Some Haematological, Biochemical and Electrophoretic Findings in Dogs with Visceral Leishmaniasis

F. KARGIN KIRAL, K. SEYREK, S. PASA, H. ERTABAKLAR and C. ÜNSAL

SUMMARY

Biochemical, haematological and electrophoretic findings were evaluated in ten dogs naturally infected by Leishmania infantum, from an endemic area in western Turkey and the results were compared with the results from eight healthy dogs. The serum concentrations of total proteins, creatinine and globulines of Leishmania group showed significant elevations (p < 0.05). However, a remarkable decreased in the mean concentration of serum albumin was recorded (p < 0.05). Electrophoretic analysis of sera from Leishmania group compared to the control animals revealed thinner albumin and thicker globulin bands. No significant alterations in serum enzyme activities (ASAT, ALAT and GGT) and in serum concentrations of urea and triglyceride were detected. Apparent haematological alterations consisted in decrease of haemoglobin concentrations, packed cell volume, and monocytes counts and increased MHCC (p < 0.05). In conclusion, these haematological and biochemical modifications suggest the occurrence of a marked immune response that can lead to renal dysfunction and would be useful for evaluating the severity of Leishmaniasis and the efficacy of treatment.

KEY-WORDS: Dog - Leishmaniasis - serum protein electrophoresis - haematology - biochemistry.

RÉSUMÉ

Données hématologiques, biochimiques et électrophorétiques chez les chiens atteints de Leishmaniose viscérale. Par F. K. KIRAL, K. SEYREK, S. PASA, H. ERTABAKLAR et C. ÜNSAL.

Dans cette étude, les paramètres biochimiques, électrophorétiques et hématologiques de 10 chiens infestés par Leishmania infantum ont été comparés aux valeurs obtenues sur 8 chiens sains. Les chiens provenaient d’une zone endémique de Leishmaniose de l’Ouest de la Turquie. Les concentrations sériques des protéines totales, de la créatinine et des globulines ont été très fortement augmentées chez les chiens malades (p < 0.05). En revanche, une diminution importante de l’albuminémie a été observée (p < 0.05). L’électrophorèse des protéines sériques sur gel de polyacrylamide 15 % a permis de confirmer la diminution des albumines et l’élévation des globulines. Les activités sériques de l’ALAT, de l’ASAT et de la GGT, l’urémie et la triglycéridémie n’ont pas été modifiées de façon significative. D’importantes modifications hématologiques ont été observées : diminution de l’hémoglobinémie, de l’hématocrite et du nombre de monocytes et augmentation de la concentration corpusculaire moyenne en hémoglobin (p < 0.05). En conclusion, ces modifications hématologiques et biochimiques suggèrent l’implication du système immunitaire dans l’induction d’un dysfonctionnement rénal et pourraient être utiles dans l’évaluation de la sévérité de la leishmaniose ou de l’efficacité du traitement.

KEY-WORDS: Leishmaniose - électrophorèse des protéines sériques - hématologie - biochimie.

Introduction

Canine visceral Leishmaniasis is a parasitic disease caused by protozoan of the genus Leishmania. This chronic and often fatal disease is a zoonosis with a wide distribution in the America, Africa, Asia and Mediterranean basin. In Mediterranean countries the prevalence of CVL ranges from 1 % to 37 % [3, 10]. Although a limited number of studies on canine visceral leishmaniasis have been conducted in Turkey, it has been established that the main causative agent in that country is L. infantum [20].

Dogs are considered as the most important vertebrate reservoir of the Leishmaniasis. The incubation period of the disease may range from 30 days to four years, so the appearance of clinical signs can not be considered seasonal [17]. In the dog, clinical features are complex, with different clinical signs depending on individual variation, type of Leishmania and phase of the disease. Clinical manifestations include non-pruritic skin lesions, such as exfoliative dermatitis and ulcerations, local or generalised lymphadenopathy, loss of weight, anaemia, splenomegaly, poor appetite, ocular lesions, epistaxis, lameness, renal failure, pancreatitis and diarrhoea [1, 4, 19].

