

ORIGINAL ARTICLE

EVALUATION OF SOME BIOCHEMICAL MARKERS AS PROGNOSTIC FACTORS IN MALIGNANT LYMPHOMAS

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ABSTRACT

This study aimed to estimate the pre- and post-treatment serum levels of glycosaminoglycans (GAGs), soluble p55 tumor necrosis factor receptor (sp55TNF-R) and soluble L-selectin in order to evaluate their prognostic significance and their role in monitoring tumor growth and host-tumor response in malignant lymphomas. Also, the work aimed to investigate the relationship between these levels with B symptoms and disease stage. For this purpose, 43 newly diagnosed patients with malignant lymphoma (12 with Hodgkin's disease (HD) and 31 with Non-Hodgkin's lymphoma (NHL) were selected from Mansoura University Hospital. Among NHL patients, 7 were in stage I/II, 13 in stage III and 14 in stage IV. In addition, 10 NHL patients were presented with B symptoms while, 21 did not. 7 apparently healthy individuals were selected as a control reference group. *Results:* 1-Pre-treatment levels of GAGs, sp55TNF-R and sL-selectin increased significantly in both HD and NHL before treatment as compared to control. Pre-treatment sp55TNF-R levels in both diseases and sL-selectin (only in HD patients) may have a significant value in predicting response to therapy, while GAGs level in both diseases and sL-selectin in NHL patients had a limited value in such prediction. 2- In contrast to sp55TNF-R and sL-selectin, post-treatment GAG levels are thought to be a good sign of remission in both HD and NHL. 3- Serum GAG levels increased significantly before treatment in stages III/IV NHL as compared to stage I/II, so serum GAGs at diagnosis could reflect tumor bulk and the disease activity. 4- Elevated serum sp55TNF-R before treatment was associated with the presence of B symptoms and such association lead to a worse prognosis. *Conclusion:* Pre-treatment sp55TNF-R levels in both HD & NHL and sL-selectin (only in HD patients) could be used as prognostic factor with respect to predicting treatment outcome. Serum GAGs at diagnosis could reflect tumor bulk and the disease activity.

Keywords: Hodgkin disease, non-Hodgkin lymphoma, glycosaminoglycans, tumor necrosis factor, L-selectin.

INTRODUCTION

The malignant lymphomas are neoplastic transformations of cells that reside predominantly with lymphoid tissues (Freedman and Nadler, 1993). They are a heterogeneous group of malignancies of B or T cells that usually originate in the lymph nodes but may originate in any organ of the body (Foon and Fisher, 1995). The term "lymphoma" emphasize the fact the hallmark of these disorders is abnormal lymph node enlargement, with disruption or replacement of the normal histologic architecture (Manner, 1998). Lymphomas have traditionally been divided into two classes: Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL).

Hematologic malignancies, such as malignant lymphoma, respond well to cancer chemotherapy. Therefore, it is important to monitor the state of response to chemotherapy as well as to determine the complete disappearance of the tumor and to predict early detection of recurrence in routine clinical work by estimation of tumor marker levels. In the past decade numerous prognostic factors have been reported

to influence response to therapy and survival of patients with malignant lymphomas. Various serum biochemical markers, such as lactate dehydrogenase (LDH), β 2-microglobulin, and albumin serum levels have most frequently been identified as prognostic factors in malignant lymphomas (Harrington *et al.*, 1993; Shipp, 1994 and Aisenberg, 1995).

Glycosaminoglycans (GAGs) are unbranched polysaccharides which, with the exception of hyaluronan, are covalently bound to a core protein forming proteoglycan (Murray and Keeley, 2000). It has been shown that human lymphocytes and monocytes produce various proteoglycan types (Kolset *et al.*, 1984).

Tumor necrosis factor (TNF) is one of the cytokines which through its p55 and p75 receptors, has been identified to participate in the development and in the function of normal lymphoid tissues and to stimulate the proliferation of T and B lymphocytes (Aggarwal and Vilcek, 1992).

L-selectin is a member of the selectin family of adhesion molecule (Bevilacqua and Nelson, 1993), which is essential for the migration of lymphocytes into peripheral lymph

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nodes and also is essential to mediate rolling of leukocytes on endothelium at sites of inflammation (Kansas, 1996).

The present study was performed to estimate the pre- and post-treatment serum levels of GAGs, soluble p55 tumor necrosis factor receptor and soluble L-selectin in order to evaluate their prognostic significance and their role in monitoring tumor growth and host-tumor response in malignant lymphomas. Also, the work aimed to investigate the relationship between these levels and some of clinico-hematological factors known to be associated with worse prognosis (such as ESR, LDH, B symptoms and disease stage).

SUBJECTS AND METHODS

Patients

The present study has been conducted on newly diagnosed 43 patients of malignant lymphoma selected from out-patient clinics of Mansoura University Hospital. The study was performed on patients attending Hematology and Oncology Unit at the time of diagnosis and then repeated after treatment with chemotherapy. According to the histopathological examination of the affected lymph node, the patients were classified into two main groups: Hodgkin's disease (HD) group (n=12) and non-Hodgkin's lymphoma (NHL) group (n=31). Within NHL group, 7 patients were in stage I/II, 13 in stage III and 14 in stage IV. In addition, 10 NHL patients presented with B symptoms, while, 21 did not. B symptoms were defined as unexplained fever with temperature above 38°C, unexplained weight loss of 10% of body weight over 6 months, and night sweats. Seven apparently healthy individuals were selected as a control reference group.

