ORIGINAL ARTICLE

Retrospective Study

Hematological picture of pediatric Sudanese patients with visceral leishmaniasis and prediction of leishmania donovani parasite load

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Abstract

BACKGROUND

Visceral leishmaniasis (VL) is a systemic protozoan infection caused by *Leishmania donovani* (*L. donovani*) and transmitted by sand flies, causing macrophage invasion in the liver, spleen, and bone marrow. Diagnosis of VL is currently based on clinical signs, symptoms, and specific *in-vitro* markers and bone marrow investigations. However, VL's specific hematological and bone marrow manifestation in

Sudanese pediatric patients is not well studied.

AIM
To examine the blood and bone marrow characteristics in pediatric patients from Sudan who have VL.

METHODS
This is a retrospective hospital-based study with a sample of 107 consecutive Sudanese pediatric patients. The data focused on hematological and bone marrow results. We included only the completed records of the pediatric patients with VL in the Tropical Disease Teaching Hospital in Khartoum, Sudan from the period of 2016 to 2020.

RESULTS
The majority of pediatric patients included in this study are below 5-years-old (n = 59, 55.2%). Moreover, anemia, thrombocytopenia, and leukopenia were among the prevalent characteristics in the population under study. To further analyze the data, we developed a machine learning model using boosted forest algorithms to predict *L. donovani* parasites load, with a mean accuracy of 0.88 for the training dataset and an accuracy of 0.46, 0.50, and 0.74 for mild, moderate, and severe *L. donovani* parasite load in the validation dataset.

CONCLUSION
This study shows that the most common bone marrow change among Sudanese VL children was increased chronic inflammatory cells (n = 88, 82.2%) with present macrophage hemophagocytes (n = 103, 96.3%). While anemia and thrombocytopenia were the most common hematological changes. These results will hopefully lead to an early diagnosis and hence better management for Sudanese pediatric patients with suspected VL.

Key Words: Machine learning; Bone marrow; Hematological changes; Tropical diseases; *Leishmania donovani*; Visceral leishmaniasis

Core Tip: Visceral leishmaniasis (VL) is a neglected tropical disease; although many parts of Sudan are hyperendemic, many centers have very limited diagnostic capabilities and data reporting. This is one of the first studies from Sudan to investigate both hematological and bone marrow changes associated with pediatric VL and *Leishmania donovani* load from bone marrow biopsies. The data were collected from tertiary and specialized centers for treating VL in the country and modern analytical methods were used to describe the result for the global audience and highlight the importance of these new findings derived from a country that is hyperendemic to VL.

INTRODUCTION

Visceral leishmaniasis (VL) or Kala-azar caused by *Leishmania donovani* (*L. donovani*) is a tropical neglected disease[1] and a major health problem in Sudan, particularly among newborns and young adults with an estimated pooled prevalence of 18% according to the meta-analysis done by Ahmed *et al*[2] in Sudan. The disease is endemic mainly in the eastern and central parts of the country, which lies between four states (white Nile State in the west, Gadarif State in the east, Blue Nile State in the south, and Kassala State in the northeast)[3]. Globally, according to World Health Organization, an estimated 50000 to 90000 new cases of VL occur worldwide annually mostly in Brazil, India, and East Africa including Sudan[4].

Due to parasite replication in the reticuloendothelial system, *L. donovani* can accumulate in the spleen, liver, and bone marrow[5], this causes hemolysis, splenic sequestration, and bone marrow suppression resulting in severe anemia. Also, edema, hypoalbuminemia, and severe cachexia are linked to advanced kala-azar[6], while ascites, jaundice, and hepatic dysfunction might appear late in the course of the illness, moreover, hemorrhagic complications can result from thrombocytopenia and hepatic dysfunction[5]. Rarely, chronic diarrhea and malabsorption can occur because of parasitic invasion of the intestine[5].