Dogs with canine visceral leishmaniasis infestation, either symptomatic or asymptomatic, will almost always demonstrate a specific humoral response. Therefore, various serological methods for the detection of anti-Leishmania antibodies have been developed. These include indirect immunofluorescence assays (IFAT), direct agglutination assays (DAT), enzyme-linked immunosorbent assay (ELISA), competitive-ELISA, Dot-ELISA, slide-ELISA, and western blot [2, 7, 11, 14, 18, 27].
The aim of this study was to monitor the biochemical, haematological and electrophoretic profiles of the dogs infected with Leishmania infantum and to find out whether these profiles can be considered as markers of the leishmaniasis evolution and help to characterise a particular phase of the disease.

Material and methods

ANIMALS AND SEROLOGICAL ANALYSIS

Ten dogs, (7 females and 3 males), whose 9 were 3-4 year old and 1 was 8 year old were infected by Leishmania infantum and developed visceral leishmaniasis (VL). Eight dogs (5 females and 3 males) whose 7 were 3-4 years old and 1 was 7 year old were used as healthy controls. All the animals were selected from an area in western Turkey in which VL is endemic. The infected dogs were given a full physical examination. They had been ill approximately for four months and were not receiving any treatment at the time of inclusion in the study. All animals with visceral leishmaniasis showed to a greater or lesser extend some of the clinical signs of the disease, including skin lesions, weight loss, lymphadenopathy and anaemia.

The clinical diagnosis was confirmed serologically by the immunofluorescence test (IFAT) for antibodies to Leishmania infantum as described in detail elsewhere [1]. Antibody titres ≥ 128 are considered as a positive test. In the control group, all animals gave a negative response. In the infested group, all dogs showed positive tests.

BIOCHEMICAL ANALYSIS

Blood was collected from a cephalic vein using EDTA-treated plasma or serum tubes for biochemical and haematological analysis. Thirty minutes after bleeding, serum was obtained by centrifugation at 1700 g for 10 minutes. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) activities, urea, creatinine, total protein, albumin, triglyceride and haemoglobin (Hb) concentrations were measured by MicroLab 200 (Merck) using commercially available kits (Biomedical Systems, Barcelona, Spain). The analyses were carried out according to the manufacturer’s instructions. Value of plasma globulins was obtained by subtracting the value of albumin from total protein concentration.

ELECTROPHORETIC ANALYSIS

To visualise the alterations in serum proteins SDS-PAGE was performed as described previously [15]. 100 µg of total serum proteins were transferred in the wells of 15 % polyacrylamide gel. The serum electrophoresis was conducted in an electrophoresis solution (pH 8.8) containing 1.46 g acrylamide, 0.04 g bisacrylamide, 2.5 ml Tris-buffer (1.5 M), 100 µl sodium dodecyl sulphate (SDS, 10 %), 50 µl N,N,N’,N’-tetramethylethylenediamine (TEMED, 10 %) in 10 ml. Gels were electrophoresed for 45 min at room temperature at 200 V using Mini-Protean gel apparatus (Bio-RAD, Munich/Germany) for vertical gels. Electrophoresis was conducted in Tris 25 mM, glycine 195 mM and SDS 0.1 % buffer. Bands were visualised by staining Coomassie Brilliant Blue R-250 (Biomol, Hamburg/Germany). The specificity of albumin bands were confirmed with the co-migration of dog serum albumin (A-3184 Sigma, Munich/Germany).

HAEMATOLOGICAL ANALYSIS

White blood cells (WBC) concentration was determined with standard method in an improved Neubauer hemocytometry by mixing blood with a solution ad modum Türk. On two separate blood films, made immediately after blood withdrawal and processed by the panoptic method ad modum Pappenheim, 200 leukocytes per animal were differentiated under light microscope, and concentrations of leukocyte types were calculated [28].

