Micronutrient levels of visceral leishmaniasis patients, a longitudinal study.

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Research article

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Abstract

Background: Micronutrients are minerals and vitamins required in small amounts, and they are essential for the normal physiological activities. The objectives of the study were to describe the progress and determinants of micronutrients level and to assess the effect of micronutrients in the treatment outcomes.

Methods: A prospective cohort study was implemented. The data were collected using interviews, measuring anthropometric indicators, collecting blood, urine and stool samples from each patient. The blood samples were collected five times: before starting anti-leishmaniasis treatments, in the first week, in the second week, in the third week and in the 4th week of anti-leishmaniasis treatments. Descriptive statistics were used to describe the profile of patients and to compare the treatment success rate of Kalazar patients. The generalized estimating equation was used to identify the determinants of serum micronutrients.

Results: The serum zinc level of Kalazar patients was affected by alcohol, DDS, family size, HIV, and sex. The serum iron level of kalazar patients was affected by alcohol, family size, malaria, hookworm, chronic disease, and HIV. The serum selenium level of kalazar patients was affected by HIV and family size. The iodine level of kalazar patients was affected by HIV, DDS, smoking, chronic illness, and regular physical exercise. The serum vitamin D level of kalazar patients was affected by HIV, alcohol, chronic illness, DDS, malaria, family size, age, residence, and MUAC. The serum vitamin D level of kalazar patients was affected by BMI, DDS, malaria, hookworm, family size, HIV, and age.

Conclusion: The Micronutrient levels of Kalazar patients were significantly low. Anti-leishmaniasis treatment did not increase the serum micronutrient levels of the patients.

Background

Leishmaniasis is a group of vector born diseases caused by the Leishmania species. The three types of leishmaniasis are; cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis [1]. Visceral leishmaniasis is the severest form of leishmaniasis affecting 90, 000 people globally [2, 3]. Visceral leishmaniasis was reported from Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan [4-6]. In the Amhara region, 5,000 incident visceral leishmaniasis patients were reported annually [7, 8].

Micronutrients are minerals and vitamins required in a small amount, they are essential for the normal physiological activities. They serve as co-factors for many important metabolic enzymes. They enhance the function of the immune system and regulate gene transcription [9]. Disease conditions especially inflammatory disease significantly decreases the micronutrient level [10]. Visceral leishmaniasis is among the disease conditions that decrease the patient’s micronutrient levels [11, 12]. The serum micronutrient concentration can be affected by chronic diseases, BMI, smoking, DDS (dietary diversification score), physical activity, intestinal parasites, MUAC (mid upper arm circumference), HIV, and ethnicity [13-26].
The impacts of visceral leishmaniasis and micronutrient deficiency were not limited to the patients and their families; it also affects the socio-economic development of the nations [27-29]. Evidence on micronutrient levels of kalazar patients was scarce and this research work conducted to answer the following objectives

- Describe the progress and determinants of micronutrient levels of visceral leishmaniasis patients.
- To assess the effects of micronutrient levels on the treatment outcomes.