In VL, peripheral hematological changes are well studied[7,8]. However, bone marrow changes have been less reported in the literature[9-11]. The alterations in peripheral hematological parameters were reported from different pediatric populations around the world[10,11]. Currently, only a few outdated studies assess and predict bone marrow cytology and histopathological biopsies in children with VL[12-14] emphasizing the need for updating the evidence. Sudan is a

hyperendemic region for VL, yet there are limited data on bone marrow changes associated with the disease, largely due to limited clinical resources in much of the country. Unlike traditional hematological changes investigated by complete blood counts, this study analyzed bone marrow histopathological biopsies associated with VL using novel machine-learning methods. Additionally, this study aims to identify fewer common changes in the bone marrow, including bone marrow aplasia, granuloma, hemophagocytosis, histiocytosis, and co-infections associated with VL. These findings could contribute to developing improved management plans for VL patients.

MATERIALS AND METHODS

Study design
This is a retrospective study adopted STROBE guideline and conducted as a collaboration between the Tropical Diseases Teaching Hospital (Pediatrics department) and the hematology and laboratory department in Mohamed El-Amin Hamid Pediatric Teaching Hospital [both are centers for leishmaniasis diagnosis and management) for children which is located in Khartoum state Omdurman, Sudan. At Tropical Diseases Teaching Hospital which is a national referral and specialized center for treating and diagnosing infectious diseases, patients underwent clinical examinations and blood diagnostic procedures to determine the extent of their medical conditions.

In regions where VL is hyperendemic like Sudan, positive VL antibody test results may be observed among asymptomatic individuals with subclinical infection or recovered VL patients. These antibody tests have false positive results[15]. In these cases, bone marrow biopsies are necessary for accurate diagnosis and treatment. These biopsies are taken by skilled medical professionals to Mohamed El-Amin Hamid Hospital for analysis and examination. The later hospital specializes in conducting detailed examinations of bone marrow samples, utilizing advanced laboratory techniques and equipment. By collaborating with Mohamed El-Amin Hamid Pediatric Teaching Hospital, we ensured that patients received accurate and reliable results from their bone marrow biopsies, enabling a clear diagnosis and effective treatment, management, and follow-up.

Sample size and sampling technique
This study was conducted among all the consecutive pediatric VL patients who have complete hematological and bone marrow reports and were diagnosed at the Tropical Disease Teaching Hospital from 2016 to 2020.

Methods of data collection
A bone marrow aspiration and trephine biopsy were performed according to the local sterile procedure guidelines. Here, the skin around the posterior iliac crest was cleaned, and 2-5 mL of 2% lidocaine was injected into the skin, subcutaneous tissue, and periosteum as a local anesthetic. After waiting 2-3 minutes for anesthesia to take effect, we selected a stainless-steel aspiration needle, size 16G or 18G, depending on the patient's age and size. For the trephine biopsy, we used a 16G needle. The patient was positioned laterally with the right knee flexed. The needle was inserted perpendicularly into the cavity of the ilium at the center of the oval posterior superior iliac spine. Once the bone was penetrated, the stiletto was removed, and marrow contents were aspirated for film preparation. Using the same opening, a trephine biopsy was obtained, with the sample size not exceeding 2 cm for pediatric patients. In terms of data collection, demographic, hematological data, and laboratory records reports (CBC and bone marrow) of patients diagnosed with VL at Tropical Disease Teaching Hospital were collected during the study period according to the following criteria:

Inclusion and exclusion criteria: Patients less than 18-years-old, who were diagnosed and confirmed with VL at the Tropical Disease Teaching Hospital laboratory unit during the study period, although patients who have incomplete forms were excluded from the study.

Ethical clearance: We obtained written ethical approval from the Tropical Disease Teaching Hospital administrative authority (TDTH/B/51/1 data: January 1, 2021). The information acquired was kept confidential and only utilized in the context of this study.

Statistical analysis
Age, sex, traveling history and *L. donovani* load are independent variables. Peripheral hematological alterations (hemoglobin level, platelets, and white blood cell count) and bone marrow aspirate changes (cellularity, hemophago-cytosis, chronic inflammatory cells, and megaloblastic changes) were dependent variables. For quantitative data, we used descriptive statistics in the form of frequency tables with percentages and graphs. The χ^2 test and Cochran-Armitage test were used to examine the associations between bone marrow alterations due to VL and the other relevant factors. Any P value ≤ 0.05 was judged statistically significant.

