RELEVANCE OF SOME SERUM BIOMARKERS (E CADHERIN, GAGS & MDA IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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ABSTRACT

This study aimed to estimate the pretreatment serum levels of SVE-Cadherin, glycosaminoglycams (GAGs) and malondialdehyde (MDA) in order to evaluate their prognostic significance and their role in monitoring tumor response and overall-survival in Non Hodgkin lymphoma (DLCL) patients. Also the work aimed to investigate the relationship between levels of these biochemical markers with LDH level, ESR and tumor stage. For this purpose pretreatment serum levels of these biochemical markers were evaluated in 40 newly diagnosed patients with non-Hodgkin lymphoma (Diffuse large cell type) and studied in relation to expression in healthy control. Our results revealed that serum levels of SVE-Cadherin, GAGs and MDA increased significantly ($P<0.05$) in NHL patients (DLCL) as compared to control, no significant relation between these parameters and ESR, LDH. However, higher level of SVE-Cadherin was found in stage II, III of the disease as compared to stage IV disease but with no statistical significance. Regarding response to therapy, only MDA showed a significant relation with response of the patient to treatment. Concerning overall survival there is no statistical significance was found between these parameters & OS in NHL patients. Elevated levels of SVE-Cadherin, GAGs and MDA in NHL patients indicate that they may have a role in the pathogenesis of the disease. High level of MDA may be used as a predictor for tumor response to systemic chemotherapy. Low level of SVE-Cadherin in stage IV participates in the invasiveness and metastasis of the disease.

Keywords: SVE-Cadherin, Glycosaminoglycans (GAGs), Malondialdehyde (MDA), Non Hodgkin Lymphoma (NHL), diffuse large cell (DLCL), overall survival (OS).

INTRODUCTION

The lymphoma is a heterogenous group of clonal (neoplastic) diseases that share the single characteristic of arising as the result of somatic mutations in a lymphocyte progenitor. The progeny of the affected cell usually carry the phenotype of B, T or natural killer (NK) cell as judged by immunophenotyping or gene rearrangement studies. Any site of the lymphatic system may be the primary site of origin of the disorder including lymphnodes; gut associated lymphatic tissue, skin or spleen. Any organ e.g. thyroid, lung, bone, brain, gonads, etc may be involved either by spread from lymphatic sites or as a manifestation of primary extranodal disease (Foon and Fisher, 2001).

The term lymphoma identifies two distinct groups of neoplasms: non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL) (Harris *et al*., 1999). Non-Hodgkin lymphoma (NHL) includes a group of more than 20 different malignant lymphoproliferative diseases that originate from lymphocytes (Grulich and Vajdic, 2005).

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), β2-microglobulin, and albumin serum levels have most frequently been identified as prognostic factors in malignant lymphomas (Shipp *et al.*, 1993 and Shipp 1994 and Aisenberg, 1995).

Glycosaminoglycans (GAGs) are unbranched poly-saccharides 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 proteo-glycan types (Kolset *et al*., 1984).

Lipid peroxidation is a radical-initiated chain reaction that is self-propagating in cellular membranes. Many of the aldehydes produced through lipid peroxidation are used as indicates of lipid peroxidation in organs, plasma, breath and urine. The most commonly used assay is that which detects thiobarbituric acid reactive species (TBARS) (Knutson *et al*., 2000).

The SVE-cadherins are a family of cell-cell adhesion molecules. These glycoprotein molecules exhibit Ca^{2+}

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dependent cell adhesion and are expressed on epithelial cells. E- Cadherin levels in serum are known to be significantly elevated in patient with epithelial carcinomas (Takubo *et al*., 2002b). Cadherins are divided into subclasses e-, p-, and n- cadherins which are distinct in immunological specifity and tissue distribution. They promote cell adhesion via a homophilic mechanism. These compounds play a role in a construction of tissues and the whole animal body.

The present study was performed to estimate the pretreatment serum levels of SVE-Cadherin, GAGs and MDA in order to evaluate their prognostic significance and their role in monitoring tumor growth and host tumor response in NHL (DLCL). Also, the work aimed to investigate the relationship between levels of these markers and other different prognostic factors (LDH, ESR) and patient's survival.

