

## Total Protein Determination and Estimation of Total Gamma Globulin in NHL Patients

Vandana Shrivastava

\*Reader, Dept. of Microbiology, ITS Centre for Dental Studies and Science, Murad Nagar, Ghaziabad, Uttar Pradesh, India.

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### Abstract

Total gamma globulin level remained almost same in pooled group study as well as in stage wise data but in stage IV significant decrease in stage IV.

Serum protein indicated significant increase in pooled as well as stage wise data.

**Keywords:** NHL = Non Hodgkin's Lymphoma; IgG = immunoglobulin G; B cell = B lymphocyte.

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### Introduction

Non Hodgkin's lymphoma (NHL) represents tumor of immune response (Lukes *et al*, 1975)

Lymphoma is generally B cell origin but some are of T cell origins. In above mentioned type humoral immunity is suppressed. The majority of NHL cases are of B cell origin.

Total No of patients Control N = 55

NHL = 60

### Material and Methods

Diagnosed cases of NHL 60

A record of clinical history of both type of lymphomas including age, Sex, clinical staging of the diseases personal habits. Like alcoholic, Tobacco chewing, smoking etc.

- 1) Total protein Determination (Lowry *et al*, 1951)

- 2) Total gamma globulin estimation by electrophoresis kohn, J (1960).

### Total Protein Determination

#### Principal

Protein react with folin ciocalteu reagent to give a colored complex. The colour is formed due to the reaction of alkaline Tyrosine and tryptophan present in the protein. The intensity of colour depends on the amount of these amino acids present in the proteins.

#### Materials

1. Alkaline sodium carbonate solution

### Photo 1: Photograph showing electrophoretic apparatus



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**Corresponding Author:** Dr. Vandana Shrivastava, 66, Kunj Vihar, Bhind Road, Near Gola Ka Mandir, Gwalior, Madhya Pradesh, India.

E-mail: shvandana@rediffmail.com

(20gm./lt/ sodium carbonate in 0.1 ml/lit sodium hydroxide)

2. *Copper sulphate*: Sodium potassium tartarate solution (5g/lit  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 10g/lit. sodium potassium tartarate) prepared fresh by mixing stock solution.
3. *Alkaline solution*: Prepared fresh on the day of use by mixing 50ml of 1 and 1ml of (2)
4. *Folin ciocalteu reagent*: Commercially available reagent was diluted with equal volume of water on the day of use. This is a solution of sodium tungstate and sodium molybdate in phosphoric acid and hydrochloric acid.
5. *Slandered protein*: Bovine serum albumin 0.2mg/ml.

Total gamma globulin estimation by electrophoresis Kohn, J (1960).

#### Materials

*Electrophoretic apparatus*: Beckman mirozone R 101 model.

- Cellulose acetate membrane
- *Buffer*: Barbitol buffer ( $\beta$  2 buffer) 0.75  $\mu\text{m}$  PH-8.6

*Staining solution*: Ponceau red solution.

*Destaining solution*: methanol: acetic acid water were mixed in ratio of 9:1:10.

#### Methods \b

Electrophoretic tank was filled to mark with buffer.

- The cellulose acetate membrane was allowed to sock with the same buffer and excess of buffer blotted out lightly.
- Now the sample was applied with the help of Microzone applicator which carries 0.25  $\mu\text{l}$  of the sample.
- Electrodes was connected to the power supply.
- Electrophoresis was carried out at a constant voltage of 200 volts for 45

minutes.

- After the run, strips was removed and stained by ponceau red for 5 minutes.
- The strips were distained in distaining solution until the back ground of the strip become clear.

#### *Estimation of Gammaglobulin – Varley (1984)*

The stained portion showing distinct protein band were cut into three region namely

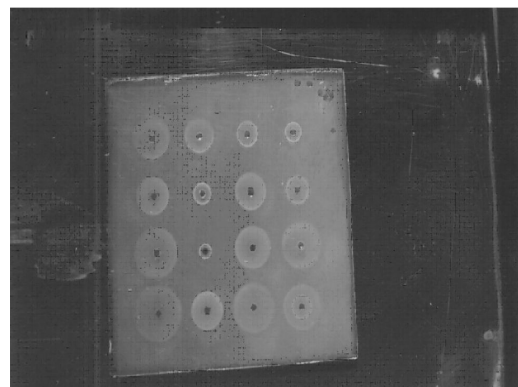
1. Albumin region
2. Gammaglobulin region

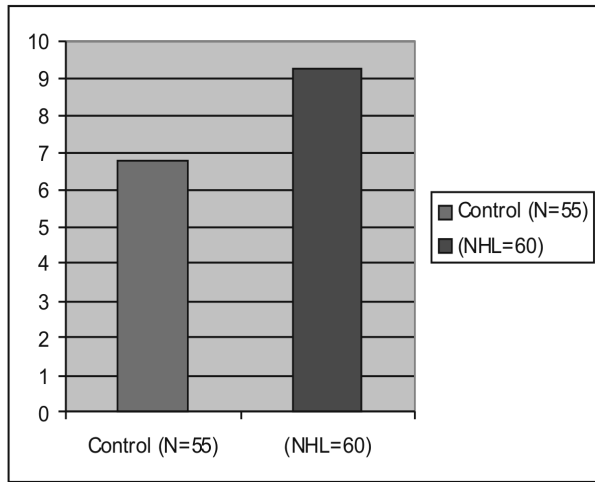
Globulin other than gamma globulin region

**Photo 7 (a): Photograph showing radial immunodiffusion of Immunoglobulin G using antihuman IgG standard of different concentration (from right to left 400 - 1600 mg / dl.)**



**Photo 7 (b): Showing radial immunodifusion in sample collected from study subjects**





Control VS NHL

the dye from each section was eluted treating with .4N NaoH solution for 20 min. each independently.