Methods

Blood samples were collected from pre- and post-treated patients and from control subjects then divided into three portions: The first portion was collected into EDTA-containing tube, and used for blood cell count within 5 hours by electronic count methods using the coulter Cell-Dyn 1700. The second portion (1.6 ml blood) was collected into a tube containing 0.4 ml sodium citrate solution (3.8 gm/dl) and used for erythrocyte sedimentation rate (ESR) determination by Westergren method. The third portion was collected into clean, dry tube, allowed to coagulate and centrifuged for 10 minutes at 3000 rpm. The clear non-hemolyzed serum was investigated for liver function and kidney function tests as well as serum LDH activity immediately. The rest of serum was aliquoted and kept deeply frozen at -20°C for further investigations of serum GAGs, sp55-TNF-R and sL-selectin levels.

Determination of serum glycosaminoglycans (GAGs)

Glycosaminoglycans (GAGs) were determined as their glucuronic acid content by the modified naphthoresorcinol method described by Mazzuchin *et al* (1971).

Reagents: Naphthoresorcinol crystals (provided from Aldrich company), glucuronic acid was provided from sigma company.

Determination of serum soluble p55 tumor necrosis factor receptor (sp55TNF-R)

Serum sp55TNF-R was determined by a solid phase Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microtiter plate according to the method of Herbelin *et al.* (1995) using a kit from "Biosource Europe S.A Company".

Determination of serum soluble L-selectin (sL-selectin)

Using a kit from "Diacclone Company", serum (sL-selectin) level was determined by a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) method described by Spertini *et al.* (1992) and modified by Seidelin *et al.* (1998). A monoclonal antibody specific for sL-selectin unknowns are pipetted into these wells. During the first incubation, the sL-selectin antigen and a biotinylated monoclonal antibody specific for sL-selectin are simultaneously incubated. After washing, the enzyme (streptavidin-peroxidase) is added. After incubation and washing to remove all the unbound enzyme, a substrate solution which is acting on the bound enzyme is added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of sL-selectin present in the samples.

Statistical analysis was performed using the Instat2 software package (GraphPad Software, Sandiego, CA). Student's t-test was used as a test of significance for comparison between two arithmetic means of two different groups. Paired t-test was used as test of significance for comparison between two arithmetic means of the same studied group before and after treatment and correlation coefficient (r) was used to measure the mutual correspondence of two quantitative variables in the same studied group.

RESULTS AND DISCUSSION

Patient characteristics

Forty three patients were included in this study; 31 with NHL and 12 with HL. Most NHL patients had diffuse large cell lymphoma (DLCL) (87%), advanced stage (81%) and positive B-symptoms (71%) at initial presentation. COP regimen was used in patients with follicular lymphoma and CHOP was used in patients with mantle cell and DLCL with CR rate of 74%. Most HL patients had Nodular lymphocyte predominance (NLP) histology (50%), early stage disease (58%) at initial presentation, positive B-symptoms (58%). C-MOPP/ABV regimen was used in all patients with HL with CR rate of 83%. Two patients with HL and 11 patients with NHL had relapsed disease after an initial response. DFS and OS probabilities were 86% and 83% respectively for patients with HL while they were 84% and 82% respectively for patients with NHL (table 1).

Table 1: Patients characteristics

	HL	NHL
Patients N (%)	12 (100)	31 (100)
Age, median (range)	25 (16-63)	40 (16-60)
Gender, Males: females	8:4	25:6
PS n, (%)		
0-1	11 (92)	26 (84)
2	1 (8)	5 (16)
Histology n, (%)		
NLP	6 (50)	-
NS	2 (17)	-
MC	3 (25)	-
LD	1 (8)	-
Follicular lymphoma	-	4 (13)
Mantle cell lymphoma	-	4 (13)
DLCL	-	23 (74)
Clinical stage n, (%)		
I	-	-
II	7 (58)	6 (19)
III	5 (42)	17 (55)
IV	-	8 (26)
Nodal versus extranodal disease	-	2 (bones)
B-symptoms n, (%)		
Positive	7 (58)	22 (71)
Negative	5 (42)	9 (29)
Chemotherapy treatment n, (%)		
C-MOPP/ABV	12 (100)	-
COP	-	4 (13)
CHOP	-	27 (87)
Response n, (%)		
CR	10 (83)	23 (74)
PR	2 (17)	8 (26)
Relapse n, (%)		
Yes	2 (17)	11 (35)
No	10 (83)	20 (65)
Fate n, (%)		
Alive	10 (83)	25 (81)
Dead	2 (17)	6 (19)
DFS probability (at 1.5 years)	86	84
OS probability (1.5 years)	83	82

HL: Hodgkin's lymphoma; NHL: non-Hodgkin's lymphoma; PS: performance status; NLP: nodular lymphocytic predominance; ND: nodular sclerosis; MC: mixed cellularity; LD: lymphocytic depletion; DLCL: diffuse large cell lymphoma; C-MOPP/ABV: cyclophosphamide, vincristine, procarbazine, prednisolone/adriamycin, bleomycin, vinblastine; COP: cyclophosphamide, vincristine, prednisone; CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone; CR: complete remission; PR: partial remission; DFS: disease free survival; OS: overall survival probability.

Malignant lymphoma ranked as the third most frequent neoplastic disease in Egypt (Bolkainy, 1991). The present study was performed to estimate the pre- and post-treatment levels of some biochemical markers (glycosaminoglycans, tumor necrosis factor receptor and soluble L-selectin) in order to evaluate their prognostic significance in monitoring tumor growth and host-tumor response in malignant lymphomas. In addition, the correlation of pre and post treatment levels of these markers with other prognostic parameters (such as ESR and LDH) was also performed.

Liver and kidney function tests as well as blood cell count and hemoglobin concentration were illustrated in table 1. Insignificant difference in serum alanine aminotransferase (ALT) activity and total bilirubin were observed in patients with HD and NHL either before or after treatment as compared to control group. In contrast, patients with HD and NHL, both before and after treatment, showed significant increase in serum AST activity as compared to control. At the time of diagnosis, patients with HD showed a highly significant decrease in serum albumin levels as compared to control, while, in NHL patients, non-significant difference was observed. Serum ALT and AST activities as well as total bilirubin and albumin level did not differ significantly after treatment as compared to before treatment indicating the absence of hepatotoxic effect of the chemotherapy protocols used. Insignificant difference in serum creatinine level was shown in patients with HD either before or after treatment as compared to control. In contrast, serum creatinine level was significantly elevated in NHL patients before and after treatment as compared to control. This result is not a predictive for renal impairment in our patients because, although significantly increased, mean serum creatinine level was still in the normal range. Anemia was detected in both HD and NHL patients either before or after treatment.