SUBJECTS AND METHODS

Patients

The present study has been conducted on newly diagnosed 40 patients of NHL (DLCL) selected from outpatient's clinic of Oncology Centre Mansoura University. The study was performed on patients attending Hematology and Oncology Unit at the period from October 2003 to October 2006. All the NHL patients had diffused large cell lymphoma (DLCL) with 28 of them with advanced stage (stage III, IV). CHOP regimen was used in all patients and DHAP regimen used in patients with partial response or no response after 4 cycles of CHOP (13 patients).

CR was achieved in 11 patients (27.5%) while PR achieved in 22 patients (55%) and NR in 7 patients (17.5%). Fifteen apparently healthy individuals were selected as a control reference group (table 1).

METHODS

Blood samples were collected from pre-treatment 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 frozen at –20ºC for further investigations of serum SVE-Cadherin, GAGs, and MDA levels.

Table 1: Patient characteristics

Patients N (%)	40 (100%)
Gender, Males : Females	26 : 14
Histology (N) (%)	DLCL (100%)
Clinical stage n (%)	
I	0 (0%)
II	12 (30%)
III	24 (60%)
IV	4 (10%)
Chemotherapy treatment n (%)	
CHOP	27 (67.5%)
DHAP after CHOP	13 (32.5%)
Response n (%)	
CR	11 (27.5%)
PR	22 (55%)
NR	7 (17.5%)
Outcome n (%)	
Alive	26 (65%)
Dead (lost)	14 (35%)
Over all survival (3ys)	
Mean survival time ± SD	14.59 ± 1.50

Determination of serum malondialdehyde (MDA)

Serum malondialdehyde was measured according to the method of Draper & Hadley (1990) in which serum proteins are precipitated by addition of trichloroacetic acid. Then thiobarbituric acid reacts with malondial-dehyde to form thiobarbituric acid reactive product that is measured at 532 nm.

Determination of serum glycosaminoglycans (GAGs)

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

Determination of serum soluble vascular endothelial Cadherin (SVE-Cadherin)

Using a kit from "IBL immuno-Biological laboratories" serum SVE-Cadherin level was determined by enzyme linked immunosorbent assay (ELISA) method described by (Tanihara *et al*., 1994) for quantitative detection of soluble human vascular endothelial (VE) cadherin.

STATISTICAL ANALYSIS

- Data was analyzed using SPSS (Statistical Package for Social Sciences) version 10.
- Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test.
- Quantitative data was tested for normality by Kolmogrov-Smirnov test.

Table 2: Relation between serum level of SVE-Cadherin, GAGs & MDA of the patients and control group

Parameters	Patients Mean ± SD	Control Mean ± SD	P value
Cadherin (ng/ml)	39.65 ± 19.80	16.98 ± 5.18	0.000
GAGs (mg/dl)	37.85 ± 16.34	16.99 ± 5.75	0.000
MDA (nmol/ml/hr)	402.99 ± 107.69	132.57 ± 78.95	0.000

Table 3: Relation between serum level of SVE-Cadherin, GAGs and MDA of the patients and sex

Parameters	Mean ± SD	T	P value
Cadherin (ng/ml) Male (n = 26) Female (n = 14)	 41.51 ± 21.70 36.20 ± 15.85	0.805	0.426
GAGs (mg/dl) Male (n = 26) Female (n = 14)	 37.26 ± 15.55 38.95 ± 18.26	0.310	0.759
MDA (nmol/ml/hr) Male (n = 26) Female (n = 14)	 412.74 ± 113.87 384.91 ± 96.50	0.776	0.443

Table 4: Relation between serum level of SVE- cadherin, GAGs and MDA of the patients and bone marrow biopsy

Parameters	Mean ± SD	T	P value
Cadherin (ng/ml) Negative (n = 36) Positive (n = 4)	 40.96 ± 20.44 27.83 ± 4.02	1.268	0.212
GAGs (mg/dl) Negative (n = 36) Positive (n = 4)	 37.89 ± 16.38 37.50 ± 18.39	0.045	0.965
MDA (nmol/ml/hr) Negative (n = 36) Positive (n = 4)	 401.34 ± 106.7 417.85 ± 132.67	0.287	0.775

Positive: there is bone marrow infiltration by lymphocytes.

- Normally distributed data was presented as mean ± SD. Paired t-test was used for comparison within groups. Student t-test was used to compare between two groups. F-test (One Way Anova) was used to compare between more than two groups.
- Pearson correlation coefficient was used to test correlation between variables.
- Cox regression analysis was used to test for survival.
- $P < 0.05$ was considered to be statistically significant.

