

## Urine Protein SDS-PAGE Reveals Different Profiles in Iraqi Children with Kala-azar

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### Abstract

Urine proteomics have been an area of interest and recently in Kala-azar as an alternative sample type for serum or plasma. Because of simplicity, noninvasiveness of collection and simpler matrix. Many studies had detected an increased protein excretion in the urine of patients with active Kala-azar due to renal involvement particularly by an immunological related mechanism(s). This study have demonstrated the presence of three different protein profiles in Iraqi children (Patients: including 60 children aged 4-60 months) with defined Kala-azar using the conventional SDS-PAGE on urine samples. Urine protein profile in Kala-azar patients revealed three groups of banding patterns: group-1(33.4%) of the patients show the pattern of 5 bands with a MW. Ranged (512.861-158.489), groups-2, 3 were (55, 11.6) % of the patients showing 2 banding proteins with a MW. ranged (512.861-199.526) , (199.526-181.97) respectively. These findings may be correlated with other epidemiological studies that revealed the presence of different clinical presentations like fever, splenomegally, hepatomegally, thrombocytopenia, and different response to leishmania therapy. Furthermore, the presence of different protein patterns may also be related to the chronicity of infection and the degree of renal involvement. The presence of a similar protein band between group-1 and 2 may be of diagnostic purpose and further studies on expanded number of patients are required to identify that kind of protein or other urine protein profiles.

**Key words:** Protein band, protein pattern, sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE), urine proteome, visceral leishmaniasis.

### الخلاصة

تشكل البروتينات في عينات البول دائرة اهتمام ومؤخراً في داء اللشمانيا الاحشائية كنموذج بديل لعينات المصل أو البلازما. بسبب البساطة في الحصول عليه وسهولة جمعه بتجنب الوسائل الباضعة كالادوات الجارحة اضفقه الى بساطة مانتة الاساسيه. اكتشفت العديد من الدراسات طرح بروتين متزايد في بول المرضى المصابين باللشمانيا الاحشائية في الفترة النشيطة للمرض بسبب التآثر الكليوي خصوصاً بما يتعلق بالالوية المناعية. كشفت هذه الدراسة وجود ثلاث مجاميع انماط بروتين مختلفة في نماذج بول الأطفال العراقيين (المرضى المشخصين باللشمانيا الاحشائية: 60 طفل بعمر 4-60 شهر) وذلك باستخدام تقنية الترحيل الكهربائي التقليدي (هلام متعدد الاكريل اميد) على عينات البول. وقد كانت كما يلي: مجموعة 1 (33.4) % من المرضى اظهرت خمس حزم بروتينية مع وزن جزيئي تراوح بين (512.861-158.489) دالتون، مجموعته 2 و 3 كانت (55 ، 11.6) % من المرضى اظهرت حزمتين من البروتين لكل منهما مع وزن جزيئي تراوح بين (199.526-512.861) ، (181.97-199.526) دالتون و على التوالي. هذه النتائج قد ترتبط بالدراسات الوبائية الأخرى السابقة والتي كشفت وجود العوارض السريرية المختلفة مثل الحمى، تضخم الكبد والطحال، قلة الصفيحات، واستجابات مختلفة لعلاج اللشمانيا الاحشائية. علاوة على ذلك، حضور أنماط البروتين المختلفة لرئياً أيضاً متعلقة بزمان المرض ودرجة التدخل الكليوي. كما إن وجود حزمة بروتين مماثلة بين المجموعة 1 و 2 قد تكونان من الفائدة بمكان للعرض التشخيصي مما يستدعي اجراء دراسات أخرى وعلى عدد موسع من المرضى لتمييز ذلك النوع من البروتين أو كشف أنماط أخرى من البروتينات في عينات البول.

### Introduction

Visceral leishmaniasis and cutaneous leishmaniasis caused by *Leishmania donovani* and *Leishmania tropica* or *Leishmania major* respectively are known endemic diseases in Iraq. Visceral leishmaniasis had been prevalent in Iraq for the last 2-3 decades causing serious problems<sup>(1)</sup>. It represents an important factor in infant's mortality in this country<sup>(2)</sup>. Diagnosis of visceral leishmaniasis generally based on clinical presentation and microscopical demonstration of the parasite in smears, aspirates of bone marrow or culture of these aspirates on certain culturing media. However, the techniques are painful,

hazardous and need skilled personnel and equipped hospitals some times difficult to be obtained<sup>(3)</sup>. Since visceral leishmaniasis is occurring in places of poor socioeconomic conditions where health services are poorly developed<sup>(4)</sup>, the diagnosis in this case is difficult to be obtained using these techniques<sup>(3)</sup>. However, presence of *Leishmania donovani* soluble antigen, corresponding antibody and component of the complement in the serum of patient with active kala-azar has been demonstrated in a number of studies<sup>(1)</sup>, Al-Bashir<sup>(5)</sup>.

