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RESEARCH ARTICLE

# Observational Study on Nephrotoxicity, Hepatotoxicity and Electrolytes Imbalance Induced by Amphotericin B in Kala-Azar Patients

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**ABSTRACT**

Leishmaniasis is a disease caused by protozoan parasites that belong to the genus *Leishmania* and is transmitted by the bite of certain species of sand fly (subfamily Phlebotominae). It is the second largest parasitic killer in the world (after malaria), responsible for an estimated 500,000 cases each year worldwide.(1) the parasite migrates to the internal organs such as liver, spleen (hence 'visceral)' and bone marrow and if left untreated will almost always result in the death of the host. Signs and symptoms include fever, weight loss, mucosal ulcers, fatigue, anemia and substantial swelling of the liver and spleen. Of particular concern, according to the world health organization (WHO), is the emerging problem of HIV/VL co-infection.(2)

**KEY-WORDS:** Amphotericin B, Visceral Leishmaniasis (kala-azar).

## INTRODUCTION:

A case of visceral leishmaniasis (VL) is a person showing clinical signs (prolonged irregular fever, splenomegaly and weight loss) with serological (at peripheral geographical level) and/or (when feasible at central level) parasitological confirmation of the diagnosis. In endemic malarious areas, visceral leishmaniasis must be suspected when fever lasts for more than 2 weeks and no response has been achieved with anti-malarial drugs. Incubation period lasts between 2 and 6 months.(3)

## RISK FACTORS:

Risk factors include cross boarder migration, socio-economic & cultural factors, poor housing condition, environmental sanitation, and lack of personal protective measures, concurrent infections associated with sub optimal or compromised immune response.(4)

## ELIMINATION OF KALA-AZAR:

Towards overall health and wellbeing of the vulnerable groups and mitigation of poverty, the National Health Policy (2002) of the Government of India has set the goal for elimination of Kala-azar by the year 2010. (5) Control of the disease can be achieved by interruption of transmission by reducing vector population through indoor residual insecticides, early diagnosis and complete treatment of Kala-azar cases; and health education programme for community awareness.(6)

## CURRENT THERAPY:

The various drugs used for treatment of VL cases are SAG, Pentamidine, Amphotericin B, Miltefosine, Amphotericin B Lipid complex (Ambisome) and Paromomycin. SAG had a cure rate of 58.5%, Pentamidine 66.7%, Amphotericin B 93.6%, Amphotericin B Lipid complex 100%, Miltefosine 97.5% and Paromomycin 93.4%. Unresponsiveness to SAG has developed, that is why its cure rate has gone down. Besides it can lead to cardio toxicity causing myocarditis. Pentamidine is not readily available and it can lead to anaphylactic shock and diabetes besides being nephrotoxic. Amphotericin B is a very good drug with a high cure rate but it is nephrotoxic and requires electrolyte monitoring most importantly potassium.

## PURPOSE OF THE STUDY:

Currently all the available therapies for the treatment of VL are associated with wide spectrum of problems and complications ranging from drug resistance to high cost of treatment. The success rate of various formulations of Amphotericin B ranges from 94% to 100% but the only problem is that it shows nephrotoxicity and electrolytes imbalance. The purpose of this study was to observe the nephrotoxicity, hepatotoxicitiy and electrolytes imbalance induced by amphotericin B for the assessment of the toxicities and side effects of the drugs during treatment of the patients of kaka azar for the effective monitoring and optimization of treatment plan and further aid some knowledge into various approaches



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used for reducing the amphotericin B induced nephrotoxicity, hepatotoxicitiy and electrolytes imbalance.

**SUBJECTS AND METHODS:**

The study was prospective observational study conducted at Nalanda Medical College and Hospital (NMCH), Patna, Bihar. This study was conducted over a period of 8 month from September 2009 to Apr 2010. Amphotericin B (Fungizone) 1 mg/kg Amphotericin B in 5% dextrose solution, 15 infusions on daily/alternate days. Data relevant to the study was collected directly from the patient profile form and laboratory investigational reports of kala azar patients at Nalanda Medical College and Hospital (NMCH) Patna, Bihar.

