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ELECTROPHORETIC DIFFERENTIAL SERUM PROTEIN PATTERN IN KALA-AZAR*

BY

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Ever since Tiselius first introduced the technique of electrophoresis in 1937, much work on protein analysis in various diseases has been undertaken—well summed up by Miller (1955) and Sherlock (1955). Kala-azar, which is widespread in India, has, however, had only a sparse and casual mention in the vast literature on the subject. The *British Medical Journal* (1957) referred to the extensive work of Feinstein and Petersdorf (1956) on the diagnostic importance of gross, moderate, and mild hyperglobulinaemia, but failed to specify kala-azar even though this disease involves a large proportion of the world's population. This failure is not surprising when it is realized that there are only six references to serum protein electrophoretic pattern in kala-azar—namely, those of Cooper *et al.* (1946), Benhamou *et al.* (1949), Le Bourdelles *et al.* (1949), Sen Gupta *et al.* (1953), Agarwal (1957), and Kunwar *et al.* (1957). The work of Cooper *et al.* was based on a study of the sera of one active case and one convalescent case of kala-azar. Benhamou *et al.* also studied two cases, Sen Gupta *et al.* studied the sera of 9 cases, and my colleagues and I (Kunwar *et al.*, 1957) published records of 19 cases.

In addition to hypo-albuminaemia and hypergamma-globulinaemia, Benhamou *et al.* (1949) reported the presence in the sera of his patients of a Factor X fraction with more rapid mobility than the albumin fraction. Other workers, however, failed to confirm the presence of such a fraction. Both Cooper *et al.* and Benhamou *et al.* identified in their cases the presence in strength of a γ -globulin of slower mobility than that of normal

γ -globulin. Sen Gupta *et al.* also found this slow-moving γ -globulin in the sera of their 9 cases. This slow-moving fraction, akin to the slow-moving γ_2 -globulin found in the sera of rabbits hyperimmunized against type I pneumococci, was held to be characteristic of kala-azar cases and had not been described in other important conditions associated with hyperglobulinaemia. Sen Gupta *et al.* also found a correlation between the complement-fixation test for kala-azar and the presence of this fraction.

Because of sparse and conflicting reports my colleagues and I (Kunwar *et al.*, 1957) considered it worth while to publish a series of 19 cases of kala-azar in which a reassessment of serum was done after the patients had been cured. Our series was larger than any hitherto published, and our findings were at variance in some respects with those of other workers. It would be interesting to compare the findings with those of other workers. I shall attempt to do that.

Our work was based on an analysis of 19 cases with a definite diagnosis of kala-azar. In each case spleen or sternal puncture was positive for Leishman-Donovan bodies. The initial assessment consisted of the clinical picture, Napier's (1921) aldehyde test, and Chopra's (1936) urea-stibamine test; cephalin-cholesterol flocculation, and thymol turbidity tests; estimation of total serum proteins; and paper electrophoretic analysis of serum protein by Durham's hanging strip electrophoretic apparatus, using Whatman No. 3 mm. filter-paper and sodium barbitone buffer at a pH of 8.6 ($U=0.075$), run for 18 hours at 6 mA. (110 volts). The strips were left in a solution of 0.01% bromophenol blue for 18 hours and were scanned by means of a photovolt scanner. Total and differential white-cell counts were done. An assessment of serum proteins was again made after a full course of urea-stibamine therapy when sternal punctures had become negative for Leishman-Donovan bodies.

Our findings (see Table I) and those of other workers, are given below.

Total Serum Proteins

Our 19 cases showed a hyperproteinaemia (average 7.58 g./100 ml.†) before the start of therapy (Kunwar *et al.*, 1957). Only two cases had an initial total serum protein level of less than 7.4 g./100 ml. Normal averages with the same technique in a separate study (Misra, Shanker, and Bajpai, unpublished) were found to be 7.07 g. It is shown below that this rise in total serum protein occurred in spite of marked hypo-albuminaemia and was mainly due to a rise in γ -globulin. However, Agarwal (1957) failed to elicit any rise in the total serum protein.

