Scientific Research Publishing

Open Journal of Epidemiology, 2024, 14, 547-564
https://www.scirp.org/journal/ojepi
ISSN Online: 2165-7467
ISSN Print: 2165-7459

Association of Nutritional Supplements with Nutritional Status and Treatment Outcomes of Visceral Leishmaniasis among Children Aged 5 - 12 Years in Baringo and West Pokot Counties, Kenya

Ann Iruata Namulen[1*], John Gachohi[2], Anselimo Makokha[3], Joseph Mutai[4]

[1]Department of Knowledge Management, Kenya Medical Research Institute, Nairobi, Kenya
[2]Department of Public Health, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
[3]Department of Food Science, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
[4]Department of Public Health, Kenya Medical Research Institute, Nairobi, Kenya
Email: *anamulen87@gmail.com

How to cite this paper: Namulen, A.I., Gachohi, J., Makokha, A. and Mutai, J. (2024) Association of Nutritional Supplements with Nutritional Status and Treatment Outcomes of Visceral Leishmaniasis among Children Aged 5 - 12 Years in Baringo and West Pokot Counties, Kenya. *Open Journal of Epidemiology*, **14**, 547-564.
https://doi.org/10.4236/ojepi.2024.144039

Received: July 10, 2024
Accepted: September 2, 2024
Published: September 5, 2024

Abstract

Introduction: Visceral leishmaniasis is a severe form of leishmaniasis that affects about 90,000 people annually worldwide. It is mainly transmitted by *Leishmania donovani* and *infantum*, which can cause damage to internal organs, such as the spleen, liver, and bone marrow. If left untreated, severe cases can be fatal, as the disease can lead to severe secondary diseases, mycological and bacterial infections, and hemorrhages. Nutritional deficiencies and concurrent infections increase the incidence of visceral leishmaniasis and the likelihood of lethality. There is limited information about the relationship between the disease and nutrition in endemic areas in Kenya. **Objective:** The study was to analyze the association of nutritional supplements with the nutritional status and treatment outcomes of visceral leishmaniasis among children aged 5 - 12 years in Baringo and West Counties in Kenya. **Methods:** A quasi-experimental study design adopting quantitative data collection method was used in this study. A total of 204 children aged 5 - 12 years were included in the study. Data on nutritional status and treatment outcomes for VL was collected using a questionnaire and consent form. Pre- and post-intervention assessments were conducted to compare BMI, fever, spleen size, splenic aspirate after treatment, and the presence of PKDL in a 3-month follow-up. The data was analyzed using R statistical software with descriptive and inferential statistics, including chi-square tests and t-tests. The impact of treatment was estimated using the difference-in-difference method to compare changes in

outcomes over time between the intervention and comparison groups. **Results:** The baseline characteristics assessed in this study were socio-demographics (age, gender, marital status, education and religion), vitamins (A, B, C, D) and minerals (zinc, Iron and Iodine). The results showed that the mean age was 8.72, children aged 5 - 9 years were 64.7%, and those aged 10 - 12 years were 35.3% in the intervention and comparison groups. There were more males than females in the study (53.9% in the intervention and 52.9% in the comparison group respectively). All the children in the study were from a Christian background, were underweight, had enlarged spleen, and were positive for VL by Splenic Aspirate. Those who presented with fever were 88.2% (88% in both intervention and comparison groups). Most children had lower levels of zinc, iron, vitamins A, B12, and D at baseline (54.9%, 91.2%, 54.4%, 57.8%, and 58.8% respectively). The majority (93.1%) were deficient in vitamin C (90.2% in the intervention and 96.1% in the comparison group). **Conclusion:** According to the study findings, the effect of administering micronutrients is significant at 5% significance level with the intervention having a positive effect. The administration of the nutritional supplement led on average to an increase of the minerals, vitamins and BMI levels in the body.

Keywords

Visceral Leishmaniasis, Micronutrients, Minerals, Vitamins, Implications

1. Introduction

Leishmaniasis is a protozoal disease found in places with extreme malnutrition and poverty. Globally, leishmaniasis is one of the top ten neglected tropical diseases, infecting around 12 million people. Visceral leishmaniasis (VL) is the fatal type of leishmaniasis. In more than 95% of cases and every year, untreated infections are fatal. It affects around 90,000 people. VL has a significant morbidity and death rate. Children are the most impacted, particularly in low-income nations, with a 50% fatality rate [1]-[3].

VL accounts for 68% of cases worldwide, with India, Sudan, Brazil, and Kenya leading the way. Kenya, a developing country in Sub-Saharan Africa, is one of the five East African countries that presently bear most of the global burden of VL. It is native to Kenya's ASAL areas. Current transmission hotspots include Baringo, Isiolo, Marsabit, West Pokot, Turkana, Kitui, Garissa, and Wajir counties. According to one estimate, there are roughly 1600 cases of VL per year, resulting in the loss of nearly 13,000 DALYs and 170 fatalities [3] [4].

