Cytokines : as useful Prognostic Markers in Lymphoma Cases

Col GS Chopra*, Lt Col PG Chitalkar[†], Maj Gen MP Jaiprakash[#]

Abstract

Cytokines are believed to be involved in the pathogenesis and enhanced expression in patients with Hodgkin's and Non-Hodgkin's lymphoma. Based on this phenomenon, a multicentric study was carried out in various lymphoma cases. The diagnosis of lymphoma was made on tissue biopsies and fine needle aspiration cytology (FNAC). Out of a total of 72 cases studied, 45 were of Hodgkin's lymphoma (62.5%) and 27 cases were of Non-Hodgkin's lymphoma (37.5%). Maximum cases of Hodgkin's disease occurred in the age group of 30-40 years and males outnumbered females. Hodgkin's lymphoma cases were predominantly of mixed cellularity histologic type (46.66%) whereas majority cases of Non-Hodgkin's lymphoma were of high grade histologic type (48.14%) with predominance in the age group 51-60 years. In both these type of lymphomas, the IL-2R and IL-6 levels were found to be increased more than four fold (as compared to healthy controls) ($p < 0.05$). The cytokine levels decreased after chemotherapy in patients showing response to therapy. However, there were few conflicting and unreliable trends in the IL-6 levels after chemotherapy where elevated IL-6 levels persisted in patients in clinical remission. Overall, it was seen that both IL-2R and IL-6 can be used as an indicator for assessing prognosis and drug therapy in lymphoma cases. IL-2R was found to be a better prognostic marker than IL-6 in assessing the response of lymphoma patient to chemotherapy, more so in Hodgkin's disease.

MJAFI 2004; 60 : 45-49

Key Words : Cytokines; IL-2R; IL-6; Lymphoma

Introduction

Malignant lymphomas display considerable histopathologic and clinical heterogeneity. This is due to biologic feature of these neoplasms and the specific lymphoid subpopulation from which they arise [1]. The lymphomas constitute neoplasm of the lymphoid system that includes distinct entities defined by clinical, histological, immunologic, molecular and genetic characteristic. They have been divided into Hodgkin and Non-Hodgkin's lymphoma and the latter is further classified into low, intermediate and high-grade lymphoma on their clinical outcome [2].

Cytokines are low molecular weight proteins and glycoproteins that are secreted principally by activated lymphocytes and macrophages and have a wide range of functions in haematopoiesis and immune responses [3]. Cytokines may act in an autocrine fashion and may also behave in a paracrine fashion by binding to receptors of cells in vicinity that produced it [4,5].

It has been postulated that characteristic clinical and histopathological features of malignant lymphomas may be due to activation of cytokines [6,7,8]. In view of above, a comprehensive multicentric study was conducted to see the role of various cytokines in disease course, prognosis and drug response in various lymphomas.

Material and Methods

Selection of patients : Fresh Hodgkin's and Non Hodgkin's

lymphoma cases referred and diagnosed at two large Military Hospitals, were studied. The diagnosis of most of lymphoma cases was made on the specimens received as tissue biopsies. In few other aspirate samples, the diagnosis and typing was confirmed by a tissue biopsy. Stains like haematoxylin and eosin, reticulin, leishman, giemsa, papinacolou were used in different clinical set ups to come to a diagnosis.

Estimation of cytokines : Serum samples from lymphoma cases were collected before start of the treatment and then periodically at 1-3 months interval. Similarly serum samples were taken from patients who had gone into clinical remission or continued complete remission for a long time. Serum samples from drug responders and treatment failure cases were also collected. These serum samples were aliquoted and kept frozen at -20°C till cytokine assay time. The serum samples were analysed for IL-2R and IL-6. An attempt was made to correlate the levels of interleukins with disease type, condition of the patient, drug response and ultimately with prognosis.

Principle of the assay for estimation of cytokines

The interleukin kits were obtained from M/s Immunotech (France). The assay employs the quantitative sandwich enzyme immunoassay technique (ELISA). A monoclonal antibody specific for the cytokine is precoated onto a microtitre plate. Defined standards, samples and acetylcholine esterase anti-interleukin monoclonal antibody were incubated in the microtitre plate along with first monoclonal antibody. The complexes were washed after the specified incubation and the bound enzymatic activity was measured by adding a chromogenic substance. The intensity of colour was

*Senior Adviser (Pathology & Immunology), Army Hospital (R&R), Delhi Cantt, [†]Classified Specialist (Medicine & Oncology), Command Hospital (Southern Command), Pune - 411 040, [#]Senior Consultant (Medicine), Office of DGAFMS, 'M' Block, New Delhi.

measured by computerized laboratory data system and the cytokine levels were calculated accordingly.

