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Review Article

Relapse Kala-azar: A potential threat to sustaining public health elimination in Bangladesh and ways to overcome it

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Abstract

Leishmaniasis is a cluster of zoonotic diseases caused by more than 20 species of Leishmania *parasites. It manifests in three primary clinical forms: visceral, cutaneous, and mucocutaneous leishmaniasis. Visceral leishmaniasis is the gravest form, which is endemic in Bangladesh. Bangladesh experiences the highest number of cases between 1993 and 2005, affecting 45 districts across the country. Over the years, Bangladesh has successfully reduced its burden through the effective implementation of the National Kala-azar Elimination Program (NKEP) since 2008, resulting in the country becoming the first in the world to achieve the historic milestone of a public health elimination certificate from the WHO. At this stage, prevention of transmission and eradication of parasites are the cornerstones in sustaining the public health elimination status. Relapse kala-azar (RKA) patients (single and multiple) are the potential threat to achieving this, as RKA patients act as an archive or repository for the* L. donovani *parasite. This review highlighted the potential risk factors of RKA, challenges associated with RKA, and possible solutions for mitigating outbreaks of RKA. Male gender, younger population, immunocompromised status, shorter regimen of treatment, splenomegaly, cytopenia, malnutrition, and poor socioeconomic status are the common risk factors. Significant challenges to be encountered are asymptomatic carriers, scarcity of parasitological confirmation, availability of drugs, potential drug resistance, and the appearance of cases (refractory KA, Para-Kala azar dermal leishmaniasis) that are difficult to treat. The increasing availability of molecular diagnosis and xenodiagnosis, longer treatment regimens (multidose, combination, and secondary prophylaxis), the availability of drugs, and the development of new molecules may be possible solutions for the effective management of RKA, which is crucial for sustaining public health elimination of KA in Bangladesh. It is now time to update the clinical case management guideline and revise the dose of LAmB to 15 mg/kg in divided doses. The provision of secondary prophylaxis needs to be added in the guideline for treating RfKA and para-KDL cases.*

Keywords: *Relapse Kala-azar, Bangladesh, Refractory Kala-azar*

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Background

Kala-azar (KA), also known as visceral leishmaniasis (VL), is an infectious disease caused by the *Leishmania* parasite when the bite of an infected sandfly transmits it. Leishmaniasis has been recognized as an epidemic worldwide for over a century.[1] The first recorded case of visceral leishmaniasis (VL) in Bangladesh dates back to 1824, and since then, significant outbreaks have occurred in the Indian subcontinent.[2] In 1990, VL contributed to 80.44 disability-adjusted life years (DALYs), ranking second only to malaria.[3] Each year, an estimated 30,000 new VL cases arise globally, with a mortality rate not exceeding 10%, with South Asia and South America accounting for nearly half of these cases.[4,5] Recognizing its impact, the World Health

Organization (WHO) has prioritized VL control as part of its initiatives to address neglected tropical diseases (NTDs).

Over 20 species of Leishmania parasites cause leishmaniasis. The disease manifests in three primary forms: (i) visceral leishmaniasis (VL), commonly known as Kala-azar, which is the most severe form. VL is endemic in Bangladesh, and L. donovani causes it.(ii) cutaneous leishmaniasis (CL), which is the most prevalent, and (iii) mucocutaneous leishmaniasis.[6,7] *L. donovani* manifests as VL in Southeast Asia (Bangladesh, India, and Nepal) and East Africa (Sudan, Somalia, Ethiopia, and Kenya) and commonly affects younger individuals due to the absence of acquired immunity [8] which is transmitted through the bites of infected female *phlebotomine* sandflies.[6] This disease predominantly affects impoverished communities in Africa, Asia, and Latin America, where malnutrition, displacement, inadequate housing, weak immune responses, and limited healthcare resources exacerbate its spread.[7,9]