Post Kalazar Dermal Leishmaniasis (PKDL) is thought to account for less than five percent of VL cases in Kenya, but research on PKDL is limited. Poverty-associated variables such as poor income, environmental conditions, and malnutrition affect immunity and host defense, increasing the risk of infection. Low micronutrient levels in undernourished individuals have been linked to altered infection resistance and host vulnerability. Inadequate micronutrient levels in the body have been shown to

be a poor predictor of leishmaniasis, particularly a lack of trace elements such as zinc, iron, and vitamins A, B, C, and D, which play an important role in the regulation of innate and adaptive immunity, cell proliferation, and human physiology [1].

Even with the aid programs designed to treat micronutrient deficiencies, patients with VL in Kenya continue to have significant levels of deficiencies in zinc, iron, iodine, and vitamins A, B, C, and D. This causes anomalies such stunted growth and development in kids, irregular metabolism, long-term inflammation, and improper nutrient absorption. Kenya is classified as one of the nations with the highest VL load in the endemic regions of West Pokot, Garissa, and Baringo. In 1935, the illness was initially reported in Kenya, namely in the Mandera and Wajir areas in the north. Since then, VL outbreaks have happened across the nation in a number of locations. It's unclear exactly how widespread VL is in Kenya [3]-[6].

Few clinical investigations have explored the relationship between low serum micronutrient levels in VL patients and their impact on treatment results. There is little evidence on VL prevalence, supplementation, burden, and spatial distribution in Kenya. According to WHO 2023, deficient micronutrients not only enhance the host's sensitivity to VL infection but also impact illness severity, presenting as a variety of physical problems, many of which include malnutrition etiologic pathways [4] [7].

The intensity of VL is affected by body mass and micronutrient availability in the blood. Vitamins A, B, C, and D, as well as zinc, iron, and iodine, have been shown to limit the development of VL, either directly or by acting as precursors for a parasite-inhibiting pathway. Given the information deficit in this area, this study will shed fresh light on the impact of micronutrients and trace elements in immunology, patient outcomes, and VL physiology. For the past 90 years, leishmaniasis research in Kenya has taken place within this framework. This intervention assesses the significance of comparable studies on leishmaniasis in Kenya, fills gaps, and suggests future study directions. This is crucial, particularly in light of Kenya's newly announced national plan (2021-2025) to expedite leishmaniasis management and ultimately eradicate it. The purpose of this study was to determine how nutritional supplementation impacts the nutritional status and treatment outcomes of visceral leishmaniasis (VL) in children aged 5 - 12 years in Baringo and West Pokot, Counties, Kenya. The study further aimed to determine any differences in nutritional status and treatment outcomes of VL of the study participants between the intervention and comparison groups, assess any differences in the recurrence and re-infection of VL among the study children between the intervention and comparison groups, and finally assess how follow-up affects the likelihood of reinfection of recurrence of VL among the study participants in the intervention and comparison groups. [4].

2. Methodology

2.1. Study Site & Study Design

A quasi-experimental study design adopting quantitative data collection method

was used in this study. The study proposal development started from December 2021 to December 2022. The data collection started from December 2022 to December 2023. The study was conducted in Kacheliba Sub-County Hospital, West Pokot County and Chemolingot Sub-County Hospital, Baringo County, Kenya. These two sites have reported high cases of VL patients who visit for diagnosis and treatment services of VL. Their locations are within the endemic areas of visceral leishmaniasis. The intervention group was selected from Chemolingot Health Facility while the comparison group was selected from Kacheliba Health Facility.

2.2. Study Population/Inclusion & Exclusion Criteria

The study population comprised children aged 5 - 12 years attending Chemolingot and Kacheliba health care facilities of Baringo and West Pokot Counties respectively.

1) Inclusion criteria were children aged 5 - 12 years with visceral leishmaniasis residing in West Pokot and Baringo Counties, children who were willing to participate in the study and after children gave assent to participate in the study and whose parents signed informed consent form for their children to participate in the study.

2) Exclusion criteria were children found not to be physically, mentally, and emotionally able to participate in the study according to a clinician's assessment and children with severe symptoms and signs of the VL such as jaundice, excessive bleeding, toxemia, edema, malnutrition, and severe anemia.

2.3. Sample Size Determination

Sample Size Determination Explanation

n = the desired sample size;

P_1 = Proportion of children aged 5 - 12 years in the study population estimated to have VL = 0.30 (KNBS 2010);

P_2 = Proportion of children aged 5 - 12 years in the study population estimated to have VL after intervention = 0.18, i.e., 30% - (40% of 30%) calculated from Lorri and Svanberg (1994);

$Z\alpha/2$ = the standard normal deviate at the required confidence level of 95% = 1.96;

$Z\beta$ = the desired power of 80% = 0.84;

$P = P_1 + P_2/2$ (Average Proportion);

$\Delta = P_1 - P_2$ (Difference between the proportions).