A highly significant rise in ESR value was detected in total HD and NHL patients before treatment as compared to control. After treatment, total HD and NHL patients showed trend of decrease as compared to total patients before treatment, while, still had a significant rise in ESR values as compared to control. This result may be ascribed to variation between patients in response to therapy. So, patients were divided according to response to therapy, and it was found that, 6 HD and 21 NHL patients attained remission, while, 6 HD and 10 NHL patients did not. It has been found that, patients with remission had normal ESR values after treatment, particularly in HD. On the other hand, patients without remission showed insignificant increase in post-treatment ESR values in comparison to their pre-treatment values and still had a significantly increased values as compared to control. This elevation of ESR after treatment in patients without remission strongly suggested aggressive or persistent disease.

Table 2: Liver and kidney function tests and blood cell count in total HD and NHL patients as compared to control (mean $\pm$ SEM).

	Control (n=7)	HD (n = 12)		NHL (n= 31)	
		Before treatment	After treatment	Before treatment	After treatment
Liver Function tests					
ALT (Units/ml)	23.3 ±2.10	32.66±3.76	32.22±3.52	28.32±2.5	26.96±1.67
AST (Units/ml)	29.9±1.20	53.77 *±0.6	48.22* ±8.93	56.67* ± 6.50	54.45 * ±6.21
Albumin (gm/dl)	4.6 ± 0.125	3.58 *±0.197	4.03 ± 0.29	4.28 ± 0.156	4.36 ± 0.093
Total bilirubin (mg/dl)	0. 6 ± 0.031	0.72 ± 0.06	0.75 ± 0.073	0.76 ± 0.054	0. 72 ± 0.044
Kidney function tests					
Creatinine (mg/dl)	0. 71± 0.044	0.79 ± 0.046	0.81 ± 0.016	0.94 ±0.042	0.91 ±0.043
Blood picture					
RBCs/ml (x10 ⁶)	4.94 ± 0.18	3.46 *± 0.38	3.74*± 0.20	3.99 *±0.096	3.97 *±0.094
Hb (gm/dl)	13.05 ± 0.3	10.52* ±0.71	10.54±0.62	11.00±0.40	11.20±0.36
WBCs/ml (x10 ³)	6.51± 0.49	7.43± 0.86	7.73± 1.15	7.018± 0.48	7.011± 0.8
Platelets/ml (X10 ³)	239± 20.25	295.88± 27.90	292.66± 28.63	239.31± 18.59	238.78± 21.99

n = number of subjects, (*) =significance against control group.

Table 3: Erythrocyte sedimentation rate (ESR) values and serum LDH activity in total HD patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM).

Groups	ESR (mm/hr)	LDH (U/L)
Control group (n=7)	5.6 $\pm$ 1.00	187.1 $\pm$ 14.7
Total HD patients before treatment (n=12)	59.1* $\pm$ 16.42	333.1 * $\pm$ 11
Total HD patients after treatment (n=12)	49.0* $\pm$ 19. 2	234.3 ^a $\pm$ 32.68
Patients with remission (n=6)		
Before treatment	30.5 * $\pm$ 4.6	301* $\pm$ 30.41
After treatment	11.8 ^b $\pm$ 0.95	195.5 ^b $\pm$ 19.9
Patients without remission (n=6)		
Before treatment	117.3 ^b * $\pm$ 19	397.6 * $\pm$ 34.86
After treatment	123.3 ^c $\pm$ 17.66	375 ^c $\pm$ 29/07

n = number of subjects, (*) = significance against control group, (a) = significance against total HD patients before treatment, (b) = significance against HD patients with remission before treatment and (c) =significance against HD patients with remission after treatment.

Many authors suggested pre-treatment ESR value to be a significant prognostic factor in HD (Tubiana *et al.*, 1984; 1989; Gobbi *et al.*, 1988 and Martinow *et al.*, 1998). These findings confirm the results obtained in this study in which patients without remission showed significantly higher ESR values either before or after treatment in comparison to patients with remission. Despite of the small HD group included in this work, our results may provide an additional support to the previous studies who proposed pretreatment ESR values as a good prognostic factor in HD with respect to achieving complete remission. On the other hand, NHL patients without remission exhibited insignificant increase in ESR values before treatment as compared to patients with remission making the suggestion of prognostic significance of ESR in predicting treatment outcome in NHL is doubtful.

In multivariate analysis, ESR was not identified as independent prognostic factor (Bremnes *et al.*, 1999). ESR also was found to have no significant relationship to survival (Hagberg *et al.*, 1984).

Lactate dehydrogenase (LDH) is perhaps the most common clinical enzyme used in cancer patients for prognostic purposes (Schwartz 1992). Its increase in various cancers can represent a good indicator of the tumoral mass's degree of growth (Schwartz, 1992 and D'Angelo *et al.*, 1989). In this study, there was a significant increase in serum LDH activity in both total HD and NHL patients before treatment as compared to control. When the patients were divided according to response to therapy it was found that, HD and NHL patients with remission demonstrated a significant

Table 4: Erythrocyte sedimentation rate (ESR) values and serum LDH activity in total NHL patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM).