Moreover, we developed an artificial intelligence algorithm to predict the *L. donovani* load from our dataset. Here, we used boosted forest algorithms with training data composed of 75% and validation data accounting for 25%. We used an early regularization technique to avoid model overfitting, and the optimal numbers of iteration and sampled splits were implemented by the algorithm. We visualized the data using the receiver operating characteristic curve (ROC), and lift curve to examine model performance compared to the baseline random classifier. In the *L. donovani* load model, entropy R Square is 0.26, -0.004 and RMSE is 0.56 and 0.66 for training and validation data, respectively. This indicates weak moderate predictability[16] for severe cases but not for mild and moderate parasitic loads. We analyzed the data using a combination of statistical software including SPSS 26, SAS JMP 14, and NCSS12.

Table 1 Background characteristics and peripheral hematological findings in visceral leishmaniasis pediatric patients

Characteristic	Subcategory	Count	%
Background characteristics			
Age group	Birth to 1 year	11	10.3
	1 to 5 years	48	44.9
	5 to 10 years	23	21.5
	10 to 18 years	25	23.4
Sex	Male	76	71.0
	Female	31	29.0
Area of the disease acquisition	Traveling to an endemic area	17	15.9
	Residence in endemic area	90	84.1
Peripheral hematological parameter			
Peripheral hematological changes hemoglobin	Less than 5 mg/dL	30	28.0
	5 to 10 mg/dL	68	63.6
	More than 10 mg/dL	9	8.4
Peripheral hematological changes platelet	Less than $50 \times 10^3/\mu L$	42	39.3
	50 to $100 \times 10^3/\mu L$	57	53.3
	100 to $150 \times 10^3/\mu L$	7	6.5
	More than $150 \times 10^3/\mu L$	1	0.9
Peripheral hematological changes white blood cells	Less than $2 \times 10^3/\mu L$	26	24.3
	2 to $4 \times 10^3/\mu L$	64	59.8
	4 to $10 \times 10^3/\mu L$	15	14.0
	More than $10 \times 10^3/\mu L$	2	1.9

RESULTS

The majority of data analyzed in this study indicate that the major part of the samples studied are male patients and are under 5-years-old, as illustrated in Table 1. Furthermore, anemia, thrombocytopenia, and leukopenia were prevalent in the study population. Additional parameters are detailed in Table 1. Notable results from the study indicate that most trephine biopsy samples showed normoplasia 57.0% (n = 61) and a high prevalence of histiocytosis 92.5% (n = 99). Additionally, bone marrow analysis revealed hypercellularity in 40.2% (n = 43) of cases, with 46.2% (n = 49) exhibiting active megakaryopoiesis. Hemophagocytosis was also highly prevalent, observed in 96.3% (n = 103) of bone marrow samples. Increased inflammatory cells in bone marrow were observed among 82.2% (n = 88) (Table 2). Moreover, a statistical model was designed and validated to further analyze the data, as depicted in Figure 1. A bone marrow biopsy sample is shown in Figure 2.

DISCUSSION

In this retrospective study, we analyzed 107 VL pediatric patient records from 2016-2020 from a tropical disease hospital. Most of the patients were male 71% (n = 76), 55.2% (n = 59) of pediatric patients with VL were below the age of five, and only 23.4% (n = 25) were between 10 years and 18 years (Table 1). This is like studies conducted in Iraq which report that most of the cases were below 5 years[17]. In this study, most of the patients live in endemic regions to VL in Sudan 84.1% (n = 90), and only 15.9% (n = 17) acquired VL through traveling.

