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Gender and Age Stratification in Visceral Leishmaniasis and Diagnostic Test Performance in Kenya

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Abstract:
Background and Objectives
Studies suggest gender and age disparities in infection rates but such data are limited for endemic regions of Kenya. This study aimed to evaluate gender and age disaggregated patterns and diagnostic test performance for visceral leishmaniasis and provide evidence for targeted interventions in endemic areas.
Methods
This study analyzed 396 patients (263 males, 133 females) presenting with VL-like symptoms at Chemolingot Hospital between July 2023 and July 2024. Patients were tested for VL using rk39, DAT, Kalazar Detect, and microscopy was used to resolve discrepancies.
Results
Out of the 396 patients, 117 (29.5%) tested positive for VL, with 105 (26.6%) showing concordant results across all four tests. Among confirmed VL cases, 74.4% were male. Age-stratified analysis revealed that young people below 20 years accounted for the highest diseases burden (43.2% for below 10yrs and 33.56% for ages 10-20). DAT showed a higher sensitivity (95.6%), NPV (98.2%) and LR- (0.05) while rk39 showed a higher specificity (98.9%), PPV (97.1%) AND LR+ (85.3) when compared. Diagnostic accuracy of the tests was 97.2, 96.7, 91.9 for rk39, DAT and Kalaazar detect respectively. Spatial analysis indicated regional vulnerability to VL infections with Silale Ward recording the highest VL burden (44.4%) and Lokis the lowest (2.6%).
Interpretation Conclusion
VL has a marked male predominance with younger population at a higher risk. Diagnostic performance had varied strengths. DAT and rk39 can used together to give high accuracy of results and reduce dependency on Splenic biopsy which is a painful and risk procedure.

Key words: Visceral Leishmaniasis, Gender disparity, Test performance, Anemia.

1. Introduction

Leishmaniasis is endemic in over 99 countries, predominantly found in tropical and subtropical regions where it majorly affects the poor population[1]. The disease is particularly prevalent in the Indian subcontinent, East Africa, the Mediterranean basin, and parts of South America. While leishmaniasis occurs in these areas, its burden varies considerably between regions, with East Africa accounting for a disproportionately large share, while prevalence in the Indian subcontinent has been on the decline[2].

Leishmaniasis in East Africa is spread by an insect vector belonging to genus, Phlebotomus. The distribution of the *Leishmania* vector, and consequently the disease itself, is influenced by a complex set of environmental, abiotic, and biotic factors. These include temperature, humidity, vegetation cover, and proximity to reservoir hosts. These conditions must support all components of the transmission cycle—vector, pathogen, and host[3,4]. Among the most critical factors affecting the spread of leishmaniasis, proper diagnosis and treatment are crucial in determining both disease distribution and morbidity and mortality. Without appropriate intervention, the mortality rate of VL can reach 95–100%, making the lack of proper diagnosis a significant issue[5].

Sex-based disparities in infectious diseases are common and may result from biological, environmental, behavioral, and socio-cultural factors. A systematic review involving 135 clinical treatment trials of visceral leishmaniasis from 1980 to 2019 Concluded that over two-thirds of the VL infections were in male patients. Similar patterns have been reported across Asia, Africa (particularly Ethiopia, Sudan, and Uganda), and South America[6,7].

Visceral leishmaniasis diagnosis is based on three major laboratory methods; microscopy (based on needle biopsy to obtain samples from spleen, lymph nodes or bone marrow) which is assumed gold standard of VL diagnosis, serological (uses blood samples to identify *leishmania* parasites) and immunological techniques (Produce artificial antibodies that the antigen)[8]. Each of the methods has limitations as highlighted by[8].

Visceral leishmaniasis is closely linked to environmental and habitat conditions including temperature, humidity, and vegetation cover that support the survival and proliferation of sandfly vectors and reservoir hosts. The environmental conditions increase human exposure to infected vectors by bringing people closer to sandfly habitats[9]. In Kenya the condition is common in arid and semi-arid areas where the prevailing conditions favor *leishmania* parasite and vector survival. The inhabitants of these regions being pastoralists are prone to exposure to the sandfly vector bites due to prolonged hours spent in the thickets taking care of their animals as well as number of hours spent outside the house due to prevailing temperatures[10].