VL has been a major public health concern in Bangladesh for decades. Before the late 1970s, VL occurred sporadically in Bangladesh. Between 1981 and 1985, cases were reported in just eight upazilas (sub-districts); however, by 2004, the disease had spread to 105 upazilas over 25 years. The epidemic reached its peak between 1993 and 2005, with reported cases rising from 3,978 in 1993 to 8,505 in 2005. While VL was once present in 45 districts, the number has since declined to 26.[10] Over the years, Bangladesh has successfully reduced the VL burden (Figure 1), achieving the national target of fewer than one case per 10,000 population. By 2016, the incidence rate had decreased to 0.14 cases per 10,000, and by 2023, only 72 new cases and one death were reported. The nearly zero mortality rate is attributed to the successful implementation of the National Kala-azar Elimination Program (NKEP), which aligns with the WHO/SEARO Regional Strategic Plan for Kala-azar Elimination (2011-2015).[11] The program emphasizes early diagnosis, effective case management, and integrated vector control strategies. The map (Figure 2) illustrates the endemicity status of VL in Bangladesh in 2023. In 2014, the incidence exceeded 2.5 cases per 10,000 population in Trishal and Fulbaria upazilas of Mymensingh district; however, by 2015, only Fulbaria remained hyper-endemic.

Achievement of historic milestone of public health elimination of leishmaniasis from Bangladesh

Elimination of a disease is a hallmark of global public health, requiring the convergence of biological, political, and socioeconomic factors. In 2005, the WHO determined that several of these factors were conducive for launching a VL elimination campaign in southeast Asia, specifically in the countries of Bangladesh, India, and Nepal. The VL elimination target was defined as the reduction to less than one case for 10,000 inhabitants in these countries by 2015. In order to that, in 2005, Bangladesh, Nepal, and India signed a Memorandum of Understanding to eliminate VL from the Indian subcontinent by 2015. Between 2007 and 2021, VL cases in these countries declined by more than 95%.[13,14]

On October 31, 2023, the WHO declared Bangladesh the first country to successfully eliminate VL as a public health problem. This validation followed three consecutive years

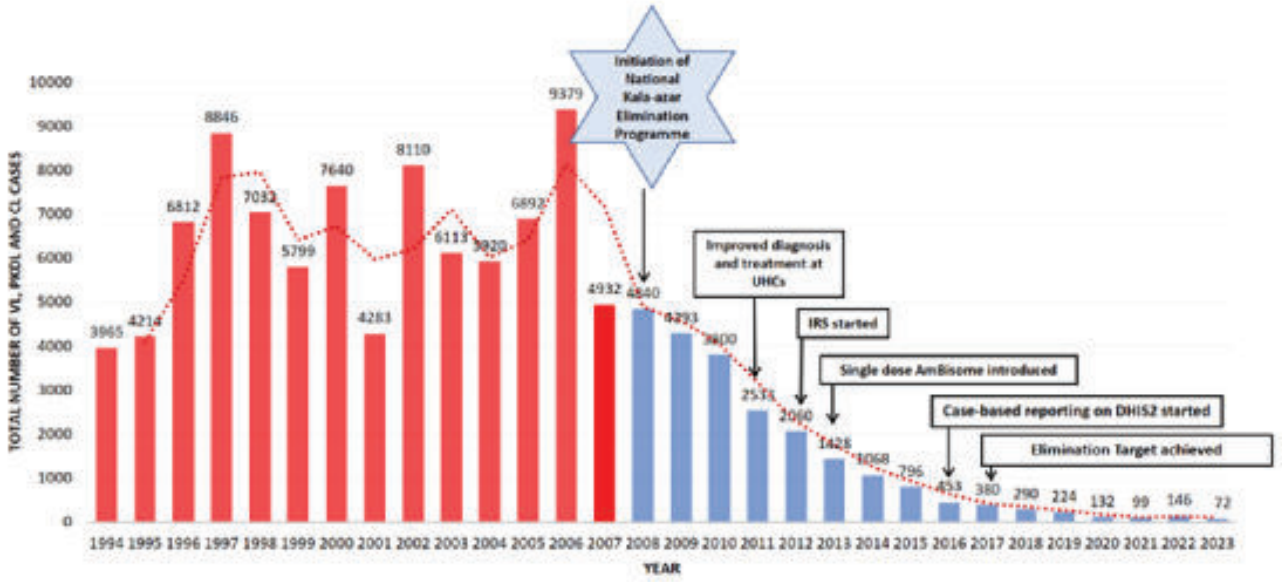

Figure 1: Current situation and historic progress of KA elimination in Bangladesh [12]

of reporting fewer than one case per 10,000 population. In 2022, Bangladesh recorded only 47 VL cases, while the entire WHO South-East Asia Region reported just 1,069 cases, the lowest ever documented. Bangladesh's success in controlling VL dates to the early 2000s, with support from WHO-endorsed integrated vector management (IVM) strategies under the Global Vector Control Response.

