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REVIEW ARTICLES

CURRENT DIAGNOSIS AND TREATMENT OF KALA-AZAR: BANGLADESH PERSPECTIVE

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Introduction:

Visceral Leishmaniasis or Kala azar is a deadly disease putting 350 million people from 88 countries at risk with an annual new case burden of 1.5 to 2 million and the number is rising.[1] Of all the cases, 90% occur in India, Bangladesh, Nepal, Sudan and Brazil. These countries have one thing in common, a substantial number of ultra poor population living substandard lives. This allows the breeding of the vector 'sand fly' (*Phlebotomus argentipes*) in the crevasses of the mud house built as shed for the cattle or dwelling of the family. Lack of awareness and economical means to protect them selves from the bite of the insect allows the leishmania parasite to enter its human host. Upon entry it invades the immune system destroys RBCs and if the situation is favorable gradually proceeds towards termination of its host.

Previous epidemiological studies show that 34 out of 64 districts of Bangladesh reported kala azar cases but 90% of them are from 10 districts.[2] The cumulative reported incidence of kala azar by district from 1994 – 2004 shows that the worst hit area in Bangladesh is Mymensingh followed by Pabna, Tangail, Jamalpur, Sirajganj, Gazipur, Natore, Naogaon, Manikganj, Rajshahi and Naawabgaj.[2] The first case of kala azar was described in Jessore district in 1824[3,4] but at present it is no longer among the list of the endemic districts. During the period 1994 to 1996 Pabna reported the highest number of cases every year but gradually it was overtook by Mymensingh, this district accounted for more than 50% of the total kala azar cases in Bangladesh reported between 2000 – 2004.[5] Kala-azar lymphadenopathy has also been recently reported in Bangladesh though it was previously thought to be nonexistent in this region. In India the state of Bihar accounts for more than 90% of the kala azar cases followed by West Bengal and Uttar Pradesh, while in Nepal the cases are mostly from the Terai region.

Diagnosis:

According to the National Guideline and Training Module for Kala-azar elimination in Bangladesh cases are suspected clinically when a patient presents with fever for more than 2 weeks from an endemic area along with one or more of the following feature, a) splenomegaly, b) anaemia and c) weight loss.[6] In a study of key clinical features of VL in Bangladesh conducted on 273 patients it was found that 81% were below 30 years of age, fever was present in 91% with double peak/ triple peak of temperature in 39%. Splenomegaly and hepatomegaly was found in 99% and 92% (Jaundice ~2%) patients respectively and anaemia was evident in 97%.[7] Ahasan HAMN et al. also demonstrated that the duration of the illness fairly correlates with the splenic size[8] making it an important marker both in clinical diagnosis and a method of assessment of treatment response.

The National Guideline recommends that the suspected kala-azar case be confirmed by a positive rK39 or demonstration of parasite in the tissue (bone marrow/ splenic puncture) or by PCR.[6]

Demonstration of the parasite from splenic aspirate, bone marrow or buffy coat of blood is the most reliable and conventional method of diagnosing kala azar. The gold standard is splenic aspirate smear which has a sensitivity of 95%.[9] Standard procedure as recommended by World Health Organization (WHO) for splenic aspiration requires the spleen to be palpable at least 3 cm below the costal margin on deep inspiration. A study in Bangladesh demonstrated the performance of splenic aspiration through the intercoastal space allowing a smear from a non palpable spleen in suspected kala azar case and there to the smears were postive.[10] In another study in Rajshahi, Bangladesh 92% sensitivity was demonstrated with splenic aspirates in comparison to 64% for bone marrow.[11] Other than that bone marrow aspiration is quite painful while splenic aspiration involves the risk of hemorrhage.

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The Aspirates can also be used to culture leishmanian protozoas in NNN media (1% defibrinated Rabbit blood + salt azar + Pencillin) and it takes about 1-3 weeks to grow. Other medias include, Grace's culture media (Growth occurs within 72 hours) and Sneader's culture media. Culture is costly, time consuming and requires sophisticated laboratory settings, so not a useful diagnostic tool.