This study aimed to analyze aggregated data on gender, age, and locality in relation to VL in Kenya. It also assessed the diagnostic performance of various tools used in VL-endemic areas to inform context-specific strategies for reducing disease burden.

2. Materials and Methods

This study analyzed clinical data from all patients presenting with VL-like symptoms—fatigue, prolonged fever, wasting, and hepatosplenomegaly—at Chemolingot Hospital in Baringo County, Kenya, between July 2023 and July 2024. This region is a known endemic area for VL.

Demographic data (including patient age, gender, village of origin) and results of VL (rk39, Direct Agglutination Test [DAT], Kalazar Detect, and microscopy), and malaria tests were collected and analyzed.

Chi-square tests were used to evaluate associations between Age, gender and visceral leishmaniasis infections while Analysis of Variance (ANOVA) was used to examine differences in haemoglobin level across age groups and assess significance of locality differences on visceral leishmaniasis infections.

3. Results

The study involved 396 patients suspected of having visceral leishmaniasis (VL) who attended Chemolingot Hospital in Baringo County. These patients presented with common VL symptoms, including fatigue, fever, body wasting, and hepatosplenomegaly. Each patient was tested for both VL and malaria. Diagnosis of VL was conducted using three tests: rk39**,** Kalazar Detect**,** and the direct agglutination test (DAT**)**. In cases where the results from the three tests were inconclusive or discordant, microscopy was employed as the confirmatory method. Hemoglobin levels were measured in patients who tested positive for VL, with 62.4% classified as severely anemic and 35% as moderately anemic, according to the World Health Organization (WHO) anemia classification guidelines (2011). **Figure 1** and **Table 1** present the key clinical characteristics of confirmed and unconfirmed VL cases.

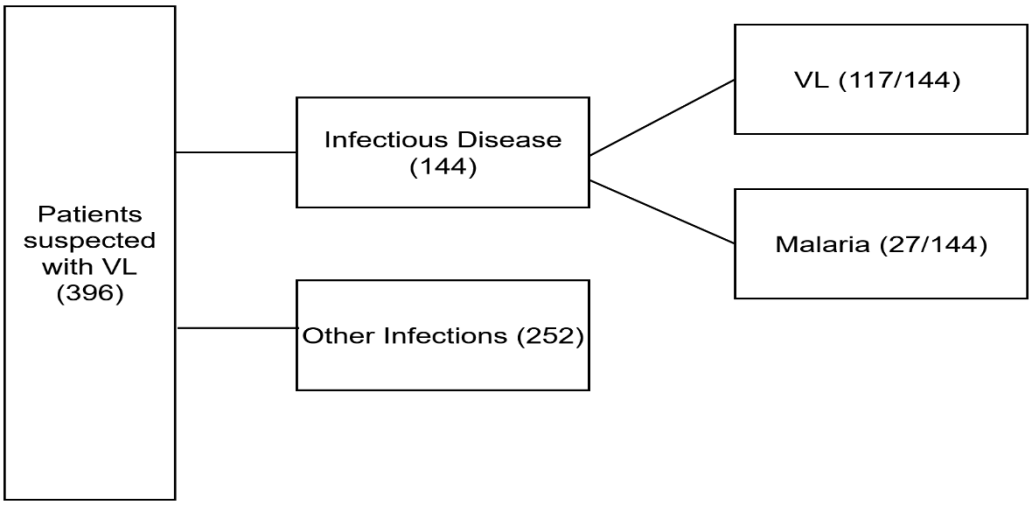

Figure 1:Flowchart showing classified study cases

Table 1: General characteristics of the VL infected study participants

Variable	**Overall (n=117)**	**Hb (0-7.9) (n=73)**	**Hb (8.0-10.9) (n=41)**	**Hb ≥11 (n=3)**
Age groups (yrs)				
0-10	52 (44.4%)	32	21	0
11-20	42 (35.9%)	27	13	0
21-30	16 (13.7%)	9	5	2
31-40	5 (4.3%)	4	1	1
Above 40	2 (1.7%)	1	1	0
Gender				
Male	87 (74.4%)	59	26	2
Female	30 (25.6%)	14	15	1
Location of residence				
Lokis	3 (2.6%)	2	1	0
Loyamorok	22 (18.8%)	10	11	1
Silale	52 (44.4%)	34	17	1
Riokwo	15 (12.8%)	12	3	0
Koloa	8 (6.8%)	6	2	0
Tirioko	10 (8.6%)	7	2	1
Korossi	7 (6%)	2	5	0

