Original Article

Updates on Cutaneous Leishmaniasis and its Association with Serum Vitamin D Status in Northwest Amhara, Ethiopia

Bizuayehu Gashaw[1*], Endalew Yizengaw[2], Birhan Mulu[3], Tsedalu Alemu[4], Fasikaw Nigatie[5], Netsanet Fentahun[2], Endalkachew Nibret[1,6*]

Abstract

Background: Cutaneous Leishmaniasis (CL) can have varying outcomes in individuals due to differences in vulnerability and severity, which can be influenced by malnutrition. Vitamin D is known to play a role in the immune system, but its specific impact on cutaneous leishmaniasis is not well understood and previous research have provided contradictory results. This study aimed to investigate the levels of serum Vitamin D in patients with cutaneous leishmaniasis and assess its relationship with the disease pattern.

Method: A cross-sectional study at Nefas Mewcha Hospital, conducted from April to July 2022. Patients suspected of CL, aged 18 and above evaluated by dermatologists and eligible for the criteria underwent skin slit tests. Consented, diagnosed and positive, all were included for the study. Completing a structured questionnaire for demographic data. Anthropometric measurements calculated body mass index (BMI). Serum samples (5 ml) were collected, stored at -80°C until processing, and analyzed for vitamin D using a MAGLIMI 800 analyzer. Statistical analysis declared when $p<0.05$.

Results: The study showed that 11/48(23%) of CL patients were underweight (BMI: <18.5). Additionally, 24/33 (72.7%) of CL patients had low serum vitamin D levels (<30ng/ml). While notable differences were observed between normal BMI and obese groups (27.4&22.5ng/ml) respectively. Severe vitamin D deficiency was associated with the chronicity of CL, with patients experiencing symptoms for less than 6 months showing higher deficiency levels of vitamin D compared to those with a longer duration of CL illness ($p<0.05$). Furthermore, patients with larger CL lesion sizes and female patients were found to have higher vitamin D deficiency compared to their counterparts. The proportion of Vitamin D measurement was lower in mucosal CL type compared to localized CL type but in the mean value, no difference was seen between the two forms.

Conclusion: Vitamin D deficiency is prevalent among CL patients. This deficiency may play a role in the disease's onset and progression, highlighting the need to incorporate in treatment plans alongside anti-leishmaniasis medications. Further research, including larger case-control studies, is essential to better understand this relationship. [*Ethiop. J. Health Dev.* 2024; 38(1): 00-00]

Keywords: Cutaneous Leishmaniasis, Nutrition, Vitamin D, Pathology of Leishmaniasis

Introduction

Cutaneous Leishmaniasis (CL) is a range of diseases caused by protozoan parasites known as *Leishmania*. While exposure and infection by *Leishmania* parasite are common, the development of disease depends on various factors such as *Leishmania* species, inoculation site, parasite dose, and individual host factors, including nutritional status, genetics, immunity, age, and overall health(1). The disease can manifest from asymptomatic to mucosal and systemic involvement, and treatment may not always completely cure the disease (1).

Malnutrition contributes to the vulnerability and severity of leishmaniasis; For example, low micronutrient levels in patients with visceral leishmaniasis can lead to poor treatment outcomes (2, 3). Malnutrition, either due to the lack of protein and energy or deficiencies in essential nutrients, is a widespread issue affecting millions of people (4). Every year, malnutrition is responsible for 300,000 deaths, with young children accounting for 50% of these fatalities (5).

Overall, malnutrition can have a significant impact on the immune system by compromising the functionality of key immune cells such as B lymphocytes, macrophages, and Kupffer cells. This can result in a weakened immune response and increased vulnerability to infections and other health complications (6, 7, 8, 9).Vitamin D functions as both a hormone and a vitamin. It is produced as a prohormone when UV light activates 7-dehydrocholesterol in the skin. Its structure is similar to steroid hormones such as estradiol, cortisol, and aldosterone (10).

Vitamin D has been found to play a crucial role in the immune system, as evidenced by the presence of Vitamin D Receptors (VDR) in various immune cells such as macrophages, dendritic cells, T lymphocytes, and B cells (11).The VDR is

[1] Department of Biology, College of Science, Bahir Dar University, Bahir Dar, Ethiopia.
[2] College of Medicine and Health Science, Bahir Dar University, Bahir Dar, Ethiopia.
[3] Nefas Mewcha primary Hospital, Lay Gayint District, South Gondar zone, Ethiopia.
[4] Addis Alem primary Hospital, Bahir Dar, Ethiopia.
[5] Tefera Hailu Memorial General Hospital,Sekota, Ethiopia.
[6] Institute of Biotechnology, Bahir Dar University, Bahir Dar, Ethiopia
***Corresponding authors email**: itisbizuayehu@gmail.com and endtg2002@yahoo.com

plentifully expressed in various cells, such as CD4+, CD8+, T-lymphocytes, neutrophils, and macrophages (12).