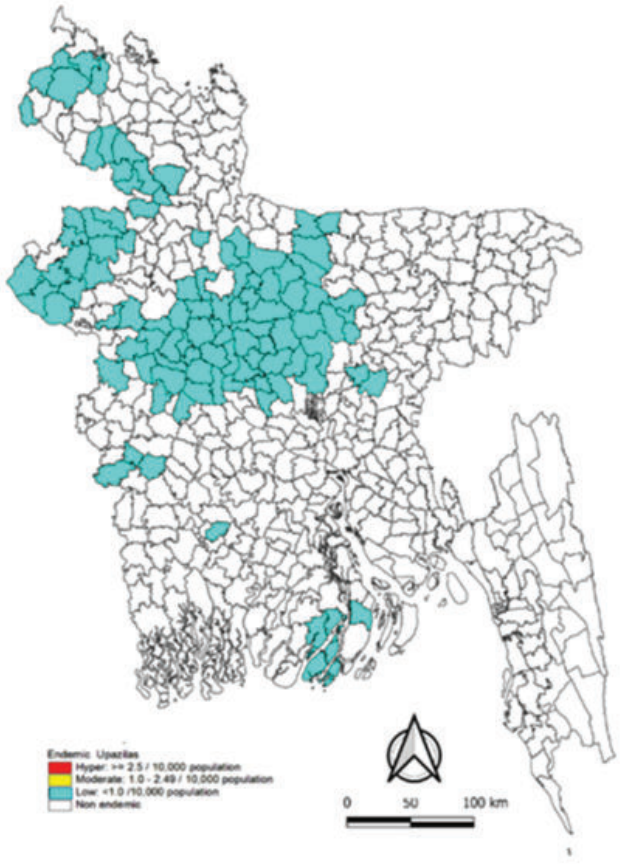

Figure 2: Endemicity of Kala-azar in Bangladesh in 2023[12]

Relapse Kala-azar: An archive for the *L. donovani* parasite

After achieving the target of eliminating new cases in Bangladesh, the main challenge now is to sustain it by preventing transmission and eradicating the parasites. Relapse kala-azar (RKA) patients pose a potential threat to stopping transmission and eliminating the parasites. RKA patients act as reservoirs for the L. donovani parasite. To prevent transmission and eradicate the parasites, preventing and treating KA relapses is essential. Therefore, understanding the risk factors related to VL relapse is crucial.

Early diagnosis and treatment prevent severe clinical manifestations and reduce death, especially in VL, which is fatal if left untreated in over 95% of cases. Diagnosis is made by combining clinical signs with parasitological or serological tests in VL. Chemotherapy is the main method for controlling the parasite in the host; however, the available treatment options are quite limited in Bangladesh, currently restricted to two drug lines: liposomal amphotericin B (L-AmB) and miltefosine. The treatment regimen (duration, dose, and schedule) is associated with treatment failures (TFs) or clinical relapses of VL. Treatment failures (TFs) and VL relapses are common. TFs occur when pharmacological therapy fails to eliminate the infection and, as a result, does not cure the disease. Conversely, cases where clinical signs and symptoms reappear after the end of treatment are referred to as relapses.[16]

Possible risk factors for RKA

Achieving complete parasite clearance in VL patients is uncommon, even with advanced treatments. It is believed that patients with a competent immune system, when treated successfully with anti-leishmanial drugs, develop an effective, lifelong cellular immune response that suppresses any remaining parasites. Relapses are more frequent in immunocompromised patients 16, such as those with HIV/AIDS, long-term steroid use, or chemotherapy. Co-morbidities like diabetes mellitus (DM), chronic liver disease (CLD), chronic kidney disease (CKD), malnutrition, anemia, and tuberculosis may also weaken the immune system. However, it is still unclear whether these conditions directly contribute to VL relapse.