**Methods**

A prospective cohort study design was implemented among visceral leishmaniasis patients taking their treatment in the Amhara national regional state leishmaniasis treatment centers. The five leishmaniasis treatment centers of the Amhara region are Felegehiwote referral hospital, Gondar University hospital, Metema hospital, Addis Zemen health center, and Abderraafi health center. From these treatment centers, more than 5,000 incident visceral leishmaniasis patients were reported annually[7, 8]. The data were collected from August 2016 - May 2019. The target population for this study was adult visceral leishmaniasis patients receiving their treatments from the five treatment centers of the Amhara regional state. Visceral leishmaniasis patients with incomplete records were excluded from the study. The sample size was calculated using Epi-info software with the assumption of a 95 % confidence interval (5 % level of significance), power of 90 %, 1:2 ratio of HIV positive kalazar patients to HIV negative kalazar patients, risk ratio of 1.2 and 15 % loss to follow up rate; finally giving 465 HIV positive kalazar patients and 930 HIV negative kalazar patients. A systematic random sampling technique was used to select both HIV positive and HIV negative visceral leishmaniasis patients. The data were collected using interviews, measuring anthropometric indicators, collecting blood, urine and stool samples from each patient. The interview and anthropometric indicators were collected by clinical nurses. Stool sample was collected to adjust for hookworm, which significantly affects the serum iron level. From each visceral leishmaniasis patient, one gram stool sample was collected in 10 ml SAF (sodium acetate-acetic acid-formalin solution). To see the presence of intestinal parasitic infection using concentration technique [30]. The blood samples were collected at five repeated times in a month: before starting anti-leishmaniasis treatments, in the first week, in the second week, in the third week and in the 4th week of anti-leishmaniasis treatments. At each spot, five-milliliter blood samples were collected from each visceral leishmaniasis patient following standard operating procedures to measure the serum zinc, iron, vitamin A, vitamin D, and selenium level. Urine samples were collected simultaneously with blood samples to measure their iodine levels. High-performance liquid chromatography was used to measure the serum vitamin A level of the patient [31], atomic absorption spectrophotometer was used to measure the serum zinc and selenium level [32, 33], Serum iron level was measured using cobas6000 (Roche kits German) instruments (Set 2015; analytics 2014), mini Vetik Immune Diagnostic Assay System (VIDAS) machine was used to measure the serum vitamin D level and urine iodine level was measured using the Sandell Kolthoff reaction. Vitamin A, iron, iodine, zinc were measured using microgram per deciliter (mcg/dl); selenium was measured using Nanogram per deciliter (ng/dl), and vitamin d was measured using
Nanogram per milliliter (ng/ml). Strict quality control measures were implemented during each laboratory procedure. CAGE tool was used to detect problematic alcohol use [34], Dietary diversification score (DDS) was measured using the world health organization (WHO) recommendations [35]. Data were entered into the computer using Epi-info software[36] and transported to SPSS for the analysis[37]. Descriptive statistics were used to describe the profile of patients and to compare the treatment success rate of visceral leishmaniasis patients. Generalized estimating equations (GEE, Autoregressive correlation matrix), with linear regression was used to identify the determinants of serum micronutrients in visceral leishmaniasis patients[38].

Ethical clearance was obtained from Bahir Dar University College of medicine and health sciences ethical review committee. Permission was obtained from the Amhara national regional state health bureau and each treatment center. Written informed consent was obtained from each leishmaniasis patient before recruitment. Visceral leishmaniasis patients with abnormal laboratory findings were referred to the curative care segment of the hospital. The confidentiality of the data was kept at each level. Study participant right to withdraw from the research was respected at any point.

**Results**

A total of 1309 visceral leishmaniasis patients was followed giving for the response rate of 94 %, 32 patients were not volunteer to participate, the medical records of 31 VL (visceral leishmaniasis) patients were incomplete and 23 patients have died. Most of the study participants were included from Gondar university hospital (305), followed by Felegehiwote referral hospital (298), Metema hospital (268), Addis zemen health center (224), and Abdereli health center (214). The mean age of study participants was 32.88 years [SD (standard deviation) ±15.95 year]. Male constitute 62.3 % of study participants and problematic alcohol use was observed in 11.5 % of the patients (Table 1).