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On the other hand, the detection of *Leishmania donovani* parasite antigen in the urine samples of patient with active Kala-azar has been demonstrated in a number of studies by immunoblotting <sup>(6)</sup> ; enzyme linked immunosorbent assay (ELISA) <sup>(7)</sup> ; latex agglutination test (LAT) <sup>(8)</sup> and direct agglutination test (DAT) <sup>(8, 9)</sup>. Furthermore, urine samples are more easily to be collected especially from young children, as well as facilitating field studies <sup>(9)</sup>. Therefore, this study aims to demonstrate the presence of variations in protein profiles in a number of Iraqi patients with defined visceral leishmaniasis using the conventional sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) in urine samples.

### Patients and Methods

This study was divided into two groups:

1. Patients: including 60 children aged 4-60 months, admitted to paediatric ward of Al-kadhimiya teaching hospital-Al-Nahrain University from December 2007-February 2009. All were proven cases of Kala-azar by: clinical manifestation of fever, hepato-splenomegally, the demonstration of the parasite - the amastigotes from indirect smear of bone marrow and serological diagnosis by indirect immunofluorescence antibody test IFAT.
2. Control: consist from 10 healthy children all were seronegative.

### Urine samples

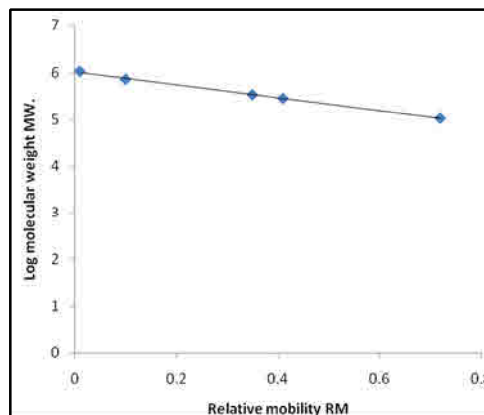
24 hours urine specimens were collected from each child in the control or the diseased group. Urine samples from all children were concentrated 100 times using Amicon Apparatus (Amicon Corporation Davers, MA) and kept at -20°C till use to avoid bacterial contamination as described by Baqir *et al.* (2002) <sup>(6)</sup> ; Al-Bashir and Ali (2003) <sup>(8)</sup>. Total protein for each sample was measured according to the method of Lowery *et al.* (1951) <sup>(10)</sup>. The protein electrophoresis was dependent on the conventional polyacrylamide gel electrophoresis PAGE as described by Fehrstrom and Morberg (1977) <sup>(11)</sup>.

### Urine protein profile

Urine protein electrophoresis was performed as described by Fehrstrom and Morberg (1977) <sup>(11)</sup>, using Coomassie brilliant blue staining. Migration distance of the calibration proteins were measured after staining the protein bands. The relative mobility (RM) was measured too according to the following equation:

$$RM = \frac{\text{Distance of protein migration}}{\text{Distance of dye migration}}$$

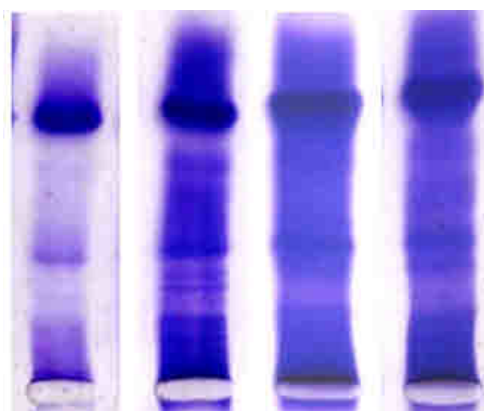
The calibration curve was constructed by plotting RMs versus log. Molecular weights for calibration proteins Fig.(1). The molecular weight of proteins of interest was determined from the position of its RM value on the calibration curve.