In the current study, most of the VL patients presented with pancytopenia, as 91.6% (n = 98) had hemoglobins less than 10 mg/dL, 92.6% (n = 99) had platelet counts less than $100 \times 10^3/\mu L$, and 84.1% (n = 90) had white blood cell counts less than $4 \times 10^3/\mu L$ (Table 1). These findings which describe the peripheral blood changes are common hematological findings in VL patients[17]. The high level of pancytopenia in this patient group is probably because of the longer course of VL and splenomegaly before presentation to the hospital and increased peripheral blood destruction[18]. The leading causes of anemia among VL patients are sequestration and destruction of RBCs in an enlarged spleen and alteration in RBC membrane permeability. Moreover, we report that only one VL patient has a platelet count of more than $150 \times 10^3/\mu L$ like Daneshbod *et al*[19] who found that thrombocytopenia occurred in 80%-90% of the VL patients. It is postulated

**Table 2 Trephine biopsy and bone marrow changes in visceral leishmaniasis pediatric patients**

Findings	Subcategory	Count	%
Trephine biopsy findings			
Trephine biopsy cellularity	Hypoplasia	15	14.0
	Normoplasia	61	57.0
	Hyperplasia	31	29.0
Trephine biopsy fibrosis	No	100	93.5
	Yes	7	6.5
Trephine histiocytosis	No	8	7.5
	Yes	99	92.5
Trephine granuloma	No	96	89.7
	Yes	11	10.3
Trephine parasite load	Mild	33	30.8
	Moderate	42	39.3
	Severe	32	29.9
Bone marrow findings			
Bone marrow cellularity	Hypocellular	14	13.1
	Normocellular	50	46.7
	Hypercellular	43	40.2
Megakaryopoiesis	Adequate	15	14.2
	Decreased	42	39.6
	Active	49	46.2
Bone marrow dysplastic changes	No	12	11.2
	Yes	95	88.8
Bone marrow megaloblastic changes	No	16	15.0
	Yes	91	85.0
Bone marrow granulopoiesis	Orderly maturation	6	5.6
	Arrest maturation	73	68.2
	Hyperactive	28	26.2
Bone marrow eosinophilic precursors	Depressed	46	43.0
	Prominent	61	57.0
Bone marrow hemophagocytosis	No	4	3.7
	Yes	103	96.3
Bone marrow chronic inflammatory cells	Depressed	19	17.8
	Increased	88	82.2

that the thrombocytopenia and leukopenia observed in the peripheral blood of VL are due to hemophagocytosis and partly because of poor platelet formation[20].

Previous studies found that there is an association between VL and bone marrow change in patients[21]. In the current study, the trephine biopsy data showed that 29% (*n* = 31) of VL patients have hyperplastic bone marrow activities, whereas 93.5% (*n* = 100), and 89.7% (*n* = 96) of them do not have trephine fibrosis and trephine granuloma, respectively. However, 92.5% (*n* = 99) have trephine histiocytosis and this is because of bone marrow hyperactivity and hepatosplenomegaly. Moreover, only 30.8% (*n* = 33) of VL patients have a mild *L. donovani* load and the remaining 69.2% (*n* = 74) (Table 2) have moderate to high *L. donovani* load. This rise in parasitic load can be explained by several immune evading mechanisms of the organism.