Breakdown of the Formula and Numbers (197, 243 and 204)

The terms and the specific values used:

n: This represents the desired sample size (what we're solving for).

$Z\alpha/2$: This value (1.96) comes from the standard normal distribution table and corresponds to a 95% confidence level (α).

$Z\beta$: This value (0.84) is derived from a power table and reflects the desired power of the study (80% in this case).

P: The average Proportion of children estimated to have visceral leishmaniasis, calculated as $(P_1 + P_2)/2$. Here, $P_1 = 0.3$ (expected prevalence) and $P_2 = 0.18$ (expected prevalence after intervention), so $P = (0.3 + 0.18)/2 = 0.24$.

Δ: This represents the difference between the two proportions we're comparing $(P_1 - P_2)$, which is $0.3 - 0.18 = 0.12$.

Plugging these values into the formula,

$$N = \frac{\left[Z_\alpha \sqrt{2\bar{p}(1-\bar{p})} + Z_\beta \sqrt{P_1(1-P_1) + P_2(1-P_2)}\right]^2}{\Delta^2}$$

$$n = \left[1.96 \times \sqrt{(2 \times 0.24)(1-0.24)} + 0.84\sqrt{(0.3(1-0.3) + 0.18(1-0.18))}\right]^2$$

This calculation initially results in a sample size of approximately 197.

Adjusting for Non-response: The study then factors in a 10% expected attrition rate (loss to follow-up). To account for this, divide 197 by $(1 - 0.1)^2$, which is 0.81 (to adjust for a 10% loss). This inflates the sample size to 197 / 0.81 ≈ 243.

Therefore, the values of 197, 243, and 204 represent:

- **197:** The initial calculated sample size is needed to detect the expected difference between the desired confidence and power, assuming no loss to follow-up.
- **243:** The inflated sample.
- **204:** The final sample size was settled on.

So why did we settle for the 204?

- A sample size of 243 would be challenging to recruit, manage, or analyze within the study's resources and timeframe.
- A sample size of 204 is sufficient to achieve the desired statistical power of 80% and a 95% confidence level.
- Our decision was guided by practical constraints such as available resources, time, and ethical considerations.

A representative sample size of 102 for each group was determined. The intervention group of 102 study children was given micronutrient powder containing vitamins A, B12, C, and D and minerals Zinc, Iron, and Iodine together with the usual VL treatment, and the other 102 study children were given no intervention but continued with their usual VL.

2.4. Sampling Method

Convenience sampling method was used in this study. Children attending the selected facilities were tested using R-K39 to determine their VL status. Those who turned positive for VL were admitted to the facility and recruited into the study. Consenting study participants were recruited to join the study after signing a written informed consent (ICF) for the guardians and assent for the children. 204 visceral leishmaniasis patients were enrolled.

Intervention & Comparison Groups

A single-blind intervention was used in the allocation of the study groups. One group at Chemolingot health facility of 102 patients received a daily dose of

micronutrient powder (MNP) containing vitamins A, B12, C, and D and micronutrients zinc, Iron, and iodine together with the usual VL treatment for one month. The comparison group at Kacheliba health facility of 102 patients received a placebo tablet together with the usual VL treatment. Both the intervention and the comparison groups were reassessed to confirm reinfection and recurrence of VL. Splenic aspirates were performed to determine if they were positive or not for VL. A body temperature of more than 37.5 Celsius was considered a fever. A spleen size of more than 15 cm was considered splenomegaly. Follow-up to determine the effects of nutritional supplements on the adverse outcomes after VL treatment of the study children was done and information on the respective signs and symptoms experienced by the children after treatment up to 3 months recorded–symptoms to include non-recovered participants re-infection, recurrence rates, Post-kala-azar dermal leishmaniasis (PKDL), need for rescue medication, relapse and in extreme cases death records, etc. These were categorized into the generally commonly accepted symptoms for exploratory data analysis.

2.5. Data Collection

A semi-structured questionnaire was designed by the principal investigator (Ann Namulen) and the trained study team administered it to the children in the study. The questionnaire had 3 sections: Section A was to collect information on the demographic characteristics of the study participants. These were age, gender, marital status, level of education, and religious affiliation. Section B was to collect information on the nutritional status of the study participants. This included: anthropometric information, BMI, vitamins and minerals test results. Section c collected information on clinical characteristics which included body temperature, spleen size, weight, height, BMI, and any signs of Post Kala-azar Dermal Leishmaniasis, Hemoglobin levels, and splenic aspirate.

2.5.1. Validity

The questionnaire was submitted to the study coordinator, the statistician, and an independent expert to evaluate the content validity and construct validity and to check for conceptual and investigative bias.

