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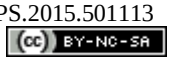
ISSN 2231-3354 

Tumor Necrosis Factor Receptor 2: Its Clinical Significance and Clinicopathologic Correlation in acute leukemias

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ABSTRACT

Objectives: To measure plasma concentrations of Tumor Necrosis Factor Receptor 2 (TNFR2) in patients with acute leukemia, and to correlate the results with clinical features.
Material and Methods: Twenty-four patients with acute myeloid leukemia (AML), sixteen patients with acute lymphoblastic leukemia (ALL) and fifteen control subjects were included in the current study. TNFR2 was measured in plasma of all subjects by enzyme-linked immunosorbent assay.
Results: Plasma TNFR2 significantly increased in ALL compared to both control and AML cases.
Conclusions: TNFR2 may be used as a biomarker for differentiating between AML and ALL patients.

INTRODUCTION

Acute leukemia refers to a group of hematologic malignancies characterized by an increase in the number of myeloid cells or lymphocytes in the bone marrow and an arrest in their maturation, resulting in hemopoietic insufficiency (Ferrara and Schiffer, 2013; Meenaghan *et al*., 2012). There are two main types of acute leukemia: acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML); these are further classified according to cellular maturity (Meenaghan *et al.*, 2012). Tumor necrosis factor α (TNF-α) is a pro-inflammatory cytokine produced mainly by macrophages and T cells after respective stimulation. During cell transformation, TNF-α can be produced by neoplastic cells or cells in the tumor microenvironment and can act as an auto- or paracrine growth factor. TNF-α signals through binding to its receptors, TNF receptor 1 (TNFR1, p55) orTNFR2 (p75) which can lead to proliferation, invasion and metastasis of tumor cells, leading to sustained tumor growth (Warzocha and Salles, 1998 and Aggarwal, 2003) as well as to apoptosis (Aggarwal, 2003). TNFR2 is expressed on certain populations of lymphocytes, including T-regulatory cells (Tregs) (Ware *et al.*, 1991 and Annunziato *et al.*, 2002), endothelial cells, microglia, neuron subtypes (Yang *et al.*, 2002 and McCoy and Tansey, 2008), oligodendrocytes (Arnett *et al.*, 2001 and Dopp *et al.*, 2002), cardiac myocytes (Irwin *et al.*, 1999), thymocytes (Tartaglia *et al.*, 1991 and Grell *et al.*, 1998), islets of Langerhans, and human mesenchymal stem cells (Böcker *et al.*, 2008). Studies have shown that patients with Fanconi anemia, Hodgkin lymphoma and non-Hodgkin lymphoma have abnormally high levels of TNFR2 and that TNFR2 plasma levels represented valuable prognostic markers in those individuals (Tian et al., 2014).

In spite of the pivotal role of TNFR2 in many types of human malignancies, little is known about its role in acute leukemia. In the present study, we measured plasma concentrations of TNFR2 in 40 patients with de novo acute leukemia and correlated the results with clinical features.

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MATERIAL AND METHODS

Patients

The study was carried out on 40 patients with acute leukemia, recruited from the outpatient clinics of the Oncology Centre, Mansoura University (Mansoura, Egypt) in the period between May 2013 and March 2014. All procedures were approved by the Ethical Committee of Faculty of Pharmacy, University of Mansoura. Patients' consent was obtained according to the regulations of the Egyptian Ministry of Health. As a baseline study, all prospective patients were subjected to history taking (personal, menstrual, past and family history) and general and local examinations. Patients included in this study were classified into two groups:

Group I consisted of 24 patients with AML with age ranged between 16-66 years with a median of 50 years. This group included 9 females (37.5%) and 15 males (62.5%). During treatment 15 patients died in this group.

Group II consisted of 16 patients with ALL with age ranged between 15-58 years with a median of 25 years. This group included 4 females (25%) and 12 males (75%). During treatment 8 patients died in this group.

Both groups were treated according to local institutional protocols.