The presence of VDR and Vitamin D activating enzymes on the nuclei of B and T cells demonstrates Vitamin D's effect on these immune cells (13). A deficiency in vitamin D in T cells leads to decreased production of IL-12, which is necessary for cell proliferation. This ultimately results in reduced levels of important molecules like IL-2 and IFN-γ, affecting recruitment and proliferation processes (14).

A lack of vitamin D can worsen the disease progression of leishmaniasis in dogs (15). The amount of vitamin D, vitamin C, zinc, and iron in the body can determine the features and severity of the disease. These micronutrients have been shown to inhibit the growth and development of both amastigotes and promastigote stages of *Leishmania* parasites (16).

Several studies have demonstrated that vitamin D plays a role in enhancing the antimicrobial activity of cells by promoting the expression of antimicrobial peptides and cathelicidin (17). Study using mice infected by *L. mexicana*, treatment with vitamin D resulted in a reduction of lesion size and a decrease of pro-inflammatory cytokine (18). Low vitamin D level correlates with the increase in the occurrence of hepatitis, AIDS, influenza, and Covid-19 (19). However, the role of vitamin D in CL is not well demonstrated in human subjects, and the dilemma remains unclear.

On the other hand, different research findings showed a low amount of vitamin D benefit for the reduction of disease severity. Repeated contact of macrophage with vitamin D inhibits the production of NO and IFN-γ as well as the production of arginase (20). In animal models of *L. mexicana* infected mice, vitamin D deficiency was laso shown to prevent disease progression (21). Vitamin D is known to help with the growth and proliferation of Th2 and T-regulatory immune responses which promote CL progression (22).

There are controversial findings and very limited data on the level and role of vitamin D in CL pathogenesis and in the development of different clinical forms of CL.Therefore, this study aimed to determine serum vitamin D levels in CL patients with different clinical manifestations and chronicity. The finding of this research could give an in-depth understanding of the relationship between vitamin D and CL that might be used for improvement of treatment modality for CL patients.

Method and Materials

Study Design, area, and period

A cross-sectional study was conducted at the Leishmaniasis Treatment Center (LTC) in Nefas Mewcha Hospital from April to July 2022. Nefas Mewcha Hospital is located in Lay Gayint District, South Gondar Administrative Zone. The altitude of this district varies from 1,500 to 3,100 meters above sea level. The annual rainfall is erratically distributed and varies from 400 to 1,100 millimeters. Based on the 2007 national census conducted by the Central Statistical Agency of Ethiopia (CSA), this district has a total population of 206,499. With an area of 1,522.43 square kilometers (587.81 sq mi), Lay Gayint has a population density of 135.64 people per square kilometer, with coordinates of 12°00'N 38°20'E. The district has nine health centers, 43 health posts, and one primary hospital providing healthcare services.

The LTC in Nefas Mewcha Hospital was established in 2019 and primarily serves CL patients in Lay Gayint district as well as those coming from districts such as Tach Gayint, Semada, and Mekeat. The vast majority of the district's population (over 95%) resides in rural areas.

Study population

The study population included all patients who received medical treatment at Nefas Mewcha Hospital between April 2022 and July 2022 G.C.

Sample size

All confirmed cases of CL during the study period at Nefas Mewcha Hospital were included in the study through purposive sampling.

Sampling procedure

Patients with skin lesions or dermatological complaints were screened and referred to a dermatology clinic for assessment. Those suspected for CL were sent for skin slit microscopy. Patients with positive results for amastigotes of the parasite were included in a study on serum vitamin D level.

Study outcome

The study could give an insight into the correlation between serum vitamin D levels and disease progression in patients with CL to better understand the potential impact of vitamin D on the manifestation of the disease.

Inclusion and Exclusion Criteria

The study participants were selected based on age; only those above 18 years old who consented to participate and tested positive for CL were included. Individuals under 18 years of age, those with known comorbidities and patients who could not provide sufficient samples were excluded.

Recruitment, diagnosis, and treatment of CL cases

Patients with signs and symptoms of skin disease were triaged at the hospital and sent to the dermatology clinic for assessment. For Those with suspected CL, skin slit was made and samples were collected and underwent microscopic examination of smears stained with Giemsa for 10 minutes (23). In confirmed cases, treatment with Sodium Stibogluconate (SSG) at 20 mg/kg/day was

administered for 28 days according to Ethiopian Ministry of Health guidelines. Treatment included both systemic and intralesional injections. The diagnostic and therapeutic procedures followed the Standard Operating Procedures (SOPs) for Leishmaniasis (24).

Data Collection and Laboratory Work

Demographic and clinical data

A structured questionnaire was employed to collect demographic information such as age, sex, and duration of illness. Additionally, the size of the lesion was assessed using a calibrated measuring scale. CL patients were clinically classified based on the site of the lesion.

Body Mass Index Measurement (BMI)

The patient's BMI can be calculated by dividing their weight in kilograms by their height in meters squared [weight/height^2]. This calculation will give the patient's BMI value, which can then be used to determine their BMI category based on the BMI classification percentile and cut-off points (25, 26). This information can be useful in assessing the client's overall health and risk for certain health conditions.