Results

All fresh Hodgkin's and Non-Hodgkins lymphoma cases diagnosed during the period of study were included. The diagnosis of lymphoma was made on tissue biopsies and FNAC.

Patients were divided into two groups; Hodgkin's lymphoma (HL) and Non Hodgkin's lymphoma (NHL) (Table 1). Out of 72 cases, 45 cases were of HL (62.5%), 27 cases were of NHL (37.5%)

Table 1

Data Analysis

Disease	No of cases	%
Hodgkins lymphoma	45	62.5%
Non-Hodgkins lymphoma	27	37.5%
Total	72	- -

Sex and age distribution

Men outnumbered women in our study. There were 42 male patients (58.3%) and 30 female patients (41.66%). This may be partly due to the study being done in a service hospital, which caters to serving soldiers mainly, thus explaining the male predominance. Age distribution varied from 2 years to 65 years. The youngest patient was a 2 year old girl while the oldest was a 65 year old female.

Amongst the HL cases, 21 were of mixed cellularity (46.66%), 8 cases of lymphocytic predominance (17.77%), 6 of lymphocytic depletion (13.33%) and 10 cases were of nodular sclerosis type (22.22%).

Estimation of IL2R & IL6

In all the cases, estimation of serum IL-2R and IL-6 was done both before and/or after chemotherapy. In few cases samples were taken repeatedly.

20 healthy individuals were taken as controls. Their serum IL-2R levels ranged from 913 to 1464 pico gm/ml with a mean of 1157 ± 180 pico gm/ml. IL-6 levels of controls ranged from 2.9 to 18.75 with mean of 7.22 ± 6.18 pico gm/ml.

IL-2R levels in Hodgkin's lymphoma

A total of 24 samples were studied before chemotherapy was started. Out of this 9 patients were found to be ultimately responders, 5 cases were diagnosed to be in continued

Fig. 1 : IL-2R levels in various phases of Hodgkins Disease

complete remission (CCR). 5 patients died during or after chemotherapy. IL-2R estimation was also done post chemotherapy in all patients. There were 20 responders who showed serum IL-2R levels ranging from 912-12500 pico gm/ml with mean of 3306 ± 1367 pico gm/ml.

17 patients with CCR had IL-2R values ranging from 776 to 3408 pico gm/ml with mean of 1966 ± 823 pico gm/ml. p values were significant (<0.05) in healthy controls when compared with responders and CCR. Responders had IL-2R ranging from 3620-26000 pico gm/ml with a mean of 10857 ± 8691 pico gm/ml. Patients with continued complete remission had IL-2R levels ranging from 4000-9275 pico gm/ml with a mean of 6451 ± 5500 pico gm/ml. Samples of 14 patients who ultimately became nonresponders, were also analyzed. Their IL-2R values ranged from 11079 ± 6710 pico gm/ml. 6 patients died during the course of treatment. Their IL-2R values ranged from 5420 to 20800 pico gm/ml with mean of 11573 ± 56637 pico gm/ml. p values were significant (<0.05) when responders were compared with non-responders and with patients who died. In few cases repeated samples were taken at different conditions ranging from non-responders, responders and CCR (Table 2).

IL-2R levels in Non-Hodgkin's lymphoma

A total of 7 samples were studied before initiation of chemotherapy. Out of these, 2 patients were found to be ultimately responders, 3 cases in CCR. Also there were 2 cases that died later on. IL-2R level estimation was also done post-chemotherapy in all cases. There were 7 responders who had IL-2R levels ranging from 1718 to 2738 pico gm/ml with a mean of 2450 ± 351 pico gm/ml. 7 patients with CCR had IL-2R levels ranging from 1651 to 2957 pico gm/ml with a mean of 2173 ± 445 pico gm/ml ($p < 0.05$). It was insignificant

Table 2

Pre and post-chemotherapy serum IL-2R levels (Hodgkin's lymphoma)

	Pre-chemotherapy (pico gm/ml)			Post-chemotherapy (pico gm/ml)		
	Total no	Mean $\pm$ SD	Range	Total no	Mean $\pm$ SD	Range
Healthy control	20	1157 ± 180	913 - 1464	20	1157 ± 180	913 - 1464
Responders	9	10857 ± 8692	3620 - 26000	22	3306 ± 1367	912 - 12500
CCR	5	6451 ± 5500	4000 - 9275	17	1966 ± 823	776 - 3408
Non-Responders	5	7321 ± 2994	3676 - 11000	14	11079 ± 6710	3166 - 25000
Death	5	7627 ± 4090	3613 - 12500	06	11573 ± 5637	5420 - 20800