Control subjects

The control group consisted of 15 apparently healthy subjects with age ranged between 27-50 years with a median of 43 years. This group included 9 females (60%) and 6 males (40%). These subjects did not show any abnormality in clinical examination and routine blood tests.

Sample collection

Fasting blood samples were collected from all subjects into ethylene diamine tetra acetic acid (EDTA) containing tubes and subsequently divided into two portions. The first portion was used for blood picture investigation within 5 h. The second portion was then centrifuged at 3000 rpm for 10 min. The isolated plasma was then transferred to a polypropylene tube and if the analysis was not performed immediately, the samples were frozen and maintained at –80 ℃ until thawed and analyzed.

Biochemical analysis

Clear non-haemolysed plasma was investigated for TNFR2 concentration using commercially available ELISA kits provided by MyBioSource Inc (San Diego, CA, USA) in accordance with the manufacturer's protocols.

Statistical analysis

Data were analyzed on a personal computer running SPSS© for windows (Statistical Package for Social Scientists) Release 15. A two-tailed p value of ≤ 0.05 was considered statistically significant. The study parameters violated the normality assumptions so non-parametric tests were used for analytic statistics. For descriptive statistics of qualitative variables, the frequency distribution procedure was run with calculation of the number of cases and percentages.

For descriptive statistics of quantitative variables, the median and range were used to describe central tendency and dispersion. Association between categorical variables was tested by the Chi Square Test; Fisher's exact test was used if the assumptions of chi-square were violated.

The Mann–Whitney U test was used to compare the distributions between two groups. Kruskal-Wallis test was used to compare the distributions between more than 2 groups. Correlations between variables were determined by Kendall's Tau non-parametric correlation coefficient.

RESULTS

Clinicopathologic features and clinical characteristics of patients

The patients' clinicopathologic features and clinical characteristics are shown in Table (1) and Figures (1-5). The study was carried out on 24 consecutive patients with AML, 16 patients with ALL and 15 healthy subjects as a control.

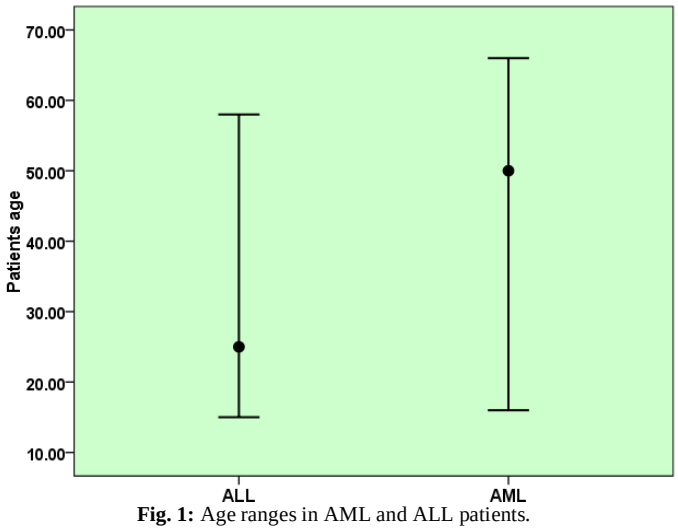

Fig. 1: Age ranges in AML and ALL patients.

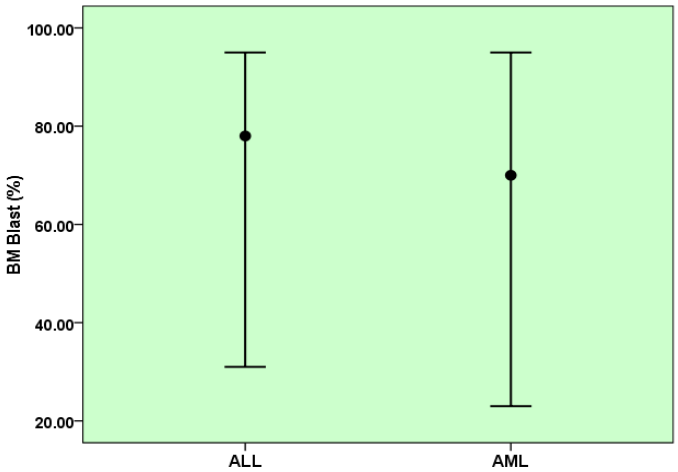

Fig. 2: BM blast % in AML and ALL patients.