| Table 1: Population profile of the study participants (n=1309) |
| Serial number | Variables                          | Frequency | Percentage |
|---------------|------------------------------------|-----------|------------|
| 1.            | Sex                                | Male      | 816        | 62.3       |
|               |                                    | Female    | 493        | 37.7       |
| 2.            | Resident                           | Rural     | 838        | 64         |
|               |                                    | Urban     | 471        | 36         |
| 3.            | Dietary diversification score      | ≥6        | 253        | 19.3       |
|               |                                    | 3-5       | 332        | 25.4       |
|               |                                    | 0-2       | 724        | 55.3       |
| 4.            | Problematic alcohol use            | Present   | 150        | 11.5       |
|               |                                    | Absent    | 1159       | 88.5       |
| 5.            | Family size                        | >4        | 669        | 51.1       |
|               |                                    | ≤4        | 640        | 48.9       |
| 6.            | Other chronic illness              | Present   | 150        | 11.5       |
|               |                                    | Absent    | 1159       | 88.5       |
| 7.            | Smoking                            | Yes       | 187        | 14.3       |
|               |                                    | No        | 1122       | 85.7       |
| 8.            | Malaria co-infection               | Present   | 831        | 63.5       |
|               |                                    | Absent    | 478        | 36.5       |
| 9.            | HIV                                | Positive  | 420        | 32.1       |
|               |                                    | Negative  | 889        | 67.9       |
| 10.           | Hookworm                           | Infected  | 530        | 40.5       |
|               |                                    | Not infected | 779     | 59.5       |
| 11.           | Body mass index                    | <18.5     | 1100       | 84         |
|               |                                    | ≥18.5     | 209        | 16         |
Table 2: Predictors of micronutrient level in visceral leishmaniasis patients (n=1309).
| Dependent Variables | Predictors                          | β [95% CI β]          | P-value |
|---------------------|-------------------------------------|-----------------------|---------|
| Serum zinc level    | Problematic alcohol use             | -2.7 [-4.01 - -1.5]   | < 0.01  |
|                     | Female sex                          | -1.28 [-2.5 - -0.07]  | 0.04    |
|                     | DDS                                 | 9.75 [7.71 – 11.79]   | <0.01   |
|                     | High family size                    | -1.63 [-2.68 - -0.58] | <0.01   |
|                     | HIV                                 | -2.95 [-4.97 - -0.92] | < 0.01  |
|                     | Age                                 | -0.043 [-0.08 - -0.01]| 0.01    |
|                     | Anti-leishmaniasis treatments       | 0.09 [-0.3 – 0.48]    | 0.66    |
| Iron                | alcohol                             | 7.6 [5.86 – 9.35]     | < 0.01  |
|                     | Chronic diseases                    | -7.44 [-9.75 - -5.13] | 0.01    |
|                     | Malaria                             | -12.69 [-14.53 - -10.87] | < 0.01 |
|                     | Hookworm                            | -4.48 [-6.82 - -2.14] | < 0.01  |
|                     | High family size                    | -5.14 [-7.01 - -3.28] | <0.01   |
|                     | HIV                                 | -5.51 [-8.23 - -2.78] | < 0.01  |
|                     | Age                                 | 0.11 [0.07 - 0.15]    | <0.01   |
|                     | MUAC                                | 0.75 [0.21 -1.29]     | < 0.01  |
|                     | Anti-leishmaniasis treatments       | 0.67 [0.08 – 1.27]    | 0.02    |
| Serum Selenium      | HIV                                 | -18.1 [-20.63 - -15.58] | <0.01 |
|                     | High family size                    | -11.36 [-13.02 - -9.7] | <0.01  |
|                     | Anti-leishmaniasis treatments       | 3.04 [2.32 – 3.76]    | <0.01   |
| Iodine              | Malaria                             | -3.78 [-6.16 - -1.39] | <0.01   |
|                     | DDS                                 | 25.84 [22.57 – 29.1]  | <0.01   |
|                     | Smoking                             | -12.34 [-15.98 - -8.7] | <0.01  |
|                     | HIV                                 | -38.02 [-41.98 - -34.06] | <0.01 |
|                     | Chronic illness                     | -5.14 [-7.82 - -2.46] | <0.01   |
|                     | Regular physical exercise           | 5.82 [0.39 - 11.26]   | 0.04    |
|                     | Anti-leishmaniasis                 | 13.67 [13.15 – 14.2]  | <0.01   |

