.47±0.91 was observed. However, after 30th and 60th day consumption, the mean potassium was 3.62±0.71 and 3.78±0.47 respectively. There was a non-significant (*p<0.05*) increase in mean value of potassium when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean potassium 3.55±0.66, 7.02±10.19 and 4.34±0.48 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean potassium 3.89±0.20, 4.34±0.44, and 4.61±0.45 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was non-significantly increased the mean values of potassium. Zinc and potassium are both minerals that contribute to healthy body functions, including the functions of the immune, circulatory, muscular and digestive systems. The use of zinc supplements can lead to decreased levels of magnesium, which also lowers the levels of potassium in the body. This negative effect can be avoided by supplementing the diet with magnesium supplements, thereby increasing the levels of potassium in the body.

Table 8. Effect of Zinc and Probiotic Supplementation on Potassium Level of Malnourished Children

Doses	Duration Mean±SD (mmol/L)			
	0 day	14th day	30th day	60th day
G-I	3.42±0.89[a]	3.47±0.91[a]	3.62±0.71[a]	3.78±0.47[a]
G-II	3.05±0.51[a]	3.55±0.66[a]	7.02±10.19[a]	4.34±0.48[a]
G-III	3.43±0.31[a]	3.89±0.20[a]	4.34±0.44[a]	4.61±0.45[a]
G-IV	3.71±0.20[a]	3.86±0.19[a]	4.07±0.37[a]	4.22±0.46[a]

K=potassium, G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

5.9. Effect on Chloride Level

Table 9 indicates that at the start of intervention day 0, the mean values for chloride doses and placebo groups were 97.10±5.64, 97.10±6.22, 97.20±5.09 and 97.87±27.53 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean chloride 97.70±5.16 was observed. However, after 30th and 60th day consumption, the mean chloride was 100.10±5.21 and 93.71±30.26 respectively. There was a significant ($p<0.05$) increase in mean value of chloride when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean chloride 87.71±28.15, 104.50±4.76 and 107.00±3.52 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean chloride 101.50±4.17, 104.90±3.72, and 106.60±2.75 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc and placebo significantly increased, while Probiotics and mixture was non-significantly increased the mean values of chloride.

Table 9. Effect of Zinc and Probiotic Supplementation on Chloride Level of Malnourished Children

Doses	Duration Mean±SD (mmol/L)			
	0 day	14th day	30th day	60th day
G-I	97.10±5.64[a]	97.70±5.16[a]	100.10±5.21[a]	93.71±30.26[a]
G-II	97.10±6.22[a]	87.71±28.15[a]	104.50±4.76[a]	107.00±3.52[a]
G-III	97.20±5.09[a]	101.50±4.17[a]	104.90±3.72[a]	106.60±2.75[a]
G-IV	97.87±27.53[a]	98.00±3.33[a]	99.50±2.17[a]	101.10±3.03[a]

Cl=chloride, G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

6. Effect on Calcium Level

Table 10 indicates that at the start of intervention day 0, the mean values for calcium doses and placebo groups were 7.88±1.11, 8.24±1.02, 7.73±0.51 and 7.92±27.08 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean calcium 7.90±0.85 was observed. However after 30th and 60th day consumption, the mean calcium was 8.14±0.59 and 17.81±29.93 respectively. There was a non-significant ($p<0.05$) increase in mean value of calcium when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean calcium 18.92±32.35, 8.90±0.64 and 8.90±0.47 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean calcium 8.22±0.72, 8.90±0.45, and 9.09±0.50 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was non-significantly increased the mean values of calcium. (Abrams and Atkinson, 2003) concluded that the strongest case can be made for calcium and vitamin D supplementation. Because excessive dietary calcium can reduce zinc absorption as a result of interactive effects within the intestine, an appropriate ratio of calcium to zinc should be used, even if this means adding zinc as a fortificant or supplement.

Table 10. Effect of zinc and probiotic supplementation on calcium level of malnourished children.

Doses	Duration Mean±SD (mg/dl)			
	0 day	14th day	30th day	60th day
G-I	7.88±1.11[a]	7.90±0.85[a]	8.14±0.59[a]	17.81±29.93[a]
G-II	7.24±1.02[a]	18.92±32.35[a]	8.90±0.64[a]	8.90±0.47[a]

Doses	Duration Mean±SD (mg/dl)			
	0 day	14th day	30th day	60th day
G-III	7.73±0.51[a]	8.22±0.72[a]	8.90±0.45[a]	9.09±0.50[a]
G-IV	7.92±27.08[a]	8.52±0.52[a]	8.70±0.34[a]	8.68±0.40[a]

Ca=calcium, G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

6.1. Effect on Weight

Table 11 indicates that at the start of intervention day 0, the mean values for weight of doses and placebo groups were 5.09±1.23, 5.61±1.16, 5.92±1.24 and 5.55±1.30 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean weight7.00±1.16was observed. However, after 30th and 60th day consumption, the mean RBC was 7.75±1.23 and 8.55±1.26 respectively. There was a significant (*p<0.05*) increase in mean value of weight when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a significant increase in the mean weight 6.27±1.18, 6.96±1.18 and 7.78±1.16 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a significant increase in the mean weight 6.78±1.14, 7.78±1.16, and 9.03±1.06 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was significantly increased the mean values of weight.

