



## Efficacy of Amphotericin-B, Clinicopathologic Variables and Oxidative Stress Markers in Three Staffordshire Bull Terrier Dogs with Visceral Leishmaniasis

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**Abstract:** This study was aimed at evaluating the effects of Leishmania infection and AMB (amphotericin-B) treatment on hematological, biochemical and oxidative stress parameters in three Staffordshire Bull Terrier dogs with visceral leishmaniasis (VL). These dogs were presented with weight loss, weakness, and cutaneous lesions. Canine kala-azar detection kit was positive in dogs with VL. AMB was administered to cases with VL at a dose of 0.6 to 1.5 mg/kg/week for 4 months. Leishmania agents caused the liver injury due to increase in the ALT level in cases 1 and 2, decrease in erythrocyte, hemoglobin, and hematocrit level and increase in the MDA, and decrease in the GSH-Px in case 3, but Leishmania agents did not affect the kidney functions due to normal urea and creatinine level in the dogs with VL. A gradual response to the AMB treatment was observed. At the end of treatment course, cases with VL were treated clinically. It was concluded that the AMB administration for 4 months might be effective to treat VL due to no clinical recurrence for 6-month follow-up period.

**Key words:** Amphotericin-B, Dog, Hematological and biochemical levels, Leishmaniasis, Oxidative stress.

## Visseral Leishmaniasis'li Üç Staffordshire Bull Terrier Köpekte Amfoterisin-B'nin Etkinliği, Klinik-patolojik Değişkenler ve Oksidatif Stres Belirteçleri

**Özet:** Bu çalışmada visseral Leishmaniasis'li (VL) üç Staffordshire Bull Terrier köpekte Leishmania enfeksiyonu ve AMB (amfoterisin-B) tedavisinin hematolojik, biyokimyasal ve oksidatif stres parametreleri üzerine etkilerini değerlendirmek amaçlandı. Bu köpekler kilo kaybı, halsizlik ve deri lezyonları ile kliniğe getirildi. Kanin kala-azar test kiti VL'li köpeklerde pozitif. Visseral Leishmaniasis'li olgulara AMB 0.6-1.5 mg/kg/hafta dozunda 4 ay süre ile uygulandı. Leishmania etkenleri olgu 1 ve 2'de ALT düzeyinde artışa bağlı olarak karaciğer hasarına, olgu 3'te eritrosit, hemoglobin ve hematokrit düzeyde azalmaya ve MDA'da artışa ve GSH-Px'de azalmaya neden oldu fakat VL'li köpeklerde normal üre ve kreatinin düzeyine bağlı olarak böbrek fonksiyonlarını etkilemedi. AMB tedavisine kademeli yanıt gözlemlendi. Tedavi seyrinin sonunda VL'li olgular klinik olarak iyileşti. Sonuç olarak 6 ay süre ile takipte klinik nüks olmamasına bağlı olarak visseral Leishmaniasis'i tedavi etmek için 4 ay süreli AMB uygulamasının etkili olabileceği kanısına varıldı.

**Anahtar kelimeler:** Amfoterisin-B, Köpek, Hematolojik ve biyokimyasal belirteçler, Leishmaniasis, Oksidatif stres.

## INTRODUCTION

*Leishmania (L.) infantum* and *L. tropica* leads to canine visceral leishmaniasis (VL) in Turkey (Toz et al., 2013). Clinical findings of leishmaniasis vary from focal cutaneous to disseminated visceralising form (Petersen, 2009). Hypoalbuminemia, hyperproteinemia, and nonregenerative anemia are the most common biochemical and hematological laboratory findings (Freeman, 2010).

Oxidative stress may occur as a host defense mechanism for killing of engulfed *Leishmania spp.* when reactive oxygen species (ROS) are produced in excess (Neupane et al., 2008). Antioxidants can function by scavenging biologically important reactive oxygen species (Halliwell, 1991; Aktas et al., 2011).

The diagnosis of VL is made by the detection of serum antibodies with a variety of techniques including indirect fluorescent antibody, enzyme-linked immunosorbent assay, immunoassays (rK39) (Freeman, 2010). Pentavalent antimonials, allopurinol, sodium stibogluconate and amphotericin-B (AMB) are used for the treatment of leishmaniasis (Petersen, 2009).

In this study, the effects of *Leishmania* infection and AMB treatment on hematological, biochemical and oxidative stress parameters were determined in three Staffordshire Bull Terrier dogs presenting clinical findings of VL.

## CASE PRESENTATION

Three male brown Staffordshire Bull Terrier dogs, about 1.5 year-old and 17-22 kg body weight, living in Antalya province were presented to the clinic with complaints of weight loss, alopecia, decreased physical activity, and ulceration on the ear and tale tips (Fig. 1) despite normal appetite. Additionally, five clinically healthy dogs, which were referred to the clinic for the purpose of routine control, were sampled as the control group.