2.5.2. Reliability

The reliability of the questionnaire was ensured by accurate and careful phrasing of each section of the questionnaire to avoid ambiguity. A test-retest technique was used. The instrument was prepared and administered to the study participants. After two weeks the instrument was administered to the same study participants. The responses to the first administration were compared to the responses of the second administration. The reliability of the responses from the two administrations was assessed to determine their reliability. A lab request form was used to collect spot urine samples for urinary iodine concentration determination. A data abstraction form was used to assess the nutritional status of the children. A blood sample was taken via venipuncture from the selected children to determine their micronutrient status. Anthropometric measurements: Height, weight for BMI of the children were

measured and recorded. The anthropometric assessments were used to compare the effect of the supplements before and after the treatment of VL weekly for four weeks and after treatment follow-up for three months.

2.6. Determining Levels of Micronutrients

About 3.5 ml of venous blood was collected. A drop of the blood was used to determine hemoglobin (Hb) by haemocue machine. The rest of the blood was separated by centrifuging and serum was stored in serum tubes in a freezer at −80˚C. Transported to KEMRI in a cooler box at −20˚C where the serum was transferred into a freezer at −80˚C for storage for analysis. The serum was used for the determination of zinc and iron.

2.7. Data Management and Analysis

The data from the questionnaire and lab request forms was coded and entered into the computer for the computation of descriptive statistics. Descriptive statistics such as frequencies, proportions, and means were computed and presented in tables and charts. For continuous data, Kruskal-Walli's test was used to assess the difference between control and intervention groups. For categorical, the Chi-square test was used. A paired t-test was used to detect whether a significant difference exists between the means within each group at a 5% level of significance. To estimate the impact of the treatment, difference-in-difference was used to compare changes in the outcomes (body temperature, spleen size, BMI, splenic aspirin, and PKDL) over time between the intervention and comparison groups.

The effect of the intervention was determined at a 5% significance level from the interaction between study groups (intervention and comparison) and the time (pre- and post-intervention).

Ethics approval and consent to participate

Before commencing the study, voluntary written consents of both parents/guardians and assent of the 5 - 12 year old were sought. The study objectives and components were explained to the respondents in a language they understood. Confidentiality of participants' information was assured.

The study was voluntary and the participants were at liberty to exit at any stage if need be. Permission to carry out the study was sought from KEMRI Scientific and Ethics Review Unit and NACOSTI, County government. Assurance of no risks associated with the study was well explained to the study participants.

3. Results

3.1. Baseline Characteristics of the Children

Table 1 shows the baseline characteristics of the children in the study. The mean age was 8.72 years and 8.72 years in the intervention and comparison groups respectively. Age did not differ significantly by group ($P > 0.05$). Children aged 5 - 9 years were 64.7% in the intervention and comparison groups. Those aged 10 - 12 years were 35.3% in the intervention and comparison groups. The age groups

did not differ by group. There were more males than females in the study (53.9% in the intervention and (52.9% in the comparison group respectively). All the children in the study were from a Christian background, were underweight had enlarged spleen, and were positive for VL by Splenic Aspirate. Those who presented with fever were 88.2% (88% in both intervention and comparison groups). Most children had lower levels of zinc, iron, vitamins A, B12, and D at baseline (54.9%, 91.2%, 54.4%, 57.8%, and 58.8% respectively). The majority (93.1%) were deficient in vitamin C (90.2% in the intervention and 96.1% in the comparison group) at baseline. Only iron levels differed by group at baseline ($P < 0.001$) (Table 1).

Table 1. Baseline characteristics of the study children.

Baseline characteristic	Total	Intervention	Comparison	P-value for the difference*
	(N = 204)	(N = 102)	(N = 102)	
AGE				0.9685
Mean (standard deviation)	8.71 (2.17)	8.71 (2.18)	8.72 (2.18)	
Age group				1
5 - 9 years	132 (64.7%)	66 (64.7%)	66 (64.7%)	
10 - 12 years	72 (35.3%)	36 (35.3%)	36 (35.3%)	
Gender				1
Female	95 (46.6%)	48 (47.1%)	47 (46.1%)	
Male	109 (53.4%)	54 (52.9%)	55 (53.9%)	
Marital status				-
Single	204 (100%)	102 (100%)	102 (100%)	
Religion				-
Christian	204 (100%)	102 (100%)	102 (100%)	
Education				1
Grade 1	30 (14.7%)	15 (14.7%)	15 (14.7%)	
Grade 2	28 (13.7%)	14 (13.7%)	14 (13.7%)	
Grade 3	25 (12.3%)	13 (12.7%)	12 (11.8%)	
Grade 4	33 (16.2%)	16 (15.7%)	17 (16.7%)	
Grade 5	24 (11.8%)	12 (11.8%)	12 (11.8%)	
Grade 6	28 (13.7%)	14 (13.7%)	14 (13.7%)	
Nursery	12 (5.9%)	6 (5.9%)	6 (5.9%)	
Standard 7	24 (11.8%)	12 (11.8%)	12 (11.8%)	
	(N = 204)	(N = 102)	(N = 102)	