Some demographic factors linked to relapse include male sex, age under 15 or over 45 years, low socio-economic status, a history of previous relapse, and the location of treatment (e.g., treatment camp vs. hospital). Studies have shown that male sex was a potential risk factor for seroprevalence, seroconversion, and incidence of new and relapses of VL.[18,19] These differences may be due to social and behavioral factors, such as increased exposure to areas of VL infection and lower health service-seeking behavior, or biological factors related to male sex, including the role of hormones in modulating the immune system, which may contribute to sex differences in the pathogenesis of leishmaniasis. Studies generated evidence that testosterone was associated with increased *L. donovani* uptake by macrophages, thereby expanding the infection rates and levels of these cells in vitro, which suggested that this hormone had a direct influence on increasing the level of infected cells.[18,19] The age group with the highest risk of VL relapse was children under 15 years. Age was identified as a significant risk factor for VL and was associated with a 5–10 times higher risk of poor treatment response in childhood than in adults.[16,20] The clinical outcome of 1016 VL patients treated with miltefosine was assessed in a

prospective trial conducted at health facilities in India and Nepal. Relapses were found to be two to three times more common in the 0–15 year age group than in the adult patient groups.[21]

Numbers of clinical factors also found to be responsible for VL relapse, such as- a short duration of symptoms before seeking treatment (<4 weeks), splenomegaly and tardy spleen size reduction after treatment (<0.5 cm/day), lymphadenopathy, leg oedema, anemia, thrombocytopenia, and the treatment regimen history, may also be associated with relapse.[9,18,22] Splenomegaly and lymphadenopathy were associated with an increased risk of VL relapse.[23,24] Leg edema, which is an indicator of malnutrition, was found to be an independent predictor of relapse in studies done in Brazil.[25,26]

The treatment regimen has a significant relation with VL relapses. Older drugs like sodium stibogluconate (SSG) and antimony compounds were related to higher relapses. A cohort study conducted in Bangladesh found that multidose LAmB (15 mg/kg BW divided into three doses) had the lowest incidence of VL relapse compared to all other treatment regimens except SSG in a 4-year follow-up period.[17] Freire et al. and some other similar studies showed that VL relapse is significantly higher in patients treated with LAmB.[16,27,28] The risk of VL relapse is lower (1.8%) in patients who received a 15 mg/kg dose of LAmB compared to a 10 mg/kg single dose (8.4%). The lower relapse rates observed at higher doses of LAmB may be due to the increased drug exposure and higher tissue concentrations achieved with higher dosages. It was also found that with LAmB, for every 1 mg/kg increase in dosage, the odds of relapse decrease by 19% [95% CI: 9%-28%].[29] Low blood cell counts (anemia, thrombocytopenia) have also been identified as risk factors for VL relapse.[28,30,31] It is reasonable to assume that these variables measure either the intensity of the splenic sequestration of blood cells or the bone marrow's ability to recover. Interestingly, while some authors identify delay in diagnosis as a predictor,[28] a robust Indian study found that subjects with a shorter interval from symptoms to therapy were more likely to relapse.[18] The possible explanation for this is unknown.

Susceptibility to relapses due to impaired cellular immunity

Since leishmaniasis is an intracellular infection, the cellular immune response plays a crucial role in disease progression, cure, and relapse. However, little is known about the specific cellular immune response associated with the treatment and cure of VL. The few studies that have examined the systemic immune response in Sudanese patients with PKDL have revealed that, when lesions are present, peripheral blood mononuclear cells (PBMCs) proliferate and produce IFN-γ and IL-10 in response to leishmanial antigens, indicating a mixed immune response responsible for both skin inflammation and the persistence of *Leish-mania.*[32–34]

The generation of protective T cell responses are necessary to control Leishmania infections. CD4+ T helper (Th) cells orchestrate immune responses in leishmaniasis and IFN-γ+ Tbet+ CD4+ T (Th1) cells are required for the activation of phagocytes to kill captured or resident parasites. In contrast, other Th cell subsets, including FoxP3+ natural regulatory T cells and Th2 cells, can promote disease progression by suppressing the activities of Th1 cells.[35]