Vitamin D analysis

Whole blood samples were collected from a vein using a 5ml syringe and blue-tip test tube. Negative pressure was used to collect the blood sample. The serum vitamin D level was measured using a MAGLUMI 800 fully automated chemiluminescence immunoassay analyzer. The analyzer's photomultiplier detected light produced in the chemiluminescence reaction within the wavelength range of 300nm to 650nm. Positive and negative controls were run daily to ensure the functionality of the machine and reagents.

Data analysis and quality control.

A second staff member at LTC double-checked the findings from the laboratory before being communicated, and the diagnosis of amastigotes was assisted by referencing atlas pictures. Giemsa stain was prepared and used to ensure the quality of the reagent. The solution prepared was kept readily available at the bench every 6 hours. The laboratory personnel involved in the study took a one-week training on microscopic investigation of CL. Data were entered and analyzed using SPSS (Statistical Package for Social Science, version 23). Descriptive analysis of the data was conducted using frequencies and proportions. The results of the Chi-square analysis (Fisher ecavt test) were used and considered statistically significant at $p<0.05$.

Operational definition:

CL patients with a duration of illness less than 6 months were classified as having acute illness, while those with a duration of illness of 6 months or more were classified as having chronic illness.

The serum vitamin D level:

- Between 30-100ng/ml was considered in the normal range,
- Between 20- 29.9ng/ml was considered moderately deficient and
- Serum value <20ng/ml was considered severely deficient.

Results

Socio-demographic profile of CL patients

The study included a total of 48 patients with CL. The mean age of the patients was 33.96 years, with a standard deviation of ± 16.7. The median age was 28 years, and the age range was between 18 and 75 years. The largest group of patients (27 out of 48, 56.2%) were between the ages of 18 and 30 years. Males made up the majority (28 out of 48, 58.3%) of the patients. The most common occupation among patients was farming, followed by students, who accounted for 16 (34%) of the cases (Table 1).

Table1. **Distribution of socio-demographic characteristics of CL patients in Nefas Mewcha Hospital Leishmaniasis Treatment Center, 2022**.

Variables	Frequency	%	P-Value
Age (Year)			
18-30	27	56.2	
31-60	18	37.5	(0.001)
61+	3	6.3	
Sex			
F	20	41.7	(0.248)
M	28	58.3	
Occupation			
Farmer	29	60.4	
Student	16	33.4	
Retired	2	4.2	(0.001)
Daily Laborer	1	2	

The clinical variables of cutaneous leishmaniasis

In the study area, the most common form of CL among patients was Localized Cutaneous Leishmaniasis (LCL), accounting for 62.5% of cases. Mucocutaneous Leishmaniasis (MCL) and Diffuse Cutaneous Leishmaniasis (DCL) were also observed in 33.3% and 4.2% of patients, respectively. The majority of CL cases (75%) had lesion sizes larger than 4 mm^2, and 73% of patients had been living with the condition for over 6 months by the time of diagnosis (Table 2).

Table 2. **Variables of CL, in Nefas Mewcha Hospital Leishmaniasis Treatment Center, 2022**

Variables'	Frequency	%	(p-Value)
CL-types			
LCL	30	62.5	(0.001)
MCL	16	33.3	
DCL	2	4.2	
Size of the lesion (mm^2)			
1-4	12	25	(0.05)
4.1-10	13	27	
>10	23	48	
Duration of illness			
1-6 month	13	27	(0.001)
>6 month	35	73	

Body Mass Index (BMI) of CL patients

Among the 48 CL patients, 23% were underweight, 6.3% were overweight, and 70.8% had a normal BMI. The mean BMI level was 20.2, with a median of 19.7 and a standard deviation of 2.3 (Figure 1).