Continued

BMI (kg/m²)				0.8803
Mean (Standard Deviation)	13.0 (0.824)	13.0 (0.888)	13.0 (0.758)	
Body Mass Index classification				
Underweight	204 (100%)	102 (100%)	102 (100%)	
Body temp				0.8987
Mean (Standard Deviation)	38.7 (0.916)	38.7 (0.915)	38.7 (0.921)	
Fever				1
No	24 (11.8%)	12 (11.8%)	12 (11.8%)	
Yes	180 (88.2%)	90 (88.2%)	90 (88.2%)	
RK39				-
Negative	0 (0%)	0 (0%)	0 (0%)	
Positive	204 (100%)	102 (100%)	102 (100%)	
Spleen Size (cm)				0.09052
Mean (Standard Deviation)	37.4 (0.849)	37.4 (0.876)	37.5 (0.816)	
SS				-
splenomegaly	204 (100%)	102 (100%)	102 (100%)	
Splenic Aspirate				-
Negative	0 (0%)	0 (0%)	0 (0%)	
Positive	204 (100%)	102 (100%)	102 (100%)	
Zinc				0.2721
Mean (Standard Deviation)	65.5 (14.6)	66.1 (14.7)	64.9 (14.6)	
Zinc levels				0.8881
deficient	92 (45.1%)	45 (44.1%)	47 (46.1%)	
normal	112 (54.9%)	57 (55.9%)	55 (53.9%)	
Iron				P < 0.001
Mean (Standard Deviation)	8.32 (1.73)	8.83 (1.63)	7.80 (1.68)	
Iron levels				0.805
deficient	18 (8.8%)	8 (7.8%)	10 (9.8%)	
normal	186 (91.2%)	94 (92.2%)	92 (90.2%)	
Iodine				0.6034
Mean (Standard Deviation)	62.3 (20.6)	62.8 (20.7)	61.8 (20.6)	
Iodine levels				-

Continued

insufficient	204 (100%)	102 (100%)	102 (100%)	
sufficient	0 (0%)	0 (0%)	0 (0%)	
Vitamin A				0.1608
Mean (Standard Deviation)	0.795 (0.554)	0.874 (0.751)	0.717 (0.200)	
Vitamin A levels				0.09162
deficient	93 (45.6%)	40 (39.2%)	53 (52.0%)	
normal	111 (54.4%)	62 (60.8%)	49 (48.0%)	
Vitamin B12				0.2647
Mean (Standard Deviation)	154 (50.8)	156 (50.4)	152 (51.4)	
Vitamin B12 levels				0.1189
deficient	86 (42.2%)	37 (36.3%)	49 (48.0%)	
normal	118 (57.8%)	65 (63.7%)	53 (52.0%)	
Vitamin C				0.154
Mean (Standard Deviation)	0.163 (0.284)	0.168 (0.285)	0.158 (0.285)	
Vitamin C levels				0.1662
deficient	190 (93.1%)	92 (90.2%)	98 (96.1%)	
normal	14 (6.9%)	10 (9.8%)	4 (3.9%)	
Vitamin D				0.3904
Mean (Standard Deviation)	24.5 (3.69)	24.6 (3.67)	24.3 (3.72)	
Vitamin D levels				0.8869
deficient	84 (41.2%)	41 (40.2%)	43 (42.2%)	
normal	120 (58.8%)	61 (59.8%)	59 (57.8%)	

3.2. Impact of the Nutritional Supplements on the Vitamins Levels Comparing Intervention and Comparison Groups

The average serum vitamin levels were assessed in the treatment period between week 0 to Week 4 comparing intervention and comparison groups. The results show that all the vitamin levels increased significantly in the entire period of study. However, the increase was greater in the intervention group. The P-values show that the vitamin levels differed significantly between the 2 groups. Table 2 shows the trend of Vitamin levels (weeks 0 to 4) Comparing intervention and comparison group.

3.3. Impact of the Nutritional Supplements on the Minerals Levels Comparing Intervention and Comparison Groups

Table 3 shows average mineral levels in the entire period of study (week 0 to week

4). P-values were presented comparing the intervention and the comparison groups. The minerals zinc, Iron and Iodine levels increased significantly throughout the study period.

The results in the table below show a greater increase in the intervention group than in the comparison group. Zinc mean levels differed by 15.1 ug/dL and 0.3 ug/dL in the intervention and comparison groups respectively. Iron levels differed by 4.17 g/dL in the intervention group and 0.33 g/dL in the comparison group. Iodine mean urine levels differed by 90.1 mcg/L in the intervention group and 0.39 mcg/L in the comparison group.

Table 2. Results of the impact of the nutritional supplements on the levels of Vitamins comparing intervention and comparison groups.