A Sudanese study revealed that a Th1/Th2/Th17 response was associated with a higher cure rate in PKDL. Patients with low IFN-γ, TNF and IL-1β before treatment are more likely to relapse. Determining IFN-γ, TNF, and IL-10 levels before treatment could help predict patients at higher risk of relapse/recovery from PKDL.[36] Lindoso et al. in a study showed that, risk of relapse and lethality was more amongst VL patients coinfected with HIV, which was determined by the preponderance of the immune response, and mainly by the CD4 + T lymphocyte count.[37]

In a review of T-cell responses in human leishmaniasis by Kharazmi et al. it was described that the outcome of human leishmaniasis infection depends on the activation of one of the two subsets of CD4+ T-cells (Th1 & Th2). The subdivision of CD4+ T-cells is done according to the cytokines they produced. IFN-γ and IL-2 are produced by Th1 cells, on the other hand, Th2 cells produce IL-4, IL-5, and IL-10. Humans typically develop immunity to leishmaniasis, which is dependent on CD4 cells with a Th1-type cytokine profile, such as IFN-γ. IFN-γ activates macrophages to kill the parasites. The killing is more effective in infections with *L. major* in patients with CL. However, macrophage killing in VL takes place less effectively; therefore, there is an increased risk of relapse.[38]

Several authors described that, in human leishmaniasis, T-cell responses are crucial for controlling the infection, with both CD4+ and CD8+ T-cells playing distinct roles. CD4+ T cells, particularly Th1 cells, play a key role in activating macrophages to kill the parasite and promoting a Th1 response. Additionally, CD8+ T cells can also contribute to parasite control and Th1 development. However, in chronic infections, T-cell exhaustion can lead to impaired T-cell function and increased susceptibility to the disease.[39] Several studies illustrated the role T-cells play in controlling VL and preventing VL relapses.

Genetic susceptibility of the host in the development of RKA

Genetic factors play a significant role in the development of RKA, particularly in the development of PKDL. Several genes and genetic variants, including those related to immune response and parasite interactions, have been identified as influencing the risk of relapse.

MBL2 Gene**:** A particular variation in the MBL2 gene's promoter region (78th position) is linked to a lower risk of developing KA. This implies that some MBL2 variations might provide protection against the initial infection and possibly reduce the risk of RKA.[40]

SLC11A1 (NRAMP1) Gene: Increased susceptibility to KA has been associated with polymorphisms in the SLC11A1 gene, which codes for a protein implicated in macrophage activation. Mutations in this gene may cause relapse by reducing macrophages' ability to clear the parasite from host tissues.[41]

IL-2 Receptor β chain gene (IL2RB): One promising gene for KA control is the IL2RB gene, located on chromosome 22q12. Mutations in this gene may affect the host immune response to the parasite, which may increase the risk of relapse.[41]

Interferon gamma (IFN-γ) Receptor Polymorphism: According to specific research, IFN-γ receptor polymorphism is related to the development of PKDL. This mutation may contribute to relapse by reducing susceptibility to IFN-γ, an essential cytokine in the immunological response to Leishmania.[42]

Alkylglycerol Monooxygenase Gene (AGMO): A recent finding demonstrates an association between AGMO mutations and kala-azar relapses.[43]

Reinfection or persistence of the parasite, which is responsible for relapses?

Many infectious diseases, such as viral infections, protozoan disorders, and tuberculosis, are characterized by the pathogen's persistence or reinfection following medical treatment.[44] In endemic areas, relapses of VL occur due to persistence of the parasite after the disappearance of the clinical features following treatment. While some cases involve the reactivation of a previously dormant infection, others may result from a new infection with the same or a different Leishmania species. To determine whether it is due to parasite persistence or reinfection, a method that utilizes genomes and proteomics to describe and compare the parasite in RKA patients with the parental type is employed. Most of the studies postulated that relapse is due to persistence of the parasite.[45,46] However, a few studies found that reinfection also occurs.[47] To eliminate, it is very crucial to identify whether it is persistence of the parasite or reinfection.