### Results and Discussion

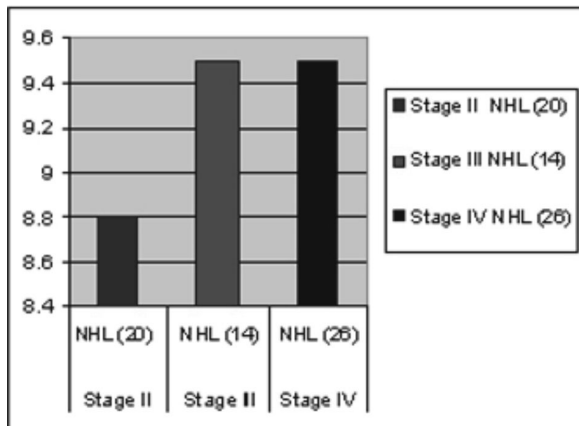
#### Total serum proteins

The optical density was read at 540 nm using  $\mu$ v Spectrophotometer against appropriate blank.

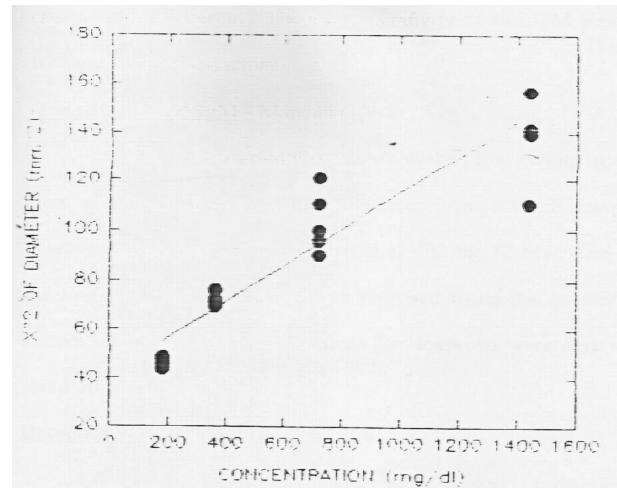
Control (N=55) 6.8 ± 0.12  
 (NHL=60) 9.3 ± 0.29 \*\*\*\*

#### Total Serum protein in various study groups.

Control  
 N=55 - 6.8 ± 0.12



**Standard Graph: Normal serum having IgG Concentration of 180 mg/dl, 360 mg/dl, 720 mg/dl, 1440 mg/dl on the X-axis and square of diameter (mm<sup>2</sup>) on the Y-axis**



Stage II NHL (20) 8.8 ± 0.39<sup>a</sup> \*\*\*\*  
 Stage III NHL (14) 9.5 ± 0.52 \*\*\*\*  
 Stage IV NHL (26) 9.5 ± 0.54

Control N=55 - 16.7 ± .80

Stage II NHL (20) 16.7 ± .80  
 Stage III NHL (14) 13.09 ± 1.45  
 Stage IV NHL (26) 11.49 ± 0.86

Total Gamma Globulin is expressed as (X ± SE)

Serum proteins indicated significant rise in pooled date of NHL patients. In stage wise data also almost in all the stages total serum protein showed significant rise then control.

NHL of B cells to be associated with abnormal serum immunoglobulin or antibody deficiency (Alexanian, 1975 and Murray 1980).

Kumar and Panny (1982) observed minimal abnormalities in immunological function but impairment of both antibody mediated and cell mediated immunity is often recorded in advance stages of the disease. However the

exact mechanism for increase or decrease of these immunoglobulin is not yet clear and it is very difficult to comment on. It is also very difficult to rule out the cause of infection which is not apparent in these subjects causing the alteration in immune globulin.

### Acknowledgement

Non Hodgkin's lymphoma are the disorders involving primarily the lymphoid tissue's they are of monoclonal origin. These may be lethal unless controlled or eradicated through therapy.

Datta *et al* (1971) reported elevated level of IgG in Lympho sarcoma (NHL) In NHL cases peripheral blood lymphocyte curing surface immunoglobulin (B. cells) function was decreased or impaired (Piessens *et al* 1973).

Lichtenstain and Taylor (1980), reported that incidence of quantitative immunoglobulin abnormalities in patients with T cells lymphomas and B cell lymphomas.

Kumar and penney (1980) reported that in early stage of non Hodgkin's Lymphoma abnormalities of immunological functions are usually minimal.

*Conflict of Interest:* Non Declared

*Source of Support:* Nil

*Ethical clearance*

College had it on ethical committee through which topic was cleared.

### References

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