Week	0		1		2		3		4	
vitamins	Intervention (N = 102)	Comparison (N = 102)	Intervention (N = 102)	Comparison (N = 102)	Intervention (N = 102)	Comparison (N = 102)	Intervention (N = 102)	Comparison (N = 102)	Intervention (N = 102)	Comparison (N = 102)
Vitamin A										
Mean (Standard Deviation)	0.874 (0.751)	0.717 (0.200)	0.931 (0.159)	0.758 (0.203)	0.881 (0.197)	0.826 (0.203)	0.896 (0.195)	0.892 (0.205)	0.937 (0.154)	0.987 (0.247)
Vitamin B12										
Mean (Standard Deviation)	156 (50.4)	152 (51.4)	316 (158)	155 (51.5)	312 (158)	161 (51.3)	313 (158)	170 (52.1)	319 (157)	177 (51.5)
Vitamin C										
Mean (Standard Deviation)	0.168 (0.285)	0.158 (0.285)	2.16 (1.73)	0.198 (0.281)	0.972 (0.520)	0.261 (0.282)	1.10 (0.553)	0.336 (0.285)	1.28 (0.580)	0.419 (0.285)
Vitamin D										
Mean (Standard Deviation)	24.6 (3.67)	24.3 (3.72)	39.9 (6.11)	24.7 (3.74)	88.3 (17.2)	25.4 (3.72)	89.4 (17.2)	26.0 (3.72)	95.8 (17.7)	26.8 (3.68)

Table 3. Results of the levels of Minerals comparing intervention and comparison groups.

Mineral/Vitamin	Intervention (N = 102)				Comparison (N = 102)			
	Pre-treatment (week 0)	Post-treatment (week 4)	Difference (week 4 - week 0)	P-value	Pre-treatment (week 0)	Post-treatment (week 4)	Difference (week 4 - week 0)	P-value
ZN								
Mean	66.1	81.2	15.1	P < 0.001	64.92	65.22	0.3	P < 0.001
FE								
Mean	8.83	13.0	4.17	P < 0.001	7.80	8.13	0.33	P < 0.001
IODINE								
Mean	62.8	152.9	90.1	P < 0.001	61.82	62.21	0.39	P < 0.001

3.4. Impact of the Intervention on the Treatment Outcomes (Clinical Characteristics) of Visceral Leishmaniasis

In Table 4, changes in the clinical characteristics were assessed between weeks 0 and 4. P-values were presented for comparison of the differences in the characteristics between the 2 groups. The clinical characteristics assessed were body temperature, spleen size, and splenic aspirate test. The clinical characteristics differed significantly between the 2 groups. The results in Table 4 below show that, the mean body temperature was 36.7˚C and 38.7˚C in the intervention and comparison groups respectively. Further, the classification of the body temperature shows that all the 102 participants in the intervention group had no fever as compared to the 90 participants in the comparison group who had fever at the end of the intervention. The table results also show that the mean spleen size was 35.7 cm and 36.6 cm in the intervention and comparison groups respectively.

The results of the splenic aspirate test show that out of the 204 participants in the study 163 tested negative for visceral leishmaniasis at the end of the intervention 102 in the intervention group and 61 in the comparison group.

Table 4. Results of the impact of the nutritional supplements on the treatment outcomes (clinical characteristics) comparing intervention and comparison group.

Clinical characteristic	Overall (N = 204)	Intervention (N = 102)	Comparison (N = 102)	P-value*
Temperature				
Mean (Standard Deviation)	**37.7 (1.49)**	36.7 (1.20)	38.7 (0.915)	P < 0.001
Fever				P < 0.001
No	**114 (46.4%)**	102 (100%)	12 (11.8%)	
Yes	**90 (53.6%)**	0 (0.0%)	90(88.2%)	
Spleen Size				P < 0.001
Mean (Standard Deviation)	**36.2 (1.40)**	35.7 (1.49)	36.6 (1.15)	
Splenic Aspirate				0.01849
Negative	**163 (80.0%)**	102 (100%)	61 (60.0%)	
Positive	**41 (20.0%)**	0 (0.0%)	41 (40.0%)	

3.5. Assessing the Effect of the Intervention

To estimate the impact of the intervention, a DID regression model was fitted using the R function to compare the change in the treatment outcomes between intervention and comparison groups for the periods pre- and post-intervention. The effect of the intervention was determined at 5% significance level from the

interaction term between study groups (intervention and comparison) and the time (pre- and post-intervention).

The results in Table 5 below, show that impact of providing the minerals; zinc (Zn), Iron, and Iodine is significant at the 5% level, indicating that the intervention has a favorable effect. The minerals supplementation increased their levels in the body improving the nutritional status of the study participants. The outcome of vitamins; B12, C, and D administration is significant at the 5% level, indicating that the intervention has a positive effect. On average, administering the nutritional supplement increased the micronutrients levels in the body to acceptable limits and further improved the nutritional status of the study participants as was seen in the increase of BMI. Body temperature decreased significantly by 3.2 and spleen size reduced by 1.3 cm.

Table 5. Assessing the effect of the intervention.

