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Immune Status of NHL Patents of Various Group

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Abstract:

Aim of Present Study, to show the immune status of Non-Hodgkin's lymphoma patients we have cauterized again them according to stage wise also to study immune status (cellular as well as Hum oral) of these patients. Our studies reveal the status of patients which is help full for there treatment as well as there improvement status.

Key words: NHL (Non Hodgkin's lymphoma); B cell (B lymphocyte); IgM (Immunoglobulin M); IgG (Immunoglobulin G); IgA (Immunoglobulin A); IgE (Immunoglobulin E); IgD (Immunoglobulin D).

Introduction

Lymphoma is malignant tumor of immune system which develops in the lymphatic system of an individual originates from lymph nodes. Enlargement – of lymph node is painless, discrete and from in other symptoms, loss of weight, fever and sweating. In this certain type of lymph cell, being to divide and multiply abnormally in the lymph nods. There cells spend and disseminated to other part of body like lungs spleen, line and bone marrow.

Non Hodgkin's lymphomas appear to

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represent tumor of immune response. (lukes et al. 1975)

The hum oral immunity, which is also called as anti body mediated immunity, needs antibody to play important role in the protection from infections. These antibodies are gamma globulins. These are live classes of immunoglobulin ie IgG, IgM, IgA, IgE, IgD Synthesized by B cells. When B cells are stimulated, they divide and transform into plasma cells. which synthesize immunoglobulin generally in lymphomas cell mediated immunity suppressed but in certain cases of NHL which are B cell origin hum oral immunity is also suppress. The majority of NHL cases are B cell origin.

Antigenic ally Stimulated B Cells undergoes blast- transformation, becoming successively plasma blasts, intermediate transitional cells and; plasma cells the mature plasma cell is the antibody secreting cell.

Plasma cell is end cells and has a short life span of two as three days. A plasma cell only makes an antibody of Single specificity of a single immunoglobulin class.

Aims and objective

Our aims of study are to identify the deficit part of the system (immune) by using different parameter. And objectives are to identify and isolate the abnormal cell which ore playing important role. Indifferent stage of disease.

Review of literature

Non Hodgkin's lymphoma is the disorders involving primarily the lymphoid tissue. NHL is originated from lymphoid tissues. They are of monoclonal origin. These disorders are lethal unless controlled or eradicated through therapy.

Data et al. (1971) reported elevated level of IgG in Non Hodgkin's lymphoma (NHL) which is an important component of humoral immunity.

Piessens, et al. (1973) reported that in NHL cases peripheral. blood lymphocyte carrying surface immunoglobulin (B cells) function was decreased or impaired.

Gajlpeczalska, et al. 1973, reported the decrease in number and functional impairment in surface immunoglobulin bearing B lymphocyte in NHL subject.

Alexanion (1975), reported that Non Hodgkin's lymphoma (NHL) of B cells may be associated with abnormal serum immunoglobulin and antibody deficiency. Same observation was reported by murray (1980).

Crispen, et al. (1976) reported a 74% reduction in different cells of all type of child cancer.

Jones, et al. Hancock, et al (1977) advani et.al (1980) reported impairment of delayed hypersensitivity and abnormalities of blood. T-Cell functions in NHL patients.

Bjorkholm, (1978) reported that cell mediated immunity is selectively impaired in virtually all entreated patients.

Lichtenstein and taylor (1980), reported that the incidence of quantitative immunoglobulin abnormalities in patients with T – cells lymphoma and B cells lymphoma.

Kumar and penney (1980), reported that in early stage of non Hodgkin's lymphoma abnormalities of immunological function are usually minimal but impairment of both antibody and cell mediated immunity is often noted in advanced disease.

Lindemalm, et al. (1983) concluded that functional abnormalities of both T and B lymphocyte in non Hodgkin's lymphoma are closely related to the presence of active disease.

Buchi et al. (1984) Studied the lymphocyte population in the blood of patient with leukaemic non Hodgkin's lymphoma. A statistically significant decrease in at both the T and B cells were detected in low grand-malignant lymphoma (LGML) as well as lymphocyte was not found to be altered in both high grade malignant lymphoma (HGML), the number of the circulating B Lymphocyte was not found to be altered in both HGML and LGML.

