

## Tumor Necrosis Factors, Interferons and Matrix Metalloproteinase-9 in Sera of Non-Hodgkin's Lymphoma Patients

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### ABSTRACT

**Objective:** In the present study, the serum levels of some cytokines and the matrix metalloproteinase-9 (MMP-9) were studied in an attempt to find suitable markers for early diagnosis of non-Hodgkin's lymphoma (NHL) and to assess their role in differentiating between disseminated and nondisseminated cases.

**Materials and Methods:** The present study was conducted on 60 patients with nondisseminated NHL, 14 patients with disseminated NHL, in addition to 10 healthy controls. Their sera were used to determine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\beta$  (TNF- $\beta$ ), interferon- $\alpha$  (IFN- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ) and Matrix Metalloproteinase-9 (MMP-9) using the ELISA technique.

**Results:** The results showed that the serum level of TNF- $\alpha$  and IFN- $\alpha$  can be used to differentiate between the control group and the group of NHL patients. However, they could not differentiate between nondisseminated NHL (nd-NHL) and disseminated NHL (d-NHL). On the other hand, the serum level of TNF- $\beta$  can be used to differentiate between nd-NHL and d-NHL, but not between the control group and nd-NHL. Each of IFN- $\gamma$  and MMP-9 were not useful in discrimination between the control group and the diseased ones. Our data revealed no correlation between serum level of the parameters investigated and the gender of the patients.

**Conclusion:** The present results revealed that TNF- $\alpha$  and IFN- $\alpha$  can be used as diagnostic tools for NHL. On the other hand, TNF- $\beta$  is useful in the differentiation between nd-NHL and d-NHL.

**Key Words:** Non-hodgkin's lymphoma (NHL) - Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) - Tumor necrosis factor- $\beta$  (TNF- $\beta$ ) - Interferon- $\alpha$  (IFN- $\alpha$ ) - Interferon- $\gamma$  (IFN- $\gamma$ ) - Matrix metalloproteinase 9 (MMP-9).

### INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of neoplastic disorders originating from the widely distributed cells of the immune system and therefore, it may originate virtually

in any organ and show disparate histological features, clinical behavior and prognosis [1]. NHL has a high incidence in the Middle East, contributing 7% of total cancer cases as compared to 4% in USA [2]. There is a high incidence of NHL in Egypt, which is possibly related to the exposure of the population at a young age to various bacterial, parasitic and viral infections as Epstein-Barr virus (EBV) [3]. Moreover, immunological deficiency, as a result of malnutrition, may be involved in the pathogenesis of lymphoma [3]. The increasing incidence of NHL makes it urgent to apply new techniques for diagnosing the disease.

Cytokines are peptides used by cells for intracellular communication [4]. They are produced by cell types that have important roles in the immune response, inflammation, hemopoiesis, healing and systemic response to injury. Cytokines can be produced by malignant cells alone, generating an autocrine loop, or they can be produced by bystander cells in a paracrine fashion. In addition to their effect on tumor cells, cytokines can also exert their activity on distant organs or functions, thus modulating the host-tumor relationship [5].

Some reports have outlined the role of cytokines and their receptors in the transformation and proliferation of tumor cells, particularly those of the haemopoietic transformation system [6]. Foss et al. [7] indicated that TNF- $\alpha$  is produced by malignant T cells and a variable proportion of reactive immunoblasts in peripheral T-cell lymphoma of the angioimmunoblastic type. Several studies have found higher plasma TNF- $\beta$  levels in lymphoma patients, where this increase was associated with numerous variables reflecting increased tumor burden. These variables include high serum values of B2-microglobulin and lactate dehydrogenase, advanced disease stage, or large tumor mass [8-9]. Hagenbeek [10] showed that IFN

maintenance treatment in the phase of minimal residual disease of patients with advanced low-grade malignant NHL increased time to progression without remarkable toxicity. MMPs have potential roles in the pathogenesis of multiple myeloma [11].

The present study was undertaken to evaluate a battery of cytokines that could be beneficial in the early diagnosis of NHL and to assess their role in the differentiation between disseminated and nondisseminated NHL (d and nd NHL). Also, the role of the collagenase enzyme MMP-9 was investigated in the same respect.

## MATERIAL AND METHODS

### A- Subjects and Selection of groups:

The present study was conducted on 84 individuals divided into the following groups:

*Group (1):* Control group: This group comprised 10 healthy individuals, 5 males and 5 females. Their ages ranged from 21 to 56 years.

*Group (2):* nd-NHL: This group comprised 60 patients, 36 males and 24 females with ages ranging from 19 to 70 years.

*Group (3):* d-NHL: This group comprised 14 patients including 10 males and 4 females. Their ages ranged from 18 to 70 years.