Page 7/20
| Vitamin A                          | Problematic alcohol use | -1.09 [-2.01 - -0.17] | 0.02 |
|-----------------------------------|-------------------------|-----------------------|------|
| Chronic illness                   | -2.56 [-3.53 - -1.59]   | <0.01                 |      |
| Urban residence                   | 0.81 [0.08 - 1.54]      | 0.03                  |      |
| DDS                               | 1.62 [0.36 - 2.88]      | 0.01                  |      |
| Malaria                           | -4.8 [-5.91 - -3.85]    | <0.01                 |      |
| High family size                  | -5.03 [-5.83 - -4.22]   | <0.01                 |      |
| HIV                               | -2.89 [-4.44 - -1.34]   | <0.01                 |      |
| Age                               | 0.09 [0.07 – 0.12]      | <0.01                 |      |
| MUAC                              | 0.86 [0.52 – 1.21]      | <0.01                 |      |
| Anti-leishmaniasis treatments     | -0.3 [-0.62 - -0.17]    | 0.06                  |      |

| Vitamin D                          | BMI                      | 1.52 [0.42 – 2.6]      | <0.01|
|-----------------------------------|--------------------------|-----------------------|------|
| DDS                               | 16.24 [14.89 – 17.58]    | <0.01                 |      |
| Malaria                           | -0.61 [-3.37 - -3.37]    | <0.01                 |      |
| Hookworm                          | -3.94 [-5.46 - -2.41]    | <0.01                 |      |
| High family size                  | -1.15 [-2.03 - -0.28]    | <0.01                 |      |
| HIV                               | -9.43 [-10.92 - -7.94]   | <0.01                 |      |
| Age                               | 0.03[0.001 - 0.06]       | 0.04                  |      |
| Anti-leishmaniasis treatments     | -0.94 [-1.25 - -0.63]    | <0.01                 |      |

**Interpretations**

Problematic alcohol use decreases the serum zinc level by 2.7 micrograms per deciliter (mcg/dl). The serum zinc level of females was 1.28 mcg/dl less than males. High dietary diversification increases the serum zinc level of leishmaniasis patients by 9.75 mcg/dl. High family size decreases the serum zinc level by 1.63 mcg/dl. The serum zinc level of HIV positive visceral leishmaniasis patients was 2.95 mcg/dl less than HIV negative visceral leishmaniasis patients. Anti-leishmaniasis treatment did not increase the serum zinc level of the patients.
Alcohol increases the serum iron level of kalaazar patients by 7.6 mcg/dl. Chronic illness decreases the serum iron level of kalaazar patients by 7.44 mcg/dl. Malaria co-infection decreases the serum iron level of visceral leishmaniasis patients by 12.69 mcg/dl. Hookworm infection decreases the serum iron level of kalaazar patients by 4.48 mcg/dl. High family size decreases the serum iron level of kalaazar patients by 5.14 mcg/dl. HIV positive visceral leishmaniasis patients had 5.54 mcg/dl less serum iron level than HIV negative kalaazar patients. Per a year increase in the age of the patient, the serum iron level increases by 0.11 mcg/dl. Per one centimeter increase on the MUAC of the patient, the serum iron level increases by 0.75 mcg/dl. Anti-leishmaniasis treatment increases the serum iron level of the patient by 0.67 mcg/dl.

HIV positive visceral leishmaniasis patient had 18.1 ng/dl less serum selenium level than HIV negative kalaazar patients. High family size decreases the serum selenium level of kalaazar patients by 11.36 ng/dl. Anti-leishmaniasis treatment increases the serum selenium level by 3.04 ng/dl.

Malaria decreases the iodine level of visceral leishmaniasis patients by 3.78 mcg/dl. High DDS increases the iodine level of kalaazar patients by 25.84 mcg/dl. Smoking decreases the iodine level of patients by 12.34mcg/dl. HIV decreases the iodine level of kalaazar patients by 38.02 mcg/dl. Chronic illness decreases the iodine level of visceral leishmaniasis patients by 5.14 mcg/dl. Anti-leishmaniasis treatment increases the iodine level of patients by 13.67 mcg/dl.

Problematic alcohol use decreases the serum vitamin A level of visceral leishmaniasis patients by 1.09 mcg/dl. Chronic illness decreases the serum vitamin A level of kalaazar patients by 2.56 mcg/dl. Leishmaniasis patients in the urban area had 0.81 mcg/dl higher serum vitamin A level than the rural patients. High DDS increases serum vitamin A level by 1.62 mcg/dl. Malaria co-infection decreases the serum vitamin A level by 4.8 mcg/dl. High family size decreases the serum vitamin A of visceral leishmaniasis patients by 5.03mcg/dl. HIV infection decreases the serum vitamin A level of visceral leishmaniasis patients by 2.89 mcg/dl. A centimeter increase in the MUAC of visceral leishmaniasis patients increases the serum vitamin A level by 0.86 mcg/dl. Anti-leishmaniasis treatment did not increase the serum vitamin A level of the patient.