Table 3**Pre and post-chemotherapy serum IL-2R levels (Non-Hodgkin's lymphoma)**

	Pre-chemotherapy (pico gm/ml)			Post-chemotherapy (pico gm/ml)		
	Total no	Mean $\pm$ SD	Range	Total no	Mean $\pm$ SD	Range
Healthy control	20	1157 $\pm$ 180	913 - 1464	20	1157 $\pm$ 180	913 - 1464
Responders	2	2110 $\pm$ 2983	2152 - 4220	7	2450 $\pm$ 351	1718 - 2738
CCR	3	1795 $\pm$ 1852	1685 - 3700	7	2173 $\pm$ 445	1651 - 2957
Non-Responders	Nil	Nil	Nil	8	8716 $\pm$ 842	6184 - 11076
Death	2	3750 $\pm$ 5303	7500 - 3200	5	12579 $\pm$ 1413	9361 - 15854

Table 4**Pre and post-chemotherapy serum IL6 levels (Hodgkin's lymphoma)**

	Pre-chemotherapy (pico gm/ml)			Post-chemotherapy (pico gm/ml)		
	Total no	Mean $\pm$ SD	Range	Total no	Mean $\pm$ SD	Range
Healthy control	20	7.22 $\pm$ 6.18	2.9 - 18.75	20	7.22 $\pm$ 6.18	2.9 - 18.75
Responders	9	187 $\pm$ 86	93.57 - 264.6	22	93.6 $\pm$ 76.9	13.97 - 204.5
CCR	5	87 $\pm$ 50.67	20.75 - 178.3	17	61.66 $\pm$ 78.73	1.57 - 163.7
Non-Responders	5	392 $\pm$ 201.8	20.85 - 780.7	14	859.7 $\pm$ 113.8	2.489 - 2500
Death	5	529 $\pm$ 487.8	329.7 - 1700	6	1146 $\pm$ 937	501.8 - 3600

($p > 0.05$) when responders were compared with CCR. 8 patients turned out be non-responders later on. Their IL-2R levels ranged from 6184 to 11076 pico gm/ml with a mean of 8716 ± 842 pico gm/ml. 5 patients died during therapy. Their IL-2R levels ranged from 9361 to 15854 pico gm/ml with a mean of 12579 ± 1413 pico gm/ml. p value was statistically significant (< 0.05) when responders were compared with non-responders. Responders when compared with patients who died had statistically significant p values ($p < 0.05$) (Table-3).

IL-6 levels in Hodgkin's lymphoma cases

A total of twenty four samples were analyzed before chemotherapy. Out of these 9 patients were found to be responders later on and had IL-6 ranging from 93.57 to 264.6 pico gm/ml with a mean of 187 ± 86 pico gm/ml. 5 cases were diagnosed to have continued complete remission. Their IL-6 ranged from 20.75 to 178.3 pico gm/ml with a mean of 87 ± 50.67 pico gm/ml. Also there were 5 patients who were non-responders. Their IL-6 ranged from 20.85 to 780.7 with a mean of 392 ± 201.8 . Five cases ultimately died during treatment and their IL-6 ranged from 329.7 to 1700 pico gm/ml with a mean of 529 ± 487.8 pico gm/ml. IL-6 estimation was also done post-chemotherapy in all patients. There were 22 responders with IL-6 ranging from 13.97 to 204.5 pico gm/ml with a mean of 93.6 ± 76.9 pico gm/ml. 17 patients went into CCR. Their IL-6 ranged from 1.57 to 163.7 pico gm/ml. 2 of them had low values such as 1.57 and 3.5 pico gm/ml while one had a value of 163.7. The mean was 61.66 ± 78.73 .

14 non-responders had IL-6 ranging from 2.489 to 2500 pico gm/ml. 4 had values of 2.489, 6, 46 and 8.32 pico gm/ml while 2 had values as high as 2200 and 2500 pico gm/ml. The mean was 859.7 ± 1138 . 6 patients died during treatment. Their IL-6 ranged from 501.8 to 3600 pico gm/ml with a mean of 1146 ± 937 pico gm/ml. Responders when compared with non-responders had significant p values ($p < 0.05$). Responders on comparison with patients who died had

significant p values ($p < 0.05$) (Table-4).

