muSerum soluble Fas in Hodgkin's disease

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Abstract:

Background: Hodgkin disease (HD) is a histologically defined B- cell neoplasm and it includes two distinct types of disease, classical and nodular lymphocyte-predominant Hodgkin's disease. Disruption of the physiological balance between cell proliferation and apoptosis is a universal feature of all cancers. Apoptosis is caused by activation of the caspases through extrinsic and intrinsic pathways. Extrinsic pathway centers on TNF family, where the ligand will bind to the cell surface receptor to induce apoptosis. Fas receptor is a member of the TNF receptor superfamily. Fas family is constituted of the receptor, ligand and soluble form. Soluble Fas will interfere with apoptosis by competing with Fas receptor for binding to legend. The aim of this study is to measure the concentration of serum soluble Fas in Hodgkin's disease patients and to determine the relation between it and certain clinical parameters and serum markers. Also to compare the concentration of serum sFas in HD and Non-Hodgkin's lymphoma (NHL) patients.

Patients & Methods: This study included 15 patients with Hodgkin's disease, 9 males and 6 females. The patients were interviewed for history and clinical examination and blood was collected for measurement of serum sFas concentration using ELISA technique. Hemoglobin (Hb) concentration, leucocytes and platelets counts, peripheral blood smear, C-reactive protein (CRP) and lactate dehydrogenase (LDH) level were all performed using standard techniques.

Results: This study revealed that the serum soluble Fas concentration was almost the same (p-value = 0.991) in both HD patients (1996.9 ± 131) and normal controls (1993 ± 125.9). However, by comparing the results of this study with the high concentration results obtained for serum sFas in newly diagnosed NHL patients (6475.9 ± 617) of another study, it revealed a highly significant difference (p-value < 0.0001) between the two groups.

Conclusions: The serum Fas concentration differs significantly between HD and NHL patients but not with control patients. Further studies including larger number of patients are recommended.

Key words: Hodgkin's lymphoma, soluble Fas

Introduction:

Hodgkin's disease (HD) is a histologically defined B-cell neoplasm. The cell of origin is a germinal centre B cell and the disease is defined by the presence of these characteristic neoplastic cells, Reed–Sternberg cells and Hodgkin's cells or their variants, in a setting of inflammatory cells with or without fibrosis [1].

Hodgkin Disease encompasses two distinct types of disease that differ in etiology, epidemiology, clinical features, pathology and prognosis. They are designated classical HD, which constitutes about 95% of the cases [2], and nodular lymphocyte-predominant HD (NLPHD), which constitutes about 5% of the cases [3].

Classical HD is further subdivided into lymphocyte-rich, mixed cellularity, nodular sclerosis and lymphocyte-depleted subtypes on the basis of the ratio between neoplastic cells and reactive cells, the specific cytological features of the neoplastic cells and the presence or absence of fibrous bands [4].

HD commences in a single lymphocyte usually in a lymph node and spreads initially by lymphatics to contiguous lymph nodes [1].

Disruption of the physiological balance between cell proliferation and apoptosis is a universal feature of all cancers [5]. Apoptosis is induced by activation of the caspases through extrinsic and intrinsic pathways [6].

Extrinsic pathway centers on TNF family, where the ligand will bind to the cell surface receptor to induce apoptosis. Fas receptor is a member of the TNF receptor super-family.

Fas family is constituted of the receptor, ligand and soluble form. Soluble Fas will interfere with apoptosis by competing with Fas receptor for binding to ligand [7].

In HD both the RS cells and the reactive cells express Fas, therefore it may be expected that what inhibits apoptosis here is serum soluble Fas [8].

Aim of the study:

To measure the concentration of serum soluble Fas in Hodgkin's patients and to determine the relation between it and certain clinical parameters and serum markers.

Also to compare the concentration of serum sFas in HD of the current study with NHL patients of another study.

Patients & Methods:

This prospective case-control study included 15 randomly selected newly diagnosed patients with Hodgkin's lymphoma, 9 males and 6 females, from May 2005 to June 2007. These patients were attending the National Center for Hematology and Baghdad Teaching Hospital.

The patients were interviewed for history and clinical examination and blood samples withdrawn for measurement of serum sFas concentration using ELISA kit (from CHEMICON). Hemoglobin concentration, WBCs and platelets counts, peripheral blood smear, serum semi-quantitative CRP and plasma LDH levels were all performed using standard techniques.

Twenty three, age and sex matched healthy control subjects were included in this study, they were 14 males and 9 females with M:F ratio of 1.56:1 and their age mean was 32 years ranging from 6-60 years. The criteria for their inclusion in this study were [9]:

1-They had no fever within 1 week.

2-They are not receiving any medication.

3-Females are not known to be pregnant.

4-They did not have a recent history of acute illness or any history of chronic illness.

The serum sFas concentration results of 14 patients with NHL were collected retrospectively from the study by Subh et al [10].