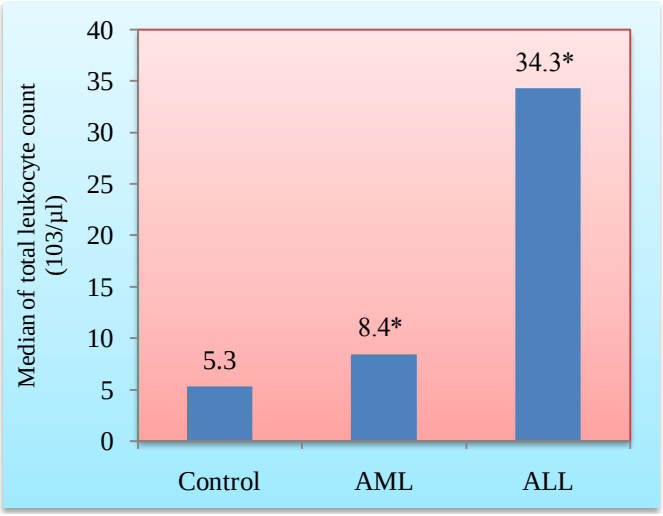

Fig. 3: Median of total leukocyte count (103/µl) in acute leukemia patients (AML and ALL) compared to control group. * Significant difference as compared with the control group at p<0.001.

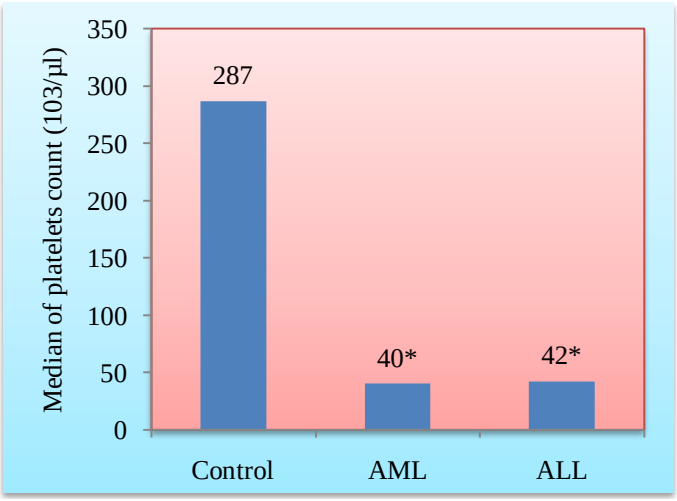

Fig. 4: Median of platelets count (103/µl) in acute leukemia patients (AML and ALL) compared to control group. * Significant difference as compared with the control group at p<0.001.

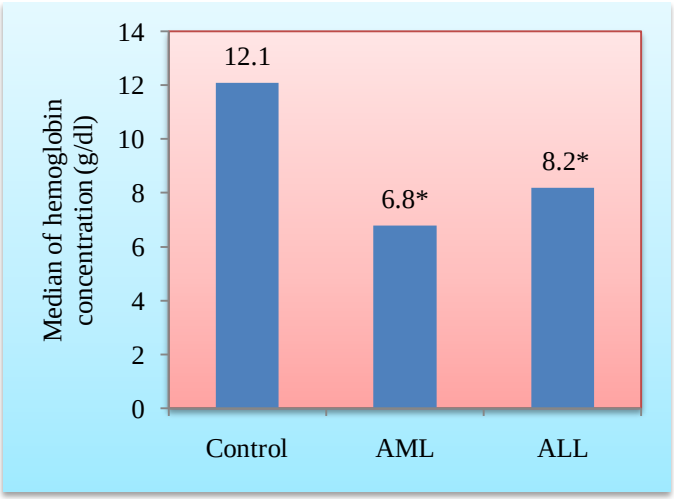

Fig. 5: Median of hemoglobin concentration (g/dl) in acute leukemia patients (AML and ALL) compared to control group. *Significant difference as compared with the control group at p<0.001.

