Serum sFas in Non-Hodgkin's Lymphoma

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Abstract

Background: Lymphomas are a heterogenous group of malignancies of B or T cells that usually originate in the lymph nodes. They are divided into Hodgkin and non-Hodgkin's lymphomas. Disruption of the physiological balance between cell proliferation and death is a universal feature of all cancers. There has been an increasing interest in the role of apoptosis in tumorogenesis of lymphoma. On the molecular base apoptosis is caused by activation of the caspases through extrinsic and intrinsic pathways. Extrinsic pathway centers on tumor necrosis factor family, where the ligand will bind to the cell surface receptor and this in turn will induce apoptosis. Fas receptor is a member of the TNF/NGF receptor superfamily. Fas family is constituted of the receptor, ligand and soluble form. Soluble Fas will compete with Fas receptor for binding to ligand, thus interfering with Fas-L-mediated apoptosis.

Objective: To measure the level of serum sFas in non-Hodgkin lymphoma (NHL) and to determine the correlations of it with certain clinical and hematological parameters and chemotherapy treatment.

Patients and Methods: This study included 30 patients with NHL (19 males and 11 females), of them 14 patients were newly diagnosed along with 30 apparently healthy controls were involved in this study. The patients were interviewed with history taking, clinical examination and aspirating blood sample for estimation of serum sFas concentration using sandwich ELISA kit from Chemicon. Also serum CRP level, plasma LDH level, hemoglobin concentration, platelets count, WBC count and peripheral blood film were all performed using standard techniques.

Results: This study revealed that the serum sFas concentration was significantly higher in NHL patients than in healthy controls ($p = 0.0001$). This increase was significantly higher in pretreated patients and closely related to the pathological grade of NHL ($p = 0.0002$ and 0.0035 respectively).

Conclusions: Serum sFas is a simple, non-invasive and clinically useful laboratory parameter .It maybe used as an auxiliary marker to assess the prognosis and the therapeutic planning in NHL.

Keywords: Fas, NHL.

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Introduction:

Lymphomas are a heterogenous group of malignancies of B or T cells that usually originate in the lymph nodes, but may originate in any organ of the body, and share the single characteristic of arising as the result of a somatic mutation(s) in a lymphocyte progenitor [1].

Within the broad group of lymphomas, Hodgkin disease (HD) is segregated from all other forms, which constitute the non-Hodgkin lymphomas (NHLs) [2]. Disruption of the physiologic balance between cell proliferation and death is a universal feature of all cancers. The mechanisms and extent of this disruption in lymphoma cells determine the characteristics and evolution of the

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tumor, and this can be achieved through the deregulation of cell proliferation or inhibition of apoptosis [3].

Apoptosis, or programmed cell death, is a genetically regulated process in which the cell is active in producing its own death, a type of cellular suicide for the sake of maintaining homeostasis in the cellular community [4].

Apoptosis is caused by the activation of caspases. Caspases are a family of intracellular cysteine proteases that lie in a latent (zymogen) state in cells but become activated in response to a wide variety of cell death stimuli [5].

Though many pathways for activating caspases may exist, only two have been elucidated in detail; one of these centers on tumor necrosis factor (TNF) family receptors, where the ligand will bind to the cell surface and this will induce apoptosis (Extrinsic). The other involves the participation of mitochondria (Intrinsic), which release caspase-activating proteins into the cytosol, thereby triggering apoptosis [6, 7, 8].

Fas family is constituted of the receptor, ligand and soluble form. Fas (APO-1/CD95) receptor is a member of the TNF/NGF receptor superfamily. It is expressed on a variety of human B and T cell lines, on various normal human tissues and on many different tumor cells [9, 10].

Triggering of Fas receptor by its ligand (expressed in cells that show cytotoxic activity, including activated T-cells and natural killer cells) results in rapid induction of apoptosis by activating the cascade of proteases (caspases) [11, 12].

While the soluble form of Fas (sFas), which is devoid of the transmembrane region, will compete with membrane-associated Fas for binding to ligand, thus interfering with FasL-mediated apoptosis. Serum sFas level is increased in various diseases, including leukemia, lymphoma and SLE [5, 6, 7, 8, 13, 14, 15, 16].