RESULTS

Table 2 shows that there is highly significant increase of levels of SVE-Cadherin, GAGs and MDA in non Hodgkin lymphoma (DLCL) patients as compared to healthy controls (p value 0.000).

Table 3 shows that there is no significant difference between levels of these markers and gender, also infiltration of the bone marrow by the disease has no significant difference with these biomarkers (table 4).

Tumor markers and stages of the disease

Serum level of E-Cadherin increased in stage II, III of the disease as compared to stage IV however no statistical significance was found, while GAGs and MDA had no significant change with the non Hodgkin lymphoma stages (table 5).

Biochemical markers and response to treatment

Mean levels of SVE-Cadhrin and GAGs in DLCL patients showed no significance difference regarding response to treatment while mean value ± SD of MDA was highly significant in patients with complete remission, partial remission as compared to patients with no remission (table 6) and these results were confirmed by using Post Hoc test (table 7).

Table 5: Relation between serum level of SVE - cadherin, GAGs and MDA of the patients and NHL stages

Parameters	Mean ± SD	T	P value
Cadherin (ng/ml) Stage 2 (n = 12) Stage 3 (n = 24) Stage 4 (n = 4)	 41.75 ± 18.48 40.42 ± 21.85 28.75 ± 2.67	0.679	0.513
GAGs (mg/dl) Stage 2 (n = 12) Stage 3 (n = 24) Stage 4 (n = 4)	 34.39 ± 17.45 40.00 ± 16.22 35.33 ± 16.56	0.512	0.604
MDA (nmol/ml/hr) Stage 2 (n = 12) Stage 3 (n = 24) Stage 4 (n = 4)	 421.30 ± 104.69 389.58 ± 109.68 428.57 ± 121.21	0.459	0.635

Table 6: Relation between serum level of SVE-Cadherin, GAGs and MDA of the patients and response to treatment

Parameters	Mean ± SD	T	P value
Cadherin (ng/ml) CR (n = 11) PR (n = 22) NR (n = 7)	 43.55 ± 21.10 39.02 ± 21.18 35.50 ± 13.72	0.365	0.696
GAGs (mg/dl) CR (n = 11) PR (n = 22) NR (n = 7)	 36.85 ± 17.56 36.30 ± 16.82 44.29 ± 13.12	0.651	0.527
MDA (nmol/ml/hr) CR (n = 11) PR (n = 22) NR (n = 7)	 462.61 ± 69.93 405.94 ± 96.24 300.06 ± 126.12	6.194	0.005*

Biochemical markers and overall survival

Using Cox Regression analysis as shown in table 9 there is no statistical significant relation between biochemical markers and overall survival in NHL patients also; there is no any significant correlation between these biomarkers and other routine laboratory tests (LDH, ESR, and hemoglobin and leukocyte count (table 8)

DISCUSSION

Malignant lymphoma ranked as the third most frequent neoplastic disease in Egypt (El-Bolkainy, 1991). The present study was performed to estimate the pre-treatment levels of some biochemical markers (SVE-Cadherin, GAGs and MDA) in order to evaluate their prognostic significance in monitoring tumor growth, host tumor response and patients' survival in NHL (DLCL) patients.

SVE-Cadherin is a transmembrane glycoprotein that mediates Ca^{+2} dependent intracellular adhesions in normal epithelial cells. SVE-Cadherin levels in serum are known to be significantly elevated in patients with epithelial carcinomas. In a study done by Takubo *et al*. (2002a). SVE-Cadherin levels in patients with acute or chronic leukemia, malignant lymphoma or myelodysplastic syndromes was determined. Significant elevation of serum SVE-Cadherin levels was detected in patients with haematological malignancies and between types of acute leukemias or subtypes of MDS, stages of malignant lymphoma and phases of chronic leukemias respectively compared with those in healthy adult volunteers. This study is in agreement with our result in table 2 that we found highly significant (P=0.000) increase in serum level of this biochemical markers in our patients and control group. These findings suggest that SVE-Cadherin might be expressed in malignant haematopoietic cells and might be useful as a diagnostic indicator in haematological malignancies (Takubo *et al.*, 2002b) determined serum E-Cadherin levels in 30 patients with non-Hodgkin's lymphoma and found that E-Cadherin antigen is expressed on the lymphoma cells in three patients with NHL, and that soluble E-Cadherin might be released into blood from lymphoma cells. Expression of E-Cadherin may contribute to the morphological appearance of some malignant lymphoma.