Aldehyde test has long been a screening method at the field level. The test has very high sensitivity near about 94% but a very low specificity as tested in Bangladesh by Chowdhury MAJ et al.[12] CFT has almost a same result compared to aldehyde test as found by the same group[12]. On the other hand counter-immunoelectrophoresis (CIE) tested by Masum MA et al has a 93.7% sensitivity with a 100% specificity[13] and there for can be a handy tool at the peripheral laboratories and for epidemiological studies. Direct agglutination test for kala-azar is a cheap, sensitive and specific test. It is quite popular in Bangladesh and Africa. Though its drawbacks include batch to batch variation, instability of the antigen, need for incubation and cumbersome procedure.[9] The recent introduction of ICT based rk-39 antibody test has rapidly gained popularity in Bangladesh. Provision of carrying out a rapid test in a field based situation with a 100% sensitivity and 93%-98% specificity[14,15] is the corner stone behind the tests popularity. Like any other antibody based test, rk-39 has the problem of remaining positive long after the disease process is over and there for cannot be used to detect relapses. In one study it was found that the IgG antibody remains positive for > 2 years but < 4 years[16,17] the same study used rK39 as a kit to diagnose asymptomatic cases, 69% of whom eventually went on to develop kala-azar.[16] So it was recommended as a tool for surveying families and to take prophylactic measures. A new antigen based test is being developed and is currently under trial in India, Nepal, Sudan and Brazil. This latex based agglutination test (KATEX) identifies the antigens in Kala-azar patients' urine.[18]

PCR based diagnostic tools are highly sensitive and specific but the cost has kept it away from the poor countries who are the victim of the disease. Recent advances have allowed the Nested PCR to be performed from blood (buffy coat) instead of splenic or bone marrow aspirates.[19] Leishmania kDNA or mini-exon has previously been amplified from blood samples for kala-azar diagnosis in India but the results were not satisfactory as it was lower than that from splenic samples.[20,21] This process is less painful and with almost no risk as the invasive procedure of splenic or bone marrow aspiration can be avoided. Along side the circulating parasites can be monitored.

Treatment:

Sodium stibogluconate (SSG) has long been the corner stone of treatment for kala-azar. Documents show its use since 1940 in India. The current recommended dose is 20 mg/kg/day (MKD) for 28 days was endorsed by WHO in 1990.[22,23] In Northern Bihar of India there is a steady rise in resistant to this regime of SSG.[24] In Darbhanga and Sitamarhi districts two villages showed 100% resistant to SSG in WHO recommended dosage. The treatment of relapse cases as suggested by WHO is 20 MKD for 40 – 60 days. In India the current recommendation is to use SSG at a dose of 20 MKD for 40 days as shown by Thakur CP et al in their large scale clinical research.[25] Unfortunately a good number of patients are unable to tolerate the drug at this dose and mortality was found to be 12%.[26] The National Guideline in Bangladesh recommends a dose of 20 MKD for 30 days to be administered through the intra-muscular (IM) route in case of treatment failure with miltefosine or when miltefosine is not available.[6] WHO recommends IM administration of the drug instead of intra-venous (IV) route to avoid the possibility of cardiovascular collapse. In one study in Bangladesh Ahasan HAMN et al. found 27 deaths out of 553 patients during treatment of SSG given IV at recommended dose[27]. The authors inference were 8 of the deaths were attributed to associated disease, 12 to hemorrhagic condition and 7 were sudden deaths, so 19 deaths were related to the treatment.[27] In another study bleeding manifestation was 5.7% prior to SSG therapy and 16.1% during treatment.[28] Data regarding relapse dose complication was not found in any studies in Bangladesh. Ahasan HAMN et al. Also reported ECG changes in 12 out of 49 cases in the form of 'T' inversion in 8 cases, sinus tachycardia in 3 and conduction defect in 1.[29] One of the major pitfalls of SSG therapy in Bangladesh is shortage of supply of drug as reported by Rajib Chowdhury and Caryn Bern in their article, where they claimed a short fall of storage of SSG since the company producing the drug in Bangladesh stopped production in 2003.[2] In Bihar the reasons behind resistance of SSG was formulated and thought to be inadequate dosage regime and treatment by

unqualified or semi qualified people.[30] Similar situations are persisting in Bangladesh. This along with the shortage of the drug lead to price hike of the product to upto 4 times in Mymensingh area, where most of the cases of kala-azar come from puting us at risk of developing a significant rise in resistance to SSG sooner or later.