### Diagnosis, Blood Collection and Analyses

Canine kala-azar detection kit (Inbios, USA) was used as a diagnostic tool. Blood samples were



**Figure 1.** Clinical appearance of ulcerous and crusty lesions on the ear before treatment.

**Şekil 1.** Tedaviden önce kulakta ülserli ve kabuklu lezyonların klinik görünümü.

collected to determine hematological, biochemical, and oxidative stress parameters. The hematological (Gen-S, ABD) and biochemical (Roche Hitachi P-800, Japan) parameters were measured. The GSH-Px activity in sera samples was measured by the method described by Pleban et al. (1982). The MDA levels were determined as TBARS (thiobarbituric acid reactive substance), in accordance with the method described by Jain (1989).

## Treatment

AMB (Fungizone; Bristol Myers Squibb) was administered to the cases 1 and 2 once a week at a dose of 0.6 mg/kg/wk in the first month, and at a dose of 1 mg/kg/wk (once only at 1.6 mg/kg/wk) in the next months (total dose of 15 mg/kg for 4 months) in 5 % of 250 ml dextrose by intravenous route. Each application lasted about 4 hours. Because the case 3 showed severe adverse effects against AMB administration, the treatment was applied at a dose of 0.6 mg/kg three times with 10 days interval in the first month, twice with 15 days interval in the second month, and at a dose of 1.5 mg/kg/wk in the third and fourth months (total dose of 15 mg/kg for 4 months).

## RESULT and DISCUSSION

Cases with VL, presented herein, were diagnosed by the physical examination findings, the positive result of kala-azar test and the efficacy of AMB treatment. In the anamnesis of cases with VL, the prominent finding was determined as a weight loss during the last three months despite good appetite. Physical examination findings were cachexia, paleness, weakness, generalized lymphadenopathy, alopecia and scurf around the body, crusty and ulcerous lesions at the tip of tale and on the ears (Fig. 2), and erythematous lesions around the mouth. Many clinical signs appeared in this study coincide with the previous reports (Abranches et al., 1991; Amusatogui et al., 2003; Petersen, 2009).

Most of the damages of membrane caused by free radicals are constituted by lipid peroxidation (Halliwell, 1991). Researchers have determined a significant increase in MDA concentration in dogs with VL (Bildik et al., 2004), and lipid peroxidation as directly related with microsomal membrane viscosity in experimental *L. donovani* infection (Bagchi et al., 1993). In consistent with this report, increased MDA and decreased GSH-Px level showed the evident development of lipid peroxidation in case 3 compared to the control group before treatment, as evidenced in Table 2.

Leishmanial infection may cause lipid peroxidation (Sen et al., 2001) and hemolytic anemia (Biswas et al., 1995). Symptomatic dogs demonstrate severe anemia, with a significant decrease in the number of erythrocytes, hemoglobin, and hematocrit (Reis et al., 2006; Freitas et al., 2012). Similarly, in this study, decreases in erythrocyte, hemoglobin, and hematocrit level due to lipid peroxidation development in case 3 were detected before treatment regarding the infection. In cases 1 and 2, erythrocyte and hemoglobin levels were within the reference range, as shown in Table 1.

In addition, a marked damage related with lipid peroxidation in liver tissue has been reported by Oliveira and Cecchini (2000). In cases 1 and 2 compared with the reference range, the ALT level increased before treatment as consistent with the finding of Heidarpour et al. (2012). With AMB treatment for leishmanial infection in cases 1 and 2, the ALT level reached to the reference range. While the ALT level in case 3 before treatment was within the reference range, as appeared in Table 2, its level increased to 63 U/L after treatment due to the dosage of AMB treatment (1.5 mg/kg/wk) during the last two months.



**Figure 2.** Normal (healed) appearance of ulcerous and crusty lesions on the ear after treatment.

**Şekil 2.** Tedaviden sonra kulakta ülserli ve kabuklu lezyonların normal (iyileşmiş) görünümü.

A marked decrease in albumin concentrations has been reported in symptomatic dogs representing clinical signs of leishmaniasis due to a reduction of hepatic synthesis (Reis et al., 2006; Heidarpour et al., 2012). In this study, albumin levels were within the reference range in dogs with VL.

Heidarpour et al. (2012) reported marked increases in the MDA, creatinine, and BUN concentrations in comparison to the control group. However, in the present study, creatinine and urea levels were within the reference range in dogs with VL before treatment, as shown in Table 2. Therefore, it is obvious that kidney function is not

impaired by causative agent of leishmaniasis. However, in this study, because of AMB treatment, an increase in urea and creatinine level in case 1 as well as increase in urea level in case 2 was detected, while creatinine and urea levels were within the reference range after treatment in cases 1 and 2. However, urea levels appeared to remain high in case 3 after treatment because of the AMB dosage.