Groups	ESR (mm/hr)	LDH (U/L)
Control group (n=7)	5.6 $\pm$ 1.00	187.1 $\pm$ 14.7
Total NHL patients before treatment (n=31)	47.74* $\pm$ 5.84	358.02* $\pm$ 29.07
Total NHL patients after treatment (n=31)	28.25*# $\pm$ 4.71	225.6# $\pm$ 18.54
Patients with remission (n=21)		
Before treatment	42.69 * $\pm$ 6.6	296 * $\pm$ 25.74
After treatment	21.6 ⁰ * $\pm$ 4.16	172.85 ⁰ $\pm$ 11.5
Patients without remission (n=10)		
Before treatment	59.9 * $\pm$ 10.0	408 ⁰ * $\pm$ 43.6
After treatment	44.9 ^d * $\pm$ 9.67	66.7 ^d * $\pm$ 37.5

n = number of subjects, (*) = significance against control group, (#) = significance against total NHL patients before treatment, (0) = significance against NHL patients with remission before treatment and (d) = significance against NHL patients with remission after treatment.

Table 5: Correlation between ESR values, serum LDH activity and serum levels of GAGs, sp55TNF-R & sL-selectin in HD and NHL either before or after treatment.

Item	Correlation with	HD		NHL	
		Before treatment	After treatment	Before treatment	After treatment
GAGs	ESR	Not significant r = 0.27	Not significant r = 0.467	Not significant r = 0.296	Sig, P<0.05 r = 0.43
	LDH	Not significant r = 0.26	Not significant r = 0.43	Not significant r = 0.28	Sig, P<0.01 r = 0.54
sp55TNF-R	ESR	Sig, P<0.01 r = 0.83	Not significant r = 0.58	Not significant r = 0.042	Not significant r = 0.045
	LDH	Sig, P<0.01 r = 0.85	Sig, P<0.01 r = 0.805	Sig, P<0.01 r = 0.54	Sig, P<0.01 r = 0.51
	GAGs	Not significant r = 0.45	Sig, P<0.05 r = 0.7	Not significant r = 0.15	Not significant r = 0.33
sL-selectin	ESR	Sig, P<0.05 r = 0.71	Sig, P<0.01 r = 0.83	Not significant r = -0.16	Not significant r = 0.29
	LDH	Not significant r = 0.57	Not significant r = 0.35	Not significant r = 0.34	Not significant r = 0.3
	GAGs	Not significant r = 0.48	Sig, P<0.5 r = 0.75	Not significant r = -0.02	Sig, P<0.05 r = 0.37
	p55TNF-R	Not significant r = 0.644	Sig, P<0.5 r = 0.7	Not significant r = 0.3	Sig, P<0.01 r = 0.53

reduction in serum LDH activity after treatment as compared to activity before treatment. On contrary, insignificant difference in LDH activity was observed in patients without remission after treatment as compared to activity before treatment. Furthermore, after treatment, patients with remission demonstrated significant decline in LDH activity in comparison with patients without remission. All these results may reflect the importance of post-treatment serum LDH as a marker of response in both HD and NHL.

LDH activity in HD patients without remission before treatment showed insignificant increase in comparison to

activity in patients with remission limiting the significance of pretreatment levels of serum LDH as a prognostic marker for predicting response to therapy in HD in our study. This finding agreed with the result of Longo *et al.* (1997) who reported that LDH did not influence the treatment outcome in massive HD of any stage. In contrast to HD, serum lactate dehydrogenase (LDH) is an important prognostic factor in patients with NHL (Schneider *et al.*, 1980 and Danieu *et al.*, 1988). It is one of the parameters of International Prognostic Index which is a strong predictor of survival in patients with NHL (Shipp *et al.*, 1993 and Shipp, 1994). In our study, NHL patients without remission exhibited significantly higher pre-treatment LDH activities

Table 6: ESR values, serum LDH activity and serum levels of GAGs, sp55TNF-R and sL-selectin in NHL patients with and without B symptoms (before and after treatment) and in control group (mean $\pm$ SEM).

Group	ESR (mm/hr)	LDH (U/ml)	GAGs (mg/dl)	sp55TNF-R (ng/ml)	sL-selectin (ng/ml)
Control (n=7)	5.6 $\pm$ 1.00	187.1 $\pm$ 14.7	6.68 $\pm$ 0.54	1.0 $\pm$ 0.1	35.61 $\pm$ 2.35
Patients without B symptoms (n=21)					
□ Before treatment	43.6* $\pm$ 6.5	319.9* $\pm$ 28.2	13.42* $\pm$ 0.7	4.1* $\pm$ 0.39	62.41* $\pm$ 2.43
□ After treatment	31.1* $\pm$ 6.1	191.1 Δ $\pm$ 15.24	9.0 Δ * $\pm$ 0.52	3.19 Δ * $\pm$ 0.38	38.6 Δ $\pm$ 1.99
Patients with B symptoms (n=10)					
□ Before treatment	56.4* $\pm$ 11.8	439.9 Δ * $\pm$ 64.42	10.95* $\pm$ 1.02	5.75 Δ * $\pm$ 0.8	59.7* $\pm$ 2.19
□ After treatment	22.2 ϕ * $\pm$ 6.8	298.1 ϕ * Ψ $\pm$ 40.09	8.4 ϕ $\pm$ 0.79	5 Ψ * $\pm$ 1.16	39.35 ϕ $\pm$ 2.25

n = number of subjects . (*) = Significance against control group.

(Δ) = Significance against NHL patients without B symptoms before treatment.

(ϕ) = Significance against NHL patients with B symptoms before treatment

(Ψ) = Significance against NHL patients without B symptoms after treatment.

Table 7: ESR values, serum LDH activity and serum levels of GAGs, sp55TNF-R and sL-selectin in different stages of NHL patients before and after treatment as compared to control group (mean $\pm$ SEM).

