

## Measurement of protein and albumin/globulin ratio in patients infected with visceral leishmaniasis

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### Abstract:

The study included 30 serum samples from people (age: 1-12 years) suffering from visceral leishmaniasis (*Leishmania donovani*) infection, which were collected from the Central Health Laboratories, and a similar number of healthy controls.

The serum level of total protein, albumin and globulin was measured, and fractions of globulin (alpha 1 globulin, alpha 2 globulin, beta globulin, gamma globulin) were also analyzed.

The results showed a significant increase ( $P \leq 0.05$ ) in total protein of patients (82.27 vs. 57.75 g/L), as compared with controls, while there was a non-significant decrease in the level of albumin (30.63 vs. 31.66 g/L). A similar significant increased level of globulin was also observed in patients (51.63 vs. 24.83 g/L), and the fractions of globulin (alpha 1 globulin, alpha 2 globulin, beta globulin, gamma globulin) were similarly increased (6.01, 13.20, 10.37, 21.64 vs. 3.74, 6.63, 7.06, 7.50 g/L, respectively).

Accordingly, the albumin:globulin ratio was decreased in patients as compared with controls (0.62 vs. 1.20).

**Key Words:** visceral leishmaniasis, albumin, globulin.

### الخلاصة:

شملت الدراسة على جمع (30) عينة مصل من أشخاص يعانون بالاصابة بالشمانيا الحشوية *Leishmania donovani* تم جمعها من مختبر الصحة المركزي وتراوحت اعمارهم ما بين (1-12) سنة، بعد ذلك تم قياس مستوى البروتين الكلي والالبيومين والكلوبيولين وتحليل اجزاء الكلوبيولين (الفا1، الفا2، بيتا، كاما)، ومقارنتها مع مصول اشخاص اصحاء.

اظهرت النتائج ارتفاعا معنويا عند ( $P \leq 0.05$ ) في قيم البروتين الكلي حيث بلغ (82.27 مقابل 57.75) غم/لتر. عند مقارنتها مع السيطرة، بينما اظهر انخفاضا غير معنوي في مستوى الالبيومين فقد بلغ (30.63 مقابل 31.66) غم/لتر. لوحظ ايضا وجود زيادة معنوية مماثلة في قيم الكلوبيولين في المرضى (51.63 مقابل 24.83) غم/لتر، وزيادة مماثلة ايضا في اجزاء الكلوبيولين الفا 1، الفا 2، بيتا، كاما (6.01، 13.20، 10.37، 21.64 مقابل 3.74، 6.63، 7.06، 7.50) غم/لتر على التوالي، ووفقا لذلك فقد انخفضت نسبة الالبيومين الى الكلوبيولين (albumin:globulin ratio) في المرضى مقارنة مع السيطرة (0.62 مقابل 1.20).

### Introduction:

Leishmaniasis is a major public health problem causing significant morbidity and mortality in Africa, Asia and Latin America<sup>[1]</sup>.

It presents mainly in three clinical forms, of which visceral leishmaniasis (VL) is the most severe form<sup>[2]</sup>. Visceral Leishmaniasis (VL) or Kala-azar is caused by *Leishmania donovani*; an obligate intracellular protozoan that parasitizes tissue macrophages.

Intra-macrophage infection by *L. donovani* potentiates a fatal visceral

infection in man and the elimination of *Leishmania* parasites by the macrophage depends upon mounting of an effective cell-mediated Immune response by the mammalian host<sup>[3]</sup>.

It is characterized by prolonged fever, anemia, leucopenia, hepatosplenomegaly, pancytopenia and emaciation. The disease is fatal if left untreated<sup>[4,5]</sup>.

Also serum albumin level was observed and a reversed albumin/ globulin ratio but most of these evaluations were carried out in dogs.

The idea of this study is to link reversed albumin/ globulin ratio and Leishmaniasis, and to find a specific laboratory tests for visceral leishmaniasis diagnosis.

**Materials and Methods:**

**Patients:**

Thirty Serum samples were collected from Leishmania positive patients who were admitted to Parasitology Unit in Central Health Laboratories during the period May-Nov/2012, their ages ranged between 1-12 years.

Thirty healthy individuals were also enrolled as control sample with a similar age range (1-12 years).

**Protein determination and protein fractions measurements:**

Total serum protein and albumin was measured according the kit manufacturer (Spinreact: BSIS02-E).

Globulin and its fractions (alpha1, alpha2, Beta, gamma) were measured in both patient and control serum samples by using Autoanalyser; Helena (Bioscience Europe) Electrophoresis for serum proteins.

All these analysis performed in Baghdad Teaching Laboratories.

**Statistical analysis:**

Comparison of paired data from patients and controls were done using ANOVA (one way analysis of variance) test. Data were expressed as (Mean ± SE). All statistical analyses were conducted with the Statistical Package for the Social Sciences (SPSS) software at significant levels of 0.05 [6].

**Results:**

The results showed a significant increase (P ≤ 0.05) in total protein of patients (82.27 vs. 57.75 g/L), as compared with controls, while there was a non-significant decrease in the level of albumin (30.63 vs. 31.66 g/L).