Material and Methods

Study done to test the immune states of the patient of non Hodgkin's lymphoma. The patients were the diagnosed cases of NHL (cancer hospital & research Institute Gwalior) nub. Of cases were – 55.

Control - Same

We had taken all records of the patients. From there.

For the blood collection – we took 5ml of patient blood for. Serum isolation as well as for Heparinied Blood for other Parameters.

Parameters for study of Immune status of patients.

- 1. Estimation of total protein.
- 2. Estimation of Gamma globulin and A, G ruto
- 3. Estimation of Immunoglobulin IgG and Igm
- 4. Absolute lymphocyte count
- 5. Quantization of T and B cells.

We separated lymphocyte, after washing, we had taken 1 ml of RPMI Medium and add to there washed cells and put 10% fetal calf serum (FCS).

B cells Detection done by EAC Rosetting (1978).

Total protein determination done by lowry et al. (1957).

Total gamma globulin estimation by electrophoresis kohn J. et al. (1960) and gamma globulin by varley (1940).

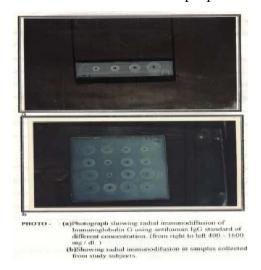
Estimation of IgG by Mancini et al 1965.

And purified the IgG as per method of Mckinney and pertinson (1987) The semipurified IgG was further purified by Colum chromatography using sephadex G 200 in a glass column of 80x2.5 cm.

Quantization of IgG done by Mancini et.al (1965).

Estimation of IgM is done in two steps.

- 1. Purification of IgM as antigen (weir 1978) by using Colum chromatography using G 200 Sephadex Colum.
- 2. Quantization of IgM done by Mancini (1965) the values for unknown were derived from the standard prepared.



Statistical Analysis

Data were analysed using various statistical formulas and expressed. As Meant se $(X \pm SE)$ and statistical significance was determined using "t" test.

Results and discussion

In NHL patients (Non Hodgkin's lymphomas) there is a malignant monoclonal proliferation of lymphoid cells usually identifiable as B cells. Occasionally T cells ate also affected. Majority of NHL ate of B cell origin.

Observation were made in 55 NHL patients by compassing the mean values of each parameter ie. Total serum protein IgM, IgG antibodies, and total leukocyte count.

In NHL patients significant decrease in total leukocyte count in stage II as compared to control (P <0.001)

Control 7609±212 N = 55 Stage II NHL (20) 5957.57±382

State III and stage IV not shares the significant decrease the leukocyte count as compare to control.

Pooled values indicated significant decreased percentage of E rosetting T cells in NHL patients. When with control. In theses patients, stage wise data of E rosetting cells decreased values nor visible in all the three stages II, III, and IV (P L 0.001).

Pooled data on EAC resetting B cells increased percentage was observed in NHL cases (P<.001).

Control = 55 20.30 ± 0.57

NHL = 55 24.3 \pm 1.15 stage III (25) 20.19 \pm 17 stage II NHL = (20) 28.35 \pm 17

Pooled data on total serum protein content was also studied in NHL patients.

Control N = 556.8 ± 0.12 NHL N = 55 9.3 ± 0.29 (Pooled)

Control	(6.8 ± 0.12)
N = 55	
Stage II NHL	$20\ 8.8 \pm 0.39$
Stage III NHL	$14\ 9.5\ \pm\ 0.52$
Stage IV NHL	$26\ 9.5\ \pm\ 0.54$

Serum albumin globulin ratio was also one parameter studied in NHL cases. The value was quite high (P <0.01).

In stage use data of serum albumin globulin ration increased in NHL patients in all the stage (II, III, IV) results was marginal high (P< 0.05) in stage II and III but in stage IV results was highly increase (P< 0.001).