A unit increase in the BMI of visceral leishmaniasis patients increases the serum vitamin D level by 1.52 ng/ml. High DDS increases the serum vitamin D level of kalaazar patients by 16.24 ng/ml. Malaria decreases the serum vitamin D level of visceral leishmaniasis patients by 0.61 ng/ml. In the presence of hookworm infection, the serum vitamin D level of kalaazar patients decreased by 3.94 ng/ml. High family size decreases the serum Vitamin D level of visceral leishmaniasis patients by 1.15 ng/ml. HIV co-infection decreases the serum vitamin D level by 9.43 ng/ml. Anti-leishmaniasis treatment did not increase the serum vitamin D level of kalaazar patients (Table 2).

The micronutrient level directly affects treatment outcomes of visceral leishmaniasis; especially the treatment outcome was not successful if the serum zinc, iron, vitamin A and vitamin D levels were lower than their first quartile. The overall treatment success rate of visceral leishmaniasis treatment was 84.7 % [95 % CI: 82.77 % - 86.67 %] (Table 3, Table 4).
Table 3: Micronutrient level versus treatment outcome

| Micronutrient       | Treatment outcome |       |       |       |       |       |       |       |
|---------------------|-------------------|-------|-------|-------|-------|-------|-------|-------|
|                     | Successful        | Frequency | Percentage | Not successful | Frequency | Percentage |
| Zinc (mcg/dl)       | ≤58               | 211   | 16.1  | 135   | 10.3  |
|                     | 59-98             | 387   | 29.6  | 36    | 2.8   |
|                     | >99               | 511   | 39    | 29    | 2.2   |
| Iodine (mcg/dl)     | ≤113              | 224   | 17.1  | 109   | 8.3   |
|                     | 114-147           | 247   | 18.9  | 72    | 5.5   |
|                     | ≥148              | 638   | 48.7  | 19    | 1.5   |
| Iron (mcg/dl)       | ≤46               | 321   | 24.5  | 200   | 15.3  |
|                     | 47-48             | 146   | 11.2  | 0     | 0     |
|                     | ≥49               | 642   | 49    | 0     | 0     |
| Selenium (ng/dl)    | ≤84               | 264   | 20.2  | 68    | 5.2   |
|                     | 85-105            | 245   | 18.7  | 71    | 5.4   |
|                     | ≥106              | 600   | 45.8  | 61    | 4.7   |
| Vitamin A (mcg/dl)  | ≤16               | 233   | 17.8  | 136   | 10.4  |
|                     | 17-31             | 209   | 16    | 56    | 4.3   |
|                     | ≥32               | 667   | 51    | 8     | 0.6   |
| Vitamin D (ng/ml)   | ≤15               | 281   | 21.5  | 112   | 8.6   |
|                     | 16-27             | 219   | 16.7  | 27    | 2.1   |
|                     | ≥28               | 609   | 46.5  | 61    | 4.7   |
Table 4: The levels of micronutrients at each week of anti-leishmaniasis treatments