All patients were inpatients or outpatients of the National Cancer Institute (NCI), Cairo University. The patients were subjected to clinical examination as well as laboratory investigations including full blood picture, bone marrow examination and cytochemical studies, in order to confirm the diagnosis. The division of groups into nondisseminated and disseminated was based on morphologic examination of the peripheral blood smears and bone marrow aspiration samples, to exclude the presence of lymphoblasts and to ensure that lymphocytes are within the normal range in the nondisseminated samples.

### B- Materials and Methods:

Sera of patients were subjected to the following biochemical investigations.

- 1- TNF- $\alpha$
- 2- TNF- $\beta$
- 3- IFN- $\alpha$
- 4- IFN- $\gamma$
- 5- MMP-9.

The kits used for determining the serum levels of TNF- $\alpha$ , IFN- $\alpha$  and IFN- $\gamma$  were purchased from Immunotech (France) and for TNF- $\beta$  from R &

D Systems (USA), while the kit used for MMP-9 was produced by Oncogene Research Products (England).

The serum levels of the mentioned parameters were determined using the ELISA technique according to the manufacturer's recommendations.

### Statistics:

Instat Graphpad Software was used for statistical analysis and data management.

Quantitative data were summarized as mean values  $\pm$  standard deviations. Comparisons between groups were done by using:

- A- One- way ANOVA test which assumes values in the same row are not related and all columns come from populations with equal SDs.
- B- Tukey- Kramer multiple comparison test which compares all pairs of columns. Microcal<sup>TM</sup> Origin<sup>TM</sup> software was used for figures.

## RESULTS

*The present study revealed the following results:*

The concentrations of the parameters tested in the sera of different investigated groups are tabulated in table (1) and graphically illustrated in Fig. (1).

When applying the ANOVA test, high significant difference in TNF- $\alpha$  concentration was observed between the different investigated groups. When the results were analyzed by the Tukey- Kramer multiple comparisons test, there was significant differences between control group and both of d- NHL and nd- NHL groups. On the other hand, there were no significant differences between the two NHL groups.

In case of TNF- $\beta$ , the ANOVA test showed highly significant differences between the different investigated groups. When using the Tukey- Kramer multiple comparisons test, significant differences between control group and d- NHL group could be elicited. On the other hand, no significant differences were observed when the nd- NHL group was compared with the control group, whereas significant differences were observed between nd- NHL and d- NHL groups.

For IFN- $\alpha$ , ANOVA test showed highly significant differences between control group and both of nd- NHL and d- NHL groups. Regarding IFN- $\alpha$ , the Tukey- Kramer multiple comparisons test showed significant differences between control group and either nd- NHL or d- NHL groups. Meanwhile, no significant differences were

observed when d-NHL group was compared to nd-NHL group.

On the other hand, INF- $\gamma$  and MMP-9 showed no significant differences when comparing the

different groups with each other. It is worth mentioning here that there is no correlation between serum level of the parameters investigated and the gender of patients, as illustrated in table (2).

Table (1): The concentrations of investigated parameters in different investigated groups.

Group	No. of Cases	$\alpha$ -TNF* pg/ml	$\beta$ -TNF* pg/ml	$\alpha$ -Interferon* IU/ml	$\gamma$ -Interferon IU/ml	MMP-9 ng/ml
Control						
Mean $\pm$ S.D.	10	29.7 $\pm$ 12.2	31.4 $\pm$ 2.9	8.2 $\pm$ 1.4	0.66 $\pm$ 0.24	87.3 $\pm$ 8.16
Range		(15-52)	(26-38)	(6.1-9.5)	(0.39-1.1)	(77-96)
nd NHL						
Mean $\pm$ S.D.	60	96.3 $\pm$ 72	45.5 $\pm$ 35	6.2 $\pm$ 2.1	0.83 $\pm$ 0.34	103.37 $\pm$ 45.6
Range		(18-395)	(23-200)	(1.1-10.7)	(0.32-1.8)	(17.6-195.8)
p-Value (1)		< 0.05	< 0.05	< 0.05	> 0.05	> 0.05
d NHL						
Mean $\pm$ S.D.	14	130.3 $\pm$ 48.9	145.1 $\pm$ 208.7	5.3 $\pm$ 1.6	0.85 $\pm$ 0.25	73.4 $\pm$ 44.8
Range		(65-235)	(26-650)	(3-8.8)	(0.61-1.44)	(13-180.4)
p-Value (1)		< 0.01	< 0.05	< 0.01	> 0.05	> 0.05
p-Value (2)		> 0.05	< 0.01	> 0.05	> 0.05	> 0.05

\* p Value  $\leq$  0.05 is significant (ANOVA test)

p-Value (1): Groups compared with control (Using Tukey-Kramer Multiple Comparison Test)

p-Value (2): d-NHL compared with nd-NHL (Using Tukey-Kramer Multiple Comparison Test)

nd NHL: Non-disseminated NHL      d NHL: disseminated NHL

Table (2): Concentration of the parameters investigated in relation to gender of patients.