Group	ESR (mm/hr)	LDH (U/ml)	GAGs (mg/dl)	sp55TNF-R (ng/ml)	sL-selectin (ng/ml)
Control (n=7)	5.6 $\pm$ 1.00	187.1 $\pm$ 14.7	6.68 $\pm$ 0.54	1.0 $\pm$ 0.1	35.61 $\pm$ 2.35
Stage I/II before treatment (n=7)	27.12* $\pm$ 3.5	249.5 $\pm$ 43.9	10.84* $\pm$ 0.55	3.86* $\pm$ 0.55	59.67* $\pm$ 3.4
Stage I/II after treatment (n=7)	13.9 ϕ * $\pm$ 1.6	191.14 $\pm$ 23.48	8.24 ϕ $\pm$ 0.54	2.8* $\pm$ 0.68	37.35 ϕ $\pm$ 2.1
Stage III before treatment (n=11)	59.18 ϕ * $\pm$ 6.7	3381.2 ϕ * $\pm$ 33.06	12.97 ϕ * $\pm$ 0.63	4.57* $\pm$ 0.61	61.45* $\pm$ 3.31
Stage III after treatment (n=11)	29.36 \$* $\pm$ 3.7	249 \$ $\pm$ 38.05	8.68 \$* $\pm$ 0.43	4.39* $\pm$ 0.78	\$38.97 $\pm$ 2.9
Stage IV before treatment (n=13)	54.23 ϕ * $\pm$ 6.8	381.3 ϕ * $\pm$ 27.07	14.1 ϕ * $\pm$ 1.18	5.04* $\pm$ 0.69	66.45* $\pm$ 3.06
Stage IV after treatment (n=13)	39.53* $\pm$ 4.16	222.4 @ $\pm$ 36.9	9.28 @* $\pm$.93	4.16* $\pm$ 0.84	@40.1 $\pm$ 1.62

n = number of subjects . (*) = Significance against control group .

(ϕ) = Significance against stage I/II before treatment. (\$) = Significance against stage III before treatment, (@) = Significance against stage IV before treatment.

as compared to patients who attained remission suggesting that, high elevated LDH at the time of therapy is often associated with a significantly lower response rate and accordingly, pre-treatment LDH may be used as good predictor for response to treatment. Many authors found this association between elevated pre-treatment LDH and the lower response to treatment (Bertini *et al.*, 1996; Bosch *et al.*, 1998 and Bosch *et al.*, 1998).

Glycosaminoglycans (GAGs) are unbranched polysaccharides which, with the exception of hyaluronan, are covalently bound to a core protein forming proteoglycans

(Meisenberg and Shimmons, 1998b and Zubay *et al.*, 1998 and Garrett and Grisham, 1999a). It has been shown that human lymphocytes and monocytes produce various proteoglycan types (Kolset *et al.*, 1984). In the current study, total HD and NHL patients before treatment showed extremely significant increase in serum GAGs levels ($p < 0.001$ and 0.0001 , respectively) as compared to control subjects. To our knowledge, this study is the first investigation of the level of serum circulating total GAGs in HD and NHL patients. According to Levitt and Ho (1983) and Morris *et al.* (1989) who reported the synthesis of GAG proteoglycans predominantly those of chondroitin sulfate

and heparan sulfate by B and T lymphocytes, it has been suggested that malignant lymphoma could synthesize higher amounts of GAG proteoglycans compared to normal cells. Serum concentration of hyaluronan (a component of total GAGs) has been found to be significantly higher in patients with malignant lymphoma than in health reference group (Hasselbalch *et al.*, 1995). Decreased uptake and degradation of hyaluronan owing to malignant lymphatic tissues was the most possible explanation to their finding. In some malignancies, serum hyaluronan was elevated because of increased synthesis. Several invasive tumors are enriched in hyaluronan, and connective tissue cells adjacent to the tumor are responsible for the production of tumor-associated hyaluronan (Knudson *et al.*, 1989). Recently, it has been found that, T-cell leukemia, Burkitt's lymphoma and acute monocytic leukemia synthesize chondroitin sulfate and heparan sulfate in both cell membrane and culture medium. Chondroitin sulfate is the major GAG in all cell lines as well as the major cell-related GAG in T-cell leukemia and Burkitt's lymphoma (Makatsori *et al.*, 2001). In our study, significant reduction in GAGs level was detected after treatment in total HD and NHL patients as compared to the level before treatment ($p < 0.01$ and 0.0001 , respectively). Insignificant increase was shown in total HD patients after treatment as compared to control, however, GAGs level was still significantly higher in total NHL patients as compared to control ($p < 0.05$). When patients were subdivided according to their response to therapy, we obtained the following results; after treatment, significant decrease in serum GAGs levels was observed in both HD and NHL patients with remission as compared pre-treatment levels ($p < 0.01$ and 0.001 , respectively), while patients without remission showed insignificant difference after treatment as compared to before treatment. The normalization of the GAGs levels in responders and the elevation in non responders after treatment in both HD and NHL enables serum GAGs to be a probable marker of remission in HD and NHL patients. Also, this observation may support the suggestion that, source of pre-treatment elevation of serum GAGs is the tumor cells. The pre-treatment serum levels of GAGs in HD and NHL patients without remission did not differ significantly from the corresponding levels in patients with remission. Accordingly, serum GAGs level at the time of diagnosis is thought to be insignificant predictor for response to therapy in both HD and NHL. This suggestion could be confirmed by the insignificant correlation before treatment between serum levels of GAGs and ESR values in HD and with LDH in NHL.

Tumor necrosis factor (TNF) is a part of a growing family of polypeptides that includes the interferons, interleukins and hematopoietic growth factors. Two receptors, p55 and p75 mediate the effects of TNF- α and TNF- β on target cells leading to cell activation, proliferation or apoptotic death. Soluble forms of both receptors have been identified in

biological fluids and modulate the effects of the cytokines (Warzocha *et al.*, 1995 and Bazzoni and Beutler, 1996).