Table 7: Post Hoc tests to correlate between different responses groups (Bonferroni).

CR versus PR	P = 0.35
CR versus NR	P = 0.004
PR versus NR	P = 0.045

Table 8: Correlation between SVE-Cadherin, GAGs, MDA of the patients and other parameters

Parameters	r	P
Cadherin (ng/ml)		
Hb (gm/dl)	0.103	0.526
WBCs	0.003	0.984
Platelets	- 0.154	0.344
ESR (2^{nd})	- 0.093	0.569
LDH	0.179	0.268
GAGs (mg/dl)		
Hb (gm/dl)	- 0.068	0.677
WBCs	- 0.108	0.506
Platelets	- 0.156	0.337
ESR (2^{nd})	0.074	0.648
LDH	0.119	0.464
MDA (nmol/ml/hr)		
Hb (gm/dl)	- 0.137	0.398
WBCs	0.286	0.074
Platelets	0.201	0.214
ESR (2^{nd})	- 0.089	0.584
LDH	- 0.048	0.769

Table 9: Cox Regression analysis of the impact of SVE-Cadherin, GAGs and MDA on the overall survival of NHL patients

Parameters	Relative Risk	SE	P value	95% CI
Cadherin (ng/ml)	1.011	0.012	0.357	0.987 – 1.036
GAGs (mg/dl)	1.014	0.019	0.462	0.977 – 1.052
MDA (nmol/ml/hr)	1.00	0.003	0.890	0.994 – 1.007

Another study done by Rabascio *et al*. (2004) found that VE-CRNA (VSVE-Cadherin RNA) was particularly increased in patients affected by haematological malignancies and decreased in patients achieving complete remission. In our study we found that serum SVE-Cadherin was elevated in patients achieved CR than those with NR but this relation is not statistically significant. This elevation in CR patients may be due to the role of SVE-Cadherin in adhesiveness of the tissue which was found to elevate in stage II, III disease rather than stage IV disease as showed in table 5. This contribute to that SVE-Cadherin is one of the marker of angiogenesis and as angiogenesis is less in early stages of the disease than in late stages (IV).

Glycosaminoglycans (GAGs) are unbranched poly-saccharides usually found in extracellular space in multicellular organisms. They provide a passageway for cell migration, supply lubrication between joints, or help maintain certain structural shapes such as the ball of the eye (Meisenberg & Simmons, 1998 and Garrett & Grisham, 1999). In the current study, NHL patients before treatment showed extremely significant increase in serum GAGs levels. According to Levitt & Ho (1983) and Morris *et al*. (1989) who reported the synthesis of GAG proteoglycans predominantly 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 healthy 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).

A recent study done by Afify *et al*. (2007) to correlate of the cytological examination with ELISA assay for hayluronon and soluble CD44v6 levels in evaluation of effusions. The purpose of this study was to correlate HA and sCD44v6 levels in effusions with the cytology diagnosis and to assess their usefulness in differentiating between malignant and non malignant effusions. In their study they evaluated HA and sCD44v6 contents in 20 effusion from cytologically positive samples (2 of them with lymphomas) and to effusion from cytologically negative sample. They concluded that the HA and sCD44v6 levels in body fluids correlate with the cytology diagnosis and could be used as an ancillary study in cytology to differentiate non malignant from malignant effusions.

In our study GAGs level was significantly higher in NHL (DLCL) patients compared to control (P=0.000). None statistically significant higher serum levels with a (mean of 44.29 ± 13.12) was seen in patients in NR comparing to CR patients (mean 36.85 ± 17.50). We agree with a previous study done by Salem *et al*. (2006) who found that serum GAGs levels increase significantly before treatment in HD and NHL as compared to control and also found higher levels of GAGs seen in association with PR to therapy. So, GAGs may be used as a prognostic factor to the response to therapy.

In our study we found high serum level of MDA in NHL (DLCL) comparing to control (P=0.000). This is in agreement with Morabito *et al*. (2004) who evaluate MDA/HNE in sera of untreated HL (Hodgkin's lymphoma) patients in advanced disease stage in order to quantify the oxidative stress, HL patients found to display significantly higher levels of both MDA/HNE as compared with healthy controls.