Serum sFas concentration was measured for the control subjects using the same technique and kit mentioned above.

Statistical analyses were done using Analyze-it version 1.71 and Microsoft Excel 2007 software. The results were expressed as Mean ± SE, with p-value of less than 0.05 was considered significant.

Results:

In this study the Male: Female ratio is 1.5:1 with a mean age of 29.8 year (range of 6-57 years).

The descriptive statistics of HD patients are listed in **table 1.** The concentration of serum sFas in HD patients in comparison with that of healthy subjects and NHL patients is listed in **tables 2 and 3** respectively. No further statistical analysis could be performed between serum sFas concentration and the clinical parameters and serum markers collected in this study because the main parameter of the study, serum soluble Fas, was almost the same in HD patients and healthy control subjects.

Table 1: Descriptive statistics of HD patients:

Parameter			HD patients (n=15)	
			No.	%
Sex		Males	9	60
		Females	6	40
Age group (years)		1-10	2	13.3
		11-20	3	20
		21-30	3	20
		31-40	4	26.7
		41-50	1	6.7
		51-60	2	13.3
B symptoms		+	10	66.7
		-	5	33.3
Extra-nodal site involvement		+	0	0
		_	15	100
Ann Arbor clinical stage		I	0	0
		II	9	60
		III	6	40
		IV	0	0
Anemia *		+	8	53.3
		-	7	46.7
Platelets count (*10^9/l)		< 150	4	26.7
		≥ 150	11	73.3
WBCs count (*10^9/l)		> 11.0	3	20
		≤ 11.0	12	80
Serum CRP (mg/l)		0	5	33.3
		6	1	6.7
		12	2	13.3
		24	3	20
		48	4	26.7
Plasma LDH (U/L)		Normal	11	73.3
		High	4	26.7
WHO classification of HD	Classical HD	Lymphocyte rich	0	0
		Mixed cellularity	10	66.7
		Nodular sclerosis	3	20
		Lymphocyte depleted	2	13.3
	NLPHD		0	0

** Anemia is defined by Hb < 13 g/dl and/or PCV < 40 % for males, and Hb < 12 g/dl and/or PCV < 36 % for females [9].*

Table 2: Concentration of serum sFas in HD patients and healthy control subjects:

Group	Serum sFas concentration (pg/ml)	
	Range	Mean ± S.E.
HD patients (n=15)	1614 - 2954	1996.9 ± 131
Control subjects (n=23)	1217 - 2849	1993.8 ± 125.9
p-value	0.991	

Table 3: Concentration of serum sFas in HD and NHL patients:

Group	Serum sFas concentration (pg/ml)	
	Range	Mean ± S.E.
HD patients (n=15)	1614 - 2954	1996.9 ± 131
NHL patients (n=14) *	3266 - 12430	6475.9 ± 617
p-value	< 0.0001	

* Subh et al [10].

Discussion:

Hodgkin's disease is a rare malignancy; nevertheless, prognosis is good with the majority of patients cured with the available therapy [1].

In HD both the RS cells and the reactive cells express Fas, therefore it may be expected that what inhibits apoptosis here is serum soluble Fas [8].

However, this is not the case, since the death-inducing signaling complex (DISC) proved not only to contain the Fas-associated death domain proteins (FADD) but also the cellular FADD-like IL1B-converting enzyme inhibitory proteins (cFLIP), which is over expressed in HRS cells [11].

The cFLIP exerts a very strong protective effect against apoptosis in HRS cells and this can be demonstrated by suppression of cFLIP which dramatically induce apoptosis in the HRS cell lines. Thus cFLIP is indeed the molecule that neutralizes the effect of activated CD95 in HRS cells and not the sFas [12, 13].

In this study the incidence of HD was higher in males than in females, and this finding is in agreement with published statistics by Iraqi Ministry of health in 2001 [14], and in 1999 [15].

The incidence of HD was not having any particular pattern with age. However, this is actually not typical, and the explanation may be related to the small HD sample size.

In this study there was no significant difference in serum sFas concentration between HD patients and healthy control subjects. This finding agrees with Sunil et al [8].

Finally, since there was no significant difference in the main parameter of this study, serum sFas concentration, between HD patients and controls, then as a result there was no statistical necessity to compare serum sFas with the rest of the parameters taken as all of them will certainly result in non-significant p-value.

Conclusions:

Serum soluble Fas concentration was very much similar in both newly diagnosed HD patients and healthy control subjects, therefore there is no role for serum soluble Fas in the disease pathophysiology and furthermore there is no place to use sFas as a tumor marker for this disease. This concluded negative significance for serum sFas in HD would raise the possibility to use this parameter to differentiate between NHL, in which serum sFas is almost always high in pretreated patients, and HD, in which serum sFas is always normal.

Further studies including larger number of patients are recommended.

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