IL-6 levels in Non Hodgkin's lymphoma

10 patients were studied for IL-6 levels before chemotherapy was started in NHL cases. Out of these 2 were responders, 3 cases showed CCR and 2 cases died later on. IL-6 estimation was also done after post-chemotherapy. 7 cases turned out to be responders later on. Their IL-6 ranged from 24 to 72.62 pico gm/ml with a mean of 47.6 ± 24 pico gm/ml. 7 patients showed CCR. Their IL-6 ranged from 39 to 87.2 pico gm/ml with a mean of 30.8 ± 27.9 pico gm/ml. Healthy controls when compared with responders had significant p values ($p < 0.05$). However, responders when compared with patients in CCR had insignificant p values ($p > 0.05$).

8 patients turned out be non-responders. Their IL-6 had a wide range from 19.45 to 2200 pico gm/ml. 2 had value of 1810 pico gm/ml and 2200 pico gm/ml while 3 had values of 19.45, 40.6 and 47.6 pico gm/ml. The mean was 822 ± 1198 pico gm/ml. 5 cases died during treatment. They had IL-6 values ranging from 43.95 to 2500 pico gm/ml. The mean was 1087 ± 987 pico gm/ml. Responders when compared with non-responders had insignificant p value ($p > 0.05$). Responders on comparison with patients who died had significant p values ($p < 0.05$) (Table 5).

Discussion

Cytokines are believed to be involved in the pathogenesis and enhanced expression of various cytokines in patients with lymphomas. In the present study a total of 72 cases were selected with 45 being of HL, 27 of NHL. Hodgkin's disease has a bimodal age distribution with two peaks in the age groups of 15-34 years and older than 55 years. In this study, maximum case of Hodgkin's disease occurred in the age group of 30-40 years. The incidence again increased in the age

Table 5**Pre and post-chemotherapy serum IL6 levels (Non-Hodgkin's lymphoma)**

	Pre-chemotherapy (Pico gm/ml)			Post-chemotherapy (Pico gm/ml)		
	Total no	Mean $\pm$ SD	Range	Total no	Mean $\pm$ SD	Range
Healthy control	20	7.22 $\pm$ 6.18	2.9 - 18.75	20	7.22 $\pm$ 6.18	2.9 - 18.75
Responders	2	40.5 $\pm$ 17.8	33.45 - 57.60	7	47.6 $\pm$ 24	24 - 72.62
CCR	3	38.5 $\pm$ 27.9	23.6 - 140.6	7	30.8 $\pm$ 27.9	39 - 87.2
Non-Responders	3	139.2 $\pm$ 75.1	107.2 - 196.2	8	822 $\pm$ 1198	19.45 - 2200
Death	2	150 $\pm$ 110.5	170 - 205.5	5	1087 $\pm$ 987	43.95 - 2500

group of 61-70 years. Maximum number of NHL cases occurred in the fourth decade.

Males outnumbered females partly due to the study being done in a service hospital with predominant male clientele. HL was the predominant disease with 62% cases and the maximum number of cases were of mixed cellularity histologic type (46.66%). This is in confirmation with the previous study [9]. The second most common type of HL was of nodular sclerosis type. In HL, the maximum number of cases were of high grade type (48.14%) occurring mostly in the age group of 51-60 years.

In our study IL-2R estimation was done in 20 healthy controls. In patients of Hodgkin's disease the IL-2R levels were found to be increased more than four fold (as compared to healthy controls). It was seen that serum IL-2R levels of controls were lower as compared to cases with HL. Gorschluter et al, in 1995 [10] suggested that cytokines are involved in the pathogenesis of Hodgkin's disease. Elevated levels of cytokines were present in patients of lymphomas and the levels decreased after therapy. In our study also, response to treatment was associated with progressive reduction of IL-2R levels. When responders and patients with CCR were compared with controls, it was found to be statistically significant ($p < 0.05$).

Significantly enhanced levels of IL-2R were found in patients who experienced a poor response or progression after treatment. The levels increased significantly in patients who died during or after therapy ($p < 0.05$). Pui et al [11,12], suggested in 1993 that very high levels of soluble IL-2R were significantly associated with a poorer treatment outcome in Hodgkin's disease. This finding corroborated with the results of our study where it was seen that higher levels of IL-2R were associated with poor prognosis and poor treatment outcome.

In NHL, patients showed two to three fold rise in IL-2R levels as compared to healthy controls. The levels decreased after chemotherapy in patients showing response to therapy. However, it was seen that IL-2R levels increased in few patients after chemotherapy that were otherwise in remission or CCR. The p values

were significant when healthy controls were compared with responders ($p < 0.05$). The results thus indicate that though IL-2R levels are significantly higher in patients they may show an unreliable trend after chemotherapy.