Serum Albumin

Hypo-albuminaemia had been reported by Chakravarty *et al.* (1949). All of our cases showed a marked fall in the serum-albumin level (average† 30.14%; range, 20.16%–36.22%). Sen Gupta *et al.* and Agarwal also found a generalized fall of serum albumin, the average value in Agarwal's cases being 25.36%. We found no oedema in spite of hypo-albuminaemia, and the presumptive conclusion is that hypo-albuminaemia is due to hepatic damage.

Alpha-globulins (α_1 and α_2).—These fractions showed only a slight rise of doubtful significance. Normal

†These average figures represent only 14 cases which were followed up.

*Work on the subject was done at the department of medicine, King George's Medical College, Lucknow, in collaboration with Dr. K. B. Kunwar and Dr. J. S. Bajpai.

TABLE I.—Showing Total and Differential Serum Protein Figures in Kala-azar before and after Treatment

Case No.	Total Serum Proteins (g.)		Albumin (%)		α_1 -Globulin (%)		α_2 -Globulin (%)		β -Globulin (%)		γ -Globulin (%)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	7.80	7.30	22.84	28.94	8.93	6.73	10.18	13.42	11.66	15.29	46.39	35.62
2	7.60	7.30	31.32	36.49	10.17	8.99	10.64	10.64	5.39	11.33	42.48	32.55
3	8.80	7.50	22.45	29.27	8.35	8.66	8.96	10.21	7.79	13.25	52.45	38.21
4	7.80	7.60	20.16	28.44	18.42	10.90	11.27	10.84	11.18	14.75	39.17	35.06
5	6.60	6.60	31.56	34.56	5.67	6.33	3.56	10.11	5.00	13.24	54.21	35.76
6	6.00	6.20	31.87	33.34	5.10	6.41	6.19	7.50	8.84	12.69	48.00	40.06
7	7.90	—	30.85	—	2.19	—	5.46	—	22.80	—	38.73	—
8	7.40	—	31.28	—	5.25	—	8.55	—	10.80	—	44.12	—
9	7.80	7.50	30.22	36.20	7.19	6.79	10.22	7.16	10.10	13.95	41.27	35.90
10	7.90	7.40	34.20	39.87	8.22	8.10	8.76	7.25	10.19	12.50	38.63	32.28
11	7.80	—	33.11	—	4.29	—	10.21	—	10.20	—	42.19	—
12	8.00	7.50	36.22	42.90	5.38	6.26	10.66	8.14	10.19	13.67	37.51	29.03
13	8.00	7.60	28.99	35.15	7.30	7.10	9.22	5.19	11.48	14.80	42.09	37.76
14	7.80	—	32.18	—	9.98	—	8.76	—	10.41	—	38.67	—
15	7.70	7.30	30.67	30.69	8.80	9.92	8.27	9.66	10.26	14.25	42.00	35.48
16	7.90	7.80	34.24	40.55	7.62	8.89	9.41	8.27	13.89	14.60	34.24	27.69
17	8.20	7.80	30.90	38.99	8.86	5.85	7.70	10.50	10.12	14.96	42.42	29.90
18	8.00	7.60	28.35	31.75	6.25	6.50	8.01	8.29	12.28	13.16	46.21	40.30
19	7.90	—	31.46	—	5.39	—	8.37	—	10.81	—	43.97	—

average values were 14.01% (Misra *et al.*, unpublished). Using the same technique, we found the average level of 17.72% before treatment and 16.76% after treatment.

Beta-globulin.—Except for one case, this fraction was not raised in any of our cases. Average value before treatment was 8.12% (range 5%–22.8%); normal average, 12.42%. The average figure at the end of therapy was 13.74% (range 11.33%–15.29%). Beta-globulin was definitely raised in only one case—No. 7 (22.8%)—and as in this patient serum cholesterol was also raised the cause may have been the raised lipid content of the blood. Unfortunately this case could not be followed up, and the interesting therapeutic result, if any, on the β -globulin level of serum could not be ascertained.

