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## Tumor Necrosis Factor in Malignant Tumours and Endotoxaemia

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### Abstract

Tumour necrosis factor (TNF), also called cachectin, has been estimated using Enzyme Amplified Sensitivity Immuno Assay (EASIA) technique in the serum of ten controls (mean 11.3 pg/ml). It was estimated also in 20 malignant patients, ten of them with breast cancer and the other ten with bladder cancer. The preoperative level mean was 28.73 and 38.06 pg/ml and the postoperative one was 29.2 and 40.68 pg/ml respectively. TNF level was found high in the malignant patients in the preoperative and postoperative samples in comparison to the control group and the difference was significant statistically ( $p < 0.05$ ). The difference between the preoperative level and the postoperative one was not significant statistically. TNF was estimated also in 20 patients with sepsis syndrome, ten of them have been subjected to indomethacin treatment and TNF level was estimated before and after its injection. TNF was found higher in septic patients compared to the control group and this increase was statistically highly significant ( $p < 0.005$ ). TNF level decreased after indomethacin injection but this decrease was not significant statistically.

### Introduction

**TUMOR** necrosis factors (TNF) are pleiotropic proteins which have a wide range of biological activities. TNF alpha (also called cachectin) is a product of macrophage and is the principal host mediator of septic shock and the cachexia of chronic diseases. A related molecule, TNF beta or lymphotoxin, is produced by T lymphocytes in response to antigen or mitogens. TNF works alongside other cytokines to stimulate and coordinate immune and in-

flammatory responses to antigenic challenges. It causes haemorrhagic necrosis of solid tumours. However, there are circumstances in which TNF itself can be a source of tissue injury. Recent scientific investigations suggest that TNF plays a prominent role in the toxic and lethal effects of bacterial sepsis and endotoxaemia [1]. Infusion of cachectin in animals led to progressive hypotension, shock and death within 3 hours. Antibodies against TNF have protected animals from lethal doses of endotoxin and *Escherichia coli* [2].

The diversity of actions of TNF appears to be the result of its multiple forms ( $\alpha$  and  $\beta$ ) and its ability to act as membrane associated and free proteins, to act locally as well as systemically, to act synergistically and to be involved in complex webs of self promotion and amplification.

TNF level in serum can be assessed using the radioimmune assay (RIA) or the enzyme linked immunosorbant assay (ELISA) test.

This study was performed to study the level of TNF in serum of patients suffering from malignant tumours and in patients suffering from endotoxic shock and to compare them with normal patients. The affect of indomethacin injection on the level of TNF will be evaluated in patients with endotoxic shock.

#### Material and Methods

The level of TNF was estimated among three groups of patients. The first group included 10 normal healthy adults and they were taken as control group with an age range from 19 to 28 years (average 23.3 Y). The second group consisted of 20 patients harboring malignant tumours, ten of them with highly suspicious breast mass. Nine of the cases were females and their ages ranged between 40 and 70 years with a mean of 52 Y and one patient was a male 60 years old. The other 10 patients had carcinoma of the urinary bladder, all are males and their ages ranged between 40 and 63 years with an average of 53.3 years. Blood samples were taken preoperatively and another one was taken one week to ten days postoperatively.

The third group were 20 patients with suspected endotoxaemia. Their ages ranged from 1.5 years to 60 years with a

mean of 27.3 years. Thirteen of this group (65%) were suffering from major burns ranging from 30% to 80% and 4 patients (20%) were cases of peritonitis following abdominal operations. Two cases had severe degloved cruch injury and one case was mediastinitis. Blood samples were taken from the patients after assessment of the general condition and signs of the sepsis syndrome (at least 2 of the signs of the sepsis syndrome were present). Ten of the 20 patients of this group selected randomly were given 2 mg/kg body weight indomethacin IV bolus, reassessment of the general condition was performed and another blood sample was taken for TNF assay, half an hour after indomethacin injection.

The 80 serum samples taken from the patients were subjected to the estimation of the level of TNF using the EASIA technique (Enzyme Amplified Sensitivity Immuno Assay).

#### Results

In the first group; the control group (N=10), the level of TNF in their blood samples ranged from 4.2 to 15.71 pg/ml with a mean of 11.3 pg/ml. Fig. (1).

In the second group; cancer patients (N=20), TNF level was assayed pre and post operatively. In breast cancer group (N=12), TNF level preoperatively ranged from 24 to 35.4 pg/ml (mean 28.73) and postoperatively it ranged from 25.2 to 38.2 pg/ml (mean 29.1 pg/ml) (Table 1 and Fig. 2). In the bladder cancer group (N=10), TNF level preoperatively ranged from 23.6 to 50.2 pg/ml (mean 38.06) and postoperatively it ranged from 28 to 45.8 pg/ml with a mean of 40.68 pg/ml (Table 2 and Fig. 3).