The 117 patients were categorized based on the wards within Tiaty Constituency from which they originated. Seven major groups were identified, with Silale Ward accounting for the largest proportion of patients (44.4%), while Lokis Ward had the fewest (2.6%). However, ANOVA results indicated no statistically significant difference in VL distribution across the regions ($p \geq 0.05$). Hemoglobin levels were pooled by age groups to obtain average values, as shown in **Table 1**. The 0–10 age group had the lowest mean hemoglobin level (7.15 g/dL), followed by the 11–20 age group **(**7.28 g/dL), as illustrated in **Figure 2**. Although hemoglobin levels showed a correlation with visceral leishmaniasis ($p \geq 0.05$), no statistically significant differences were observed between the different age groups ($p \geq 0.05$).

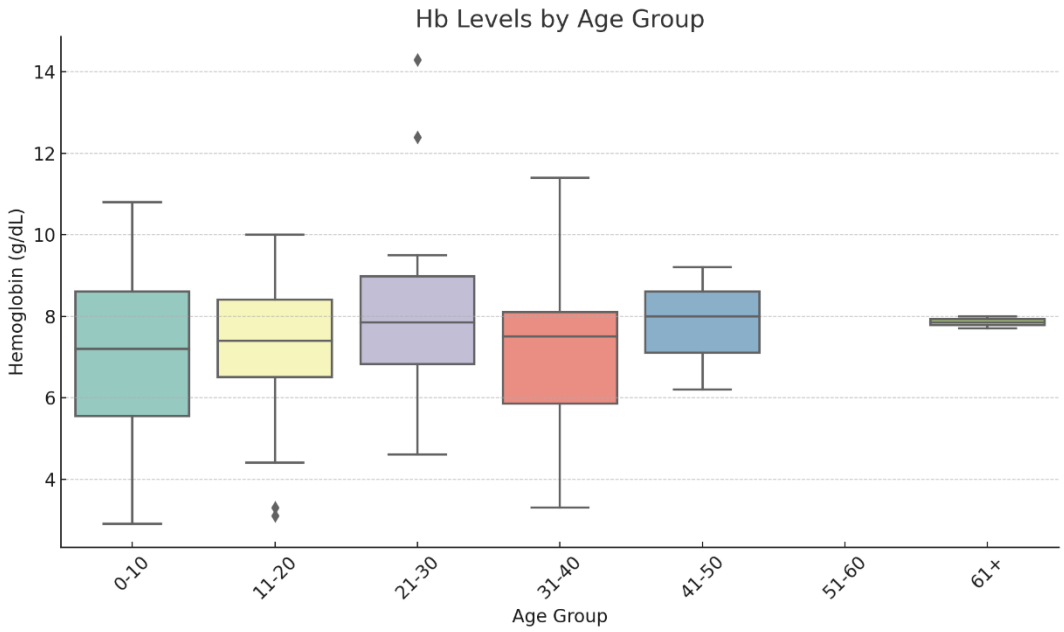

Figure 2: Average haemoglobin level per age group among the infected cases (ANOVA, P=0.808)

To determine the diagnostic utility of the testing methods sensitivities (probability of testing positive when a disease is present), specificity (Probability of testing negative when a disease is absent), positive predictive values (PPV), negative predictive values (NPV), positive likely-hood ratio (LR+) and the negative likelihood ratio (LR-) were calculated as indicated in table 2.

Table 2: Sensitivity, Specificity, PPV, NPV, LR+ and LR- of the diagnostic tools

	Test Result	**VL cases**	**Non-VL cases**	**Sensitivity % [95% CI]**	**Specificity % [95% CI]**	**PPV % [95% CI]**	**NPV % [95% CI]**	**LR+**	**LR-**	**Accuracy %**
rK39	Positive	TP-114	FP-3	93.4(87.5-97.1)	98.9(96.8-99.8)	97.4(92.5-99.2)	97.1(94.6-98.5)	85.3	0.07	97.2
	Negative	FN-08	TN-271							
DAT	Positive	TP-109	FP-8	95.6(90.1-98.6)	97.2(94.5-98.8)	93.2(87.3-96.4)	98.2(95.9-99.2)	33.7	0.05	96.7
	Negative	FN-5	TN-274							
Kalaazar Detect	Positive	TP-90	FP-27	94.7(88.1-98.3)	91.0((87.2-94.0)	76.9(69.9-82.7)	98.2((95.9-99.2)	10.5	0.06	91.9
	Negative	FN-5	TN-274							