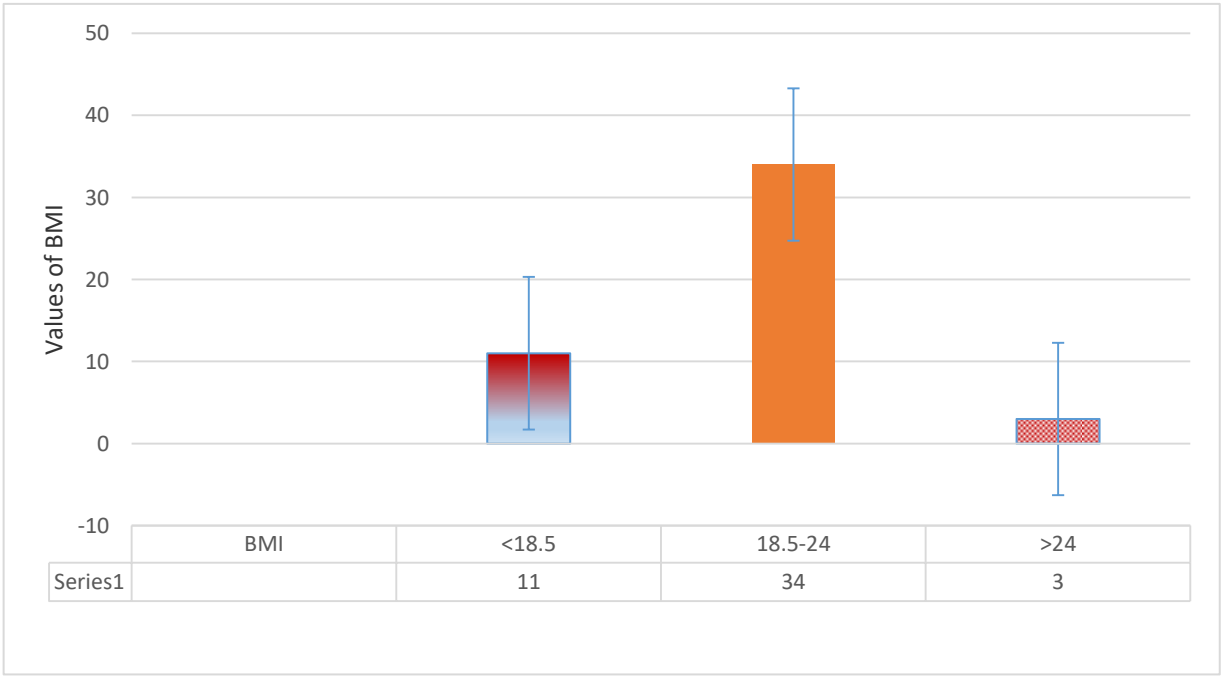

Figure 1. **Body Mass Index of CL patients at Nefas Mewcha Hospital, 2022**

Serum Vitamin D Level of CL patients

Serum vitamin D analysis was conducted from the serum of CL patients and it was found that 6(18.2%) were severely deficient and 18(45.5%) were moderately deficient. In summary, as per the standard reference, 24/33 (72.7%) of the CL cases were all living with vitamin D deficiency (Figure 2).

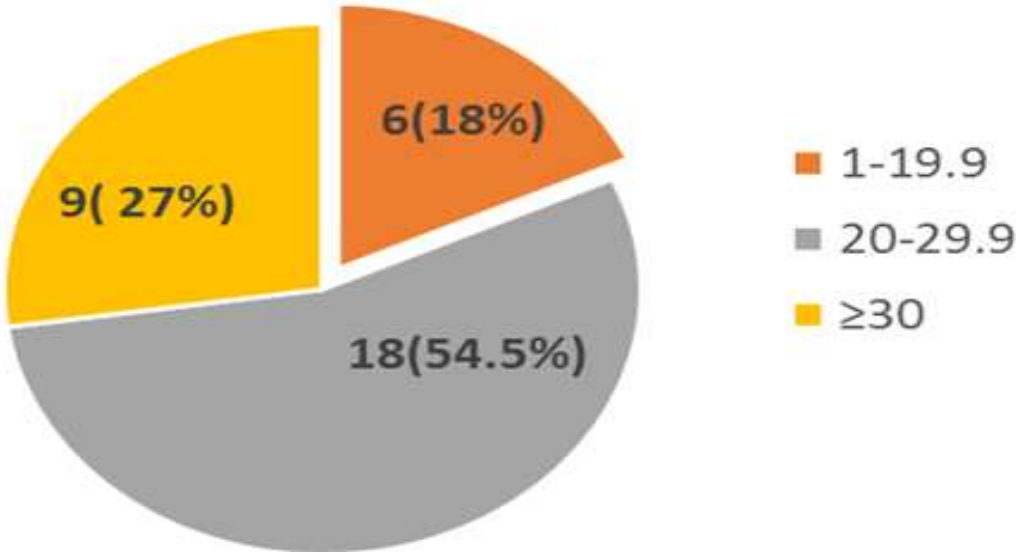

Figure 2. **Serum Vitamin D level (ng/ml) in CL patients in Nefas Mewcha Hospital, 2022**

The mean serum Vitamin D levels for CL patients were calculated in various categories, including age groups, sex, CL types, and BMI. As age increased, the mean vitamin D level decreased. However, statistical relationship between age and Vitamin D was not seen. Males had higher mean vitamin D levels compared to females.

Vitamin D levels also varied significantly among different types of CL groups ($p<0.05$), with no difference in the mean value of Vit D, between LCL and MCL types but in DCL it was higher than the two forms. While notable difference were obsereved between normal BMI and obese groups (27.4 & 22.5) respectively (Table3).

Table 3. **Mean Serum Vitamin D level with age group, sex, CL type, and BMI of CL patients in Nefas Mewcha Hospital Leishmaniasis Treatment Center, 2022**.