As for leukocyte levels, in contrast to leukopenia report in symptomatic dogs (Amusatogui et al., 2003; Reis et al., 2006), leukocytosis in case 3 is consistent with the report of Freitas et al. (2012). Thus, one could speculate that leukocytosis and leukopenia might be found in canine leishmaniasis related with clinical stage, severity of the infection that affects organs, the treatment choice, etc.

**Table 1.** Hematological levels in the cases with VL.

**Tablo 1.** VL'li olgularda hematolojik düzeyler.

Parameters	Case 1		Case 2		Case 3		Reference <sup>16</sup>
	BT	AT	BT	AT	BT	AT	
RBC (x10 <sup>6</sup> /μL)	6.1	7.02	6.49	7.12	3.8	6.6	5.5-8.5
WBC (x10 <sup>3</sup> /μL)	8.9	10.94	12.2	10.44	17.1	6.8	6-17
HT (%)	54.2	49.9	42.4	46.7	25.7	44.3	37-55
Hb (g/dL)	16.2	17.6	13.7	16	8.7	14.5	12-18
MCV (fL)	71	71.1	65.3	65.6	68.2	67.6	60-77
Plt (x10 <sup>3</sup> /μL)	318	399	397	404	509	565	200-500

RBC: Red Blood Cells; WBC: White Blood Cells; Ht: Hematocrit; Hb: Hemoglobin; MCV: Mean Corpuscular Volume; Plt: Platelet. BT: Before Treatment; AT: After Treatment.

**Table 2.** Serum biochemical levels and oxidative stress markers in the cases with VL.

**Tablo 2.** VL'li olgularda serum biyokimyasal düzeyleri ve oksidatif stres belirteçleri.

Parameters	Case 1			Case 2			Case 3			Reference <sup>16</sup>
	BT	DT	AT	BT	DT	AT	BT	DT	AT	
Urea (mg/dL)	38	129	41	20	67	45	16	52	66	21-60
Creatinine (mg/dL)	0.51	1.97	0.95	0.26	1.10	0.87	0.25	0.87	0.97	0.5-1.5
ALT (U/L)	91	46	28	78	35	25	47	46	63	23-66
AST (U/L)	28	30	24	24	29	26	35	18	28	23-66
Albumin (g/dL)	3.1	3.4	3.5	2.7	3.4	3.5	2.7	2.9	3.4	2.6-3.3
Total Protein (g/dL)	6.2	6.2	6.6	5.9	6.5	6.6	5.6	5.4	6.2	5.4-7.1
MDA (nmol/ml)	0.69	1.11	0.62	0.78	1.28	0.79	0.85	0.66	1.65	0.76±0.05*
GSH-Px (U/ml)	1.74	2.68	2.62	2.5	2.32	1.9	2.1	2.68	3.02	2.36±0.15*

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; MDA: Malondialdehyde; GSH-Px: Glutathione Peroxidase; BT: Before Treatment; DT: During Treatment; AT: After Treatment. \*: MDA and GSH-Px levels of the control group (mean±SEM).

Different formulations of AMB are preferred to treat VL instead of pentavalent antimonials because of the resistance and immune complex glomerulonephritis (Baneth and Shaw, 2002). Recent reports suggest using different AMB formulations, highly effective oral amphotericin B formulation against the murine VL (Wasan et al., 2009) and mannosylated liposomes bearing amphotericin B for effective management of VL (Rathore et al., 2011). AMB is reported to be more

effective as a treatment choice than antimonial preparations in human beings (Croft and Coombs, 2003). Accordingly, in this study, AMB was used because of its cost-effectiveness and satisfactory efficacy of treatment against leishmaniasis.

In addition, Lamothe (2001) have reported that 17 dogs receiving a total dosage of more than 10 mg/kg are clinically treated by the end of treatment. In this study, a gradual response to AMB administration was observed as a weight gain,

normal physical activity, and improvement of skin lesions in the dogs with VL during treatment. However, adverse effects due to AMB administration, such as tremor, vomiting, and diarrhoea were observed during and after the administration in the first month of treatment. Following the total dose of 15 mg/kg of AMB for 4 months was administered, all the infected cases gained their normal body weight, normal mucosal appearance, and healthy skin. Any recurrence has not been observed during 6-month follow-up period of the treated cases.

It was concluded that, the administration of AMB for 4 months may be effective to treat VL with no clinical recurrence for 6-month follow-up period and that the AMB treatment with a dosage of 0.6-1.5 mg/kg/wk may be tolerated based on serum ALT and creatinine levels determined in cases with VL.

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