



VARIATION IN SERUM PROTEIN AND ITS COMPONENT IN DIFFERENT STAGES OF KALA-AZAR

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ABSTRACT

The problem of Kala-azar has importance because of its epidemic outbreak in some parts of our country, India. It has been observed clearly that there is definite variation in the values of different plasma protein in various stages of Kala-azar cases. Objective is to assess the severity of damage of liver in kala-azar by testing Serum protein and its component in different stages of illness. This study was done on 100 selected diagnosed cases of kala-azar from RMRIMS & NMCH, Patna and 25 healthy individuals were taken as control. Appropriate statistical tests were used whenever necessary. The mean value of total serum protein, albumin and globulin ratio were low in comparison to mean value of control group. It was concluded that Total and Differential Protein test can give guideline to know the prognosis of the disease as well as it may also help in treatment plan.

KEYWORDS : A: G Ratio, Albumin, Kala-azar, Total Protein,

INTRODUCTION

Kala-azar is a protozoal disease characterized by fever of alternating / remittent or intermittent type. The problem of kala-azar assumes considerable importance today in view of epidemic outbreak in our country. This disease has made abode in the plains of Ganges and Brahmaputra. It has been known to occur epidemically and endemically in well defined areas in the eastern sector of the country viz., Assam, West Bengal, Bihar, eastern districts of Uttar Pradesh, foothills of Sikkim and to a lesser extent in Tamil Nadu and Orissa. (Park, PSM, 23rd Edn., 2015). [16] In affected districts, patient presents with almost proportionate enlargement of the liver & spleen in kala-azar. M.N.De (1933) studied 25 cases of kala-azar in post-mortem. He studied distribution of parasite in different organ and its changes and observed that in liver, the parasites were present enormously in the kupffer cells, causing their hypertrophy but parenchymal cells of the liver were free from them. [1]. George Cheever Shatuck (1951) also mentioned hepatic enlargement in kala-azar cases. [4]. Napier (Brit. Encyclopedia of Medical Practice, 2nd edn, 1951) was able to show parasitization of the kupffer cells of the liver in kala-azar cases. These involved reticuloendothelial tissue invades the lobules, separates the hepatic parenchymal cells of the central zone, compress them, and thus cause their atrophy. At a later stage, such reticuloendothelial tissue was found to be partly replaced by fibrous tissue producing interlobular cirrhosis. [5]. Upon liver biopsy on 10 cases of kala-azar, Sen Gupta, P.C. et al (J. Ind. Med. Assoc. 1953) found intense degree of reticuloendothelial cell proliferation, and cloudy swelling & marked inequality in size of hepatic parenchymal cells. In all the cases, collection of lymphocytes, plasma cells and histiocytes around some parasitized cells were seen resembling microscopically granuloma in the substance of liver. [6].

In study of 118 cases of kala-azar, S.N. Chatterjee (1954) found that about 28% of cases showed proportionate enlargement of liver and spleen, while high percentage of case showed disproportionate enlargement. [7]. According to report of Sen Gupta P.C., Chakravarty N.K., Roy H.N. and Dasgupta B. (1956), histological finding in liver was abnormal in Visceral Leishmaniasis. [9]. Biochemical aspect of such liver abnormality was first described by Napier (1946) who found marked reduction in serum albumin with corresponding increase in the globulin, i.e. reversal in the albumin: globulin ratio. Serum proteins show an increase in globulin (mainly IgG component of γ - globulin) which may be as high as 9 mg/dl, the ratio of globulin to albumin is typically high. (SEMJ, vol.2, no. 6). [2].

The Trisettes electrophoresis apparatus was employed to analyse the serum proteins in 9 cases of Kala-azar (Sen Gupta et al, 1953). [6]. They found profound increase of γ -globulin in all the cases. In chronic cases, the increase was more marked and it was found that porphyrin of γ -globulin was about 5 times the value of albumin fraction. This increase of γ - globulin was also detected in another series of 9 cases of kala-azar by paper electrophoresis (Banerjee et al,

Bull. Cal. Sch. Trop. Med., 1955). [8]. A case report, published in J. of Clinical Path, June 1994, 47 (6), 547-551 on 9 patients of kala-azar, mentioned biochemical evidence of low total protein before starting of treatment. [11]. A case report by O P Dhakal, JEMDS, May 2013 also mentioned totally deranged total protein in kala-azar patients from Sikkim. [13]. According to Manson's Tropical Diseases (23rd Edition 2014), kupffer cells are heavily parasitized, hypertrophied & hyperplastic and push out into the sinusoids in leishmaniasis. Patchy necrosis of groups of hepatocytes may also be found in leishmaniasis if venous congestion is severe. [15]

For a better observation and clear study, the kala-azar cases have been classified into below mentioned 3 stages (based on worldwide accepted classification of N.K.Chakravarty and P.C.Gupta in 1976), with duration of illness being the main criteria for classification: [10]

- (A) Early stage: duration of illness below 3 months
- (B) Mid stage: duration of illness between 3-5 months
- (C) Advanced stage: duration of illness above 5 months.