Gamma-globulins (normal, 23.18%: Misra *et al.*, unpublished).—These were markedly raised in all our cases. Before treatment the average figure in 14 cases was 45.4% (range 39.24%–54.29%). After treatment the average value in these 14 cases was 34.71% (range 27.69%–40.3%). This seems to be the most significant finding in kala-azar cases. Sen Gupta *et al.* (1953) demonstrated that the γ -globulins from the sera of their kala-azar cases showed a mobility significantly slower than normal γ -globulin or even the γ_2 -globulin of hyperimmunized serum. Earlier, both Cooper *et al.* and Benhamou *et al.* had reported the presence of slow-moving γ -globulin in their small series. In the cases of Sen Gupta *et al.* the average mobility was 0.4 volt/sq. cm./sec., as compared with normal γ -globulin mobility of 1 volt/sq. cm./sec. at pH 8.6 and ionic strength 0.1. In our series no unusual peak was noticed in the electrophoretic pattern after running it for 18 hours.

Total Globulins

There is a definite hyperglobulinaemia in kala-azar. The average value before treatment in our cases was 5.32 g./100 ml., and by the end of treatment it fell to 4.82 g. As already stated, the *British Medical Journal* (1957) drew attention to the findings of Feinstein and Petersdorf (1956), who used the salting-out technique of Cohn and Wolfson (1948), which gives results that closely agree with electrophoretic findings. They conclude that with a gross hyperglobulinaemia of over 5 g./100 ml., there is a one-in-two chance that the patient is suffering from multiple myeloma, sarcoidosis, or collagen disease. In view of our findings kala-azar must be added to the list in this part of the globe, and must be regarded as an important cause of hyperglobulinaemias of over 5 g./100 ml. From our findings it also becomes apparent that, as with multiple myelomas, sarcoidosis, or collagen

diseases, the chances of diagnosing kala-azar would become bleak with degrees of hyperglobulinaemia lower than 5 g./100 ml.

Fraction X

As already stated, Benhamou *et al.* detected in the sera of their two kala-azar cases a protein fraction of more rapid mobility than the albumin fraction, and they called this factor "X." They concluded that the presence of Fraction X was of greater value in initial diagnosis and also in assessing the ultimate prognosis of the case. Cooper *et al.*, Sen Gupta *et al.*, and Agarwal failed to detect the presence of any fraction with faster mobility than the albumin fraction. We also could not find any unusual peak in the electrophoretic patterns of our 19 cases.

Correlation with Aldehyde and Chopra's Serological Tests for Kala-azar

Levinson and MacFate (1956) very admirably summed up the diagnostic importance of the aldehyde test thus: "de Vries (1939) stated that a positive formol-gel reaction points to a globulin value of 3.7 g./100 ml. or more . . . it is of diagnostic importance in multiple myeloma (Bauer, 1955), in leishmaniasis (Napier, 1922), in lymphogranuloma inguinale (Benhamou and Gills, 1935), in leprosy (Haseltine, 1924), in endocarditis lenta (Kurten, 1929), and in acute rheumatism (Green *et al.*, 1939)."

Cooper *et al.* (1946) carried out the aldehyde test with separated γ -globulin fraction from the sera of kala-azar patients, but failed to get positive results. They concluded that abnormal γ -globulin could not have represented the whole of the material responsible for the positive reaction with the aldehyde test.

In our series of 19 cases (Kunwar *et al.*, 1957) the aldehyde test was positive in 11 and Chopra's test in 16. Table II shows the correlation between the average protein values and the aldehyde test results as reported by us.

It will be seen that the total serum proteins are the same in the aldehyde-positive and the aldehyde-negative groups, but serum albumin is a little lower and γ -globulin a little higher in the positive group. This is in conformity with the general belief that aldehyde gelation is due to hyperglobulinaemia (Levinson and MacFate, 1956).

Complement-Fixation Test, and Anticomplementary Nature of Sera

Kala-azar serum has also been found to be markedly anticomplementary. Sen Gupta and Adhikari (1952) showed that 16% of aldehyde sera of kala-azar cases

TABLE II

	No.	Total Serum Proteins (Average)	Albumin (Average)	α - and β -Globulins (Average)	γ -Globulin (Average)
Aldehyde-positive ..	11	7.7 g.	28.63%	25.96%	45.41%
.. negative	8	7.7 "	32.24%	27.33%	40.43%

were anticomplementary to the extent of about 2 M.H.D. or more. Aldehyde-negative sera of kala-azar cases have rarely been found to be anticomplementary (Sen Gupta *et al.*, 1953). It has been said that the serum changes responsible for positive gelation in the aldehyde test are also responsible in some way for the anticomplementary nature of such sera. It may be noted that a cold precipitable globulin was described in kala-azar serum by Wertheimer and Stein (1944), and a false-positive Wassermann reaction may be obtained in about 10% of cases (Sen Gupta *et al.*, 1953).