Abbreviations: TP= true positive, FP=false positive, FN=false negative, TN=true negative, CI = Confidence Interval, NPV = negative predictive value and PPV = positive predictive value, LR+= positive likelihood ratio, LR-= negative likelihood value.

4. Discussion

4.1 Age, gender and Leishmaniasis

This study found that males account for a significantly higher proportion (74.4%) of visceral leishmaniasis (VL) cases compared to females (25.6%). Notably, 76.71% of infected males were under the age of 20, indicating a heightened risk in young males. These findings are consistent with studies such as Dahal et al., (2021) which suggests an adjusted male to female infection percentages as 53.0% for males and 47.0% in females in India and unadjusted percentages of 61.4% for males and 38.6% for females in Bihar, India.

The gender disparity observed in this study is likely due to greater male exposure to the sandfly vector, particularly in pastoral communities where men and boys are primarily responsible for herding livestock. In Kenya, these regions, typically arid or semi-arid, are characterized by thick bush vegetation and numerous anthills which are ideal habitats for sandfly breeding (Figure 3).

Figure 3: An anthill Chemolingot, Baringo County, Kenya

Beyond the socially constructed roles for males, biological factors also contribute to the gender disparity in the infections. Differences in social lifestyles including access to healthcare, immune responses, and hormonal differences may explain the gender bias[11,12]. Studies also suggest that interaction between the sex hormones (especially testosterone) and the immune effectors may drive the differences in gender susceptibility to leishmania infections [13-15]

A study on juvenile male hamsters observed that, prior to puberty the hamsters were less susceptible to VL than adults, indicating that age and hormonal maturity influence infection risk[12]. Other similar studies have found that males are more prone to infection than the females while infection risk in males is slightly higher in infancy, hence gender susceptibility level by late childhood[14,15].

Other risk factors include the patient age, location of residence, and livestock ownership. In the endemic regions including Kenya, Male children and young men are culturally mandated to take care of the family animals hence spend prolonged periods outdoors herding, often sleeping outside, further increasing vector contact[16]. Environmental changes such as the replacement of grassland with bush vegetation—more conducive to termite activity and anthill formation—have expanded vector habitats[10]. Additionally, reduced school attendance and long distances between homes and anthill zones exacerbate risk a situation familiar with Silale ward-a region with the highest infections in the region[17,18]. Regions where soils are fair for agriculture has allowed farming hence reducing dependency on livestock as well as reducing the presence of termite hills, a situation that has not been fully realized in the rocky regions [10]

4.2 Haemoglobin levels and Leishmaniasis

In this study Haemoglobin levels were classified using WHO classification of 2011 where, values below 7g/dL were considered severe anemia, levels 8-10.9g/dL considered moderate and levels above 11 considered normal. In this study, 62.4% of the patients were severely anemic, 35.0% moderately anemic while only 2.6% had close to normal haemoglobin levels. Children and adolescents aged 1–20 years had the lowest mean hemoglobin levels (7.15 and 7.28 g/dL, respectively)[19].

Visceral leishmaniasis has been associated with low haemoglobin count with over 99.1% of the leishmania positive patients exhibiting haemoglobin levels below thresholds[20-22] Anemia severity correlates with disease progression, parasite burden, nutritional status, and availability of iron supplements[23-25].

Additionally, VL can induce hemolysis through immune and mechanical pathways, releasing extracellular hemoglobin (free hemoglobin) into plasma. Normally, 80–90% of RBCs are destroyed in the liver without releasing hemoglobin. However, in VL, immune-mediated destruction can elevate plasma hemoglobin, affect cellular function and signaling[26-28]. Leishmania has been sen as one of the health conditions that exert mechanical pressure on the patient body leading leakage of haemoglobin to plasma[20,29]. The

leishmania driven haemolysis is due to multiple mechanisms which needs to be considered and managed during VL treatment and management[30].