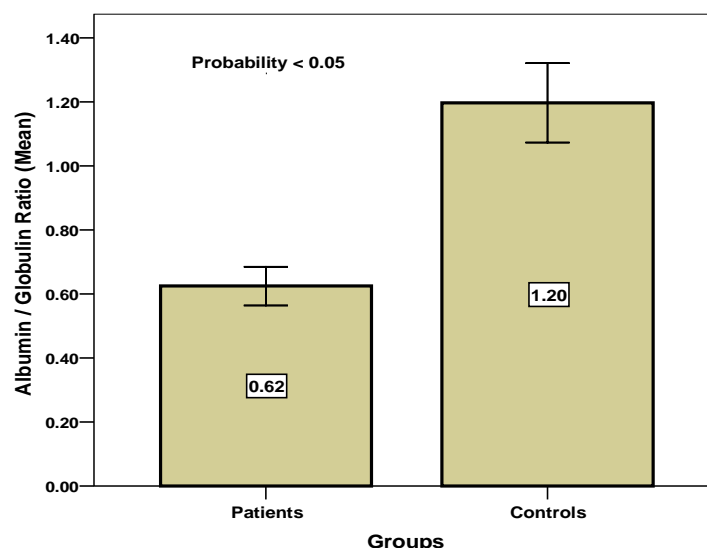
A similar significant increased level of globulin was also observed in patients (51.63 vs. 24.83 g/L) (Table 1), and the fractions of globulin (alpha 1 globulin, alpha 2 globulin, beta globulin, gamma globulin) were similarly increased (6.01, 13.20, 10.37, 21.64 vs. 3.74, 6.63, 7.06, 7.50 g/L, respectively) (Table 2). Accordingly, the albumin/globulin ratio was decreased in patients as compared with controls (0.62 vs. 1.20) (Figure 1).

**Table-1: Total level of protein, globulin and albumin in sera of visceral leishmaniasis of patients and controls.**

Parameter	Mean ± S.E. (g/L)		P-value ≤
	Patients (No.= 30)	Controls (No.= 30)	
<b>Proteins</b>	82.27 ± 2.58	57.75 ± 1.88	0.05 (significant)
<b>Globulin</b>	51.63± 3.40	24.83± 0.74	0.05 (significant)
<b>Albumin</b>	30.63± 1.72	31.66± 1.49	Not Significant

**Table-2: Globulin fractions of serum electrophoresis in visceral Leishmaniasis patients and controls.**

Globulin fractions	Mean ± S.E. (g/L)		P value ≤
	Patients (No= 30)	Controls (No= 30)	
<b>Alpha1</b>	6.01± 0.44	3.74± 0.34	0.05 (significant)
<b>Alpha2</b>	13.20±0.91	6.63± 0.51	0.05 (significant)
<b>Beta</b>	10.37±0.71	7.06± 0.58	0.05 (significant)
<b>Gamma</b>	21.64± 3.26	7.50± 1.06	0.05 (significant)



**Figure-1: Reverse albumin/ globulin ratio in a human infected with *Leishmania donovani* compared with healthy controls.**

### Discussion:

In visceral Leishmaniasis, the organs of the reticuloendothelial system (liver, spleen and bone marrow) are the most severely affected organs; causing reduced bone marrow activity coupled with cellular destruction in the spleen, and results in anemia, leukopenia and thrombocytopenia.

This leads to secondary infections and a tendency to bleed. The spleen and liver become markedly enlarged, and hypersplenism contributes to the development of anemia, Lymphadenopathy, hepatosplenomegaly, pancytopenia and emaciation can also occur.

The disease is fatal if left untreated<sup>[4, 5]</sup>. Also, it was found that an increase in production of globulin results in hyperglobulinemia but most of these researches were done in dogs. Therefore, we found a linkage between reversed albumin:globulin ratio and Leishmaniasis, interdependence as specific laboratory tests for Visceral Leishmaniasis diagnosis.

Mishra *et al*<sup>[7]</sup> manifested high serum globulin levels associated with decrease T-Helper1 (Th1) lymphocyte and increased T-Helper2 (Th2) Lymphocyte response and the hypothesis that patients who progress to visceral leishmaniasis were not able to produce an adequate Th1

response, and reversal of the albumin to globulin ratio<sup>[7,8,9]</sup>.

A number of studies relating to CVL (Canine Visceral Leishmaniasis) have concerned the biochemical- haematological alteration in dogs that had been either naturally or experimentally infected with Leshmania.

The finding include normocytic/ normochromic, anemia, leucopenia, thrombocytopenia, an increase in total serum proteins with hyperglobulinemia and hypoalbuminemia, decreased albumin/ globulin ratio<sup>[5,10,11]</sup>. Byadgi<sup>[12]</sup> and Ettinger<sup>[13]</sup> also found raised serum proteins with reversal of albumin:globulin ratio in patients infected with visceral Leishmaniasis. Dubreuil *et al*<sup>[14]</sup> showed that dogs infected with amastigote, had elevated total serum protein and gamma-globulin, and an inverted albumin/ globulin ratio. Dunan *et al*<sup>[15]</sup> showed elevated alpha 1 globulin ( $\alpha_1$ ), alpha2 globulin ( $\alpha_2$ ), Beta globulin ( $\beta$ ) and gamma globulin fractions<sup>[15]</sup>. Also in the dogs. White Keenan *et al*<sup>[16]</sup> reported in all dogs, the largest increase was in gamma globulins, although there were also increases in alpha globulin and Beta globulins.

It long has been considered that this increase in gamma globulins<sup>[17]</sup> predominantly immunoglobulin G) is not

productive or protective [18,19,20]. The present results were close to results for Senior Gopal whose showed an unusual of Visceral Leishmaniasis (VL) in a 7-year-old-male Hypergama- globulinemia with albumin globulin ratio of 0.3 was seen with hypoalbuminemia mainly (IgG from polyclonal B cell activation)<sup>[21]</sup> and look like from result Singhs and Sivakumar Confirm use of a laboratory diagnosis is required to the clinical suspicion.

The diagnostic tools used for each leishmania syndrome, the laboratory parameters include increase of total and albumin fraction of the serum protein.<sup>[22]</sup>

However, measuring reversible albumin:globulin ratio can give some indication to infection with Visceral Leishmaniasis and can helpful as clinical factors for detection, thereby reducing the mortality of Visceral Leishmaniasis.

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