| Micronutrients      | Before treatments | At 1<sup>st</sup> week | At 2<sup>nd</sup> week | At 3<sup>rd</sup> week | At 4<sup>th</sup> week |
|---------------------|-------------------|------------------------|------------------------|------------------------|------------------------|
|                     | Mean          | SD         | Mean          | SD         | Mean          | SD         | Mean          | SD         | Mean          | SD         |
| Zinc (mcg/dl)       | 86.43         | 25.80      | 91.31         | 18.98      | 98.37         | 11.28      | 93.94         | 17.85      | 85.56         | 23.69      |
| Iodine (mcg/dl)     | 85.21         | 43.81      | 86.71         | 27.49      | 94.63         | 24.85      | 110.98        | 28.75      | 141.44        | 35.52      |
| Iron (mcg/dl)       | 63.61         | 38.10      | 58.61         | 26.04      | 53.92         | 22.37      | 58.72         | 22.80      | 66.92         | 35.35      |
| Selenium (ng/dl)    | 92.00         | 49.30      | 93.46         | 32.83      | 101.76        | 29.64      | 101.61        | 27.08      | 103.14        | 27.39      |
| Vitamin A (mcg/dl)  | 35.45         | 21.73      | 38.44         | 17.19      | 32.89         | 19.81      | 36.35         | 26.26      | 34.99         | 18.70      |
| Vitamin D (ng/ml)   | 34.84         | 19.49      | 32.55         | 17.39      | 33.86         | 21.72      | 31.30         | 16.31      | 30.79         | 17.87      |
Discussion

Problematic alcohol use decreases the serum zinc level by 2.7 mcg/dl and the serum vitamin A level by 1.09 mcg/dl. This finding was in line with previous scholars results [39, 40]. This is because alcohol interferes with the absorption and metabolism of zinc [41]. However, alcohol increases the serum iron level of kalazar patients by 7.6 mcg/dl. This is due to the fact that alcohol increases the absorption of iron from the intestine [42].

High dietary diversification score increases serum zinc level of leishmaniasis patients by 9.75 mcg/dl, the iodine level by 25.84 mcg/dl, the serum vitamin D level by 16.24 ng/ml, and the serum vitamin A by 1.62 mcg/dl. This finding agrees with previous findings [43]. This is due to the reason that, high dietary diversification score increases access to enough quality and quantity of micronutrients [44].

High family size decreases the serum zinc level by 1.63 mcg/dl, the serum iron level by 5.14 mcg/dl, the serum zinc level by 11.36 ng/dl, the serum vitamin A by 5.03mcg/dl, the serum vitamin D level of visceral leishmaniasis patients by 1.15 ng/ml. This finding was in line with previous researches [45-47]. This is due to the sharing of the limited micronutrient-rich foods to the unbalanced household numbers [48].

The serum zinc level of HIV positive visceral leishmaniasis patient was 2.95 mcg/dl less than HIV negative visceral leishmaniasis patients, HIV positive visceral leishmaniasis patients had 5.54 mcg/dl less serum iron level than HIV negative kalazar patients, HIV positive visceral leishmaniasis patient had 18.1 ng/dl less serum selenium level than HIV negative kalazar patients, HIV decreases the iodine level of kalazar patients by 38.02 mcg/dl, the serum vitamin A level of visceral leishmaniasis patients by 2.89 mcg/dl, the serum vitamin D level by 9.43 ng/ml. This finding agrees with previous research findings [21, 49]. This is due to the reason that HIV infection reduced the intake of food and absorption and increased utilization and loss of micronutrients [50].

Chronic illness decreases the serum iron level of kalazar patients by 7.44 mcg/dl, the iodine level by 5.14 mcg/dl and the serum vitamin A level by 2.56 mcg/dl. This finding agrees with the 2019 published research finding [51]. This is because the homeostasis of micronutrients especially iron will be disturbed by chronic illnesses [52].

Malaria co-infection decreases the serum iron level of visceral leishmaniasis patients by 12.69 mcg/dl, the iodine level by 3.78 mcg/dl, the serum vitamin A level by 4.8 mcg/dl and the serum vitamin D level by 0.61 ng/ml. This finding was supported by other research results [53-55]. This is due to the multiple effects of malaria on serum micronutrient levels like ingestion of the nutrients by the parasites, decreases the intake from the host, increases the execration of the nutrients through vomiting, perspiration, etc [56-58].

Hookworm infection decreases the serum iron level of kalazar patients by 4.48 mcg/dl; also, hookworm infection decreases the serum vitamin D level of kalazar patients by 3.94 ng/ml. This finding agrees with
previous research outputs [59]. This is due to the fact that the hookworm parasite ingests the micronutrient of the host [60].