The PCV (Packed Cell Volume) was obtained by sedimentation of erythrocytes by centrifugation in a microhematocrit centrifuge and after by measuring the obtained height of packed red cells reported to the total height of blood. The MHCC (Mean Haemoglobin corpuscular concentration) was calculated by the formula : MHCC = (100 X Hb) / PCV (%) where Hb was the blood haemoglobin concentration expressed in g/l.

STATISTICAL ANALYSIS

Differences between the two study groups were established by means of Student’s t-test, with p < 0.05 as limit of significance.

Results

Clinical findings were consistent with Leishmaniasis, based on skin lesions such as alopecia, dry desquamation and/or ulceration. Especially, skin lesions, weight loss, lymphadenopathy and anaemia were observed in all of the infested animals.

As summarised in table I the haemoglobin concentration, the percentage of packed cell volume (PCV) and monocytes of affected dogs showed remarkable declinations in comparison to control dogs (p < 0.05). However, the mean haemoglobin corpuscular concentrations (MHCC) in diseased animals showed significantly elevations (p < 0.05). No significant alteration in the number of total leukocytes and eosinophils was observed. Nevertheless, infested dogs showed an increase of lymphocyte counts although this haematological change was not significant from control values.

Biochemical results are presented in Table II. Compared to the control group, significant increases (p < 0.05) in the mean total serum protein (from 53.4 ± 1.5 g/l to 66.1 ± 4.3 g/l) and creatinine (from 66 ± 2 µmol/l to 82 ± 6 µmol/l) concentrations of the infested dogs were evidenced. The alterations in serum albumin and globulin concentrations of Leishmanian group were highly significant (p < 0.001). A remarkable decline (from 27 ± 1.1 g/l to 17.0 ± 0.8 g/l) in the mean values of serum albumin concentrations of dogs with visceral leishmaniasis was noticed. On the contrary, the serum globulin concentrations of ill dogs showed significant increases (from 26.4 ± 0.2 g/l to 48.8 ± 3.8 g/l). In the affected dogs with clinical signs the albumin/globulin ratio was significantly lower than that of the controls (p < 0.05). Moderate but not significant elevations were observed for GGT, ASAT, ALAT serum activities and for triglyceride concentrations.
In SDS-PAGE analysis (Fig. 1) albumin bands of infested animals were remarkably thinner than that of healthy animals. On the other hand, the globulin bands from leishmanian animals were thicker.

**Discussion**

Canine visceral leishmaniasis caused by *Leishmania infantum* is a major health problem for the canine population in countries bordering the Mediterranean Sea [23]. Visceral leishmaniasis in dogs is a progressive systemic disease characterised by chronic wasting. Vague clinical signs may be observed initially, including weight loss, exercise intolerance, fever, and anorexia. More specific clinical signs indicative of systemic involvement include lameness, peripheral lymphadenopathy, epistaxis, and non-pruritic skin lesions [16]. In this study, the affected dogs also showed the same clinical signs. Anaemia was the most frequently observed haematological abnormality in Leishmania group. Haematological findings indicate that the anaemia developed according to the hyperactivity in the reticulo-endothelial system and also to the failure in haemoglobin synthesis. Both the concentrations of haemoglobin and PCV showed remarkably decrease, whereas the MHCC increased significantly in diseased animals. This increase is a result of the stronger decrease in PCV compared to haemoglobin concentrations in diseased dogs. The increase of MHCC points out that there is a hyperchromasia in leishmanian dogs. These findings are in agreement with the previous reports [6, 13, 22, 25, 26]. Contrary to the expectations, the number of eosinophils in infested animals showed no significant increase which is however characteristic for parasitic diseases. The number of leukocytes may increase or decrease in dogs with visceral leishmaniasis [25]. In this study, with respect to the number of leukocytes, we observed no marked difference between the infested dogs and the control animals although a moderate (but not significant) increase of lymphocyte counts was noticed. Furthermore, it is well known that a specific immune response and sometimes auto-immune reactions occurred during leishmaniasis [11, 12, 23, 26]. Because monocytes play an important role in the initiation of immune response and because they are able to synthesize cytokines (for example IL1 and IL6) and consequently participate to recruitment of lymphocytes, the increases of monocytes counts would be related to immune response.