Variable	Mean Vit D level	(p-Value)
Age Category		
18-30	27.2	(0.418)
31-60	25.5	
61+	20	
Sex category		
F	24	(0.36)
M	28	
CL-type		
LCL	26.1	(0.04)
MCL	26.2	
DCL	32.5	
BMI Category		
Undernourished<18.5	26.3	(0.69)
Normal 18.5-24	27.4	
Obese >24	22.5	

The majority (77.8%) of CL patients with a lower BMI (<18.5) had a deficiency in vitamin D (<30ng/ml). Likewise, 15(68%) of the CL patients were deficient in vitamin D. Among CL patients with chronic lesions (>6 months), five (83.3%) had a severe deficiency in vitamin D levels, compared to those with acute lesions (≤6 months). The MCL type of the disease was getting more deficient in Vitamin D than the LCL type (85% Vs 70.5%) respectively. Although the mean didn't show the difference. Additionally, more females 11(78.5%) had vitamin D deficiency than males 13 (68.2%). More acute cases face a deficiency of Vitamin D in their serum than chronic ones (90% Vs 65.3%) respectively (Table 4).

Table 4. **Serum Vitamin D levels of CL patients across different variables in Nefas Mewcha Hospital, 2022**

Variables	Vitamin D level (ng/ml)/Deficiency			(p-Value)
	Sever (1-19.9)	Moderate (20-29.9)	Normal (≥ 30)	
Duration of illness				
Acute (1-6month)	1(10)	8(80)	1(10)	
Chronic(>6month)	5(21.8)	10(43.5)	8 (34.7)	(0.04)
CL category				
LCL	2(11.7)	10(58.8)	5(29.5)	
MCL	4(26.6)	8(53.3)	3(20)	(0.40)
DCL	0	0	1(11.2)	
Sex				
F	5(35.7)	6 (42.8)	3(21.5)	
M	1(5.2)	12(63)	6(32)	(0.08)
BMI				
Under (<18.5)	1(11.2)	6 (66.6)	2(22.2)	
Normal (18.5-24)	4(18.)	11(50)	7(32)	(0.64)
Obese (>24)	1(50)	0(0)	1 (50)	

Lesion size and Serum Vitamin D level

Out of 24 patients with Cutaneous Leishmaniasis, 16 patients with lesion size (≥4mm^2) had lower levels of Vitamin D (<30ng/ml) compared to 8 patients with lesion sizes (<4mm^2). When the lesion size increases the proportion of cases having low vitamin D increased (Table 5).

Table 5. **Lesion size and Vit D level in CL patients in Nefas Mewcha Hospital, 2022**

Variable	Vit D <30ng/ml	Vit D ≥30ng/ml	(p-Value)
Lesion size in mm^2			
1-4	8 (33.3)	1(11.1)	(0.302)
4.1-10	6(25)	3 (33.3)	
>10	10(41)	5 (55.6)	

Discussion

A high percentage (72%) of CL patients is found to have lower serum vitamin D levels. This suggests that vitamin D deficiency may facilitate the development of the disease by weakening the immune system and increasing vulnerability. Nevertheless, many individuals and animals infected with L*eishmania* remain asymptomatic (27).

A study has shown that serum vitamin D levels are affected by seasonal variation, with higher levels observed in summer and lower levels in winter (28). However, our study, conducted in the summer, revealed higher rates of deficiency compared to the previous study. Both studies (28 and ours) found that as BMI increases, the mean vitamin D levels decrease. In our study, the mean vitamin D levels were 26.3 in individuals with normal BMI and 22.5 in obese individuals.

A previous study found that 50% of children with Visceral Leishmaniasis (VL.) had vitamin D deficiency (29). In contrast, our study on CL patients showed that nearly two-thirds of them had lower levels of vitamin D, which was higher than the findings in the study conducted on Iranian (30) CL patients, where only 23% had low vitamin D levels. In our case, the deficiency surpasses the VL patients.

The variation in vitamin D deficiency levels could be attributed to the underlying causes of CL beyond skin color. In Ethiopia, the majority of CL cases are caused by *L. aethiopica*, whereas in Iran, other species are responsible for causing CL. In a specific region in Southern Ethiopia, 14.8% of individuals had vitamin D levels below 30 nmol/L, putting them at risk for deficiency (31). The discrepancy in findings could be attributed to the variations in study subjects. In the case of Southern Ethiopia, the study focused on pregnant mothers rather than on *Leishmania*-infected patients. Therefore, the development of CL is influenced by factors such as the type of parasite, the immune response of the host, and nutrition (32, 33). Our study found that patients with CL suffer from vitamin D deficiency, which might impact the effectiveness of microbial treatment and slow down the recovery process. Specifically, a significantly higher percentage of patients who had been living with the lesion for over 6 months (62.5%) were found to have vitamin D deficiency compared to those with a duration of less than 6 months (37.5%). Previous research also demonstrated that vitamin D can inhibit the growth of the *Leishmania* parasite in macrophages (34), highlighting the importance of addressing this deficiency in CL patients.

This suggests that patients with CL who had a deficiency in serum vitamin D may experience prolonged symptoms and a higher likelihood of developing chronic conditions. Vitamin D plays a key role (35) in enhancing the body's natural immune response, particularly through the production of cathelicidin, which acts as an antimicrobial agent. Additionally, vitamin D is essential for promoting skin health.