Challenging issues with RKA in sustaining VL elimination in Bangladesh

The persistence of a significant number of PKDL and RKA patients (many with multiple relapses) and ongoing transmission, as evidenced by new infections, threatens the sustainability of the post-elimination phase. Emphasis should be given to eliminating the pathogen to prevent the resurgence of VL epidemics—several challenges must be addressed to sustain the elimination status.

a. **Asymptomatic carrier:** Studies show large asymptomatic carriers of VL parasites among humans and other vectors such as dogs and sandflies.[48] Studies in northeastern Brazil found concerning levels of leishmania infection among asymptomatic blood donors. Similar findings were also found in Bihar, India.[49,50] In Bangladesh, such reports are lacking, but the delay in diagnosis of RKA makes them carriers and is crucial for transmission. KA is prevalent among socially poor and marginalized populations. This group of the population typically seeks healthcare support at a late stage. Late diagnosis of RKA exposed them as a source of the leishmania parasite.

b. **Parasitological confirmation of RKA:** Diagnosis of RKA is confirmed by detecting parasites in tissue samples from affected sites such as bone marrow, spleen, liver, blood, lymph nodes, or skin. Microscopic examination of aspirates is the traditional confirmatory test for VL, with bone marrow and spleen aspirates being the most used, despite their invasiveness. The presence of amastigote forms of the parasite, typically seen inside mononuclear phagocytes, confirms the diagnosis. Although the sensitivity varies (93–99% for spleen aspirates and 53–86% for bone marrow), it remains an essential diagnostic method.

   Molecular techniques such as PCR and culture can enhance sensitivity and provide species identification, especially in complex cases.[51] Immunological tests like the rK39 immunochromatographic test (rK39 ICT) is used for screening, but it is not useful in RKA.[52,53] PCR-based tests offer high sensitivity and specificity, particularly for HIV/Leishmania co-infected patients, and hold promise for monitoring disease progression during treatment. However, PCR remains costly and requires advanced infrastructure, limiting its routine

application.[54–56] Although qPCR is now more accessible, the cost of reagents and technology required for DNA extraction and/or amplicon detection limits the use of PCR for routine VL diagnosis. Recently, loop-mediated isothermal amplification (LAMP)[57] has become available in other countries (not in Bangladesh) and is successfully employed in the diagnosis of VL and PKDL. It is rapid, simple, and peculiar. Diagnosis of KA using peripheral blood in Asia and Africa showed high sensitivity (>90%) and excellent specificity, with >90% sensitivity and specificity.[57,58]

c. **Treatment of RKA- achieving parasite clearance with available drugs**: At present in Bangladesh, only two drugs are available for the treatment of VL, these are liposomal amphotericin B (LAmB) and miltefosine (MF). Paromomycin is the other drug indicated for VL treatment available in India. For new kala-azar (NKA) monotherapy (either LAmB 10mg/kg single dose or MF 2.5 mg/kg/day 28 days) is recommended. A combination of two of the three available drugs is recommended for treating RKA. In Bangladesh, LAmB 5 mg/kg as a single dose on the first day and MF 2.5 mg/kg/day orally from the second day to the eighth day are used for treating RKA. Since there is no vaccine for VL, chemotherapy is the only method of controlling the disease. There are few satisfactory therapeutic options for VL because of issues with efficacy, side effects, rising drug resistance, high expense, and the requirement for hospitalization to complete the entire course of treatment. To preserve the effectiveness of the few anti-leishmanial medications already on the market and to optimize treatment regimens, clinical trials have been conducted in this subcontinent so far.

d. **Treatment failure and drug resistance**: Treatment of RKA cases is challenging because patients usually present in a late and advanced stage. The primary determinant of the treatment outcome of RKA is the interaction between drugs, the parasite, and the human host.[59] According to recent reports, the Indian subcontinent is experiencing a number of SSG resistance mechanisms, such as reduced drug uptake because of decreased expression of aquaporins in various experimental models, loss of metal reduction, overexpression of thiol metabolism enzymes, and multidrug-resistant transporters.[60,61] Despite being uncommon, LAmB resistance has been shown to alter the sterol profile by replacing ergosterol in the parasite's membrane with its precursor, cholesta-5,7,24-trien-3-ol, which lowers the drug's affinity. Resistance phenotypes have been directly linked to extrachromosomal DNA amplification.[62] Emerging resistance against miltefosine is a matter of serious concern as it is the only available oral antileishmanial drug. Incomplete treatment and the long half-life of this drug in circulation have been thought to be one of the factors driving the parasite's machinery for adaptability against drug-induced stress. Its precise mechanism may be attributed to mutations in P-glycoprotein-LdRos3 and LdMT that are specific to particular alleles.[63] Significant drug resistance to LAmB and miltefosine has become more prevalent, especially in endemic areas where outbreaks are occurring.[64] Even though these drugs have shown clinical effectiveness in curing the condition, total parasitological eradication is still needed, indicating that the disease persists in the community. Although it is not that very common, in some regions of India, resistance of the parasite to antimonials as well as MF and LAmB has been documented.[60] The outbreak of drug resistance and its dissemination can significantly impact chemotherapy-based VL control campaigns, as seen in malaria. Treatment failure and the establishment of drug resistance are believed to be primarily caused by incomplete treatment, either in terms of drug regimen or duration.[65] This issue sparked calls for pharmacovigilance, ongoing drug development, and the relevant policymaking to track drug resistance.