**MATERIALS:**

The study was prospective observational study conducted at Nalanda Medical College and Hospital (NMCH), Patna, Bihar. This study was conducted over a period of 8 month from September 2009 to Apr 2010. Amphotericin B (Fungizone$^{(R)}$) had given 1 mg/kg in 5% dextrose solution, 15 infusions on daily/alternate days. Data relevant to the study was collected directly from the patient profile form and laboratory investigational reports of kala azar patients at Nalanda Medical College and Hospital (NMCH) Patna, Bihar.

**METHODS:**

**STUDY CRITERIA:**

**INCLUSION CRITERIA:**

- Patient of either sex of VL
- Age 15-65 years.
- Patient confirmed diagnosis of kala-azar.
- Patient receiving Amphotericin B

**EXCLUSION CRITERIA:**

- Renal failure patient.
- Suspected or confirmed pregnancy.
- Age <15 or > 65 years.
- Serum creatinine level of 2 mg/dl
- Hb < 5g/dl
- Platelet count < 50000platelet/cmm.
- Malaria, Tuberculosis, HIV and diabetics patient excluded from the study.

**BASELINE ASSESSMENT AND INVESTIGATIONS:**

- Specific confirmatory diagnostic tests like rk-39, DAT (Direct Agglutination Test) and Splenic aspiration for LD bodies (Leishmania Donovani bodies).
- Complete blood count (CBC).
- Serum Bilirubin & liver enzymes (SGOT, SGPT, ALP).
- Serum creatinine and blood urea.
- Serum electrolyte analysis (Sodium and potassium).

**STUDY PROCEDURE:**

Patients of either sex with confirmed diagnosis of visceral leishmaniasis, who satisfied study criteria were enrolled into the study after the nature of the study was explained to them. After enrolment complete blood count, ESR, serum bilirubin, liver enzymes (SGOT, SGPT), serum creatinine, blood urea and serum electrolytes (sodium, potassium and chloride) analysis was carried out before treatment. Patients were received 1mg/kg Amphotericin-B 15 infusions on daily/alternative days for 30 days. Adverse Drug Reactions, clinical improvement of patient was accomplished by daily ward round participation. Compliance of patient was improved by counselling. After completion of the treatment, again complete blood count, serum bilirubin, liver enzymes (SGOT, SGPT), serum creatinine, blood urea and serum electrolyte analysis (sodium and potassium) and splenic aspiration was carried out to evaluate the toxicities and Adverse Drug Reactions of Amphotericin B in the treatment of kala azar patients.

**DATA COLLECTION:**

Patient details were collected and recorded in the patient profile form. All the required data of the study patients including demographic details such as name, sex, age, address; clinical data such as diagnosis, previous disease history, other co-morbid diseases, other diagnosis; therapeutic data such as name of the drug, dose, route, duration, tand other relevant details were collected from treatment chart, patient's case and laboratory investigational reports noted down.

**OBJECTIVE MEASURES:**

Once the patient fulfill all the inclusion and exclusion criteria, blood sample from the enrolled patient were collected, from the serum analysis hematological and biochemical parameters were estimated before and end of the treatment. Splenic aspiration for LD bodies and rk-39 dip stick test was done before the treatment to confirm the diagnosis. The patients clinically confirmed kala azar enrolled in the study and observed the treatment and improvement of patient on the basis of symptomatic relief, fever, weakness, differential blood count, total count and Hb. The patient data will collect from the patient profile form and laboratory investigation data of patient for the treatment of kala azar.

**RESULTS:**

A total of 14 patients included into the study and received 1 mg/kg Amphotericin B in 5 % dextrose solution 15 infusions in alternative/daily days. No pregnancy or deaths occur during the treatment period or during follow-up. The initial complaints reported by family members or patients and at the time of diagnosis were showed in the table.