NHL $(N = 55) 2.30$	± 0.16	(P< 0.001)
Control		
N = 55	=	1.59±0.06
Stage II NHL (20)	=	2.43±0.40
Stage III NHL (14)	=	2.18 ±0.29
Stage IV NHL (21)	=	2.27±0.15
(P<0.001)		

A/G ration control 1.59±0.06

Pooled value of Serum Immunoglobulin G. Control

$$N = 55$$
 1199.7±48.5

Serum Immunoglobulin IgM% is expensed as $X \pm SE \ NHL = 1451.6 \pm 58.7$

P<0.01 = significant

Serum IgG level was increased in NHL cases of stage II, III patients, but in stage IV value was significantly high when compare to control (P<0.01).

Serum IgM level was raise in NHL patients but not significant.

Control	116.05	±3.5		
N = 55		Pool	ed data	
NHL (55)	112.5±2	2.0		
Serum Immunoglobulin (IgM)				
Control		116.05±3.5		
N = 55				
Stage II (2	20)=	NHL (20)	112.9±3.6	
Stage III ((14)	=	114.4±4.4	

Stage IV (21)	=	11.25±3.02
Pooled values of	total Gai	mma Globulin
Control $N = 55$		16.07 ± 0.80

NHL = 55 12.56±0.76 (Total Gamma Globulin % 15 expressed as

(P<0.01)

 $(X - \pm SE)$

Stage use Total gamma Globulin

Control = $(N 55)$ =	16.07±0.80
Stage II NHL (20)	13.09±1.45
Stage III NHL (14)	13.78±1.98
Sate IV NHL (21)	11.49±0.86

Discussion

The present study was under taken of NHL patient stage wise as well as pooled data. The observation were mode according to stage of the patients. Control war. (Pooled data)

In NHL cares the Severity of immune deficiency may vary and is not necessarily relatable to extent of the disease. (lany and Kaplan 1974). The nature of immune defect and mechanism under lying the immunological dysfunction in partly exposed, some fact of immune deficiency seems to be altered with active disease.

T Cell indicated significant decrease in NHL patients. In the stage wise data also indicated significant decrease in T cells. The clinical manifestation of NHL might well dependant on degree of T cell immunocompetance.

Hancock, et al 1977, Jones et al. 1978 and advani, et al. (1980) reported impairment of delayed hypersensitivity and abnormality in T cells function of NHL patients. Cur results in agree meet with pieces et al. (1973) which indicate decrease in numbers and functional impairment in surface immunoglobulin bearing B lymphocyte in NHL patients which was also supported by Gajl – peczlska (1973) and simonsson, et al. (1983). In this study pooled data of T cells indicated significant decreases in Tells.

In this study Igm level remained same in pooled data of NHL when compared with control subject but IgG level was increased in these cases Datta et al (1971)

In stage uses data gradual increase in IgG level with advancement of studies showed maximum increase in IV stage. With hyper gamma globulinemia may be due to rise in globulin. Similar observations were reported by data et al. (1971) and Lichtenstein and taylor (1980). Other investigators reported NHL of B cells to be associated with abnormal serum immune globulin. A antibody deficiency (alexanian 1975 and marray, 1980) kumar and panny (1982) observed minimal abnormalities in immunological function but impairment of both antibody mediated and cell mediated immunity is recorded in advance stages of the the disease. However the exact mechanism for increase or decrease of there immunoglobulin is not yet clear. It very difficult to comment on it. It is also very difficult to rule out of the cause of infection, which is not apparent in these subjects causing the alteration immunoglobulin.

Serum proteins indicated significant rise in pooled data of NHL patients. In stage wise data also almost show the same result in all stages, Total serum proteins showed significant rise then control.

Summary and Conclusion

In NHL patients T cells decreased significantly in all stages as well as in pooled data B cells are also increased in NHL patients of all stages then control.

Serum IgG level indicated gradual rise which was at its peak Laval in stage IV and as also in pooled group patients.

Total gamma globulin percentage was significantly decreased in NHL patients in polled as well as in stage uses data.

Serum proteins indicated significant increase in polled as well as stage uses data.