In lymphoma the Fas superfamily can be used as tumor markers in the fields of tumorogenesis, clinical course, treatment and prognosis [7, 9, 10, 11, 12].

Aim of the Study:

To measure the level of serum soluble Fas in non-Hodgkin's lymphoma patients. Furthermore, to determine the relation between serum sFas concentration and certain clinical parameters, hematological markers, stage, classification, prognostic parameters and chemotherapy treatment of non-Hodgkin's lymphoma.

Patients,Control,Methods And aterial:

This study was conducted prospectively on 30 NHL patients, from October 2004 to June 2005, 19 were males and 11 were females, M:F ratio was 1.73:1.

The criteria for inclusion of the patients in this study were the followings:

1. No limitation of age or sex.
2. The patients were randomly selected.
3. The patients were diagnosed, clinically and histopathologically, by a consultant physician and an experienced histopathologist.
4. The patients were either newly diagnosed or already on treatment.

Twenty of these patients were attending the National Center for Hematology; while the rest were attending Al-Khadimiya Teaching Hospital and Baghdad Medical City.

A total of 30 apparently healthy control with an age range of 16-65 year were selectively included in this study. The criteria for inclusion of the control in this study were the followings [9]: They were selected in a way to be age (mean and range) and sex ratio matched for the patient group.

1.They had no fever within 1 week.
2.They are not receiving any medication.
3.They are not known to be pregnant.
4.They did not have a recent history of acute illness or any history of chronic illness.

A sample of venous blood was collected in plain and EDTA tubes.

Serum sFas present in the serum was measured by using sFas ELISA detection kit [from CHEMICON].

Serum CRP was estimated by using semiquantitative technique kit [from BIOKIT]. Plasma LDH was measured by using colorimetric method kit [from RANDOX]. Hemoglobin concentration, platelet count, WBC count, and peripheral blood film were all were performed using standard techniques [17].

The patient is considered anemic if Hb < 13g/dl (PCV < 40%) for males and if Hb < 11.5 g/dl (PCV < 36%) for females [1].

The patient is considered to have thrombocytopenia if Platelet count < $150x10^9$ and to have leucocytosis if WBCs count > $11.0x10^9$ [18].

Results:

Thirty patients with malignant lymphoma, with an age range of 14-67 year and age mean of 46.2 year, were enrolled in this study. 14 of the NHL patients were newly diagnosed. The Descriptive statistics of NHL patients are listed in the following table 1:

Table 1: Descriptive Statistics of NHL patients

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Parameter		NHL patients (n=30)	
		No.	%
B symptoms*	+	12	40
	-	18	60
Number of extranodal sites	0	19	63.33
	1	6	20
	2	5	16.67
Ann Arbor clinical stage	I	0	0
	II	4	16
	III	10	40
	IV	11	44
Anemia	+	24	80
	-	6	20
Platelet count ($x10^9$/l)	< 150	7	23.3
	≥ 150	23	76.7
WBCs count ($x10^9$/l)	> 11	6	20
	≤ 11	24	80
Serum CRP** (mg/l)	0	4	13.33
	0.6	2	6.67
	1.2	5	16.66
	2.4	3	10
	4.8	16	53.34

Plasma LDH* (U/l)**	**Normal**	**5**	**16.66**
	High	**25**	**83.34**
NCIWF** classification for NHL**	**High grade**	**15**	**50**
	Intermediate grade	**8**	**26.7**
	Low grade	**7**	**23.3**
IPI score***	**0**	**0**	**0**
	1	**8**	**26.67**
	2	**9**	**30**
	3	**9**	**30**
	4	**4**	**13.33**
	5	**0**	**0**
Age-adjusted IPI score	**0**	**0**	**0**
	1	**8**	**34.8**
	2	**11**	**47.8**
	3	**4**	**17.4**

* **B** symptoms: fever, night sweat and weight loss
CRP: C-reactive protein
***LDH**: Lactate dehydrogenase
**** **NCIWF**: National Cancer Institute Working Formulation
***** **IPI**: International Prognostic Index

Serum soluble Fas concentration and NHL patients:

The results reported in this study revealed that there was a highly significant difference (p = 0.0001) in serum sFas level between NHL patients mean ± S.E. (4961.9 ± 422pg/ml) with range (1657 to 12430 pg/ml) and normal control mean ± S.E. (1993.8 ± 125.9pg/ml) and range (988–2849 pg/ml) (Table 2).