e. **Refractory (difficult to treat) KA (RfKA) cases:** Some of the RKA who experienced three or more relapses are not responding with conventional treatment. This group of patients is refractory to standard treatment[66]. This poses a significant challenge for sustaining public health elimination status in Bangladesh. There is a growing concern about the increasing number of RfKA cases that are unresponsive to standard therapy. RfKA can lead to prolonged illness, increased risk of relapse and transmission, and potentially higher mortality if not managed effectively.[66] They may require an alternative and potentially more complex treatment regimen. There are reports of giving monthly secondary prophylaxis for such cases with good outcome.[67]

Prospects

Despite significant scientific advancements in the field of leishmaniasis over the last decade, including the genome sequencing of several pathogens that cause distinct forms of leishmaniasis, the quality of clinical care for VL in the field has not yet been improved by these advancements, as there are currently very few antileishmanial medications available for treatment. With the current background information, the

following advancement can play an essential role in sustaining VL elimination status.

a. **Increasing availability of molecular diagnosis:** Among the molecular methods, PCR has been categorized as the most promising technique for the clinical diagnosis of PKDL. The detection of Leishmania DNA is possible in various clinical samples, and maximum sensitivity can be achieved by multicopy PCR amplicon.[68] Several genes, including ribosomal RNA, kinetoplast DNA (kDNA), mini-exon-derived RNA (med RNA), β-tubulin, and gp63, have been extensively targeted for PCR-based diagnosis of VL. Furthermore, PCR-based methods can detect Leishmania parasites in patients with low levels of parasitemia and even a few weeks before the appearance of clinical symptoms. Specimens like skin, peripheral blood (mostly buffy coat), splenic aspirate, bone marrow, or lymph node aspirates can be used for diagnosis.[69] Quantitative real-time PCR (qPCR) allows continuous monitoring of the patient's response to therapy. The real-time PCR assay is a reliable and noninvasive tool for the diagnosis of VL. qPCR is more sensitive than microscopy, but it requires a well-equipped laboratory.[70] Real-time PCR, or qPCR allows detection and quantification of the parasite number, thus playing an essential role in monitoring prognosis and parasite clearance.[55]

b. **Xenodiagnosis:** Xenodiagnosis is the only recognized proof for reservoir infectiousness, which consists of feeding lab-reared sandflies on the presumptive reservoir, resulting in the infection in the fly.[71,72] Several xenodiagnosis studies performed over two to three decades lead to the finding that PKDL patients constitute a crucial interepidemic reservoir of Leishmania. In the scope of local elimination of VL, Mondal and colleagues presented quantitative data on the significance of PKDL patients as potential reservoirs by reporting the results of xenodiagnosis from 47 PKDL and 15 VL patients.[73] Although Bangladesh achieved VL elimination status, RKA stands as a major obstacle as it is a major source for transmission of the parasite.

c. **Immunology and RKA:** The disease onset, appearance, and self-healing properties greatly vary depending upon the intensity of immune responsiveness and the region to which the patient belongs. VL is an immunological disease where innate as well as adaptive immune cells undergo dysfunctions. It is already described that T-cells are a crucial determinant of immunoregulation during early immune responses; therefore, the immunotherapeutic strategy aiming towards reinvigorating the T-cell potency needs further attention.[74]