Table 1: Clinicopathologic features and clinical characteristics of studied cases.

		AML		ALL	
		N	%	N	%
Gender	**Male**	**15**	**62.5%**	**12**	**75%**
	Female	**9**	**37.5%**	**4**	**25%**
Bleeding	Absent	19	79.2%	9	56.3%
	Present	5	20.8%	7	43.8%
Lymphadenopathy	Absent	16	66.7%	9	56.3%
	Present	8	33.3%	7	43.8%
Splenomegally	Absent	9	37.5%	5	31.3%
	Present	15	62.5%	11	68.8%
Central Nervous System (CNS) leukemia	Absent	23	95.8%	13	81.3%
	Present	1	4.2%	3	18.8%
Hepatitis B surface antigen (HBsAg)	-ve	22	95.7%	14	93.3%
	+ve	1	4.3%	1	6.7%
Hepatitis C Virus (HCV)	-ve	17	73.9%	11	73.3%
	+ve	6	26.1%	4	26.7%
Bone Marrow (BM) Cellularity	Normo	7	41.2%	0	0%
	Hyper	10	58.8%	10	100%
French-American-British (FAB) classification	M0	3	12.5%		
	M1	5	20.8%		
	M2	1	4.2%		
	M3	1	4.2%		
	M4	8	33.3%		
	M5	2	8.3%		
	M6	3	12.5%		
	M7	1	4.2%		
	L2			8	50%
	L3			3	18.8%
	T-ALL			5	31.3%

Measurement of plasma TNFR2 concentrations

Patients with ALL showed a significant increase in plasma TNFR2 level when compared with the control group as well as the AML patients (p<0.05). While patients with AML showed no significant difference in plasma TNFR2 level when compared to the control group as shown in **Figure (6).**

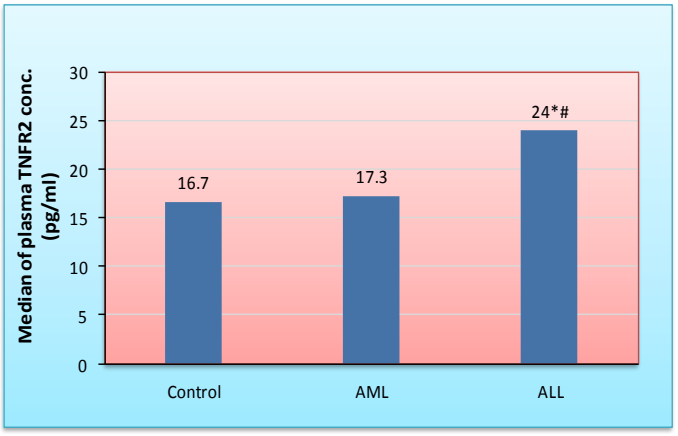

Fig. 6: Median of plasma TNFR2 conc. (pg/ml) in acute leukemia patients (AML and ALL) compared to control group. *Significant difference as compared with the control group at p<0.05. #Significant difference as compared with the AML group at p<0.05.