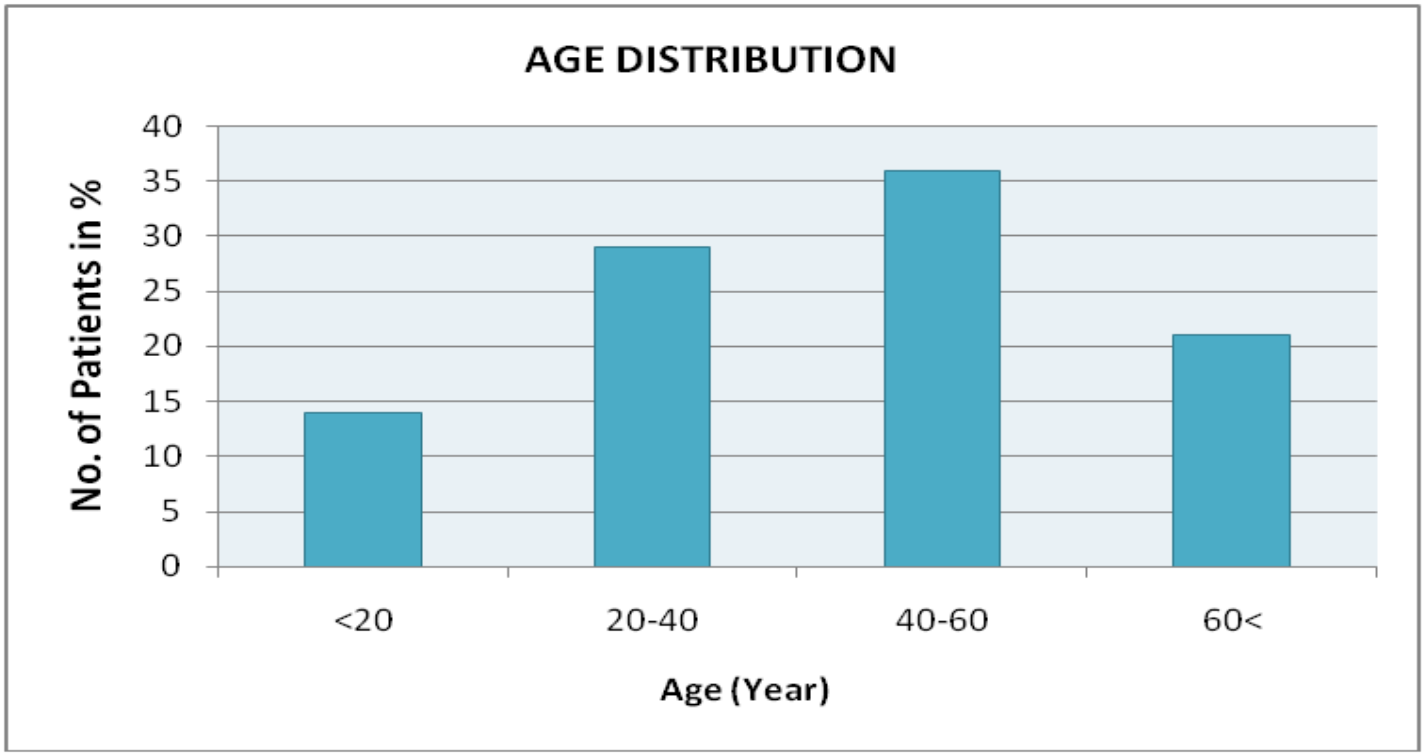


**Figure No. 1: Age distribution graph**

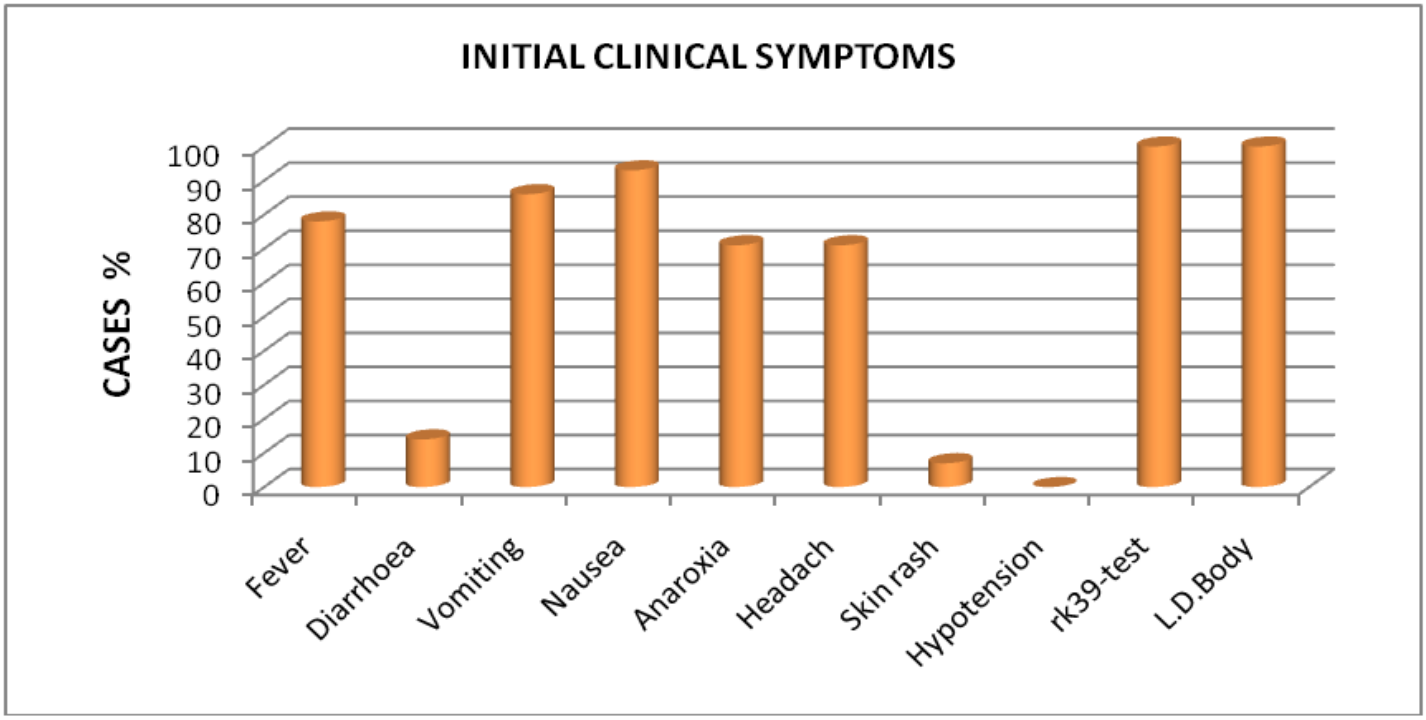


**Figure No. 2: Initial clinical symptoms**

**CHANGES IN HEMATOLOGICAL PARAMETERS:**

The mean hemoglobin levels, the total leucocyte count, neutrophil count, eosinophil count of patients before and after the treatment were recorded. Statistical test applied here was a parametric Paired T-Test by using statistical software Minitab 15. Inferences: There was not significant decreases in Mean leucocyte count(P=0.117), Mean Polymorphs count(P=0.027), Mean Lymphocytes count(P=0.658), Mean Eosinophils count(P=0.002), raise in hemoglobin levels up to end of the treatment (P=0.017), raise in ESR (mm) levels up to end of the treatment (P=0.73).