Table 11. Effect of zinc and probiotic supplementation on weight of malnourished Children.

Doses	Duration Mean±SD (kg)			
	0 day	14th day	30th day	60th day
G-I	5.09±1.23[cde]	7.00±1.16[bcde]	7.75±1.23[abc]	8.55±1.26[ab]
G-II	5.61±1.16[e]	6.27±1.18[cde]	6.96±1.18[bcde]	7.78±1.16[abc]
G-III	5.92±1.24[de]	6.78±1.14[cde]	7.78±1.16[abc]	9.03±1.06[a]
G-IV	5.55±1.30[e]	6.18±1.28[cde]	6.89±1.20[bcde]	7.65±1.14[abcd]

G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

6.2. Effect on Height

Table 12 indicates that at the start of intervention day 0, the mean values for height of doses and placebo groups were 70.30±6.23, 70.70±6.88, 70.70±7.42 and 70.40±5.68 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean height 71.30±6.23 was observed. However, after 30th and 60th day consumption, the mean height was 71.90±5.76 and 72.80±5.69 respectively. There was a non-significant (*p<0.05*) increase in mean value of height when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean height 68.70±6.88, 69.70±6.29 and 70.60±5.77 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean height 70.70±7.42, 70.80±7.25, and 71.50±7.36 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was non-significantly increased the mean values of height.

Table 12. Effect of zinc and probiotic supplementation on height of malnourished children.

Doses	Duration Mean±SD (cm)			
	0 day	14th day	30th day	60th day
G-I	70.30±6.23[a]	71.30±6.23[a]	71.90±5.76[a]	72.80±5.69[a]
G-II	70.70±6.88[a]	68.70±6.88[a]	69.70±6.29[a]	70.60±5.77[a]
G-III	70.70±7.42[a]	70.70±7.42[a]	70.80±7.25[a]	71.50±7.36[a]
G-IV	70.40±5.68[a]	69.40±5.68[a]	69.40±5.68[a]	70.20±5.61[a]

G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

6.3. Effect on Z-Score Level

Table 13 indicates that at the start of intervention day 0, the mean values for z-score level of doses and placebo groups were 3.50±0.52, 3.60±0.51, 3.50±0.52 and 3.60±0.51 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean Z-score 2.50±1.35 was observed. However, after 30th and 60th day consumption, the mean Z-score was 2.50±1.78 and 3.80±1.93 respectively. There was a non-significant (*p<0.05*) increase in mean value of z-score when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean z-score 2.70±1.41, 2.50±1.58 and 3.20±1.93 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean z-score 2.00±1.05, 3.00±2.10, and 5.00±0.00 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was non-significantly increased the mean values of Z-score.

Table 13. Effect of zinc and probiotic supplementation on z-score level of malnourished children.

Doses	Duration Mean±SD (SD)			
	0 day	14th day	30th day	60th day
G-I	3.50±0.52d	2.50±1.35cd	2.50±1.78bcd	3.80±1.93abcd
G-II	3.60±0.51abcd	2.70±1.41abcd	2.50±1.58abcd	3.20±1.93abcd
G-III	3.50±0.52abc	2.00±1.05abc	3.00±2.10ab	5.00±0.00a
G-IV	3.60±0.51abcd	3.00±1.05abcd	2.30±1.25abcd	2.40±1.83abcd

6.4. Effect on OFC Level

Table 14 indicates that at the start of intervention day 0, the mean values for OFC of doses and placebo groups were 44.30±2.16, 44.30±3.43, 44.70±1.88 and 44.70±1.94 respectively. In G-I, after consumption of zinc syrup for 14 days, an increase in the mean OFC 43.40±2.05 was observed. However, after 30th and 60th day consumption, the mean OFC was 43.70±2.00 and 44.35±2.06 respectively. There was a non-significant (*p<0.05*) increase in mean value of OFC when compared with the mean value at day 0 for this group. In G-II, after consumption of probiotics (Enterogermina) 1 ampule per day a non-significant increase in the mean OFC 45.30±4.43, 45.30±4.43 and 45.90±3.18 was observed at day 14, 30th and 60th respectively. In G-III, after consumption of both zinc and probiotics supplementation a non-significant increase in the mean OFC 46.70±1.88, 46.90±1.79, and 47.10±1.82 was observed at day 14, 30th and 60th respectively. The present study demonstrated that zinc, probiotics, mixture and placebo was non-significantly increased the mean values of OFC.

Table 14. Effect of Zinc and Probiotic Supplementation on OFC Level of Malnourished Children

Doses	Duration Mean±SD (cm)			
	0 day	14th day	30th day	60th day
G-I	44.30±2.16d	43.40±2.05cd	43.70±2.00bcd	44.35±2.06abcd
G-II	44.30±3.43abcd	45.30±4.43abcd	45.30±4.43abcd	45.90±3.18abcd
G-III	44.70±1.88abc	46.70±1.88abc	46.90±1.79ab	47.10±1.82a
G-IV	44.70±1.94abcd	45.70±1.94abcd	45.70±1.94abcd	46.10±1.9abcd

OFC=occipital frontal circumference, G-1=zinc, G-2=probiotics, G-3=mixture, G-4=placebo.