Moreover, among VL patients, 88.8% (*n* = 95), 85% (*n* = 91), 96.3% (*n* = 103), and 82.2% (*n* = 88) have bone marrow dysplastic, megaloblastic, hemophagocytosis activities and presence of chronic inflammatory cells, respectively. The bone
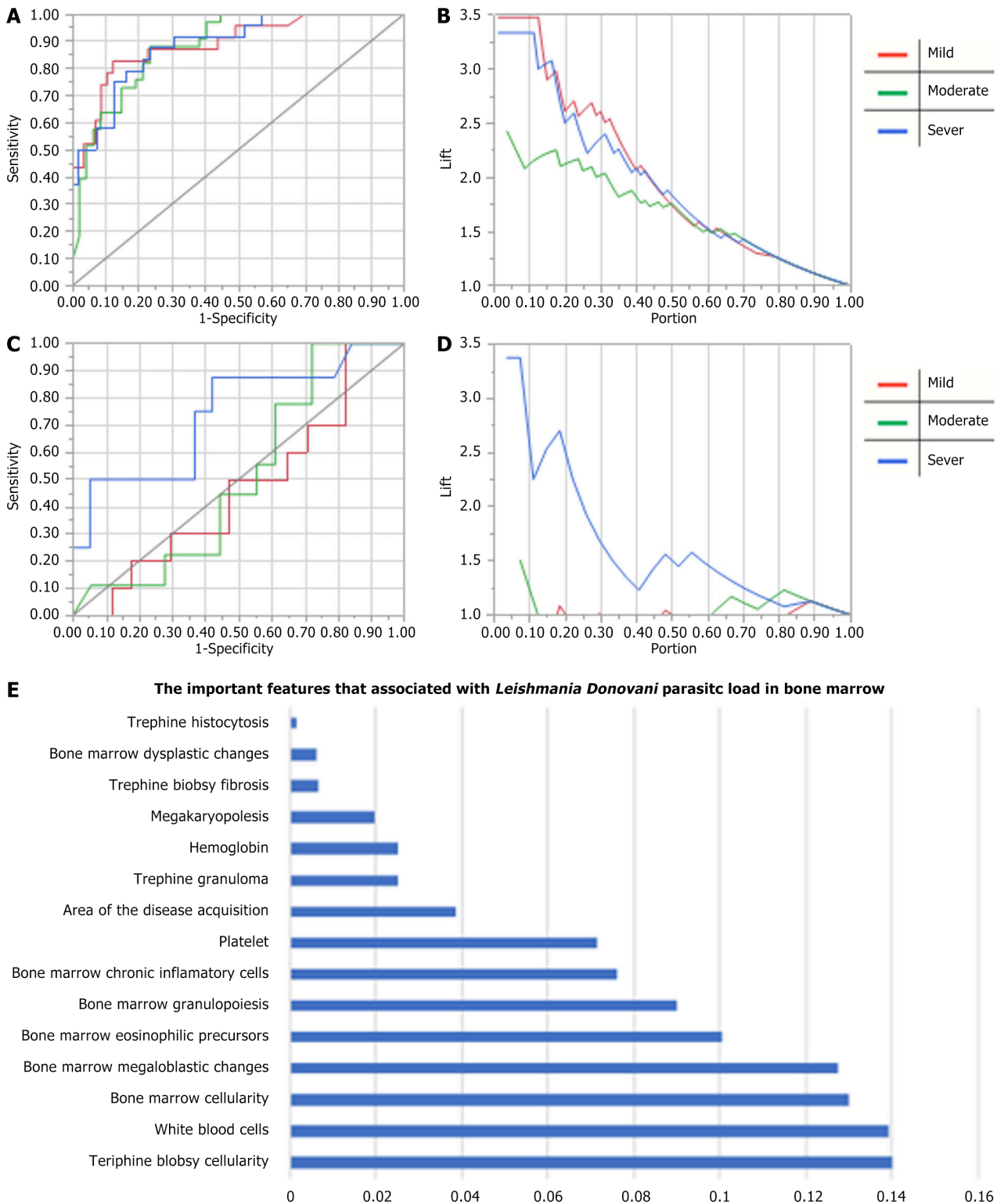
Figure 1 Model for predicting parasitic load. A-D: Performance of the parasite load model with three target categories (mild, moderate, severe); A: ROC for training data; B: Lift curve for training data; C: ROC for validation data; D: Lift curve for validation data; E: Important features that are associated with lei*shmania donovani* parasites load.

marrow data showed hypercellularity and granulopoiesis hyperactivity in 40.2% (*n* = 43), and 26.2% (*n* = 28), respectively, (Table 2). Children below 5 years are more frequently affected to have trephine biopsy changes, with an increase in *L. donovani* parasite load ranging from moderate to severe[22,23].