It has been shown that in most of the cases of kala-azar, there was evidence of altered serum protein secondary to liver involvement. Also, it has been observed clearly that there is a definite variation in the values of different serum protein in above mentioned stages of kala-azar cases. Hence, for a better appreciation of ensuing liver damage, different components of serum protein level eg. Total serum protein, albumin, globulin and A: G ratio have been observed in diagnosed cases of kala-azar. We have found decrease in total protein, serum albumin and albumin-globulin ratio with the progression of disease and increase in serum globulin as the disease advances. These values have provided the evidences of definite liver damage.

The main aim of this work is to assess the severity of liver damage in Kala-azar by means of estimating total protein & its component : serum albumin, serum globulin and serum albumin-globulin ratio in different stages of illness and thus apply this information related to prognosis in kala-azar.

METHODOLOGY

The study was conducted among 100 selected diagnosed cases of kala-azar admitted in RMRIMS, Patna and 25 healthy individuals selected in NMCH, Patna between study period of September 2015 to November 2015.

1. Selection of Study group:

- **Case group:** One hundred diagnosed cases of kala-azar were selected from RMRIMS, Patna. The stage of disease was found out. The blood samples were taken within the first week of their admission.
- **Control groups:** Twenty five healthy individuals were selected in NMCH, Patna as control from volunteers. The control groups

were non-alcoholic, non-smoker and had no past history of hepatitis/jaundice, Tuberculosis, altered sensorium, haematemesis, melaena, blood transfusion, drug or tobacco addiction and prior hospitalization for any disease.

2. Collection of Specimen: About 10 ml of blood was collected in a clean dry test tube using a dry sterilized disposable syringe and was allowed to clot. The serum was separated and centrifuged in a Kahn's test tube. Finally the clear serum was collected in a dry test tube. That test tube was used for the estimation of various serum protein levels.

Serum Protein Estimation: This includes total protein, albumin and globulin estimation.

Total protein and albumin estimation was done by Biurette method. We used gm% as its unit. (Globulin = Serum Protein – Albumin).

Statistical Calculation: The data were entered in MS excel spread sheet and result was analyzed by SPSS 16 version. Mean value, Standard Deviation (S.D.), Standard Error of Mean (SEM), t-value and P-value were calculated.

RESULTS

The study was based on 100 diagnosed cases of kala-azar and 25 number of normal subjects were kept as control. Different types of observations were obtained during the course of study which have been tabulated in tables 1,2,3,4 & 5 given below.

Table 1: Showing Comparative Mean Value Of Serum Protein In Control Group And Kala-azar Cases

Types of cases	No. of cases	Serum Protein in Gm %						Remarks
		Range	Mean	S.D.	SEM	t	P	
Control group	25	6.3-8.4	7.24	0.57	0.11	7.56	<0.001	Significant
Kala-azar	100	4.0-8.0	6.01	0.76	0.077			

Types of cases	No. of cases	Serum Albumin in Gm%						Remarks
		Range	Mean	S.D.	SEM	t	P	
Control group	25	4.0-5.4	4.84	0.37	0.069	15.89	<0.001	Significant
Kala-azar	100	1.0-4.4	2.41	0.74	0.074			

Table 2: Showing Comparative Mean Value Of Serum Albumin In Control Group And Kala-azar Cases

Types of cases	No. of cases	Serum globulin (in gm%)						Remarks
		Range	Mean	S.D.	SEM	t	p	
Control group	25	1.8-3.4	2.40	0.35	0.068	12.04	<0.001	Significant
Kala-azar	100	2.6-5.2	3.61	0.47	0.047			

Table 3: Comparative Mean Value Of Serum Globulin In Control Group And Kala-azar Cases

Types of cases	No. of cases	Albumin-Globulin (A:G) Ratio						Remarks
		Range	Mean	S.D.	SEM	t	P	
Control group	25	1.4-2.8	1.98	0.29	0.058	13.2	<0.001	Significant
Kala-azar	100	0.25-1.69	0.68	0.47	0.047			

Table 4: showing Comparative Mean Value Of Albumin & Globulin Ratio (a: G Ratio) In Control Group And Kala-azar Cases

Types of cases	No. of cases	Total Serum Protein (in Gm%)						Remarks
		Range	Mean	S.D.	SEM	t	P	
Control group	25	6.3-8.4	7.24	0.57	0.11	7.56	<0.001	Significant
Kala-azar	100	4.0-8.0	6.01	0.76	0.077			

Table 5: SHOWING COMPARATIVE MEAN VALUE OF TOTAL AND DIFFERENTIAL SERUM PROTEIN, ALBUMIN-GLOBULIN RATIO IN DIFFERENT STAGES OF KALA-AZAR CASES AND CONTROL GROUP