Group	Parameter				
	TNF- $\alpha$ pg/ml	TNF- $\beta$ pg/ml	IFN- $\alpha$ IU/ml	IFN- $\gamma$ IU/ml	MMP-9 ng/ml
Control					
Male	29.6 $\pm$ 12.97 (20-52)	32.6 $\pm$ 3.05 (31-38)	8.26 $\pm$ 1.753 (6.6-10.7)	0.762 $\pm$ 0.3066 (0.48-1.1)	93.19 $\pm$ 3.04 (89.1-96.6)
Female	29.8 $\pm$ 12.98 (15-50)	30.2 $\pm$ 2.49 (26-32)	8.06 $\pm$ 1.11 (6.1-8.8)	0.562 $\pm$ 0.1203 (0.39-0.72)	81.46 $\pm$ 7.39 (77-94.6)
nd-NHL					
Male	87.25 $\pm$ 75.54 (18-395)	46.29 $\pm$ 33.42 (23-200)	6.11 $\pm$ 1.99 (3.4-10.7)	0.9063 $\pm$ 0.4032 (0.32-1.8)	108.86 $\pm$ 45.17 (17.6-195.8)
Female	116.91 $\pm$ 68.582 (47-255)	43.78 $\pm$ 39.52 (26-180)	6.405 $\pm$ 2.45 (1.1-9.6)	0.7184 $\pm$ 0.202 (0.35-1.02)	93.86 $\pm$ 46.12 (24.2-163.9)
d-NHL					
Male	122.75 $\pm$ 50 (65-235)	123.44 $\pm$ 169.85 (26-550)	5.737 $\pm$ 1.82 (3.3-8.8)	0.8075 $\pm$ 0.2054 (0.61-1.19)	80.62 $\pm$ 47.07 (13.2-180.4)
Female	150.66 $\pm$ 48.85 (120-207)	193.75 $\pm$ 304.67 (26-650)	4.55 $\pm$ 0.755 (3.7-5.5)	0.935 $\pm$ 0.3472 (0.65-1.44)	49.3 $\pm$ 31.36 (17.6-80.3)

p-Value > 0.05: There are no significant differences among males and females of all investigated groups according to Tukey-Kramer Multiple Comparison Test.

- Results are expressed as mean values  $\pm$  S.D.

- ( ) :range

nd NHL: Non-disseminated NHL

d NHL: disseminated NHL

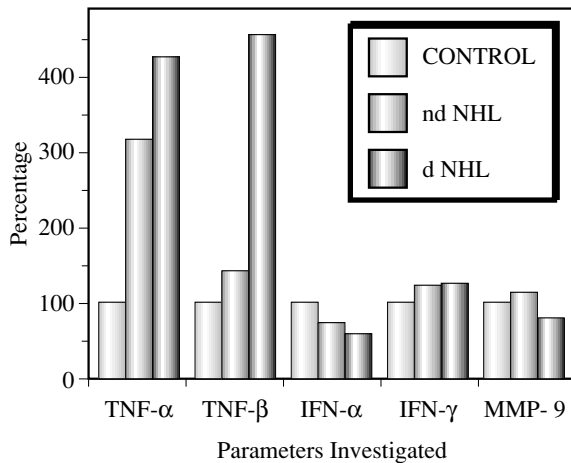


Fig. (1): Values of tested parameters (calculated as percentages taking control values as 100%).

## DISCUSSION

TNF- $\alpha$  is a polypeptide cytokine, produced primarily by mononuclear phagocytes, initiating its multiple effects on cell function by binding to specific, high-affinity cell surface receptors [12]. It has been shown to promote the differentiation of dendritic cells *in vitro* [13], but its excessive production may hamper their ability for effective antigen presentation [14]. Also, it has been demonstrated that increased endogenous TNF- $\alpha$  production by tumor cells could contribute to chemotherapeutic drug resistance [15]. In the present study, a highly significant increase was found in serum levels of TNF- $\alpha$  for both nd-NHL and d-NHL groups compared with the control group. This is in agreement with previous reports [16-18]. It also coincides with the report of Chaperot et al. [19] who detected significant levels of TNF- $\alpha$  in cytotoxic T-Lymphocytes of NHL. These high levels indicate that TNF- $\alpha$  is produced in lymphoma patients either by the malignant cells themselves or the reactive bystander cells, which may be the major source of TNF- $\alpha$  [20]. TNF- $\alpha$  producing cells were observed in all lymph nodes from the angioimmunoblastic lymphadenopathy type T cell lymphoma [21]. It has been shown previously that TNF- $\alpha$  messenger RNA can be found in lymph node sample of patients with various lymphoid malignancies [22]. The present study, however, did not show any significant difference between the levels of TNF- $\alpha$  in serum of d-NHL patients as compared with nd-NHL patients. Also, no significant differences were observed between both genders of patients.