**Figure 1: Standard curve for approximate estimation of molecular weights of various proteins in urine samples.**

### Results

Conventional polyacrylamide gel electrophoresis has been used to differentiate between protein patterns in serum of normal and patients with Kala-azar. Coomassie brilliant blue stain has been used to visualize the band separated on the gel <sup>(12)</sup>. Urine of the control group has a specific protein pattern with one protein bands fig.(2).



**Control group (1) group (2) group (3)**  
**Figure 2 : Urine protein profiles in normal and kala-azar children.**

The relative mobility value (RM) was 0.48 with a molecular weight 199.525. Urine protein profile in Kala-azar patients revealed three banding patterns ranged from (2-5) protein

bands. The (RM) values were ranged from (0.22-0.52). See fig.(2). The first protein pattern demonstrates five bands. The RM values were ranged from (0.22-0.52), with molecular weights ranged from (512.861 - 158.489)  $\times 10^3$  Dalton respectively. Four out of these bands were shown to be abnormal from the control these are bands (1, 2, 3 and 5) as shown in table (1). This band picture was found in 20 patients (33.4 %) Table (2). The second protein pattern shows two bands the RM values were ranged from (0.22-0.48), with

a molecular weights (512.861 and 199.526)  $\times 10^3$  Dalton respectively. One of these two bands (band-1) was shown to be different from the control as shown in table (1). This profile was found in 33 patients (55 %) Table (2). The third protein shows two bands the RM values were ranged from (0.48-0.50), with a molecular weights (199.526 and 181.970)  $\times 10^3$  Dalton respectively. One of these two bands (band-2) was shown to be different from the control as shown in table (1). This profile was found in 7 patients (11.66 %) Table (2).

**Table 1 : RM values and molecular weight for each band in different urine protein profile groups.**

Band No.	Control RM	Control MW $\times 10^3$ Dalton	G1-RM	G1-MW $\times 10^3$ Dalton	G2-RM	G2-MW $\times 10^3$ Dalton	G3-RM	G3-MW $\times 10^3$ Dalton
1	0.48	199.526	0.22	512.861	0.22	512.861	0.48	199.526
2			0.32	316.227	0.48	199.526	0.50	181.970
3			0.37	236.026				
4			0.48	199.526				
5			0.52	158.489				

RM: relative mobility, MW: molecular weight in Daltons.

**Table 2 : Different urine protein profiles with the percentage of patients in each group.**

Group	Total No. bands	No. abnormal bands	No. Patients	% patient
1	5	4	20	33.4
2	2	1	33	55
3	2	1	7	11.6

## Discussion

The detection and identification of trace amounts of proteins in complex samples is a major challenge in biomarkers discovery and validation (13, 14). Samples of interest for detection of protein biomarkers are typically serum or plasma. However, with the rapidly growing interest in human urine proteome (15, 16), because of simplicity and noninvasiveness of collection, making urine an alternative sample type for many diagnostic tests (17). Furthermore, urine contains a relatively small number of proteins typically present at low concentrations and thus simpler matrix for detecting proteins as compared to serum (17). Some human diseases, excess proteins are found in the urine as can occur in patients with compromised kidney function. As a result, many of the proteins normally present in blood will also be excreted in to the urine. This condition is known as proteinuria, is often observed in acute inflammation, acute urinary tract infection, amyloidosis, diabetic nephropathy, kidney failure, multiple myeloma, nephrotic syndrome and severe yeast infection (18, 19). Kidney involvement

has been demonstrated in patients with Kala-azar with significant proteinuria (20). The detection of *Leishmania donovani* soluble antigen and antibody (IgM and IgG) has also been shown in urine samples of kala-azar patients (21, 22). Abnormal renal functions particularly, glomerular filtration rate, urinary concentration, acidification that may be correlated to an immunological mechanisms and the evidence of renal proximal tubular damage have been demonstrated recently (23, 24). Lima et al. (2009) (24) have also detected the presence of different kinds of proteins in the urine of patients with Kala-azar particularly, albumin, IgG,  $\beta$ 2-microglobulin,  $\alpha$ 1,  $\alpha$ 2,  $\beta$  and  $\gamma$ -globulins. All these studies had confirmed renal involvement during active Kala-azar disease. However, to the best of our knowledge, no study till now tried to demonstrate the types of protein patterns or profiles that may be observed in patients with the active disease. Therefore, in this study we tried to investigate-using urine samples- the presence of different protein profiles in a number of defined children with Kala-azar in