Correlation between MDA and response to therapy in our study shows a significant higher level of MDA in patients achieving CR, PR with a P value of (0.004, P 0.04) respectively versus patient with NR which mean that higher level of MDA may be used as prognostic factor for a tumor response to chemotherapy. To our knowledge we are the first who try to correlate between MDA and response to therapy. Other studies showed that there is significant increase in MDA level after high dose of chemotherapy (Cetin *et al*., 2004). Bergman *et al*. (2004) founds also elevated urinary excretion of MDA level after high dose of radio chemotherapy preceding stem cell transplantation.

Cox regression analysis as shown in table 9 showed the impact of SVE-Cadherin, GAGs, MDA on overall survival but the result is not statistically Significant.

CONCLUSION

High levels of SVE-Cadherin, GAGs and MDA were found in NHL patients comparing to control. So, they may have a role in the pathogenesis of the disease. High level of MDA may be used as a predictor for significant tumor response to systemic chemotherapy. Low level of SVE-Cadherin in stage IV participates in the invasiveness and metastasis of the disease.

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List of abbreviation

CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone
COP: cyclophosphamide, vincristine, prednisone
CR: complete remission
DFS: disease free survival
DLCL: diffuse large cell lymphomes.
DHAP:Dexamethasone,cytarabine,cisplatin
ESR **:**Erythrocyte sedimentation rate
GAGs: Glycosaminoglycans,
HD: Hodgkin's lymphoma
NHL: Non-Hodgkin's lymphoma
LD: lymphocyte depletion
LDH: Lactate dehydrogenase
OS: overall survival probability.
PR: partial remission
NR: No remission
PS: performance status
MDA: Malondialdehyde.
SVE Cadherin: Soluble human vascular endothelial Cadherin.

REFERENCES

Afify LC, Lynne and Howell L (2007). Correlation of cytologic examination with ELISA assays for hyaluronan and soluble CD44v6 levels in evaluation of effusions. *Diagn. Cytopathol.*, **35**(2): 105-110.

Aisenberg AC (1995). Coherent view of non-Hodgkin's lymphoma. *J. Clin. Oncol.*, **13**(10): 2656-2675.

Bergman V, Leanderson P, Starkhammar H and Tagesson C (2004). Urinary excretion of 8-hydroxide-oxyguanosine and malondialdehyde after high dose

radiochemotherapy preceding stem cell transplantation. *Free Radic. Biol. Med*., **36**(3): 300-306.

Cetin T, Arpaci F, Yilmaz MI, Saglam K, Ozturk B, Komurcu S, Gunay M, Ozet A, Akay C, Kilic S and Ulutin C (2004). Oxidative stress in patients undergoing high dose chemotherapy plus peripheral blood stem cell transplantation. *Biol. Trace. Res.*, **97**(3): 237-247.

Draper HH and Hadley M (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.,* **186**: 421-431.

El-Bolkainy N (1991). Relative frequency of cancer. *In*: General Pathology of Cancer. 1st edition, Cairo, p.51.

Foon KA and Fisher RI (2001). Lymphomas. *In*: Beutler E, Lichtman M, Coller B and Kipps T (eds). William's Hematology. Sixth Edition McGraw-Hill, New York, p.1237.

Garratt RH and Grisham CM (1993). Carbohydrates. *In*: Biochemistry. Saunders College Publishing, Philadelphia, New York, Toronto, London, Sydney, pp.209-237.

Grulich AE and Vajdic CM (2005). The epidemiology of non-Hodgkin lymphoma. *Pathology*, **37**(6): 409-419.

Harris NL, Jaffe ES and Diebold J *et al.* (1999). World Health Organization classification of neoplastic diseases of hematopoietic and lymphoid tissues: Report of the Clinical Advisory Committe Metting, Airlie House, Virginia and November 1997. *J. Clin. Oncol.*, **17**: 3835.

Hasselbalch H, Haggard D, Nissen N and Junker P (1995). Serum hyaluronan is increased in malignant lymphoma. Am. J. Hematol., **50**(4): 231-233.

Knudson W, Biswas C, Li Q, Nemec XRE and Toole BP (1989). The role and regulation of tumor-associated hyaluronan. *Ciba Found Symp.,* **143**: 150-159, discussion 159-169, 281-285.

Knutson MD, Handelman GJ and Viteri FE (2000). Methods for measuring ethane and pentane in expired air from rats and humans. *Free Radical Biol. Med.*, **28**(4): 514-519.