Mineral/Vitamin	DID value	P-value
Zinc (ug/dL)	14.8	P < 0.001
Iron (g/dL)	3.84	P < 0.001
Iodine (mcg/L)	89.71	P < 0.001
Vitamin A (μmol/L)	2.17	P < 0.001
Vitamin B12 (pmol/L)	138.1	P < 0.001
Vitamin C (mg/dL)	0.85	P < 0.001
Vitamin D (nmol/L)	68.76	P < 0.001
Body Mass Index (BMI)	2.72	P < 0.001
Body Temperature	−3.2	P < 0.001
Spleen size	−1.3	P < 0.001

Impact of Follow-Up on the Likelihood of Reinfection of Recurrence of Visceral Leishmaniasis

The impact of follow-up on the likelihood of reinfection and recurrence of vicseral leishmaniasis after the intervention was assessed among the study participants after intervention comparing the intervention and comparison groups. The reinfection and the recurrence of viceral leishmaniasis was confirmed by presence of any signs and symptoms of Post-Kala-azar Dermal Leishmaniasis (PKDL). The results of the 3-months follow-up showed that there was no presence of PKDL in the intervention group which is usually an indication of recurrence and relapse of visceral leishmaniais. However, in the comparison group, 2 children (2%) turned positive for viceral leishmaniasis (Table 6).

Table 6. PKDL changes during follow-up period to show recurrence/relapse of VL.

	Overall (N = 204)	Intervention (N = 102)	Comparison (N = 102)	P-value*
PKDL				0.4773
Positive	2 (2.0%)	0 (0%)	2 (2.0%)	
Negative	202 (98.0%)	102 (100%)	100 (98.0%)	

PKDL—Post-Kalazar Dermal leishmaniasis; VL—visceral leishmaniasis.

4. Discussion

The aim of this study was to determine the impact of nutritional supplementation on the nutritional status and treatment outcomes of visceral leishmaniasis among children aged 5 - 12 years in Baringo and West Pokot Counties, in Kenya. The results of the study revealed that the nutritional supplements improved the nutritional status of the study participants as was seen by the increase of in Body Mass Index, increased in the levels of vitamins and increased levels of minerals. These improvement of the nutritional status was more in the intervention group than in the comparison group.

On assessing the impact of the supplements on the treatment outcome of viceral leishmaniasis, clinical characteristics of visceral leishmaniais were assessed. The study findings showed that there were significant changes in the body temperature, spleen size and splenic aspirate test. The characteristic observed were body temperature, spleen size, and splenic aspirate test. The results revealed that the body temperature dropped significantly between week 0 to week 4. This control of the body temperarture was greater in the intervention group than the comparison group, All the 102 study participants in the intervention group had a reduction in the spleen size as compared to the comparison group. Also, all the 102 participants in the intervention group tested negative for viceral leishmaniais as compared to the comparison group which had 41 participants who tested positive for visceral leishmaniais at the end of the intervention.

To estimate the impact of the intervention, a DID regression model was fitted using the R function to compare changes in the outcomes of body temperature, spleen size, BMI, splenic aspirate and any signs of PKDL over time between the intervention and comparison groups. The results showed that body temperatures decreased significantly by 3.2, Spleen size decreased significantly by 1.3, BMI had an increase of 15.2 kg/m^2 in the intervention group and 13.6 in the comparison group. All vitamins, and all minerals had a significant change (positive increase) between pre- and post-treatment periods. Splenic aspirate results showed that at the end of intervention, all 102 (100%) participants in the intervention group turned negative for VL compared to 71 (70%) participants who turned negative and 31 (30%) who turned Positive for VL in the comparison group. The study findings also showed that intervention prevented the likelihood of recurrence and reinfection of VL as indicated by the percentage of VL reinfection and recurrence

which was 0% in the intervention group as compared to 15.7.% and 31% respectively among VL participants in the comparison group. There was also no recurrence of VL in the intervention group at the month 3 follow-up period. In the comparison group, 2 children (2%) turned positive for PKDL.

The findings also agree with previous researchers' outputs (Mashayekhi Goyonlo V, 2020) indicating that administering the anti-leishmaniasis treatment alone will not yield a favorable treatment outcome in visceral leishmaniasis patients and a study by Nweze JA, Nweze EI (2019), malnourished patients with VL had an unfavorable outcome, which was recurrence in 21.4% and also that serum micronutrients levels favor good treatment outcome in visceral leishmaniasis.

5. Conclusions

The association between the intervention and the nutritional status of the study participants had a statistical significance as seen in the increase in BMI, vitamins, and minerals greater in the intervention than in the comparison group. The p-value of 0.001 was less than the level of significance at alpha (α) equal to 0.05. This is a sufficient conclusion that the nutritional supplements increased the levels of the vitamins, minerals, and BMI.

The intervention had a statistical significance in the treatment outcomes as was seen in the control of body temperature, BMI increase, and spleen size reduction more in the intervention than in the comparison group at alpha (α) equal to 0.05 ($P = 0.001$, $P < 0.05$).

The intervention accelerated the recovery of the study participants by accelerating the control of the body temperature from high fever to normal body temperature below 37.50 C which was below the acceptable limits. This is a statistical significance between the intervention and the treatment outcome variables.