There was no relation between serum sFas level and sex (p = 0.5338) or age (p = 0.67) of NHL patients.

The level of sFas in pretreated patients was significantly higher than in treated patients (p = 0.0002) (Fig. 1). Also the level of serum soluble Fas concentration was significantly decreased with the number of chemotherapy hours (p<0.0001)(Fig. 2).

There was no relation between serum sFas concentration and the plasma LDH, serum C-reactive protein, the number of extranodal sites, the presence of B symptoms, the Hb concentration, WBC and platelet count, bone marrow involvement, the performance status score, the international prognostic index (IPI) and the age-adjusted IPI score for all NHL patients and for the 14 pretreated patients.

Moreover, there was a highly significant difference between serum sFas level in NHL patients with high and intermediate grade versus those with Low grade lymphoma (p = 0.0035) and although the serum sFas level was higher in the in the high grade compared to the intermediate grade but it did not reach the level of significance (p=0.1786) (Figure 3).

Table2: Concentration of serum sFas in NHL and control groups:

Group	Serum sFas concentration (Range) (pg/ml)	Serum sFas concentration (Mean ± S.E.) (pg/ml)
NHL (n=30)	1657 - 12430	4961.9 ± 422
Control (n=30)	988 - 2849	1993.8 ± 125.9
p-value	0.0001	

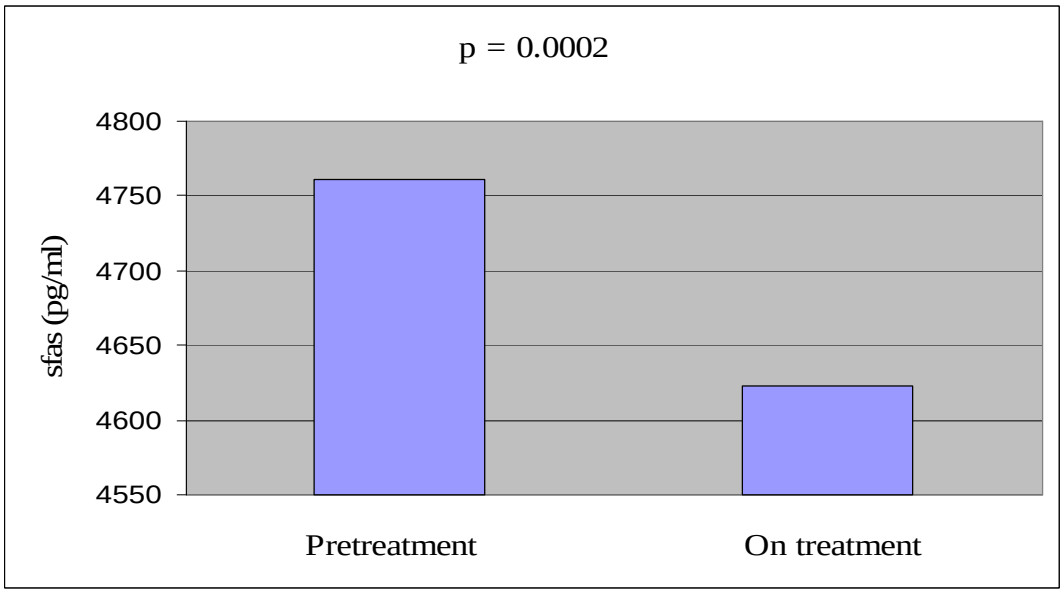

Figure 1: Mean of concentration of serum sFas in pretreatment and on treatment NHL patients.