Albumin: globulin ration indicated gradual rise which was at its peak Laval in stage IV

patients.

References

- Alexanian, R. Monoclonal Immunopathy in lymphoma. Arch Intern Med. 1975; 135: 62-72.
- 2. Advani SH, Dinshaw KA, Nair CN, Gopal R, Talwalkar GV, Lyyer YS, Bhatia HM and Desai PB. Immunodys function in non Hodgkin's lymphoma. *Cancer*. 1980; 45: 2843-2848.
- 3. Bjcarkhalm M. Immunodeficieny in Hodgkin's disease and its relation to prognosis. Scand.J Haematol suppl. 1978; 33.3.
- 4. Buchi G, Girotoo M, Termire G, Gario S, Grosso E, Antino R, Zappala C and Ferrero I. *Acta Haematol.* 1984; 71: 322-28.
- 5. Crispen RG and Rosenthal SR. Vaccination and cancer mortality. *cancer Immurnol Immunother*. 1976; 1: 139–142.
- Datta U, Aikat BK and Sehgal S.
 Immunoglobulin's in cases of leukemia's and lymphomas in north India (Chandigarh). *Indian J med Res.* 1971; 59: 1950–1958.
- 7. Gajl-Peczalska KJ, Hensen JA, Bloomfield CD and Good RA. Blymphocytes in untreated patients of malignant lymphoma and Hodgkin's disease. *J Clin Invest*. 1973; 52: 3064-3073.
- 8. Han Cock BW, Bruce L, Dunsmore JR. Follow-up Studies on the immune status of Patients with Hodgkin's disease after splencetomy and treatment in relapse and remission. *Br J Cancer*. 1977; 36: 347-354.
- 9. Jones SE, Griffith K, Domborowski P and Geinee JA. Immunodeficiency in patients with Non Hodgkins Lymphoma. *Blood.* 1977; 47: 335-344.
- 10. Levy R and Kaplan HS. Impaired Lymphocyte function in untreated Hodgkin's disease. *N Eng J Med.* 1974; 290: 181.
- 11. Lichtenstein A and Taylor CR. Serum Immunoglobulin levels in patients with non Hodgkin's Lymphoma. *Am J Clin Pathol.* 1980; 74: 12-17.
- 12. Lindemalm CS, Biberfeld P, Bjorkholm M, Holm G, Johansson B, Mellstedt H, Nilsson B and Ost A. Longitudinal studies of Blood lymphocytes functions in non Hodgkin's lymphomas. *Eur J Can Clin Oncol.* 1983; 19: 747-755.
- 13. Lowry OH, Roseborongh NJ, Farr PL and Randill RJ. *J Biochemistry*. 1951.

- 14. Lukes RJ and Collins RD. New approaches to classification of the lymphoma. *Br J can (Suppl-2)*. 1975; 31: 1-28.
- 15. Kohn J. Cellulose Acetate electrophoresis and Immunodiffusion technique edit by Ivar smith Vol. II. London: Willum Heinman medical book Ltd.; 1960, 56.
- 16. Kumar KR and Penny R. Cell medicated immune deficiency in Hodgkin's disease. *Immunology Today.* 1982; 3: 269-273.
- 17. Mancini G, Vaerman JP, Carbonara AO and Heremans JF. Immunochemical quantization of antigen by Single radial immune diffusion. *Immunochem.* 1968; 2: 235-254.

- 18. Mckinney MM and Parkinson A. A simple non chromatographic procedure to purify immunoglobulin full serum and asitic fluid. *J Immune method.* 1987; 96: 221-278.
- 19. Murray JL, Hurtubise PE, Young DC, Baleerzak SP, Lobuglio AF. Correlation of Prognostic factors and blood lymphocyte sub type in non Hodgkin's lymphoma. *Cancer*. 1980; 46: 1817-1824.
- Piessens WF, Schur PH, Maloney WC and Churchill, WH. Lymphocyte surface immunoglobulin distribution and frequency in lymphoprolifertive disease. *New engl J med.* 1973; 288: 176-180.

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