7. Summary

This was a randomize control study to find out the effect of zinc and probiotics supplementation on health outcomes in under 5 malnourished hospitalized children with diarrhea visiting children ward A, Nutrition Rehabilitation Unit, Lady Reading Hospital Peshawar. It was more suitable site for study because children from both rural and urban areas visit hospital for medical and nutritional problems. Total sample size was 40 children and the patients were divided in four groups. Each group was containing 10 children. One group was receiving only zinc supplement (ZS), another group were given probiotic supplement (PS), third group was receiving zinc and probiotic (ZPS) in combine form and the last group were kept as control. Selection criteria were that, the children whose Z score value becomes < -3sd or above were admitted and put on research protocol. The subject who have Z score value < -2sd or below and having edema are also a part of research study. The children who have some chronic illness including liver, kidney, heart and other metabolic diseases were not admitted in the research study. Defaulter subjects were also excluded from study. The children were assessed at nutrition assessment room and blood test for serum electrolytes and complete blood count level were also done. Anthropometric assessments of children were determined through weight, length/height and occipital frontal circumference (OFC). The standardized equipment's and methods were used for physical assessment. Socio-demographic status of child includes (Guardians occupation, parent's education, family size and type along with their monthly income), medical history of the child, knowledge and awareness level of mothers/care providers and hygiene and sanitation of houses was recorded.

Mean age in months of the children were 22.6000±15.88990. More than half of the families were living in joint family system (n=25; 63.4%), the literacy rate of the mother was very low, majority of mother were illiterate 35 (85.4%). The selected families were from low socio-economic status and the mean income per month was 12000 PKR. Medical history and clinical assessment were taken on questionnaires. Medical history includes diarrhea n=40 (100%), Consistency of stool, watery 35 (85.4%) Semi-watery 5 (12.2%), Blood in stool n=5 (12.2%), Mucous in stool n=6 (14.6%), vomiting n=4 (9.8%) and Dehydration status, n=31 (75.6%).

Results on anthropometric and nutritional status of children are also shown in tables 11, 12, 13 and 14 respectively. Means weight, height/length, Z-score and occipital frontal circumference (OFC) of the children were 6.09±1.23, 71.30±6.23, 3.50±0.52 and 43.30±2.16 respectively. The negative z-scores of children for all anthropometric measurements indicate that our children had mean weight, height and (OFC) lower than the median values of the WHO standards. Unadjusted analysis shows a very significant association between risk factors socio-demographic and nutritional factors. Bottle feeding and improper dilution was found a strong predictor for causing diarrhea; with the increase in episodes of diarrhea and more chances of infections were exposed. Finding on breast feeding showed a positive relation for the occurrence of diarrhea within the families. Mother education was found negatively associated with the likeliness of diarrhea. A very strong positive increase was found in weight of children and nutritional status as assessed by taking their current body weight and consecutive three follow ups.

Child health status as indicated by incidences of diarrhea was also found in association with malnutrition in children. Children with improper intake of nutrient were more likely to be malnourished as compared to the rest. Among the dietary factors, dietary behavior and intake of unhygienic food and improper diluted feed were found in association with diarrhea of malnourished children. A few confusions were found in malnourished children in contrast with all around supported, similar to greater part of mothers were found for not giving breast milk to their children during a half year which is much critical for youngster wellbeing, they were nourished with bottle feeding and found weakening and cause serious looseness of the bowels while a few children were found fussy nature toward complementary food.

8. Conclusion and Recommendations

8.1. Conclusion

1. Consumption of zinc and probiotic supplements increase the RBC's mean level significantly ($p<0.05$) compare to control group.
2. Changes in mean levels of WBC's and HB were non-significantly recorded in all the groups.
3. Consumption of zinc and probiotic supplements increase the Platelets and sodium mean level significantly ($p<0.05$) compared to mixture group respectively, while changes in potassium and calcium level were non-significantly and chloride level was significantly recorded in all the groups.
4. Weight of malnourished children was changed significantly ($p<0.05$), while height, Z score level and Occipital frontal circumference (OFC) was non-significantly recorded in all groups.
5. Zinc and probiotics therapy could stimulate the body immune system and thereby improve the health outcomes.

8.2. Recommendations

1. Zinc and probiotic supplementation help in reducing the health complications related to malnutrition in children, therefore this therapy can be recommended by Nutritionist and health care provider.
2. Zinc and probiotic rich diet should be beneficial because it could play a vital role in management of malnutrition by enhancing immune system.
3. Provide hygienic condition to the children to prevent diarrhea.
4. Nutritionists and Dietitians should play their role by educating mothers about proper diet preparation and serving to their children.
5. Seminars should be arranged for mothers to create awareness regarding unhygienic conditions, acute malnutrition and risk factors associated with them.

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