| Amphotericin–B | | | | | |
|---|---|---|---|---|---|
| Variable | | N | Mean | Sd | P-value |
| T.C(Leucocytes) | BT | 14 | 9257 | 3509 | **0.117** |
| | AT | 14 | 7707 | 1695 | - |
| D.C(Polymorphs) | BT | 14 | 51.22 | 16.98 | 0.027 |
| | AT | 14 | 39.14 | 18.01 | - |
| Lymphocytes | BT | 14 | 43.50 | 16.86 | **0.658** |
| | AT | 14 | 45.5 | 15.65 | - |
| Eosinophils | BT | 14 | 4.22 | 5.26 | 0.002 |
| | AT | 14 | 10.85 | 3.41 | - |
| Monocytes | BT | 14 | 1.129 | 1.741 | 0.002 |
| | AT | 14 | 4.5 | 1.69 | - |
| Basophils | BT | 14 | 0.05 | 0.21 | **0.336** |
| | AT | 14 | 0 | 0 | - |
| Hb (Gm/dl) | BT | 14 | 8.87 | 1.66 | 0.017 |
| | AT | 14 | 10.42 | 3.28 | - |
| ESR(mm) | BT | 14 | 40.85 | 19.70 | **0.73** |
| | AT | 14 | 38.14 | 19.93 | - |

**Table N. 1: Changes in Hematological Parameters**

Statistical test applied – Paired T-Test by using statistical software Minitab 15.

**BT** = before treatment , **AT** = after treatment, **n** = Number of patients, **SD** = standered deviation

**CHANGES IN BIOCHEMICAL PARAMETERS:**

The mean of Blood urea, Serum creatinine, $Na^+$, $K^+$, $Cl^-$, SGPT (ALT) and Serum Bilirubin of 14 patients before and after the treatment were recorded. Statistical test applied here was a parametric Paired T-Test by using statistical software Minitab 15.