The third group included 20 patients with septic shock. In the 10 patients that did not receive indomethacin, the level of TNF ranged from 26.4 to 767.5 pg/ml (mean 188.88 pg/ml). In the 10 patients that received indomethacin, the level of

TNF before the drug administration ranged from 21.78 to 2814.7 pg/ml (mean 862.284 pg/ml) and after indomethacin therapy the level ranged from 11.78 to 2823.5 pg/ml with a mean of 821.154 pg/ml (Table 3 and Fig. 4).

Table (1): The Second Group: TNF in Patients with Breast Cancer (N=10).

Pathological type	Stage of disease	No. of patients	Age (mean) (years)	Sex	Mean TNF level preop postop. (pg/ml)	
Infil.	T2 N0 M0	1	45	F	25.4	25.2
duct	T2 N1 M0	4	47.5	F	28	27.8
carcin.	T2 N2 M1	1	60	M	26	30.2
	T4 N1 M1	2	45	F	34.6	29.5
	T4 N1 M1	2	60	F	27.2	32.8
Mean			53		28.73	29.1
Median			52.5		27.95	28.7
S.D.			10.05		3.77	3.2

Table (2): The Second Group: Patients with Bladder Cancer (N=10).

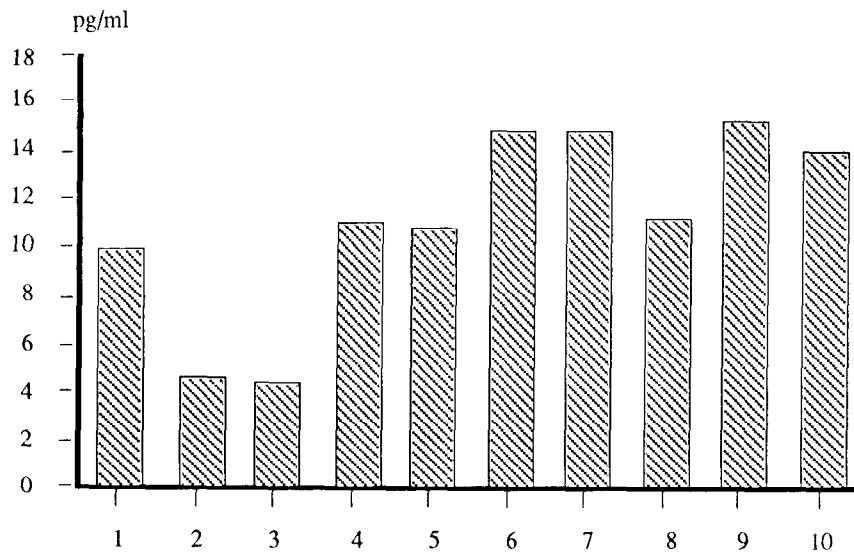
Pathological type	Stage of disease	No. of patients	Age (mean) (years)	Sex	Mean TNF level preop postop. (pg/ml)	
Squamous	3B	9	48	M	38.9	42.1
cell carc.	3A	1	50	M	31.8	28
Mean			48.2		38.06	40.68
Median			47.5		38.8	37.8
S.D.			7.85		7.9	15.0

Table (3): Effect of Indomethacin Injection in Septicaemic Patients (N = 10).

Patient	Age	Sex	Cause	Pulse		B.P.		Temp.		Resp. rate		TMF level	
				Before	After	Before	After	Before	After	Before	After	Before	After
1	26 y	M	Mediastinitis	160/min	110/min.	Not detectable	100/60	36.5	36.5	35/min.	24/min.	2732.35	2317.14
2	8 y	F	Degloved both LL	160/min.	120/min.	70/50	110/70	37.0	37.0	50/min	30/min.	2814.70	2823.50
3	1.5 y	M	20% burn	180/min.	110/min.	100/60	110/70	38.5	38.0	40/min.	38/min.	35.00	27.50
4	28 y	F	85% burn	130/min	110/min.	90/60	110/70	37.0	37.0	39/min.	34/min.	69.28	43.20
5	37 y	M	75% burn	180/min.	110/min.	Not detectable	100/70	37.0	37.0	45/min.	25/min.	50.70	35.70
6	5 y	M	50% burn	150/min.	100/min.	90/60	110/80	37.0	37.0	40/min.	30/min.	21.78	11.78
7	25 y	F	100% burn	130/min.	100/min.	90/60	110/70	39.5	38.0	40/min.	30/min.	68.92	70.00
8	31 y	M	crushed both LL	130/min.	100/min.	100/60	120/80	38.0	37.5	40/min.	30/min.	2779.40	1808.80
9	4 y	M	40% burn	170/min	110/min.	100/60	120/80	38.5	37.5	45/min.	30/min.	28.21	26.42
10	30 y	M	50% burn	140/min	100/min.	90/60	110/70	38.5	38.0	45/min.	30/min.	22.50	47.50

Table (4): Statistical Evaluation of Results of TNF Study.