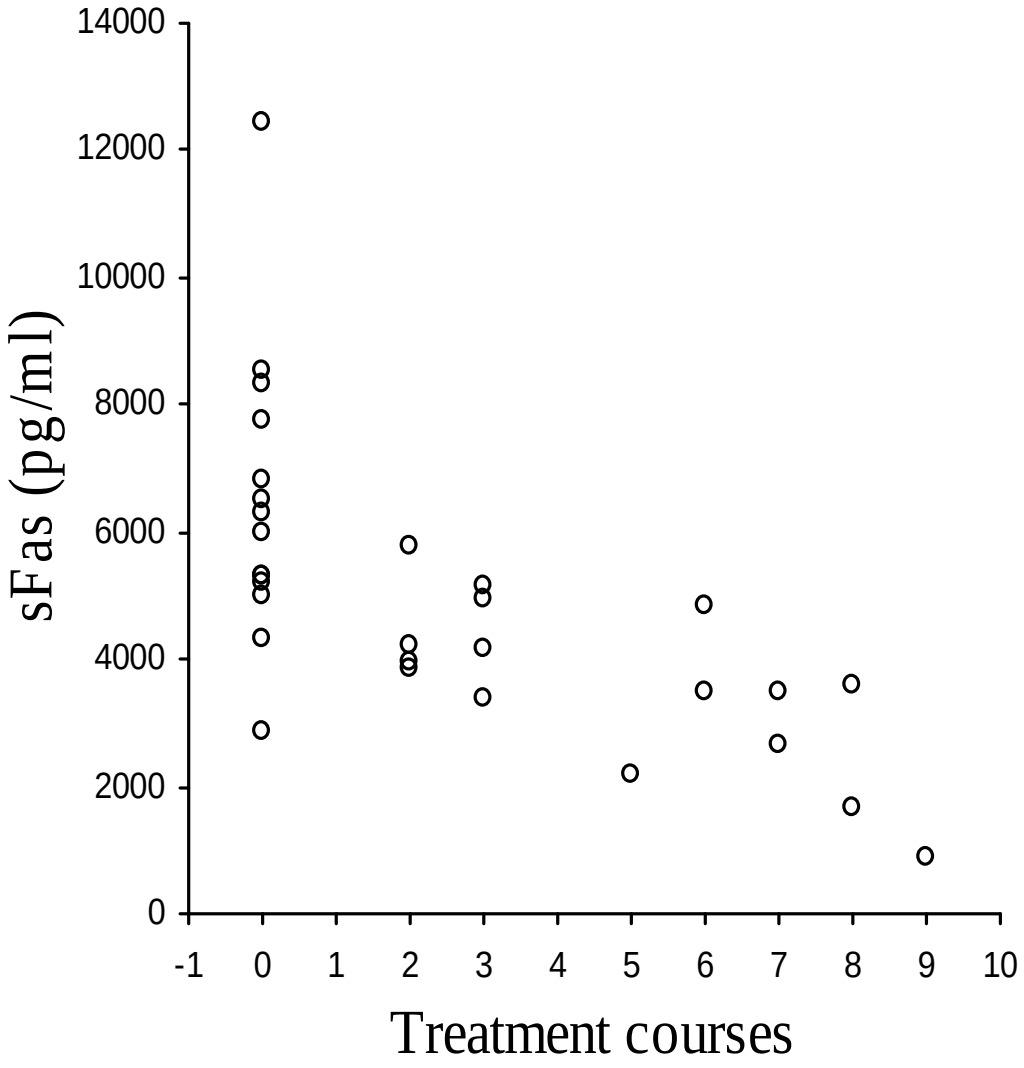

Figure 2: Relation between number of treatment courses and serum sFas level of NHL patients.