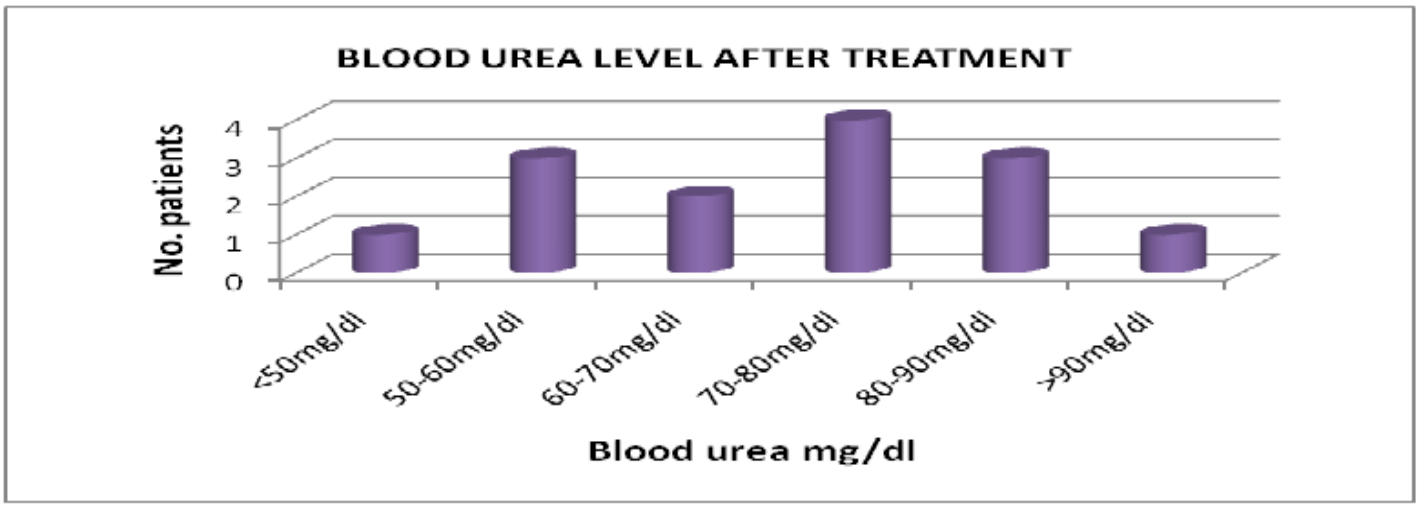


**Figure No. 3: Blood Urea Level**

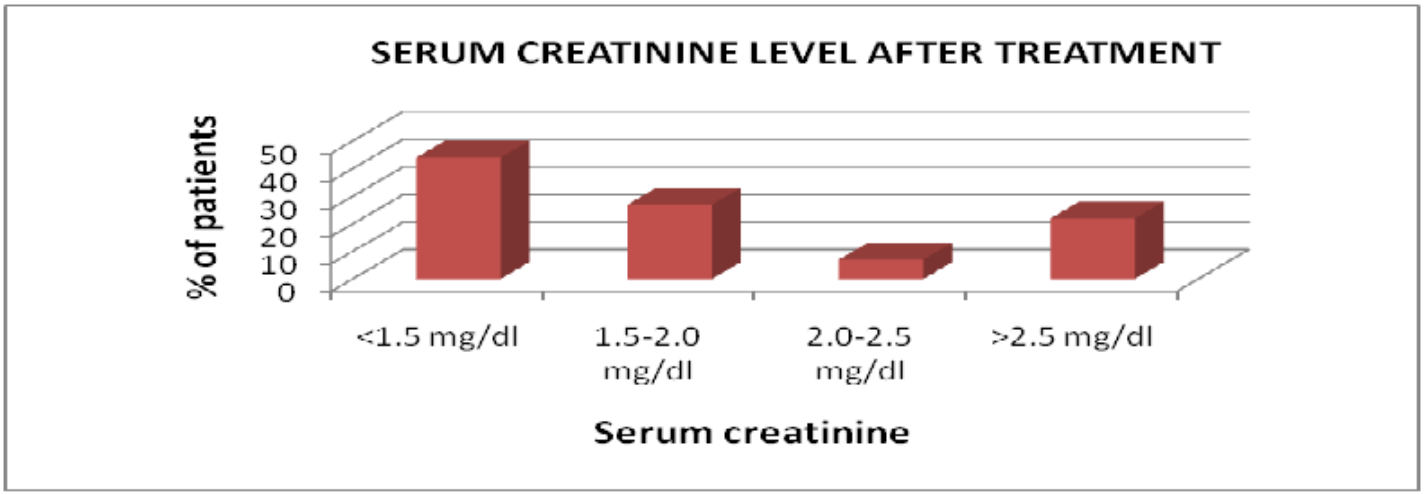


**Figure No. 4: Serum Creatinine Level**

**INFERENCES:**

The change in the blood urea level in the patients before and after treatment shown in figure as follows: There was significant raise in Blood urea levels up to end of the treatment (P=0.001), raise in Serum creatinine levels up to end of the treatment (P=0.009), raise in $Na^+$ levels up to end of the treatment (P=0.808), decrease in $K^+$ levels up to end of the treatment (P=0.002), raise in $CL^-$ levels up to end of the treatment (P=0.913), raise in SGPT (ALT) levels up to end of the treatment (P=0.041), raise in Serum Bilirubin levels up to end of the treatment (P=0.547).

| Amphotericin –B | | | | | |
|---|---|---|---|---|---|
| **Variable** | | N | Mean | Sd | P-value |
| Blood urea | BT | 14 | 39.71 | 9.3 | 0.001 |
| | AT | 14 | 71.24 | 16.43 | - |
| Serum creatinine | BT | 14 | 1.23 | 0.41 | 0.009 |
| | AT | 14 | 1.87 | 0.74 | - |
| $Na^+$ | BT | 14 | 132.14 | 10.45 | **0.808** |
| | AT | 14 | 132.78 | 5.35 | - |
| $K^+$ | BT | 14 | 4.1 | 0.48 | 0.002 |
| | AT | 14 | 3.14 | 0.62 | - |
| $CL^-$ | BT | 14 | 105.07 | 5.66 | **0.913** |
| | AT | 14 | 105.21 | 3.64 | - |
| SGPT (ALT) | BT | 14 | 26.78 | 5.80 | 0.041 |
| | AT | 14 | 32.85 | 9.07 | - |
| Serum Bilirubin | BT | 14 | 0.82 | 0.06 | **0.547** |
| | AT | 14 | 0.83 | 0.04 | - |