Kolset SO, Seljelid R and Lindahl U (1984). Modulation of the morphology and glycosaminoglycan biosynthesis of human monocytes, induced by culture substrates. *Biochemistry Journal*, **219**(3). 793-799.

Levitt D and Ho PL (1983). Induction of chondroitin sulfate proteoglycan synthesis and secretion in lymphocytes and monocytes. *J. Cell Biol.,* **97**(2): 351-358.

Makatsori E, Karamanos NK, Papadogiannakis N, Hjerpe A, Anastassiou ED and Tsegenidis T (2001). Synthesis and distribution of glycosaminoglycans in human leukemic B- and T-cells and monocytes studied using specific enzymic treatments and high-performance liquid chromatography. *Biomed. Chromatogr.*, **15**(6): 413-417.

Manner CE (1998). The lymphomas. In: Clinical Hematology: Principles, Procedures, and Correlations. Stiene-Martin EA, Lotspeich-Steininger CA and Koepke JA (eds). Lippincott Company, Philadelphia, New York, pp.490-502.

Mazzuchin A, Walton RJ and Thibert RJ (1971). Determination of total and conjugated glucuronic acid in serum and urine employing a modified naphthoresorcinol reagent. *Bioch. Med*., **5**(2): 135-157.

Meisenberg G and Simmons WH (1998). Plasma proteins-integration. *In*: Medical Biochemistry. Mosby Company, New York, Philadelphia, London, Sydney, Tokyo, pp.511-546.

Morabito F, Cristani M, Saija A, Stelitano C, Callea V, Tomaino A, Minciullo PL and Gangemi S (2004). Lipid peroxidation and protein oxidation in patients affected by Hodgkin's lymphoma. *Mediators Inflamm.,* **13**(5-6): 381-383.

Morris AJ, Dexter TM and Gallagher JT (1989). Metabolic properties of a homogenous proteoglycan of a hematopoeitic stem cell line, FDCP-mix. *Biochemistry Journal*, **260**(2): 479-486.

Murray RK and Keeley FW (2000). The extracellular matrix. *In*: Harper's Biochemistry, Murray RK, Granner DK, Mayes PA and Rodwell VW (Eds). Appelton & Lange Company, California, pp. 695-714.

Rabascio C, Muratori E, Mancuso P, Calleri A, Raia V, Foutz T, Cinieri S, Veronesi G, Pruneri G, Lampertico P, Iavarone M, Martinelli G, Goldhirsch A and Bertolini F (2004). Assessing tumor angiogenesis: increased circulating VE-Cadherin RNA in patients with cancer indicates viability of circulating endothelial cells. *Cancer Res*., **64**(12): 4373-4377.

Salem HA, Eissa LA, Rabbie AM, El-Helw IM and El-Gayar AM (2006). Evaluation of some biochemical markers as prognostic factors in malignant lymphomas. *Pak. J. Pharm. Sci.*, **19**(3): 219-230.

Shipp M, Harrington D and Anderson J *et al.* (1993). A predictive model for aggressive non-Hodgkin's lymphoma. *N. Engl. J. Med.*, **329**: 987-994.

Shipp MA (1994). Prognostic factors in aggressive non-Hodgkin's lymphoma: who has "high risk" disease? *Blood*, **83**(5): 1165-1173.

Takubo T, Kanashima H, Erada Y, Aoyama Y, Nakamae H, Yamamura R, Shima E, Makita K, Tanaka K, Ohta K, Yamane T, Hino M, Kamitani T and Tatsumi N (2002a). Clinical significance of serum E- cadherin levels in patients with haematological malignancies. *Haematologia* (Buadap). **31**(4): 319-325.

Takubo T, Kanashima H, Terada Y, Shibata H, Aoyama Y, Nakamae H, Yamamura R, Shima E, Makita H, Tanaka K, Ohta K, Yamane T, Hino M, Kamitani T, Tasumi N and Ohshima K (2002b) E-Cadherin expression in lymph nodes of three patients with non – Hodgkin's lymphoma. *Haematologia* (Buadap) **32**(1): 67-72.

Tanihara H, Kido M, Obata S, Heinark RL, Dandson M, St John T and Suzuki S (1994). Characterization of Cadherin – 4 and Cadherin 5 reveals new aspects of Caherins. *J. Cell Sci.,* **107**(pt 6): 1697-1704.