Types of Cases	Total Serum Protein (in Gm%)				Serum Albumin (in Gm%)				Serum Globulin (in Gm%)				Albumin-Globulin Ratio			
	Range	Mean	S.D.	SEM	Range	Mean	S.D.	SEM	Range	Mean	S.D.	SEM	Range	Mean	S.D.	SEM
Early stage	6.0-8.0	6.58	0.60	0.10	2.0-4.4	3.01	0.67	0.11	2.6-4.4	3.56	0.46	0.08	0.50-1.69	0.87	0.28	0.05
Mid stage	4.2-7.7	5.93	0.57	0.08	1.5-3.6	2.31	0.45	0.07	2.7-5.1	3.62	0.45	0.07	0.38-1.16	0.65	0.16	0.02
Advanced stage	4.0-6.5	5.24	0.62	0.14	1.0-3.0	1.56	0.44	0.10	2.9-5.2	3.67	0.57	0.13	0.25-0.85	0.44	0.15	0.03
Control Group	6.3-8.4	7.24	0.57	0.11	4.0-5.4	4.84	0.37	0.07	1.8-3.4	2.40	0.35	0.07	1.4-2.8	1.98	0.29	0.05

DISCUSSION

Liver is a vital organ of the body. It performs various functions in regulating internal metabolism, e.g. synthesis of protein – albumin; conversion of non-glucose substances into glucose and glycogen; esterification of cholesterol (cholesterol metabolism) and also some detoxicating function. It is very clear that this organ is almost invariably involved in kala-azar cases.

In the present study, one hundred diagnosed (based on finding of Leishmania donovani in the bone marrow or splenic aspirate smear examination) patients of kala-azar were selected from RMRIMS, Patna, and they were classified in three stages on the basis of classification made by N.K. Chakarvarty and P.C. Gupta (1976) [10]:

- (A) Early stage –Duration of illness below 3 months.
- (B) Mid stage –Duration of illness between 3 to 5 months.
- (C) Advanced stage – Duration of illness above 5 months.

Values of serum protein and their components were estimated in these diagnosed cases of kala-azar and was compared with those of control groups. The total protein in control group ranged between 6.3 – 8.4 gm % with a mean value of 7.24±0.57 gm% and in kala-azar cases range of total protein was 4.0 to 8.0 gm% with a mean value of 6.01±0.76 gm% (Table-1). The serum albumin in control group

ranged between 4.0 to 5.4 gm% with a mean value of 4.84±0.37 gm% and in kala-azar cases it ranged between 1.0-4.4 gm% with a mean value of 2.41±0.74 (Table-2). The serum globulin level in the control group ranged between 1.8-3.4 gm% with a mean value of 2.40±0.35 gm% where as in kala-azar cases it ranged between 2.6-5.2 gm% with a mean value of 3.61±0.47gm%(Table-3). The serum albumin-globulin ratio in the control group ranged between 1.4-2.8 with a mean value of 1.98±0.29 whereas in kala-azar cases it ranged between 0.25-1.69 with a mean value of 0.68±0.47 (Table-4). The serum protein level, serum albumin level and albumin-globulin ratio is definitely low in comparison to control group and its level decreases as the disease advances. Serum globulin level shows that the level is definitely high in comparison to control group and level increases as the disease advances (Table-5).

CONCLUSIONS

The above mentioned findings indicate that the mean value of total serum protein and serum albumin was significantly low in kala-azar cases in comparison to control group. The mean level in serum globulin was significantly high in kala-azar cases in comparison to control group. The albumin-globulin ratio was low in kala-azar cases in comparison to control group – the finding being in close conformity with the work of P.C. Sen Gupta and Chatterjee (1948).[3]. S.R. Naik, P.N.Rao, D.V.Datta, S.K.Mehta, R.C. Mahajan have

reported serum albumin less than 2.50 gm%, serum globulin greater than 3.0gm% in their study on kala-azar, which compares well with the findings of the present observation.[19]

In my study, the total serum protein, serum globulin and albumin globulin ratio were also studied according to severity of the disease. The results here in study clearly showed that the total serum protein was decreasing with the progress of the disease. The serum albumin levels were also decreased proportionally with the progression of the disease, while serum globulin showed increasing trend with the advancement of the disease process and the albumin-globulin ratio were also altered with the advancement of the disease. The results in this study were also close with the work of Sen Gupta and Chatterjee (1948).[3] According to Davidson (22nd edition 2014)[14] & Harrison's principle of Internal Medicine (19th edition 2015)[17], functioning status of liver can be assessed by serum protein level.

The disorder in hepatic function is an inability on the part of the liver to convert the amino acid derived from the food protein into serum albumin. So in this way there is decrease in protein synthesis. The hypoalbuminemia in kala-azar, therefore can be regarded as an indication of hepatic dysfunction and failure of liver to synthesize albumin either due to parasitisation of the liver cells or possibly due to direct or indirect effect of the toxic metabolic products of parasite on liver cells.

The serum proteins showed lower level in 69% of total cases and serum albumin was lowered significantly in 97% of kala-azar cases. Serum globulin was significantly increased in 67% of total cases as the disease progress. Hyperglobulinaemia that was observed was probably a relative response to some immune complexes stimulated by heavy parasitisation in the liver cells. The albumin-globulin ratio was altered in almost all the cases of kala-azar.

From above results, it can be concluded that the variation in the serum protein is an early evidence of hepatic dysfunction in kala-azar.

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