There was a highly significant increase in serum sp55TNF-R in total HD and NHL patients before treatment ($p < 0.01$) as compared to control. The same findings were observed by Nadali *et al.* (1997) and others (Nadali *et al.*, 1997; Warzocha *et al.*, 1997b; 1998; Metkar *et al.*, 2000 and Vener *et al.*, 2000) with respect to HD and by Denz *et al.* (1993) and others (Denz *et al.*, 1993; Salles *et al.*, 1996; Warzocha *et al.*, 1997a and Dumontet *et al.*, 1999) with respect to NHL. These findings also confirm the finding of Aderka *et al.* (1991) who reported that the concentrations of sp55TNF-R were elevated in cancer patients as compared to control.

Elevated sp55TNF-R may reflect the generalized immune activation associated with the presence of malignancy (Warzocha *et al.*, 1997b). It is suspected that the excessive amounts of receptors in the sera of cancer patients are produced, at least in part, by the tumor cells (Aderka *et al.*, 1991). The raised levels of soluble p55TNF-R in HD before treatment could be attributed to the increased cytokine production from malignant cells during the course of the disease. HD is associated with an abnormal or unbalanced production of a large number of cytokines and soluble cytokine receptors by Hodgkin and Reed-Sternberg (H-RS) cells (Nadali *et al.*, 1994; Gruss and Dower, 1995). A recent study carried out by Warzocha *et al.* (2000) demonstrated constitutive expression of genes coding for TNF-related ligands and receptors in NHL samples. In addition to this finding, overexpression of TNF and sp55TNF-R was detected in a type of T-cell lymphoma and this overexpression may play a role in controlling T-cell proliferation (Yamaguchi *et al.*, 2000).

Insignificant difference in serum sp55TNF-R levels was detected in total HD and NHL patients after treatment in comparison to levels before treatment. In addition, the levels in total HD and NHL patients after treatment were still significantly increased as compared to control ($P < 0.01$). It is of interest that, in both HD and NHL, serum levels of sp55TNF-R increased significantly after treatment as compared to control not only in patients without remission but also in those with remission ($p < 0.0001$ and < 0.01 , respectively). This finding was similar to that of Gruss *et al.* (1993). This elevation in remission may be attributed to the believed role of TNF in persistent immune activation (Barnes and Karin, 1997) and may point to the defect in cellular immunity characteristic for HD patients (Gruss *et al.*, 1993). In comparison with levels before treatment, HD and NHL patients with remission showed significant decrease in sp55TNF-R levels ($P < 0.05$) as compared to their pre-treatment levels, while, insignificant difference was observed in patients without remission after treatment as compared to before treatment.

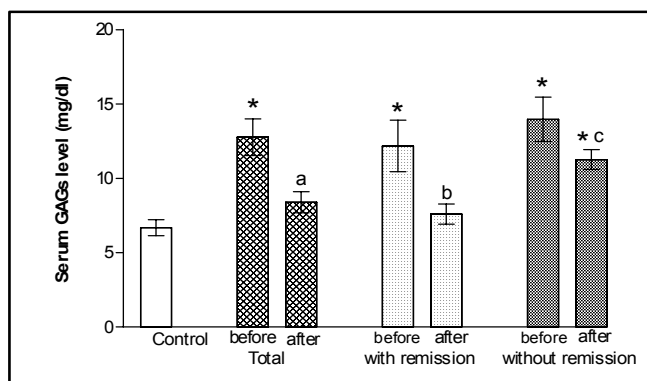


Fig. 1: Serum glycosaminoglycans (GAGs) levels in total HD patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM). (*) = significance against control group, (a) = significance against total HD patients before treatment, (b) = Significance against HD patients with remission before treatment and (c) = Significance against HD patients with remission after treatment.

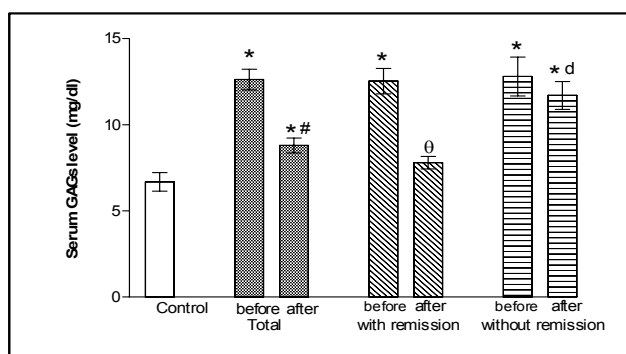


Fig. 2: Serum glycosaminoglycans (GAGs) levels in total NHL patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM). (*) = significance against control group, (#) = significance against total NHL patients before treatment, (θ) = significance against NHL patients with remission before treatment and (d) = significance against NHL patients with remission after treatment.

In both HD and NHL, the pre-treatment serum sp55TNF-R levels in patients without remission were significantly higher as compared to the corresponding levels in the responders ($p < 0.05$). This result may suggest the significant value of pre-treatment levels of p55 TNF-R in predicting treatment outcome with respect to response to therapy. This result could be confirmed by strong positive correlation before treatment between serum sp55 TNF-R with ESR values in case of HD and with LDH in case of NHL. Significantly reduced levels were detected after treatment ($P < 0.0001$) in patients with remission as compared to those without remission.

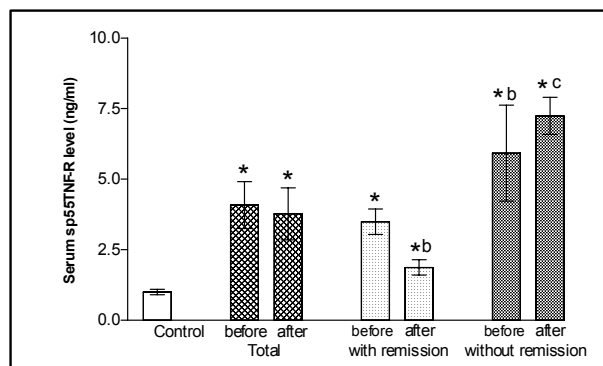


Fig. 3: Serum soluble p55 tumor necrosis factor receptor (sp55TNF-R) levels in total HD patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM). (*) = significance against control group, (b) = significance against HD patients with remission before treatment and (c) = significance against HD patients with remission after treatment.