Our study found that even among patients with normal BMI, 15 out of 22 (68%) still had hidden hunger vitamin D. This suggests that BMI may not be a reliable indicator of vitamin D levels in the body and should not be used as the sole measure for identifying hidden hunger in patients with CL. This is consistent with previous research (35), which has shown a negative correlation between BMI and 25(O.H.) D3 levels, indicating that as BMI increases, vitamin D levels decrease.

Our study found that severe serum vitamin D deficiency (<20 ng/ml) was more common in the MCL type of disease (66.6%) compared to the LCL type (33.3%). This is consistent with previous research (36), which showed that a vitamin D level below 20 ng/mL can inhibit the synthesis of cathelicidin and defensin β2, important antimicrobial chemicals for host protection. This suggests that vitamin D levels may play a role in the pathogenesis of CL, particularly in cases with larger lesions like the MCL type.

A substantial number of patients with LCL (70%) and MCL (80%) in our study exhibited lesion sizes exceeding 4mm^2. Conversely, a different study (30) did not show a connection between serum levels of vitamin D, disease severity, and healing duration in individuals with mucosal leishmaniasis. Having a minimum 25(OH)-D level of 32 ng/ml is essential for supporting immune response and physiological function (32). Vitamin D plays a direct role in supporting the function of monocytes, so having insufficient levels may adversely affect monocyte activity (33).

The interaction between vitamin D and its receptors on immune cells has been found to enhance innate immunity (37) by promoting the production of cathelicidin, an antimicrobial peptide. This process also aids in the differentiation of innate cells. Failure to effectively control and eliminate parasites can result in the development of extensive lesions and the progression to a more severe form of disease.

Our research found that individuals with lesions larger than 4mm^2 had lower levels of vitamin D compared to those with smaller lesions. Specifically, 66% of individuals with larger lesions had vitamin D deficiency, while only 33.3% of those with smaller lesions were deficient. This suggests a correlation between vitamin D levels and the severity of the disease. Additionally, patients with multiple lesions also had lower vitamin D levels compared to those with a single lesion, supporting our findings (38). In an animal model study, it was observed that lower levels of vitamin D were linked to the progression of the disease. The dog exhibiting severe clinical symptoms had a vitamin D level of 19.6 ng/ml, while the non-infected and asymptomatic groups had levels of 31.8 ng/ml and 29.6 ng/ml, respectively (27).

A population-based study conducted in various countries, including Ethiopia, indicated that there is a significant need for attention to vitamin D levels, with hypovitaminosis being common in over 50% of the population in tropical regions (39). In Europe, 17.7% of the population had vitamin D deficiency in the Winter, while 8.3% had a deficiency in the summer (40). In Ethiopia, a study conducted in a sunlight area in the southern part of the country found that vitamin D deficiency was 14.8% among participants with levels below 30 nmol/L(41). This suggests that the high deficiency seen in leishmaniasis patients in our study may be linked to the progression of the disease.

In our study, we observed a difference in the mean serum level of vitamin D between female and male CL patients. The mean serum vitamin D level was found to be lower in females compared to males. This discrepancy may be attributed to the fact that more females tend to stay indoors, thus reducing their exposure to sunlight, which is essential for the conversion of precursor vitamin D into its active form. While the body can obtain vitamin D from food sources (20%), the majority (80%) is produced in the skin with the help of sunlight. However, the amount of vitamin D produced in the skin can vary seasonally (40).

Underweight was a significant issue in this study, with 11 out of 48 (23%) of the CL patients having a BMI <18.5, indicating malnourishment. Poverty may be a contributing factor to this finding. A different study on nutritional status of VL patients found that 48 out of 109 (44%) were malnourished (42), which is more serious than the CL form of the disease. The World Health Organization has recognized that the risk of leishmaniasis is exacerbated by poverty, inadequate housing conditions, and nutritional deficiencies (43).

Conclusion

In this study, CL patients are in a serious problem of malnutrition which is explained by energy-protein deficiency and starvation of hidden hunger like vitamin D. All these conditions might lead to complicated, serious, and chronic form of clinical features of CL. This fact, deficiency of vitamin D, can be explained by developing MCL form of the disease, larger lesion size, and extended time for living with the disease that might give a clue for considering vitamin D beyond antimicrobial treatment. These clinical forms may influence the daily activity of the patients and later negatively influence the productivity of those citizens.So, considering CL treatment with vitamin D might be the focus for the future case management approach that benefits the system and the patient at large. A study using a larger sample size to control the case and controls is quite important to conduct an in-depth analysis that helps to see clearly, the association between vitamin D and CL.

Ethical considerations and consent

The study was done on the acceptance and approval of Amhara Public Health Institute. The hospital managers and clinical staff were aware of the study. Informed consent was taken from participants of the study APHI/03/1691.