**Table No. 2: Changes in Biochemical Parameters**

Statistical test applied **–** Paired T-Test by using statistical software Minitab 15

**BT** = Before treatment, **AT** = After treatment, **n** = Number of patients, **Sd** = Standered deviation

**DISCUSSION:**

In present study 86% (n=12), 14% (n=2), 93% (n=13), 71% (n=10), 7% (n=1) of patients showed vomiting, diarrhea, nausea, anaroxia and skin rash respectively. There was raised serum SGPT level in 35% (n=5) of patients during 3$^{nd}$ week of the treatment and they came to normal up to end of the treatment, 1 month follow up. In present study no significant (P=0.547) raise in serum bilirubin was observed during and after the treatment of patients. The transient elevation in liver enzymes during treatment is attributed to a moderate effect of Amphotericin B on hepatocytes. Mean results on renal-function tests were

substantially altered by Amphotericin B and mild-to-moderate elevations of serum creatinine, blood urea were founded. The incidence of Amphotericin-B nephrotoxicity is very high. Acute renal failure is common. Previous studies suggest that acute renal failure associated with amphotericin-B is between 49% and 65%. In the study by wingard et al >50% of patients had significant increase in serum creatinine compared with baseline. Specifically serum creatinine doubled in 53% of patients and 29% had a serum creatinine of >125 mmol/L representing a decreased renal function of 70%. Usually the literature on acute renal failure (ARF) in patients using Amphotericin-B defines ARF as an increase in the serum creatinine level above 2.0 mg/dl. In our study, I observed this increase in the serum creatinine level of 2.0 mg/dl. In the present study there are a significant raise in serum creatinine (P=0.009) above 2.0 mg/dl and blood urea (P=0.001) above 70 mg/dl levels in 28.5% (n=4) and 57% (n=8) patients respectively. There is a significant decrease in serum electrolyte potassium $K^+$ (P=0.002) where as no significant decrease in sodium $Na^+$ (P=0.808) and chloride $Cl^-$ (P=0.913). Also, There is found significant rise in liver enzymes SGPT (P=0.041) and no significant raise in Serum Bilirubin (P=0.547). This treatment also requires daily injections for 28 to 30 days. Amphotericin B is greater than 95 percent effective but is almost always associated with fever, frequently causes renal dysfunction, and requires intravenous injections for 15 to 20 days. The most widely used new formulation of amphotericin B (liposomal amphotericin B) is also more than 95 percent effective but requires injections on six days over a three-week period of medical attention, it may have immediate side effects, and its cost precludes its use in more than 99 percent of patients.

**CONCLUSION:**

The main objective of the present study was to assess the incidence and risk of toxicity associated with the use of Amphotericin B , used as a first line drugs in the treatment of Visceral Leishmaniasis (kala-azar). Amphotericin B related Nephrotoxicity is an important cause of morbidity, occurring to some degree in up to 80% of patients treated with this drug. Amphotericin B Nephrotoxicity is a severe complication. It results in excess mortality and higher hospital stay cost. The recognition of risk factor and early intervention are much more effective than treatment of established acute renal failure. A change in creatinine level, however small, should be regarded as consequential and should trigger review and possible intervention. In our study, We concluded that Amphotericin B is not a 100% safer drug to treat the patients of the Visceral Leishmaniasis (kala-azar) due to its nephrotoxicity and it also slightly induced electrolyte imbalance mainly hypokelamia. Therefore, it required electrolytes monitoring very closely during treatment. It also raised the some liver enzymes (SGOT, SGPT & ALP) which were normalized after completion of treatment.

There are some limitations of our study. First, it was the measurement of renal function which relied on objective laboratory test, not on subjective evaluations of the well being of the patients. Second, the sample size of the study people was very small to show the significant difference between the renal function parameters. Finally, we did not have sufficient funds include mopre number of patients and to assess the histopathological changes to confirm the alteration in the glomerular membrane due to Nephrotoxicity induced by the Amphotericin B.

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