TNF- $\beta$  is a cytokine that is distantly related to TNF- $\alpha$  and is produced mainly by lymphocytes [23]. It acts on target cells via the same binding

sites and is considered to exert a similar spectrum of biologic activities [24]. It exerts antiangiogenic effects on tumor blood vascular endothelial cells, resulting in hemorrhagic necrosis [25]. Our results revealed highly significant elevations in serum levels of TNF- $\beta$  for both nd-NHL and d-NHL groups. These results are confirmed by Verzocha et al. [26], who reported that lymphoma patients presented the rare TNF- $\beta$  allele, which is involved in increased TNF gene transcription, and, thus, higher TNF plasma levels at the time of diagnosis. They added that the presence of at least two TNFs or lymphotoxin (LT) high producer alleles constituted an independent risk factor for first-line treatment failure, shorter progression free survival and overall survival of the patients. Sappino et al. [22] found elevated levels of LT mRNA in the majority of NHL patients. Both TNF- $\alpha$  and TNF- $\beta$  stimulate the growth of malignant B cells [27]. Also, they are polymorphic markers which may be considered as candidates to design new prognostic models for NHL based on biological factors [28]. They may also help in selecting the patients for whom new treatment approaches are recommended, especially those based on immunomodulation [29] or TNF inhibitors [30].

The present study, showed no significant difference concerning the serum TNF- $\beta$  between males and females of all the investigated groups.

IFN- $\alpha$  is produced by monocytes, macrophages and lymphocytes. Different types of virus-activated cells may produce this cytokine. Many other cells can also produce this cytokine, including hemopoietic cells [31]. IFN- $\alpha$  is an antiproliferative molecule for certain tumors, where the inhibition of cell growth and proliferation may result from the induction of the same enzymes that inhibit viral replication and, possibly, others that prevent synthesis of essential amino acids. Inhibition may also result from the inhibitory effect in the production of hematopoietic growth factors that originate in the bone marrow micro-environment [32]. Aviles [33] had shown that interferon should be considered as part of the therapeutic process in patients with low grade lymphomas. However, in another study [34] it was not recommended that IFN- $\alpha$  be used out of prospective trials for treatment of NHL and in particular as maintenance treatment following induction chemotherapy. Some reports have shown that IFN- $\alpha$  dose is probably critical for obtaining a longer survival in patients affected with low risk multiple myeloma [35].

The present study revealed significant decrease in serum levels of IFN- $\alpha$  in both nd-NHL and d-

NHL when compared with the control group. On the other hand, no significant difference was observed between nd-NHL and d-NHL. It also revealed no significant difference between male and female patients.

IFN- $\gamma$  is produced by activated T cells and probably natural killer (NK) cells. It is known to exert pleiotropic effects on monocytes and macrophages [36]. It induces major histocompatibility complex class II and crystallizable fragment receptor expression and has been shown to enhance the antimicrobial, antiviral and antitumor activity of human monocytes. IFN- $\gamma$  exerts therapeutic effectiveness in the treatment of cutaneous T cell lymphoma [37]. Raziuddin et al. [38] reported the immunoregulatory aberration in lymph node derived malignant T cells that produce IFN- $\alpha$  in high levels.

The present study indicated no significant differences in serum levels of IFN- $\gamma$  in NHL patients, whether nd-NHL or d-NHL groups, when compared with the control group. These results are in agreement with Stasi et al. [39] who reported that serum IFN- $\gamma$  levels were not elevated in lymphoma patient. On the other hand, Melichar et al. [40] found that serum IFN- $\gamma$  levels in malignant lymphoma patients had been elevated. Novelli et al. [41] had shown that the combination of chemotherapeutic drugs with IFN- $\gamma$  may provide a more effective way of inhibiting the progression of human malignant T cells through synergistic induction of apoptosis. No significant differences between the concentration of IFN- $\gamma$  in sera of male and female patients could be found in our study.

Many enzymes are capable of proteolytic degradation of extracellular matrix and basement membranes and have been implicated in tumor progression including the matrix metalloproteinase, which is capable of degrading several connective tissue components [42]. Our results showed no significant changes concerning MMP-9 among the three investigated groups. This indicates that this enzyme has no role in the development or the dissemination of NHL [36].

In conclusion, the present results highlight the role of both TNF- $\alpha$  and IFN- $\alpha$  in diagnosis of NHL. They also indicate that TNF- $\beta$  could be used as a marker to differentiate between nd-NHL and d-NHL.

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