Acknowledgments

We extend our sincere gratitude to the participants of this study, whose cooperation and commitment were vital to the success of this research. We also express our deep appreciation to the dedicated staff at Nefas Mewcha Hospital, Lay Gayint District Health Office, Amhara Public Health Institute, and Bahir Dar University for their invaluable support and expertise in facilitating the smooth execution of this study. Their unwavering dedication and collaboration were instrumental in enabling us to conduct this important research.

Reference

1. Smith AB, Johnson CD. Leishmaniasis: Cutaneous and Visceral. *J Infect Dis*. 2022; 215(3):456-468.
2. Ali A. Visceral leishmaniasis in Southern Ethiopia II. Nutritional risk factors. *Ethiop J Health Dev*. 1997; 11(2):139-144.
3. Feleke BE, Feleke TE. Micronutrient levels and their effects on the prognosis of visceral leishmaniasis treatment, a Prospective cohort study. *BMC Infect Dis*. 2020; 20(1):1-9.
4. Müller O, Krawinkel M. Malnutrition and health in developing countries. *CMAJ*. 2005;173(3):279-86.
5. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? *Lancet*. 2003;361(9376):2226-34.
6. Redmond HP, Shou J, Kelly CJ, Schreiber S, Miller E, Leon P, Daly JM. Immunosuppressive mechanisms in protein-calorie malnutrition. Surgery. 1991; 110(2):311-317.
7. Petro TM, Schwartz KM, Chen SSA. Production of IL2 and IL3 in syngeneic mixed lymphocyte reactions of BALB/c mice is elevated during a period of moderate dietary protein deficiency. Immunol Invest. 1994; 23(2):143-152.
8. Honda M, Kamiyama Y, Kawamura K, Kawahara K, Shishido S, Nakai H, Kawamura T, Ito H. Growth, development and nutritional status in Japanese children under 2 years on continuous ambulatory peritoneal dialysis. Pediatr Nephrol. 1995;9(5):543-548.
9. Stapleton PP, Fujita J, Murphy EM, Naama HA, Daly JM. The influence of restricted calorie intake on peritoneal macrophage function. Nutrition. 2001; 17(1):41-5.
10. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr. 2008; 88:491S-499S.
11. Hewison M, Freeman L, Hughes SV, Evans KN, Bland R, Eliopoulos AG, Kilby MD, Moss PAH, Chakraverty R. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. J Immunol. 2003; 170(11):5382-5390.
12. Baeke, F., Takiishi, T., Korf, H., Gysemans, C., and Mathieu, C. Vitamin D: modulator of the immune system. Curr. Opin. Pharmacol. 2010; 10, 482–496.
13. Provvedini, D.M.; Tsoukas, C.D.; Deftos, L.J.; Manolagas, S.C. 1,25-Dihydroxyvitamin D3 receptors in human leukocytes. Science 1983, 221, 1181–1183
14. Wei, R.; Christakos, S. Mechanisms underlying the regulation of innate and adaptive immunity by vitamin D. Nutrients 2015, 7, 8251–8260
15. A. Rodriguez-Cortes, C. Martori, A. Martinez-Florez, A. Clop, M. Amills, J. Kubejko, J. Llull, J. M. Nadal & J. Alberola.Canine Leishmaniasis Progression is Associated with Vitamin D Deficiency. Scientific Reports 3346 2017; 7:3346
16. Kumar U, Favas M, Sharma A, Bisht P, Dhingra S, Ravichandran V, Ramesh M, Murti K. The Possible Role of Selected Vitamins and Minerals in the Therapeutic Outcomes of Leishmaniasis V. Biological Trace Element Research. 2023; 201(7):1672-1688.
17. Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Introduction Introduction of cathelicidin in normal and .F.CF bronchial epithelial cells by 1,25 Dihydroxy Vitamin D3. J cyst Fibros. 2007; 403-410.
18. Ramos-Martinez E, Villasenor-Cardoso MI, Lopez-Vancell MR, Garcia-Vazquez FJ, Perez-Torres A, Salaiza-Suazo N, Perez-Tamayo R. Effect of 1,25,(OH)2D3 on BALB/c.2013
19. Siddiqui M, Abdulrahman HA, Manansala JS, Nasrallah GK, Smatti MK, Younes N. Immune Modulatory Effects of Vitamin D on Viral infections. Nutrients. 2020; 12(9):2879
20. Ehrchen J, Helming L, Varga G, Pasche B, Loser K, Gunzer M, Sunderkotter C, Sorg C, Roth J, Lengeling A. Vitamin D receptor signaling Contributes to susceptibility to infection with Leishmania major. FASEB J. 2007; 21(12):3208-3218.
21. Ramos-Martinez E, Gutierrez-Kobeh L, Villasenor-Cardoso MI. The role of Vitamin D in the control of Leishmania infection. Can J Physiol Pharmacol. 2015;93(5):369-376
22. Sassi F, Tamone C, D., Amelio P. Vitamin D: Nutrient, home and immune modulator. Nutrients. 2018; 10 (11):1656.
23. Chulay J, Bryceson A. Quantitation of amastigotes of Leishmania donovani in smear of splenic aspirates from patients with Visceral Leishmaniasis. Am J Trop Med Hyg 1983; 32(4); 759.
24. Ethiopian Federal Democratic Republic, Ministry of Health (2013). Guideline for the treatment of Leishmaniasis.
25. Weir CB, Jan A. BMI Classification Percentile And Cut Off Points. 2023 Jun 26. In: StatPearls [Internet]. Treasure Island (F.L.): StatPearls Publishing; 2024 Jan–. PMID: 31082114.
26. Marchese, R., Hill, A. (2005). The Essential Guide to Fitness: for the fitness instructor. Pearson: New South Wales