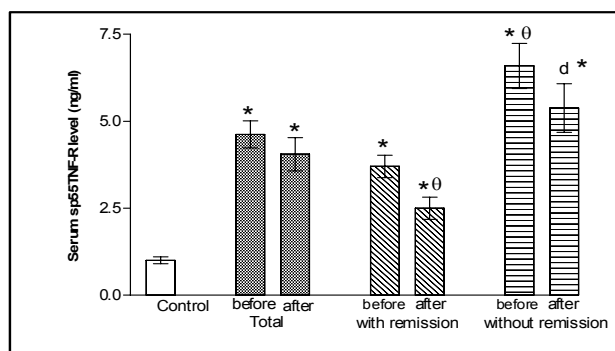


Fig. 4: Serum soluble p55 tumor necrosis factor receptor (sp55TNF-R) levels in total NHL patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM). (*) = significance against control group, (θ) = significance against NHL patients with remission before treatment and (d) = significance against NHL patients with remission after treatment.

L-selectin is a member of the selectin family of adhesion molecule. It is expressed normally on the surface of most leukocytes including lymphocytes (Tatewaki *et al.*, 1995). It is essential for the migration of naïve lymphocytes into peripheral lymph nodes by facilitating adhesion to high endothelial venules within lymph nodes and also essential to mediate rolling of leukocytes on endothelium at sites of inflammation (Kansas, 1996).

In our study, pre-treatment levels of serum sL-selectin increased significantly in total HD and NHL patients as compared to control ($p < 0.001$). Our results, concerning HD and NHL, agreed with the results obtained by Haznedaroglu

et al. (2000a). With the exception of his study, no further data are available about circulating L-selectin in HD and NHL until the time of writing this paper. High levels of soluble L-selectin in lymphoma may suggest the shedding of this molecule from malignant cells (Gu *et al.*, 1998). In addition, the increments of sL-selectin in NHL may be simply as a result of leukocytes activation related to the inflammatory process (Haznedaroglu *et al.*, 2000b).

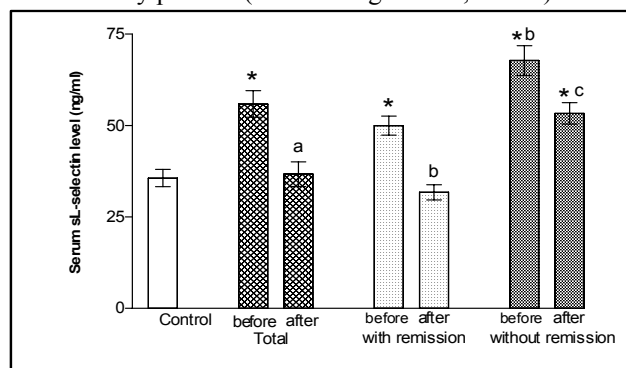


Fig. 5: Serum soluble L-selectin levels in total HD patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM). (*) = significance against control group, (a) = significance against total HD patients before treatment, (b) = significance against patients with remission before treatment and (c) = significance against patients with remission after treatment.

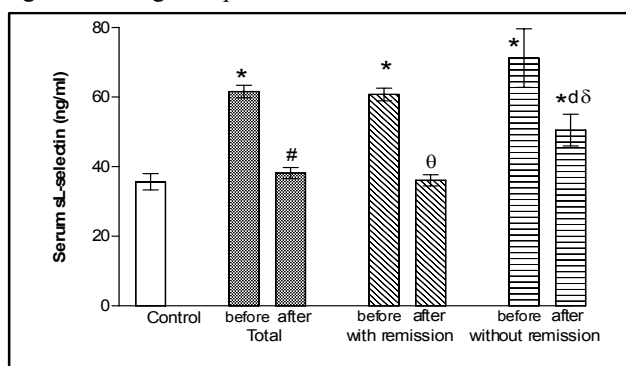


Fig. 6: Serum soluble L-selectin levels in total NHL patients and in patients with and without remission (before and after treatment) as compared to control group (mean $\pm$ SEM). (*) = significance against control group, (#) = significance against total NHL patients before treatment, (θ) = significance against NHL patients with remission before treatment, (d) = significance against NHL patients with remission after treatment and (δ) = significance against NHL patients without remission before treatment.

After treatment, total HD and NHL patients exhibited significant decrease ($p < 0.001$) in serum sL-selectin as compared to levels before treatment, while, no significant difference was detected in comparison with control. HD patients with remission showed highly significant decrease

($p < 0.01$) in serum sL-selectin after treatment in comparison with pre-treatment levels, whereas, insignificant decrease was seen in patients without remission. Also, after treatment, HD patients with remission had significantly reduced levels ($p < 0.01$) as compared to those without remission. Unlike HD, serum sL-selectin levels in NHL patients tended to be reduced significantly ($p < 0.01$) after treatment in comparison to levels before treatment even in patients without remission, but, serum sL-selectin levels after treatment significantly decreased ($p < 0.01$) in patients with remission as compared to those without remission. Besides, after treatment, patients with remission showed insignificant difference as compared to control whereas those without remission still attained high levels which were significantly raised than control ($p < 0.01$) indicating that reduction after treatment still dependent on response to therapy. The strong positive correlation of serum sL-selectin levels with GAGs and sp55TNF-R levels after treatment may support this finding. The fact that serum sL-selectin levels dropped after therapy, even in patients without remission suggests that malignant cells are not the sole source of elevated L-selectin in NHL.

