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.

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.

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.

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 cardonate solution

Photo 1: Photograph showing electrophoretic apparatus
(20gm./lt/ sodium carbonate in 0.1 ml/lit
sodium hydroxide)

2. Copper sulphate: Sodium potassium
tartarate solution (5g/lit cusO4. 5H2O in
10g/lit. sodium potassium tartarate)
prepared fresh by mixing stock solution.

3. Alkaline solution: Proposed 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: Barbital buffer (β 2 buffer) 0.75 µm
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 soak 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 µl 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.

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
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 µv 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

Stage II NHL (20) 8.8 ± 0.39 ****
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