In the series of Sen Gupta *et al.*, the complement-fixation test was positive in all the cases, and it was demonstrated that, even when the complement-fixation test alone was positive, there was an increase in slower-moving γ -globulin, associated with decrease of albumin. These authors suggest that the antibody involved in the complement-fixation test for kala-azar is associated chiefly with the slow-moving γ -globulin.

We did not perform the complement-fixation test in our 19 cases.

Thymol Turbidity and Cephalin-cholesterol Flocculation Tests

The biochemical basis of these tests is far from being solved with certainty. But MacLagan (1944), Kunkel and Hoagland (1947), and Martin (1949), have tried to elucidate the part played by γ -globulin in the thymol turbidity reaction; while Hanger (1954) has adequately discussed the role of γ -globulin in the positive cholesterol flocculation test. Thus, although the last word has not been said, the part played by raised γ -globulin is generally agreed upon. Of the 19 cases reported by us (Kunwar *et al.*, 1957), the liver-function tests reveal abnormality in every one. The thymol turbidity reaction varied from 6 to 18 units; cephalin-cholesterol flocculation was ++ in eleven cases and +++ in eight.

Leucocyte Count; Splenomegaly

Total and Differential Leucocyte Count.—Although leucopenia with relative lymphocytosis was found in all the 19 cases, no correlation could be established between the degree of leucopenia or lymphocytosis and the protein fraction abnormality.

Splenomegaly.—No correlation could be established between the size of the spleen and the alterations in the differential serum protein pattern in cases reported by us.

Effect of Therapy

In 14 of our 19 cases, from a laboratory point of view cure was thought to have been attained after a full course of antimony therapy, when sternal smear had become negative for L.D. bodies and when the patient had been relieved of his symptoms. At the end of therapy in these 14 cases there was a slight rise in serum albumin level (the average figure rose from 29.76% to 34.79%), but it was still much below the normal level (50.39%); γ -globulin showed a great fall (on average from 45.40% to 34.71%), but was above the normal average value of 23.18% (Misra *et al.*, unpublished). Total serum protein values also showed a fall (average from 7.58 g./100 ml. to 7.4 g), but was still above the

normal value of 7.07 g (controls). These estimations in cases reported by us were done in only 14 cases, as 5 patients had left the hospital prematurely. The changes in total and differential serum protein levels in these 14 cases, before and after therapy, are shown in Table I, which illustrates such changes individually. Unfortunately a complete follow-up of these cases could not be done for practical reasons, but it would have been worth while if we could have followed them up long enough to assess the length of time required for the total and differential serum protein levels to return to normal.

Summary

The literature on total and differential serum protein analysis in kala-azar cases is reviewed, and a comparison is made with results obtained by myself and two colleagues.

Characteristic changes in the serum protein pattern consist of the following. (a) Usual rise in total serum proteins. (b) Universal and marked fall in serum albumin. (c) Gross hyperglobulinaemia of more than 5 g./100 ml. is a characteristic feature. In this respect kala-azar should rank as a major cause of severe hyperglobulinaemia along with multiple myeloma, sarcoidosis, and collagen diseases. (d) There is an insignificant rise in alpha- and beta-globulins. (e) Aldehyde-positive cases show a higher degree of hyperglobulinaemia than aldehyde-negative cases. (f) Literature on slow-moving γ -globulins, and their relationship to the complement-fixation test and aldehyde test, are discussed. (g) With treatment, various protein components have a tendency to return to normal. However, biochemical (protein pattern) correction is still incomplete when a clinical and laboratory cure has already been achieved. (h) The presence of factor "X" could not be confirmed by most workers.

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