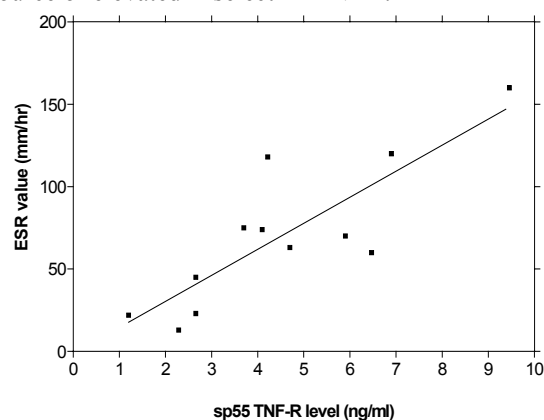


Fig. 7: Significant positive correlation between serum soluble p55 tumor necrosis factor receptor (sp55TNF-R) levels and erythrocyte sedimentation rate (ESR) values in HD patients before treatment ($r = 0.83$, $p < 0.01$).

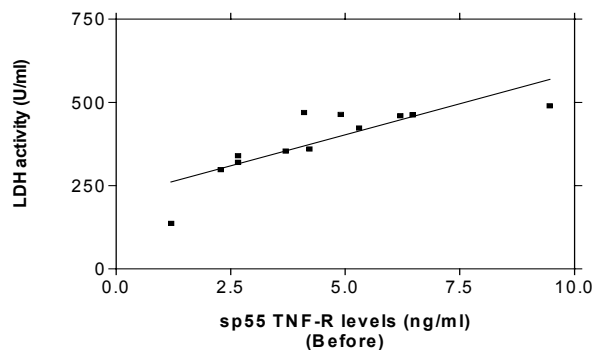


Fig. 8: Significant positive correlation between serum soluble p55 tumor necrosis factor receptor (sp55TNF-R) levels and lactate dehydrogenase (LDH) activity in HD patients before treatment ($r = 0.85$, $P < 0.01$).

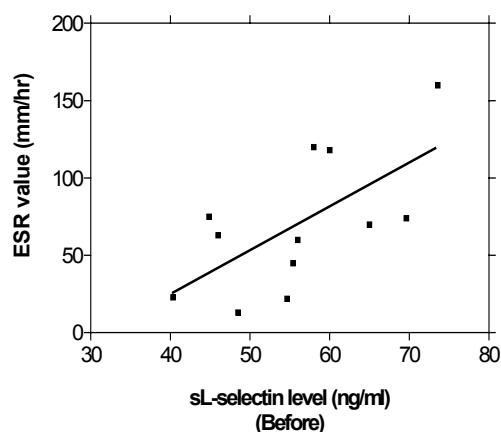


Fig. 9: Significant positive correlation between serum soluble L-selectin levels and erythrocyte sedimentation rate (ESR) values in HD patients before treatment ($r = 0.7$, $P < 0.05$).

HD patients without remission showed significant increase ($p < 0.01$) in serum sL-selectin levels before treatment as compared to patients with remission. This result may suggest the prognostic significance of L-selectin in HD and this could be confirmed by the strong correlation with ESR values before treatment. However, the prognostic significance of L-selectin in HD needed to be validated by further studies on a larger group of patients. In contrast to HD, NHL patients without remission showed insignificant increase in serum sL-selectin levels before treatment as compared to patients with remission. This result together with the insignificant correlation between pre-treatment serum sL-selectin level and other adverse prognostic factors such as LDH activity, sp55TNF-R levels and ESR values, all may reflect the minor value of serum sL-selectin in prognosis of NHL.

Tables 5 and 6 illustrated the correlation of the five parameters (ESR, LDH, GAGs, sp55TNF-R and sL-selectin) with both B-symptoms and disease stage in NHL. As shown in table 5, NHL patients with B symptoms showed higher pre-treatment serum LDH activity and sp55TNF-R levels as compared to those without B symptoms while no significant differences were observed in case of ESR, serum GAGs and sL-selectin levels. Patients without B symptoms showed a significant reduction in serum p55TNF-R after treatment as compared to the level before treatment, while patients with B symptoms exhibited insignificant difference after treatment as compared to before treatment. In addition, sp55TNF-R levels in patients without B symptoms reduced significantly after treatment as compared to those with B symptoms. This result reflects that, the association of elevated sp55TNF-R with B symptoms may result in a poor status of the patient and may influence the patient's ability to tolerate therapy and, as a consequence, result in a worse prognosis.

The correlation between the biochemical parameters in our study and tumor burden (as determined by clinical stage at presentation) was also evaluated in NHL patients (table 6) and it has been found that, before treatment, advanced stages III/IV revealed significantly higher ESR values, serum LDH activity and GAGs levels than early stages I/II. Therefore, the levels of these parameters at diagnosis could help to assess the disease activity. On the other hand, pre-treatment serum sp55TNF-R and sL-selectin levels revealed insignificant differences in stages III/IV as compared to stages I/II. From these findings we can conclude that:

1. Pre-treatment levels of serum GAGs increased in HD and NHL patients and these levels may have no prognostic value in predicting response to therapy both in HD and NHL. However, the pre-treatment levels could reflect disease activity. Post-treatment levels reduced significantly in remission, while persistent elevation was observed in patients without response, so post-treatment GAGs level may be a good marker of remission.
2. Pre-treatment levels of serum sp55TNF-R increased in both HD and NHL and these levels could be used as a prognostic marker to predict treatment outcome and response to therapy in both HD and NHL. As their levels were still significantly high after treatment as compared to control even, in patients with remission, so post-treatment levels may not be a perfect sign of response to therapy.
3. Pre-treatment levels of serum sL-selectin elevated in both HD and NHL. High pre-treatment sL-selectin levels may suggest bad prognosis in HD but not in NHL. As the post-treatment levels reduced significantly in NHL as compared to before treatment, even in patients without remission, so post-treatment level may have little value as a marker of remission.

Our findings, particularly those with HD, are needed to be validated and extended on a larger group of patients.

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