**TABLE 1.** — Haematological findings in healthy and Leishmania infected dogs. Data expressed as mean ± Standard deviation (x ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=6)</th>
<th>Leishmania dogs (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/l)</td>
<td>123.6 ± 3.2</td>
<td>126.8 ± 7.5</td>
<td>0.05</td>
</tr>
<tr>
<td>HCV (%)</td>
<td>45.32 ± 1.4</td>
<td>34.5 ± 2.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCHC (g/l)</td>
<td>34.84 ± 0.86</td>
<td>38.60 ± 1.62</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Leukocytes (x10^9/l)</td>
<td>2.60 ± 0.73</td>
<td>9.43 ± 0.59</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/l)</td>
<td>3.98 ± 0.37</td>
<td>6.28 ± 0.81</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils (x10^9/l)</td>
<td>5.64 ± 0.11</td>
<td>2.39 ± 0.47</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophils (x10^9/l)</td>
<td>0.55 ± 0.28</td>
<td>0.43 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Monocytes (x10^9/l)</td>
<td>0.25 ± 0.24</td>
<td>0.14 ± 0.03</td>
<td>&lt;0.08</td>
</tr>
</tbody>
</table>

**TABLE II.** — Biochemical findings in dogs with canine visceral leishmaniasis and control group. Data expressed as mean ± Standard deviation (x ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=6)</th>
<th>Leishmania dogs (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>191.12 ± 9.3</td>
<td>28.34 ± 17.2</td>
<td>NS</td>
</tr>
<tr>
<td>ALAT (U/l)</td>
<td>26.76 ± 1.5</td>
<td>42.5 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>2.98 ± 0.17</td>
<td>4.87 ± 0.78</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>56 ± 2</td>
<td>82 ± 6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tryptophan (mg/l)</td>
<td>0.52 ± 0.06</td>
<td>0.39 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>53.4 ± 2.5</td>
<td>66.4 ± 4.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Albunin (g/l)</td>
<td>27 ± 1.1</td>
<td>17 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Globulins (g/l)</td>
<td>26.4 ± 0.2</td>
<td>48.6 ± 3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.07</td>
<td>0.39</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**FIGURE 1.** — Electrophoresis of sera dogs with leishmaniasis (lanes 3-6,8) or healthy dogs (lanes 2, 7, 9) in 15 % polyacrylamide gel. Figuration of Albumines (Alb), Globulins (Glob) and standard (lane 1, St) are indicated by arrows.
The most common biochemical abnormalities of dogs with visceral leishmaniasis were a hyperproteinemia associated to a hyperglobulinemia and hypoalbuminemia. This finding is in agreement with previous data [21]. Hyperproteinemia would be related to dehydration. However, it is not the case in the present study. Because PCV of dogs with leishmaniasis was not elevated, but on the contrary decreased and the serum concentrations of albumins, the major serum proteins, were remarkably diminished, hyperproteinemia in dogs with leishmaniasis probably resulted from an important globulin production. This fact was confirmed by electrophoretic findings. To further substantiate the biochemical findings we separated the serum proteins electrophoretically. SDS-PAGE analysis of serum proteins in dogs infected with L. infantum showed changes, such as strong globulin and weak albumin bands with strong accuracy in comparison to classical serum proteins separated the serum proteins electrophoretically. SDS-PAGE was not useful in the diagnosis of canine visceral leishmaniasis. In conclusion, severe alterations in haematological parameters associated with strong modifications of serum proteins (hypoalbuminemia and hyperglobulinemia) and a probable renal failure were evidenced in the group in Leishmanian dogs. These findings would result from an exaggerated immune response and these haematological and biochemical markers would probably help to characterise the state of Leishmaniasis and/or control the efficacy of treatment.

References