4.3. Diagnostic performance of Visceral leishmaniasis test

All diagnostic tests assessed in the study exhibited varied strong performance indicators with DAT having a higher sensitivity (95.6%), NPV (98.2%) and LR- (0.05). On the other hand, rk39 showed a higher specificity (98.9%), PPV (97.1%) AND LR+ (85.3) when compared to other tests. Overall, the three diagnostic tests had test accuracy of 97.2, 96.7, 91.9 for rk39, DAT and Kalaazar detect respectively.

These metrics demonstrate the tests' ability to accurately identify positive and negative cases. Sensitivity of a test tool refers to the proportion of true positives while specificity refers to the proportion of true negatives in test samples. PPV is the probability that following a positive test result, the patient will indeed have the infection/condition while NPV is the probability that following a negative test result, the patient will indeed be free of the infection/ condition. Positive predictive value and Negative predictive values give the test being used its clinical relevance. The two variables, unlike sensitivity and specificity, they use the prevalence of the condition under test to determine the possibility of the diagnostic test picking out the specific infection[31-34].

Predictive values are prevalence dependent. As prevalence increases, PPV increases while NPV decreases. In this case, 1% prevalence, PPV may be 8% and NPV 99%, but at 50% prevalence, both values rise to 90%[31,35].

Likelihood ratios provide additional clinical utility. Positive likelihood ratio is possibility of a patient testing positive in a test divided by possibility of a non-patient testing positive in the same test, while negative likelihood is the possibility of a patient testing negative divided by possibility of a non-patient testing negative in the same test. A positive likelihood ratio (LR+) >10 and negative likelihood ratio (LR–) <0.1 are considered strong indicators of diagnostic accuracy[36,37]. A valid test cutoff should be compared to a "gold standard" diagnostic tool. In Kenya, microscopy remains the gold standard, with reported sensitivity of 93–99% and near 100% specificity when using splenic aspirates[38].

4.4 Patient locality and Leishmaniasis

Infection distribution in the study was uneven, with Silale ward accounting for 44.4% of all positive cases, whereas Lokis had only 2.6%. Silale's high burden may be attributed to environmental and socioeconomic factors which are essential in the disease distribution, with decline in grassland cover in North-West Kenya, replaced by bushy vegetation that supports termite proliferation and anthill formation, thereby increasing vector habitats [10]. As residents travel farther for pasture, exposure to sandflies increases. Long distances between homes and anthill areas, combined with outdoor sleeping and herding, exacerbate infection risk, particularly among children. Reduced school enrollment further increases

vulnerability[17,18]. In contrast, agriculturally productive regions have less dependence on livestock and fewer anthills, lowering VL risk[10].

Conclusions

Visceral leishmaniasis is strongly influenced by gender, age, and locality. Young males in pastoralist communities are at greater risk due to socio-cultural roles and environmental exposure. Hemoglobin levels are significantly affected, with high rates of severe anemia among patients.

Although microscopy and rk39 tests are widely used in Kenya, rk39's lower sensitivity and the risk of splenic biopsy raise concerns. DAT used along with rk39 shows promise as a supplementary test especially in rural areas where expertise for splenic biopsy is limited.

Recommendation

Targeted public health interventions should address high-risk groups, promote early diagnosis using reliable tools, and consider environmental factors in vector control strategies. A balanced diagnostic approach incorporating multiple tools (specifically DAT and rk39 in tropical regions) may be preferable given the risks associated with invasive microscopy.

Ethical clearence:

The study was conducted with ethical approval obtained from the Kisii University Institutional Review Committee (Ref No. KSU/ISERC/0009/7/24), Baringo County (Ref No. BCG/HS/RES/VOL.1/2/24) and a permit issued by the National Commission for Science, Technology, and Innovation, Kenya (Permit No. 180763).

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Informed consent:

Informed consent was waived due to the retrospective nature of the study. Ethical clearance by the ethical committee was obtained.

Use of AI:

The authors confirm that AI tools were used in language refinement, grammar correction, and formatting during the preparation of this manuscript. These AI tools were not used in any other step during the development, analysis, or interpretation of findings.

Data availability:

Data collected to support the findings of this study is available from the corresponding author on request.

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