The intervention reduced the likelihood of reinfection and recurrence, as shown by the results that all the 102 study participants in the intervention group had no signs and symptoms of VL including the PKDL, at the end of treatment and month 6 follow-up, whereas in the comparison group 2 (2.0%) study participants turned positive for VL.

6. Recommendations

The study recommends educating the Counties in this study on the use of the available nutrients in the locally available foods that contain the micronutrients required by the VL patients. The study also recommends the strategy for the VL elimination Programme in endemic areas through early diagnosis & complete treatment, Integrated Vector Management including Indoor residual spraying (IRS) Advocacy, Communication for Behavioural Impact and Inter-sectoral convergence, Capacity Building, Supervision, Monitoring and Evaluation.

This study recommends the integration of nutritional supplements to all visceral leishmaniasis patients as a routine to hasten recovery and prevent recurrence and re-infection of VL.

The study recommends the surveillance of PKDL to prevent recurrence of VL as PKDL cases serve as a reservoir for disease transmission during the inter-epidemic period and also because treatment of PKDL is prolonged. Also, the recurrence of VL may cause the spread of drug resistance, especially in anthroponotic leishmaniasis settings. Research should focus on the use of combination therapy in these patients to reduce the number of recurrences, prevent resistance, and reduce toxicity, avoiding the use of highly cardiotoxic pentavalent antimonials.

Acknowledgements

The authors would like to thank the study participants and the health care facilities, the research and data team for the whole process of this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] World Health Organization (2022) Leishmaniasis Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis

[2] Malafaia, G. (2009) Protein-Energy Malnutrition as a Risk Factor for Visceral Leishmaniasis: A Review. *ParasiteImmunology*, **31**, 587-596. https://doi.org/10.1111/j.1365-3024.2009.01117.x

[3] Ngere, I., Gufu Boru, W., Isack, A., Muiruri, J., Obonyo, M., Matendechero, S., *et al.* (2020) Burden and Risk Factors of Cutaneous Leishmaniasis in a Peri-Urban Settlement in Kenya, 2016. *PLoS One*, **15**, e0227697. https://doi.org/10.1371/journal.pone.0227697

[4] (2021) The Strategic Plan for Control of Leishmaniasis 2021-2025. Republic of Kenya Ministry of Health.

[5] Ali, A. and Ashford, R.W. (1994) Visceral Leishmaniasis in Ethiopia. IV. Prevalence, Incidence and the Relation of Infection to Disease in an Endemic Area. *Annals of Tropical Medicine and Parasitology*, 88, 289-293. https://doi.org/10.1080/00034983.1994.11812869

[6] Grifferty, G., Shirley, H., O'Brien, K., Hirsch, J.L., Orriols, A.M., Amechi, K.L., *et al.* (2023) The Leishmaniases in Kenya: A Scoping Review. *PLOS Neglected Tropical Diseases*, **17**, e0011358. https://doi.org/10.1371/journal.pntd.0011358

[7] Eshetu, B. and Mamo, H. (2020) Cutaneous Leishmaniasis in North-Central Ethiopia: Trend, Clinical Forms, Geographic Distribution, and Determinants. *Tropical Medicine and Health*, **48**, Article No. 39. https://doi.org/10.1186/s41182-020-00231-w

Appendix

Study Questionnaire

Association of nutritional supplementation with nutritional status and treatment outcomes of visceral leishmaniasis among children aged 5 - 12 years in Baringo and West Pokot Counties in Kenya.

Questionnaire Number…..……..…

Name of the researcher…….. Date……..

SECTION A: SOCIAL DEMOGRAPHIC CHARACTERISTICS

Socio-Demographic Characteristics

1) Child's age

5 – 9 years ( )

10 – 12 years ( )

2) Gender

Male ( )

Female ( )

3) Marital status

Married ( )

Not Married ( )

4. Level of education.

a) Grade 1 - 3 ( ) b) Grade 4 - 6 ( ) c) Nursery ( )

d) Standard 7

5) Religion.

a) Christian ( ) b) Muslim ( ) c) Traditionalist ( )

d) No religious affiliation ( )

SECTION B: NUTRITIONAL STATUS

1) Anthropometric information of the child.

Gender (M/F): _____

Age (years): _____

Current weight: _____kg

Height: _____cm

BMI: _____kg/m^2

2) Vitamins test results:

A_____

B12_____

C_____

D_____

3) Minerals test results:

Iron_____

Zinc _____

Calcium. _____

SECTION C: Data Abstraction Form

Clinical characteristics Assessments: Date……..

Date/Visit	Body Temperature	Spleen size	Weight	Height	BMI	PKDL	Others e.g. jaundice

LABORATORY TESTS

i) Hemoglobin concentration g/dl

a) Week 1: ……

b) Week 2: ……

c) Week 3: ……

d) Week 4:……..

ii) Splenic Aspirate:

e) Week 1: ……

f) Week 4……..

Time interview ends……..