Per a year increase in the age of the patient, the serum iron level increase by 0.11 mcg/dl. This finding agrees with other scholar’s work [61]. This is due to the fact that serum iron decreasing factors like chronic diseases and other unhealthy lifestyles were prevalent as the age increases [62].

Per a centimeter increase on the MUAC of the patient, the serum iron level increases by 0.75 mcg/dl and the serum vitamin A level by 0.86 mcg/dl. This finding was in line with previous researches [63]. This is due to the reason that, higher MUAC groups have good nutritional support [64].

Smoking decreases the iodine level of kalazar patients by 12.34mcg/dl. This finding agrees with previous scholar’s work [65]. This is due to the effect of smoking in disturbing the iodine metabolism by disrupting the normal thyroid gland functions [66, 67].

Leishmaniasis patients in the urban area had 0.81 mcg/dl higher serum vitamin A level than the rural patients. This finding agrees with finding from Nepal [68]. This is because of the higher awareness of the urban population about vitamin A [69].

A unit increase in the BMI of visceral leishmaniasis patients increases the serum vitamin D level by 1.52 ng/ml. This finding disagrees with finding from Norway [70]. This might be due to the cultural difference between the two populations.

The serum zinc level of females was 1.28 mcg/dl less than the males. This finding agrees with previous literature [71]. This is because of women losses their serum zinc level during their pregnancy and menstruation cycle [72].

Anti-leishmaniasis treatment did not increase the serum zinc, the serum vitamin A, the serum vitamin D, the serum iron level of visceral leishmaniasis patients. Anti-leishmaniasis treatment increases the serum selenium level by 3.04 ng/dl and the iodine level of patients by 13.67 mcg/dl.

The overall treatment success rate of visceral leishmaniasis treatment was 84.7 % [95 % CI: 82.77 % - 86.67 %]. A systematic review and meta-analysis estimates also support this finding [73].

Possible limitation for this study was a failure to address all the vitamins and minerals status of visceral leishmaniasis patients, but since practically it is very difficult to address all of them this study gives the baseline evidence on main vitamins and minerals levels.

**Conclusion**

The serum micronutrient level of visceral leishmaniasis patients was low. Problematic alcohol use affects the serum zinc, iron, vitamin A levels. DDS affects the serum zinc, iodine, vitamin A and vitamin D level. Family size affects the serum Zinc, iron, selenium, vitamin A and vitamin D level. HIV infection affects the
serum zinc, iron, selenium, iodine, vitamin A and vitamin D levels. Anti-leishmaniasis drug slightly increases the serum iodine and selenium level, but it doesn’t increase the serum iron, zinc, vitamin A and vitamin D levels. The serum level of zinc, iron, vitamin A and vitamin D significantly affects the treatment outcomes of visceral leishmaniasis.

**Recommendations**

The visceral leishmaniasis treatment guideline should incorporate supplementing the micronutrients as part of anti-leishmaniasis intervention.

**Abbreviations**

B – Beta coefficient

BMI – Body mass index

CI – Confidence interval

DDS – Dietary diversification score

GEE – Generalized estimating equations

HIV – Human immune deficiency virus

MCG/DL- Micrograms per deciliter

Mg/dl – Milligram per deciliter

MUAC – Mid upper arm circumference

Ng/dl – Nanogram per deciliter

SD – Standard deviation

VL – Visceral leishmaniasis

WHO – World health organization

**Declarations**

*Ethics approval and consent to participate*
Ethical clearance was obtained from Bahir Dar University College of medicine and health sciences ethical review committee. Permission was obtained from the Amhara national regional state health bureau and each treatment center. Written informed consent was obtained from each leishmaniasis patient before recruitment. Visceral leishmaniasis patients with abnormal laboratory findings were referred to the curative care segment of the hospital. The confidentiality of the data was kept at each level. Study participant right to withdraw from the research was respected at any point.

**Consent for publication**

Not applicable

**Availability of Data and Materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declares that they have no competing interests

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**Author contribution**

BEF conceived the experiment; BEF and TEF performed the experiment, plan the data collection process, analyzed and interpreted the data. BEF and TEF wrote the manuscript and approved the final draft for publication.

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