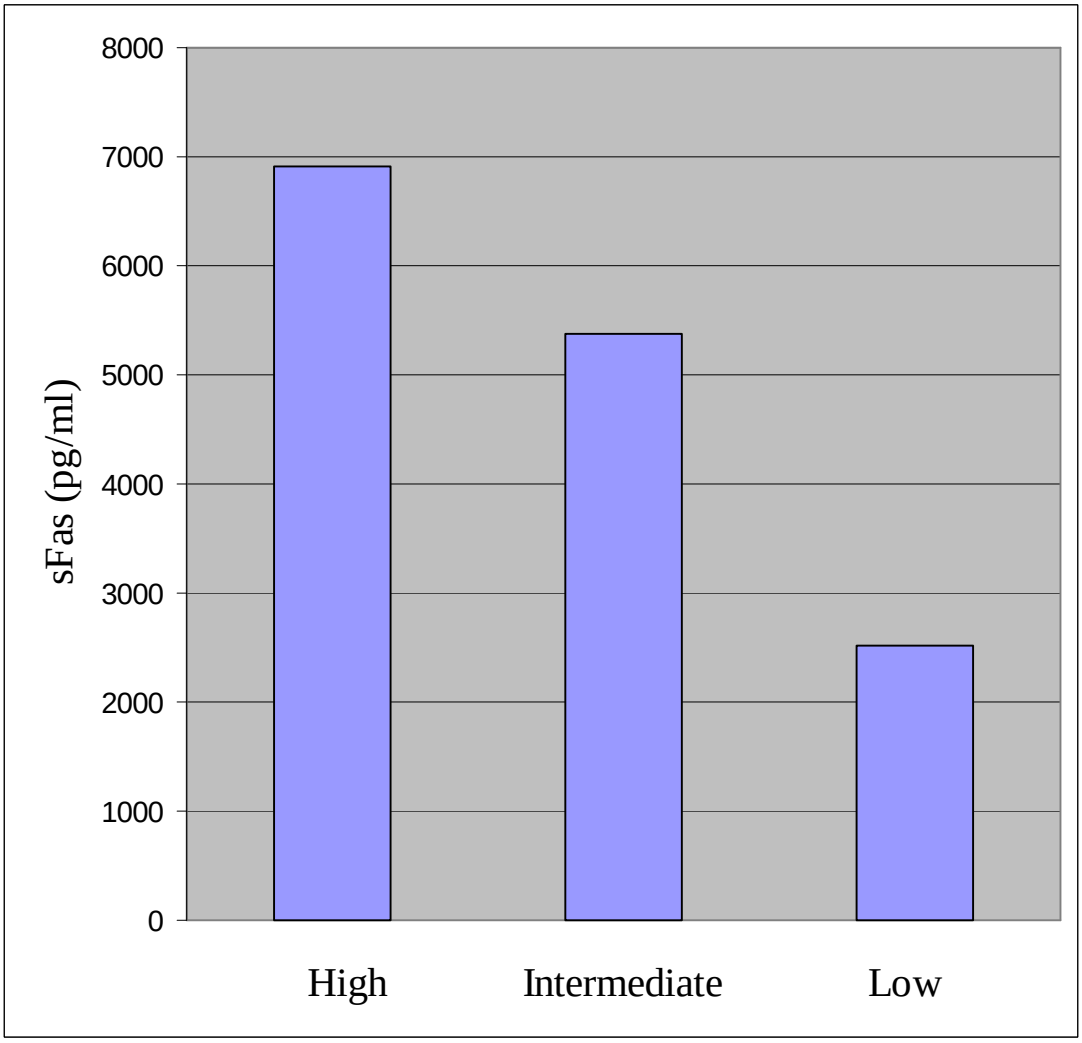

Figure 3: The mean serum concentration of s-Fas in High, Intermediate and low-grade NHL patients.

Discussion:

The present study is a prospective one aiming at evaluating the significance of the apoptotic inhibitor sFas using ELISA technique in patients with non-Hodgkin's lymphoma. The study included the comparison of serum sFas to a number of clinicopathological variables of well-known significance.

The incidence of NHL was higher in males than in females and it was rising with age. These results are similar to those reported by Ministry of health cancer registery [19, 20] and Yasir [21].

However, serum sFas level of NHL patients did not depend on either age or sex, and these findings were in agreement with Niitsu et al [9].

This study demonstrated that the serum sFas level was significantly

higher in NHL patients than in healthy control (Table 2). Similar findings were reported by Sunil et al [7], Niitsu et al [9],Takeshi et al [11], Yufu et al [22], Munker et al [23] and Knipping [24].

The soluble Fas (which is a competitive inhibitor of apoptosis) compete with Fas receptor for binding to Fas ligand, thus interfering with Fas ligand-mediated apoptosis [16,25,26]. Many studies had revealed that the defects in the Fas-mediated apoptotic pathway were associated with lymphoproliferative disorders and particularly lymphoma [27, 28,29], therefore we may proposed that this increase in serum soluble Fas level is part of the disease process and may explain the tumorogenesis of NHL.