27. Rodriguez-Cortes A, Martori C, Martinez-Florez A, Clop A, Amills M, Kubejko J, Llull J,Nadal JM, Alberola J. Canine Leishmaniasis Progression is associated with Vitamin D. Sci. Rep 2017;7:3346.
28. Lagunova Z, Porojnicu AC, Lindberg F, Hexeberg S, Moan J. The dependency of vitamin D status on body mass index, gender, age, and season. Anticancer Res. 2009; 29:3713-3720.
29. Diro E, Lynen L, Gebregziabiher B, Assefa A, Lakew W, Belew Z, Hailu A, Boelaert M, Van Griensven J. Clinical aspects of pediatric visceral Leishmaniasis in North West Ethiopia. Trop Med Int Health. 2015 20(1):8-16,
30. Figueiredo LP, Cerqueira-Silva T, Magalhães A, Lago EL, Lessa MM. Brief communication: Vitamin D serum levels in American tegumentary Leishmaniasis from an endemic area in Northeast Brazil. Braz J Infect Dis. 2023 Jan-Feb;27(1):102720. doi: 10.1016/j.bjid.2022.102720. Epub 2022 Dec 2. PMID: 36463934; PMCID: PMC9730050.
31. World Health Organization. Report ofa fifth consultative meeting on Leishmania/HIV coinfection, Adddis Ababa, Ethiopia, 2007; WHO/CDS/NTD/IDM/2007.5.
32. Khazai N, Judd SE, Tangpricha V. Calcium and vitamin D: skeletal and extraskeletal health. Curr Rheumatol Rep. 2008;10(2):110-7. doi: 10.1007/s11926-008-0020-y. PMID: 18460265; PMCID: PMC2669834.
33. Liu, P.T.; Stenger, S.; Li, H.; Wenzel, L.; Tan, B.H.; Krutzik, S.R.; Ochoa, M.T.; Schauber, J.; Wu, K.; Meinken, C. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006, 311, 1770–1773.
34. Machado Pde A, Escrivani Do, Gomes Dco, Rossi-Bergmann B, Chaves SP, Coimbera ES,de Matos Guedes HL. Vitamin D increases Killing of intracellular Leishmania amazonensis in invitro independently of macrophage oxidative mechanisms. Parasitology. 2020; 147:1792.
35. Jim B., "Vitamin D: Emerging Roles in Infection and Immunity." *Expert Review of Anti-Infective Therapy* 8, no. 12 (2010): 1359–69. doi:10.1586/eri.10.102.
36. Sudfeld C.R.; Wang M.; Aboud, S.; Giovannucci, E.L.; Mugusi, F.M.; Fawzi, WWW Vitamin D, and HIV progression among Tanzanian adults initiating antiretroviral therapy. PLoS ONE 2012, 7.
37. Wang TT.; Nestel FP.; Bourdeau V.; et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol. 2004; 173:2909–12
38. Shabandoust H.; Sharifi I.; Raiesi O.; Getso MI.; Saedi ED.; Afgar A, Shirvani G, Salarkia E, Babaei Z. Serum 25-hydroxyvitamin D level and vitamin D receptor (VDR) polymorphisms in patients infected with Leishmania tropica: a case-control study. J Parasit Dis. 2020;44(1):40-48.1):40-48.
39. Mandarino NR, Monteiro J unior FC, Salgado JVL, Lages JS, Salgado Filho N. Is vitamin D deficiency a new risk factor for cardiovascular disease? Open Cardiovasc Med J. 2015;9:40–9.
40. Urashima, M.; Segawa, T.; Okazaki, M.; Kurihara, M.; Wada, Y.; Ida, H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am. J. Clin. Nutr.2010, 91, 1255–1260.
41. Gebreegziabher T, Stoecker BJ. Vitamin D insufficiency in a sunshine-sufficient area: Southern Ethiopia. Food and Nutrition Bulletin. 2013;34(4).
42. Bantie K, Tessema F, Massa D, Tafere Y. Factors associated with visceral leishmaniasis infection in North Gondar zone, Amhara region, North West Ethiopia, case-control study. *Science Journal of Public Health*. 2014; 2(6):560-568.
43. Leishmaniasis;(2023).https://www.who.int/news-room/fact-sheets/detail/leishmaniasis