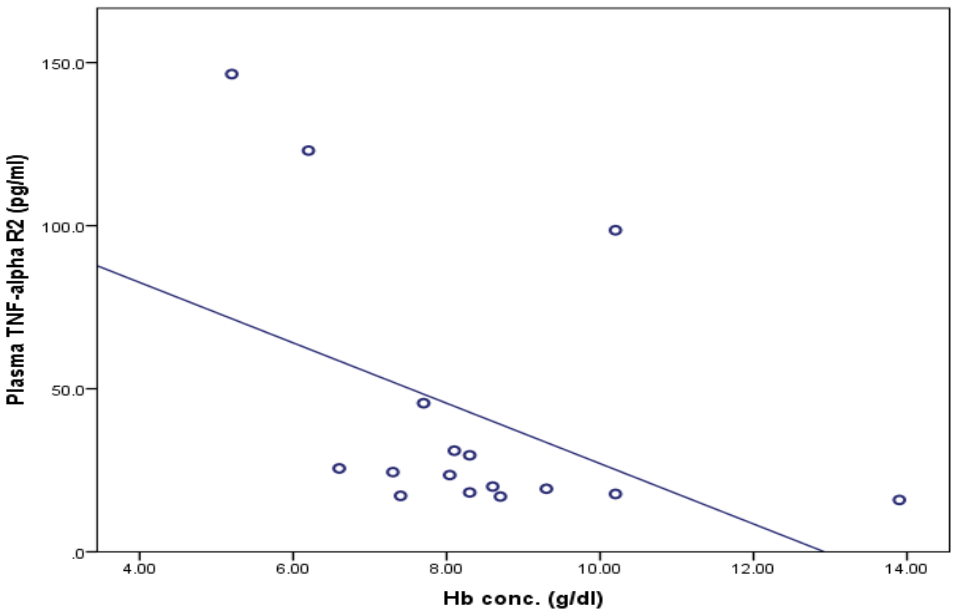

Fig. 7: Significant negative correlation between plasma TNFR2 level (pg/ml) and Hb concentration (g/dl) in ALL patients (r = -0.563, p=0.023).

Correlations between studied parameters in acute leukemia patients

In ALL patients, the plasma TNFR2 level correlated negatively (r = -0.563, p=0.023) with hemoglobin concentration as shown in **Figure (7).**

DISCUSSION

A major unmet need in the field of oncology is the ability of biomarkers to allow for early characterization of tumors according to their aggressive potential. Tumor necrosis factor receptor 2 (TNFR2) is a type I transmembrane glycoprotein and one of the two receptors that orchestrate the complex biological functions of tumor necrosis factor (TNF, also designed TNF-α). TNF-R2 expression is induced upon inflammatory stimulation and is limited to hematopoietic lineage cells (Aggarwal, 2003).

In this study, plasma levels of TNFR2 were found to be significantly higher in ALL patients than in healthy subjects, clearly suggesting its increased production in the course of the disease. This was in consistent with Aguayo *et al.,* (2000). ALL patients showed a significant increase in plasma (NOT plasma and leukocytes) TNFR2 levels when compared to AML patients. Therefore, it is a promising biomarker for ALL.

The balance between TNF-α and its two receptors is tightly regulated. Rising levels of TNF-α induce shedding, especially of TNFR2 from the membrane. This can lead to a desensitization of the cell to TNF-α mediated signaling. In healthy individuals, the shedding is limited to a maximum, which might permit the elimination of excess unbound TNF-α (Aderka *et al.,* 1998). This balance might be deregulated in acute leukemia patients, leading to the observation that elevated circulating levels of TNFR2 are characteristic for acute leukemia. However, it cannot be ruled out that elevated levels of soluble cytokine receptors solely reflect an active immune response against malignant cells and is a sign for a chronic inflammation process not controlled by the host (Aderka *et al.,* 1998).

These observations have stimulated the idea that TNF is an autocrine growth factor for ALL cells and is potentially important in the pathogenesis and expansion of this disease. However, the present study demonstrates that two contradictory principles are present with respect to the effects of TNF.

On one hand, the cells express increased numbers of TNF receptors that facilitate TNF-induced proliferation. On the other hand, the presence of soluble TNF receptors in plasma implicates an endogenous TNF neutralizing activity that counteracts proliferation. There was a significant negative correlation between plasma level of TNFR2 and hemoglobin concentration in ALL patients, reflecting the fact that decreased hemoglobin concentration and increased TNFR2 showed an increase in severity of the disease. Elevation of plasma concentrations of soluble TNFR2 receptor is not unique to ALL patients. Aderka *et al.,* (1998) have reported that patients with cancer in the gastrointestinal tract, breast, lung, and other locations have increased concentrations of TNFR2 receptor. They also demonstrated that patients with advanced stages of the disease had a tendency toward higher receptor concentrations.

CONCLUSION

The results of this study provide a strong evidence that TNFR2 can be used as a biomarker for differentiating between AML and ALL patients.

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