Earlier studies have suggested that IL-6 is involved in the pathogenesis of several diseases including Hodgkin's disease [13,14]. In our study, IL-6 was estimated in the serum of healthy controls as well as patients. It was seen that higher IL-6 levels were seen in patients of HL as compared to controls. In majority of patients IL-6 levels decreased after therapy. However, elevated IL-6 persisted in some patients who showed good response to therapy clinically. This correlates with the study carried out by Gorschluter et al [10]. It was seen that IL-6 levels increased in non-responders ($p < 0.05$). Responders on comparison with cases who died had statistically significant p values. Rieckman et al, in their study concluded that serum IL-6 levels are elevated in lymphoma patients and correlate with survival in Hodgkin's disease [15].

In NHL, the patients were seen to have higher values of IL-6 than controls. The levels showed an unreliable trend after chemotherapy as they increased in most responders ($p < 0.05$). The levels decreased in most patients in CCR ($p > 0.05$). Patients showing non-response to therapy had higher IL-6 levels ($p > 0.05$). The highest levels were obtained for patients who died ($p < 0.05$).

Overall, it was seen that both IL-2R and IL-6 can be used as an indicator for assessing prognosis and drug therapy in lymphoma. IL-2R was a better prognostic marker as the levels of IL-6 had an unreliable trend after chemotherapy. The levels of IL-2R increased maximum in cases of HL. It was also seen that patients with lower IL-2R levels had a better event free survival. Serial assays of serum IL-6 levels may be useful in indicating the efficacy of treatment or the activity of the disease. Though there are varying trends of IL-6 after chemotherapy, it still remains a reasonably good prognostic marker.

To conclude, these results indicate that there is a role for the estimation of IL-2R and IL-6 in predicting the

clinical course and response to treatment in patients of Hodgkin's disease and NHL. Thus, these assays may be carried out in lymphoma patients at pre and post chemotherapy periods.

References

1. Macon WR. Cytokine expression in malignant lymphomas. *Hum Pathol* 1993;24:1039-41.
2. Skarin AT, Dorfman DM. Non-Hodgkins Lymphomas : Current classification and management. *Cancer J Clin* 1997;47:351-72.
3. Balkwill FR, Burke F. *Immunol Today* 1989;10:299-304.
4. Su Minmg HSU, Waldron JW, Hsu P et al. Cytokines in malignant lymphomas. *Hum Pathol* 1993;24:1040-56.
5. Hansson M. Growth and differentiation factors for B and T cell. *Leuk Res* 1990;14:705-10.
6. Enblad G, Sundstrom C, Gronowitz S et al. Serum levels of IL-2 receptor in patients with Hodgkins disease with special reference to age and prognosis. *Ann-Oncol* 1995;6:65-70.
7. Kurzrock R, Redman J, Cabanillas F et al. Serum IL-6 levels are elevated in lymphoma patients and correlate with survival in advanced Hodgkins disease and with B symptoms. *Cancer Res* 1993;53:2118-22.
8. Pul CH, Hip S, Shulahifiah et al. Serum IL-2 receptor levels in childhood acute lymphoblastic leukaemia. *Blood* 1988;71:1135-7.
9. Jeffery L, Timothy M, Greiner C et al. Hodgkins disease. *Cancer* 1995;75:357-69.
10. Gorschluter M, Bohlen H, Hasenclever D et al. Serum cytokine levels correlate with clinical parameters in Hodgkin's disease. *Ann Oncol* 1995;6:477-82.
11. Pui CH, IpP SH, Kung P et al. High serum IL-2 receptor levels are related to advance disease and a poor outcome in childhood Non-Hodgkin's lymphoma. *Blood* 1987;70:624-8.
12. Pui CH, Hudson M, Luo X et al. Serum IL-2 receptor levels in Hodgkin's disease and other solid tumours of childhood. *Leukaemia* 1993;7:1242-4.
13. Jucher M, Abb H, Liw et al. Expression of IL-2 and IL-6 in Hodgkins disease. *Blood* 1991;77:2413-8.
14. Freeman GJ, Freedman AS, Rabinowe SN et al. IL-6 gene expression in normal and neoplastic B cells. *J Clin Invest* 1998;83:1512-8.
15. Rieckman P, Alessandro F, Nordon RP et al. IL-6 and tumour necrosis factor , autocrine and paracrine cytokines involved in B cell functions. *J Immunol* 1991;146:3462-8.

A SHORT HISTORY OF MEDICINE: "Doctor, I have an ear ache."

2000 B.C. - "Here, eat this root."

1000 B.C. - "That root is heathen, say this prayer."

1850 A.D. - "That prayer is superstition, drink this potion."

1940 A.D. - "That potion is snake oil, swallow this pill."

1985 A.D. - "That pill is ineffective, take this antibiotic."

2000 A.D. - "That antibiotic is artificial. Here, eat this root!"