Iraq using the conventional SDS-PAGE. The results of this study revealed the presence of one protein band in the control healthy children and the presence of three different protein patterns groups in the defined children with Kala-azar. As shown in the results, patients involved in this study were divided into three groups according to their urine protein profile (see the above results). Groups 2 and 3 showed only 2 banding proteins in 55 and 11.6 % of the studied patients, while group 1 showed 5 different banding proteins in 33% of the studied patients. These differences in the number of bands for each banding pattern group may be attributable to the chronicity of infection (24) and to the difference in the degree of renal tubular dysfunction with differences in the types and amounts of proteins excreted in the urine (23, 24). Furthermore, clinical and epidemiological studies in Iraq showed a large differences in the clinical picture of the disease in terms of fever, hepatomegally, splenomegally (2, 25, 26), anaemia, thrombocytopaenia and response to therapy (sodium stibogluconate) (26). Similar findings have been reported in other endemic areas with Kala-azar like Sudan (27), India (28) and Iran (29). This may explain the different protein patterns among different groups in this study. An interesting finding was the presence of common band between group 1 and 2 with a molecular weight ( $512.861 \times 10^3$ ) Dalton. Further investigations with expanded number of patients as well as identifying the types of protein for each banding pattern group especially for the common band which could be of diagnostic value.

## References

1. Kharazmi, A., H. R. Rezai, M. Fani, and N. C. Behforouz. Evidence for the presence of circulating immune complexes in serum and C3b and c3d on red cells of Kala-azar patients. *Trans Roy. Soc. Trop. Med. Hyg.* 1982;76:793-6.
2. Atia, L. A.. Hepatic involvement in visceral leishmaniasis and its relation to the severity of infection. Msc. Theses College of science Baghdad-university.1995.
3. Jensen, A. T. R., A. Gaafar, A. Ismail, C. B. V. Christensen, M. Kemp, A. M. El-Hassan, and T. Theauder. Serodiagnosis of leishmania donovani infections. *Trans Roy. Soc. Trop. Med. Hyg.* 1999;93:157-60.
4. Cerf, B. J., T. C. Jones, R. Badaro, D. Sampaio, R. Teixeira, and, W. D. Jr Johnson. Malnutrition as a risk factor for severe visceral leishmaniasis. *J. Inf. Dis.* 1987;156:1030-33.
5. Al-Bashir, N. M. T. Plasma protein profiles in Iraqi children with Kala-azar. *Indian Journal of Clinical Biochemistry.*(in press).
6. Baqir, H. I., T. I. Al-Jeboori, and N. T. Al-Bashir. Detection of parasite antigen in the urine of patients with active visceral leishmaniasis. *Diagnostic potential.* 2002;2(1):22-5.
7. Islam, M.Z., M. Itoh, S. M Shmsuzzaman, R. Mirza, F. Matin, I. Ahmed, A. K. M. S. Choudry, M. A. Hossain, X. G. Qiu, N. Begam, M. Furuya, J. L. Leafasia, Y. Hashinguchi, and, E. Kimura. Diagnosis of visceral leishmaniasis by ELISA using urine samples. *Clinical diagnostic laboratory immunology.* 2002;9:789-94.
8. Al-Bashir, N. M. T., and S. H. Ali. The use of urine as a sample for the diagnosis of visceral leishmaniasis. *The Iraqi journal of medical sciences.* 2003;2(3):332-39.
9. Islam, M. Z., M. Itoh, R. Mirza, I. Ahmed, A. R. M. S. Ekram, A. H. Sarder, S. M. Shamsuzzaman, Y. Hashiguchi, and E. Kimura. Direct agglutination test with urine samples for the diagnosis of visceral leishmaniasis. *Am J Trop Med Hyg.* 2004;70: 78-82.
10. Lowery, Q. L., N. S. Rosenbrough, A. L. Farr, and R. J. Randall. Protein measurement with Folin-Phenol reagent. *Journal of Biochemistry.* 1951;193:265-75.
11. Fehrnstrom, H., and U. Morberg. SDS and conventional polyacrylamide gel electrophoresis with LKB2117 multiphor. LKB application note 306. Bromam, Sweden 1977.
12. Johnson, A., E. Rohfs, and M. Siverman. Protein. In. *Tietz textbook of clinical chemistry.* Eds. Burtis, C. A. & Ashwood. 3rd ed. R. E. Saunders W. B. CO. London. 1999;pp.477-530.
13. Echan, L. A., H. Y. Tang, N. Ali-Khan, K. Lee, and D.W. Speicher. Depletion of multiple high-dependence protein improves protein profiling capacities of human serum and plasma. *Proteomics.* 2005;5:3292-303.
14. Sigdel, T. K., K. Lau, J. Schilling, and M. Sarwal. Optimizing protein recovery for urinary proteomics, a tool to monitor renal transplantation. *Clinical transplant.* 2008;22(5):617-23.
15. Soldi, M., C. Sarto, and C. Valsecchi. Proteome profile of human urine with two-dimensional liquid phase fractionation. *Proteomics.* 2005;5:2641-47.