Moreover, a higher *L. donovani* load is associated with a decrease in megaloblastic maturation activities, eosinophilic precursors, and chronic inflammatory cells *P* value < 0.05. This inverse relation has been noted before in previous studies in both humans and animals, especially in mice and dogs. The increased eosinophils affect the parasite count by either directly phagocytizing the parasite or improving the antimicrobial activity of the macrophage[24]. Figure 1A-D illustrate the accuracy matrix for predicting the *L. donovani* load from the study variables. Figure 1A and B show the ROC, and fit
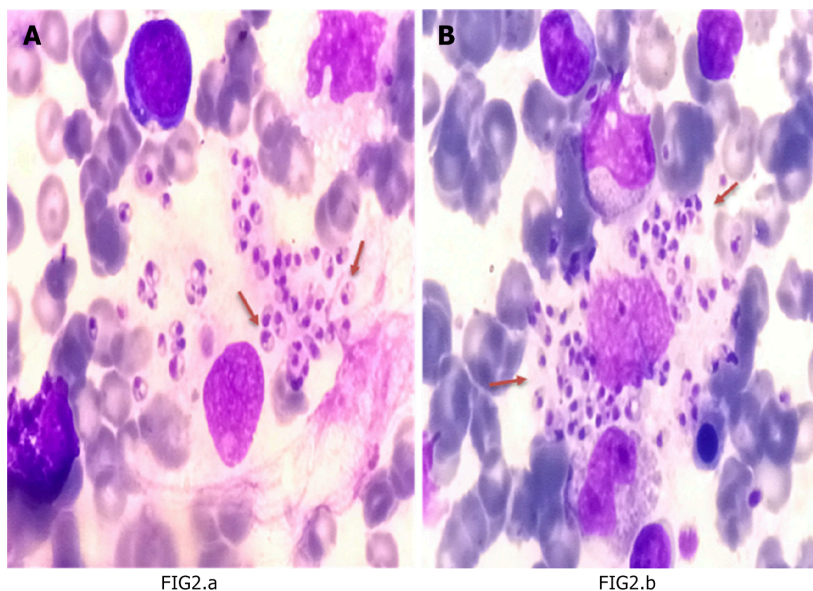
Figure 2 Bone marrow smears (Giemsa stain × 100). A: Reputed macrophage shedding Extracellular amastigotes, each with a prominent nucleus and kinetoplast (red arrows); B: *Leishmania donovani* bodies as intracytoplasmic, non-flagellated leishmania parasites (amastigotes), located intracellularly in macrophages (red arrows).

curves for the training data, respectively, with a mean of 0.88 AUC for all *L. donovani* load categories, however, the area under the curve (AUC) for the validation set varies considerably as the AUC is 0.46, 0.50 and 0.74 for mild, moderate, and severe *L. donovani* load in the validation set. This variation indicates that the model can predict severe *L. donovani* load better than mild or moderate load, Figure 1C shows that the validation prediction for mild and moderate parasite load is weak, and below the random classifier, as shown in Figure 1C and D. Only severe cases were predicted better than the random classifier. The variables and features that are associated with *L. donovani* load prediction include bone marrow cellularity, white blood cells, and megaloblastic changes (Figure 1E). However, a high misclassification rate is present in the training dataset 29% and 62% in the validation dataset, despite using a robust algorithm. It is hard to predict the parasitic load with good accuracy indicating the need of more samples to tune the algorithm again for better generalization and predictability. There are several limitations to this study. To begin with, this study was a retrospective single-center study. Single-center studies may have significant bias due to insufficient data heterogeneity[25]. To further verify the accuracy and clinical applicability of these prognostic models, more patients from other centers are required. In this case, we emphasize the need for international collaborative cohort studies[26] from all the VL treating centers in the world. This approach can generate generalizable results that can inform better diagnostic models and enhance the VL research evidence.

CONCLUSION

This study shows most patients with VL are living in the disease's endemic regions in Sudan. In addition to peripheral pancytopenia, bone marrow normoplasia and histiocytosis are common. Additionally, common bone marrow changes associated with VL include hypercellularity, megakaryopoiesis, hemophagocytosis, and increased inflammatory cells and eosinophilic precursors. The mean accuracy for predicting the *L. donovani* load is 0.88 for the training dataset, and accuracy of 0.46, 0.50, and 0.74 for mild, moderate, and severe parasite load in the validation dataset.

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FOOTNOTES

Institutional review board statement: We obtained written ethical approval from the Tropical Disease Teaching Hospital administrative authority (TDTH/B/51/1 data: 1st Jan 2021). The information acquired was kept confidential and only utilized in the context of this study.

Informed consent statement: Informed consent statement was inapplicable in retrospective study.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

Data sharing statement: Data are available upon request to the corresponding author.

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