Amphotericin B and liposomal Amphotericin B are recommended as 2nd line drugs in Bangladesh at a dosage schedule of 1 MKD 20 days through infusion.[6] The drug is reserved for 1) patients who do not respond to the first line drugs or if those drugs have been discontinued due to side effects 2) women during preagnancy 3) Lactating mothers 4) infants <2 years of age and kala-azar patients with liver or kidney disease.[6] Primary unresponsiveness and relapses to this drug is uncommon.[31-33] Relapse could though be effectively re-treated with the same drug.[34,35] The major pitfall remains availability and affordability. Hospitalisation is mandatory and side effects are quite common. As per Sundar S. in Bihar side effects such as thrombophlebitis and infusion reaction was found in almost every patient.[30] Apart from this hypokalaemian, thrombocytopenia, myocarditis and death were occasional findings. All of this has been an obstacle to this drugs becoming the primary treatment option.

Comparison study to see the efficacy of SSG and Amphotericin B was conducted by Mishra M et al. on 80 patients dividing them in groups of 40 where one group receive SSG at the dose of 20 MKD in two divided doses for 40 days and the other half received amphotricin B at a dose of 0.5 MKD in infusion for 14 doses at alternate days. All 40 patient receiving amphotericin B was cured, while 25 of the patients receiving SSG showed definitive cure.[36]

Liposomal Amphotericin B is available at three different preparations. These are liposomal amphotericin B (LAMB) Ambisome (Gilead Sciences, Inc San Dimas, CA), Amphotericin B lipid complex (ABLC), and amphotericine B cholesteryl dispersion (ABCD) Amphotec (Intermune corp, Brishane, CA). Ambisome is used at a dosage of 3-5 MKD for 5 days or 15 MKD as single infusion and is as effective as amphotericin B and with minimal untoward effects 90 to 100% cure can be achieved in all refractory cases of VL.[37] The recommended dose as per the national guideline is 3 MKD IV for 5 days. The major drawback is its very high cost.

Miltefosine at a dose of 2.5 MKD divided in two doses for 28 days has been given the honour of being the first line of treatment by the national guideline,[6] there for obviously deserves special attention. The oral route of administration along with minimal side effects and good efficacy has given this drug a cutting edge over the others. In a phase 3 trial on 291 adult patients in India, it was found that the cure rate was 97% at 6 months of follow up.[38] While another study on 80 Indian children (age, 2-11years) with similar dosage schedule showed a 94% response.[39] Mahmood et. al. of Bangladesh in his study on 1007 patients showed that 970 (96.3%) completed the 28 day course, initial cure rate was 100% with miltefosine given at 2.5 MKD. At two months follow up the rate was 95.7%. The relapse rate was 1.4% with 6 deaths during the study.[40] In spite of the good response the drug has few major draw backs. First of all it is teratogenic and acts as an abortifacient[41] as a result cannot be given to women at child bearing age until or unless preagnancy is ruled out and vigorous contraceptive measures are taken for atleast 8 weeks after the completion of the therapy. The major concern for us is the 3.7% drop out in Mahmood et.al.'s study[40] as the drug has a long half life in humans[41] making it prone to development of drug resistance at the presence of low drug concentration until or unless the course is completed.[42] Other side effects of the drug includes mild-to-moderate gastrointestinal disturbance which occurs in 25% patients[38,39] and reversible elevation of aspartate amino transferase (ASAT) level at the early phase of treatment.[38,39]

Paromomycin is registered in India for treatment of kala-azar. It is reported to have a 93% cure rate at 16 MKD IM therapy for 21 days.[43] Unfortunately the company that used to produce this drug in India abandoned its production and prevented the process of this drug being chosen as the first line therapy. Sitamaquine another orally active drug has not seen the light due to slow progress and only one phase I/II pilot study has been completed.[44] A study was conducted on 16 kala-azar patients to see their response to ketoconazole but the response was very disappointing with only 4 patients achieving parasitological cure.[45]

Drug resistance and invasive confirmatory methods remain as the dark cloud in the horizon. Until or unless measures are taken to stop drug resistance and to prevent drop outs it will not be possible to

eliminate kala-azar. It is high time that we think of combination therapy to prevent resistance. Measures regarding prevention, though not the domain of this paper is also an important factor.

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