   Use of a monoclonal antibody-based approach targeting the immune checkpoint molecules and/or immunoregulatory cytokines can be another avenue that can open possibilities for therapeutic intervention. Immunomodulatory therapy aims to relieve the immunosuppressive microenvironment and instigate protective Th1 responses that can be used together with chemotherapeutic regimens during VL treatment to prevent the emergence of relapses. Further immunological research is required urgently including the multiple relapse cases in Bangladesh to have a better understanding of the problem.

d. **Omics in RKA:** Recent developments in high-throughput technologies have transformed biomedical research, particularly in omics tools. Proteomics, metabolomics, and genomes working together create a pathway to a deeper comprehension of illness origins and functional consequences. Leishmania's phenotypic plasticity has been examined using genomic, proteomic, and metabolomic methods, and the results have given us a wealth of knowledge regarding its critical function in dictating the course of infection. Understanding the complexities of host-pathogen interaction through omics research and bioinformatics could help improve the effectiveness of current treatment plans and comprehend the transmission cycle.[75,76] The therapeutic intervention of VL will benefit greatly from the use of omics techniques to improve the pharmacological characteristics and phenotypic screening of future candidate medications. Despite the potential therapeutic benefits of omics technologies, their increased cost prevents them from being used effectively in drug discovery.

e. **Therapies:** The increased cost of drug discovery and development limits its application in the field of neglected tropical diseases; therefore, judicious use of existing drugs remains the need of the hour. In this context, combination therapies have emerged as a viable option for preventing the emergence of drug-resistant parasites, improving drug efficacy, and potentially reducing drug toxicities.

LAmB is the first choice of treatment in Bangladesh. It was given as a 10 mg/kg single dose for treatment of NKA and in combination with MF for the treatment of RKA. Several clinical trials conducted in Indian subcontinent demonstrated that LAmB causes substantially less toxicity than other conventional amphotericin B formulations. It can be used in

very high doses such as 40mg/kg in divided doses. LAmB has a concentration-dependent activity against the parasite, where higher concentration of drug results in greater parasite load reduction and longer persistence of elevated drug levels in liver and spleen.[31] Therefore, increased parasite clearance can be achieved by using increased doses of LAmB. Furthermore, for refractory cases secondary prophylaxis with low doses of LAmB found effective in curing diseases.[67] The relapse cases we have experienced in recent years are all single dose LAmB treated patients. Therefore, it is now high time to update the clinical case management guideline and revise the dose of LAmB to 15 mg/kg in divided dose. The provision of secondary prophylaxis needs to be added in the guideline for treating RfKA and para-KDL cases.

Paromomycin (PM) is a broad-spectrum aminoglycoside antibiotic with activity against a variety of Gram-positive and negative organisms, mycobacteria, and protozoa. The anti-leishmanial activity of paromomycin was first demonstrated in the 1960s. Several studies have reported that paromomycin, when used as a single agent or in combination with other anti-leishmanial drugs, is highly efficacious and well-tolerated in the treatment of VL. High efficacy rates for paromomycin (IM) injection (dose: 11 mg/kg for 21 days) have been reported, with an initial cure rate of 98.4% and a final cure rate of approximately 94.6%.[77,78] The availability of PM will add significant strength in sustaining VL elimination status in Bangladesh. Sitamaquine is another oral drug, developed by the Walter Reed Army Institute of Research (WRAIR, USA) in collaboration with GlaxoSmithKline (UK), following the development of miltefosine. Clinical trials using this drug have been completed in India, Kenya, and Brazil with a cure rate ranging from 27 to 87 %.[79] Different case management guidelines now recommend multidrug therapy for VL for several reasons: to shorten the duration and cost of therapy, slow the emergence of parasite resistance, as the mode of action of the drugs will be different, and increase the efficacy rate, even in cases of coinfection.

Conclusion

In the absence of an effective vaccine, control of VL is exclusively dependent on chemotherapy. The available drugs are costly and may require hospitalization, which involves monitoring, causing substantial loss of income for affected families. The emergence of drug resistance further complicates the treatment of disease. All follow the development of RKA and refractory diseases. Therefore, effective treatment of RKA and close follow-up are crucial to sustain VL elimination status in Bangladesh.

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