The serum sFas level was significantly higher in the more aggressive high and intermediate grade than in the low-grade lymphoma (Figure 3). Similar findings were reported by Sunil et al [7], Niitsu et al [9], Takeshi et al [11], Munker et al [23 and Knipping[29].Morover John CR. and Ian TM. had proposed that the more the aggressive the lymphoma the more the antiapoptotic phenomenon [6, 35], and this may explain the high level of sFas in the more aggressive grade .

This study had revealed that the level of serum soluble Fas concentration was significantly decreased with the number of chemotherapy courses (Figure 3) and it was significantly higher in pretreated patients compared to treated patients (Figure 2) . This was in agreement with Takeshi et al [11] and Niitsu et al(9) who cocluded that serum sFas concentration may be used as a valuable clinical tool and since it is well correlated to the clinical course and to the remission and relapse state therefore it may help in generating a more appropriate treatment plan. Similarly Niitsu et al [9] and Kondo et al [36] had found that NHL patients with a low pretreated serum sFas level had achieved complete remission while those with high serum sFas level were considered resistant to chemotherapy.

In this study there was no relation between B symptoms and serum sFas concentration. This finding was supported by Munker et al [23] and Knipping[24], who concluded that serum sFas level was not related to the clinical symptoms. Also no relation was found between the performance status and serum sFas concentration, which was in agreement with Sunil et al [7].

Moreover, LDH level which is a part of the IPI score that corresponds well with the tumor burden, extent of disease, response to treatment and relapse [32,33], was not related to the serum sFas concentration (p=0.1839). This finding was in agreement with Sunil et al [7], Takeshi et al [11], Munker et al [23] and Knipping et al [24].

Furthermore no relation was found between number of extranodal sites and serum sFas level (p=0.197) which was in agreement with Niitsu et al [9].

Also similar to Takeshi et al[11] study the serum sFas level was not affected by the clinical stage (Ann Arbor staging system).

Since this study had revealed that the serum sFas concentration was not related to the parameters that indicate growth and invasive potential of the tumor, including plasma LDH level, extranodal disease involvement and clinical stage of the tumor, therefore we might propose that sFas was not produced by the tumor cells. This finding is supported by Niitsu et al [9], Takeshi et al [11] and Munker et al [34]. However the cells that produce serum soluble Fas are unclear yet. Takeshi et al [11] had proposed that it may be produced by T lymphocytes, which may reflect a responsive change, or immune activation in lymphoma and

this may protect lymphoma cells from apoptosis [11].

In agreement with Sunil et al [7] and Niitsu et al [9] this study had showed that the IPI score and age-adjusted IPI score were not correlated with the level of serum sFas (p=0.3102 and p=0.5130 respectively).

In this study 32% of NHL patients had bone marrow involvement and this was in agreement with Julie et al [35] and Raad [36], however no relation was found between the bone marrow involvement and serum sFas concentration (p=0.337), which was supported by Sunil et al [7] study.

Also there was no relation between hemoglobin concentration, platelet count or WBCs count and serum sFas concentration (p=0.945 ,p=2456 and p=5457 respectivel) , these findings were in agreement with Takeshi et al [11].

Serum CRP is another simple and valuable prognostic factor for NHL. Its increment is closely related to worsening of clinical course and increase LDH level [37]. In this study there was no relation between CRP and serum sFas concentration (p=0.1456) which was in agreement with Takeshi et al [11], Munker et al [23] and Knipping et al [24].

Conclusions:

1. Since the antiapoptotic serum soluble Fas level was significantly higher in NHL patients, especially those who are pretreated and having the more aggressive disease. Therefore we may concluded that the serum sFas concentration has the prospect to become a clinically useful, simple, noninvasive, time-saving, prognostic and therapeutic planning auxiliary tumor marker test for the more aggressive non-Hodgkin's lymphomas.
2. Serum sFas maybe considered as a complementary independent test to the international prognostic index scores (IPI) for NHL.

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