16. Thongboonkerd, V., T. Semangoen, and S. Chutipongtanate. Enrichment of basic/cationic urinary proteome using ion exchange chromatography and bath adsorption. *Journal of Proteome Research*.2007;6:1209-14.
17. Kushnir, M. M., P. Morzinski, A. L. Rockwood, and D. K. Crockett. Depletion strategy for improved detection of human proteins from urine. *Journal of Biochemical Techniques*.2009;20: 101-08.
18. Biesenbach G., K. Hommer, G. Stadler, R. Kramar, G. Syré, and J. Zazgornik. ["Diabetic" proliferative retinopathy and nodular glomerulosclerosis without diabetes mellitus]. *Dtsch Med Wochenschr*. 1988;16,113(50):1968-71.
19. van Ypersele de Strihou, C., and Y. Pirson. Indications for dialysis and trasplantation in end-stage renal diseae. *Contrib Nephrology*. 1989;71:1104-10.
20. Weisinger, José R., A. Pinto, G. A. Velazquez, I. Bronstein, J. J. Dessene, J. F. Duque, J. Montenegro, F. Tapanes, and A. R. de Rouse. Clinical and Histological Kidney Involvement in Human Kala-Azar. *Am. J.Trop. Med. Hyg.*1978 ;27(2) : 357-359.
21. Kohanteb, J., S. M. Ardehali, and H. R. Rezaei. Detection of *Leishmania donovani* soluble antigen and antibody in the urine of visceral leishmaniasis patients. *Trans R Soc Trop Med Hyg*. 1987; 81(4):578-80.
22. Mebrahtu, Y. B., L. D. Hendricks, C. N. Oster, P. G. Lawyer, P. V. Perkins, H. Pamba, D. Koech, and C. R. Roberts. *Leishmania donovani* parasites in the nasal secretions, tonsillopharyngeal mucosa, and urine centrifugates of visceral leishmaniasis patients in Kenya. *Am J Trop Med Hyg*. 1993;48(4):530-5.
23. Lima Verde, F. A., F. A. Lima Verde, I. A. Lima Verde, G. B. Silva Junior, E. F. Daher, and E. M. Lima Verde. Evaluation of renal function in human visceral leishmaniasis (kala-azar): a prospective study on 50 patients from Brazil. *J Nephrol*. 2007;20(4):430-6.
24. Lima Verde, F. A., F. A. Lima Verde, E. F. Daher, G. M. Dos Santo, and E. M. Lima Verde. Renal tubular dysfunction in human visceral leishmaniasis (Kala-azar). *Clinical Nephrology*, 2009;71(5):492-500.
25. Abas, M. Z., and A. M. Jabri. Clinico-epidemiological study of kala-azar in Iraq. A thesis submitted to the scientific council of Paediatrics. Baghdad.1998.
26. Ali, S. H., N. T. Al-Bashir, and D. M. Thamiri. Clinical and epidemiological study of visceral leishmaniasis in a group of hospitaized children in Baghdad. *Iraqi paediatric Medicine Journal*, 2003;2(4): 371-77.
27. Zijlstra, E. E., M .S. Ali, A. M. el-Hassan, I. A. el-Toum, M. Satti, H.W. Ghalib, E. Sondorp, and A. Winkler. Kala-azar in displaced people from southern Sudan: epidemiological, clinical and therapeutic findings. *Trans R Soc Trop Med Hyg*. 1991;85(3):365-9.
28. Mittal, V., R. Bahatia, and S. Sehgal. Clinocoepidemiological profile of Kala-azar patients in Delhi. *Journal of Communicable Diseases*, 1989;21(4) : 255-61.
29. Soleimanzadech, G., G. H. Edrissian, A. M. Movahhed, Danesh, and A. Nadim. Epidemiological aspect of Kala-azar in Meshkin-Shahr, Iran (human infection). *Bull WHO*. 1993;71(6): 759-62.