Variable 1	Variable 2	Significance
Breast cancer (preop.)	Control	Sig. ( $p < 0.05$ )
Breast cancer (postop.)	Control	Sig. ( $p < 0.05$ )
Breast cancer (preop.)	Breast cancer (postop)	Not sig.
Bladder cancer (preop.)	Control	Sig. ( $p < 0.05$ )
Bladder cancer (postop.)	Control	Sig. ( $p < 0.05$ )
Bladder cancer (preop.)	Bladder cancer (postop)	Not sig.
Septicaemic pt. (before indometh.)	Control	Highly sig. ( $p < 0.005$ )
Septicaemic pt. (after indometh.)	Control	Highly sig. ( $p < 0.005$ )
Septicaemic pt. (before indometh.)	Septicaemic pt. (after indometh.)	Not sig.



Mean 11.317 median 11.59 S.D. 4.12  
 Fig. (1): TNF level in the control group.

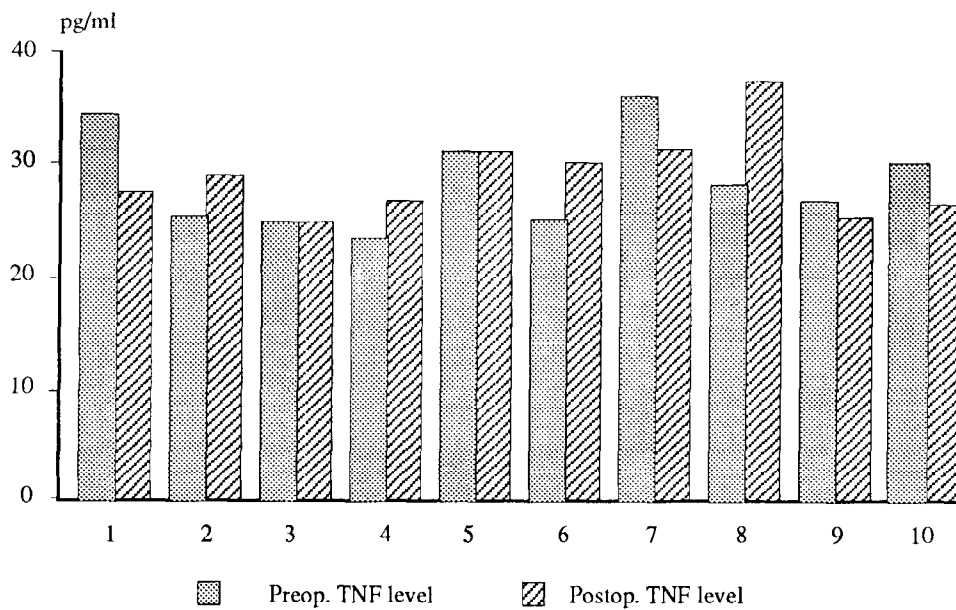


Fig. (2): TNF level in breast cancer patients pre and post operatively.

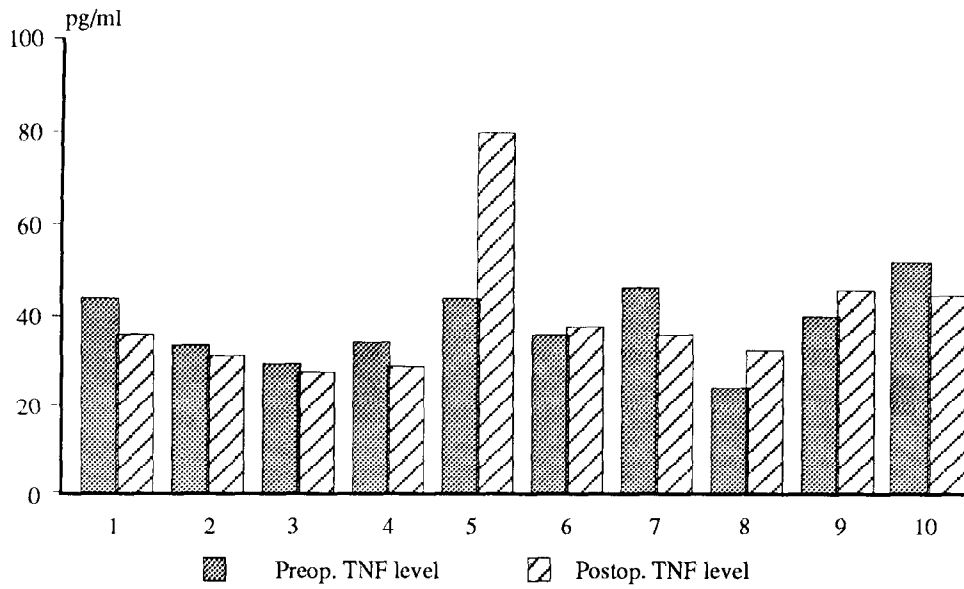


Fig. (3): TNF level in bladder cancer patients pre and post operatively.

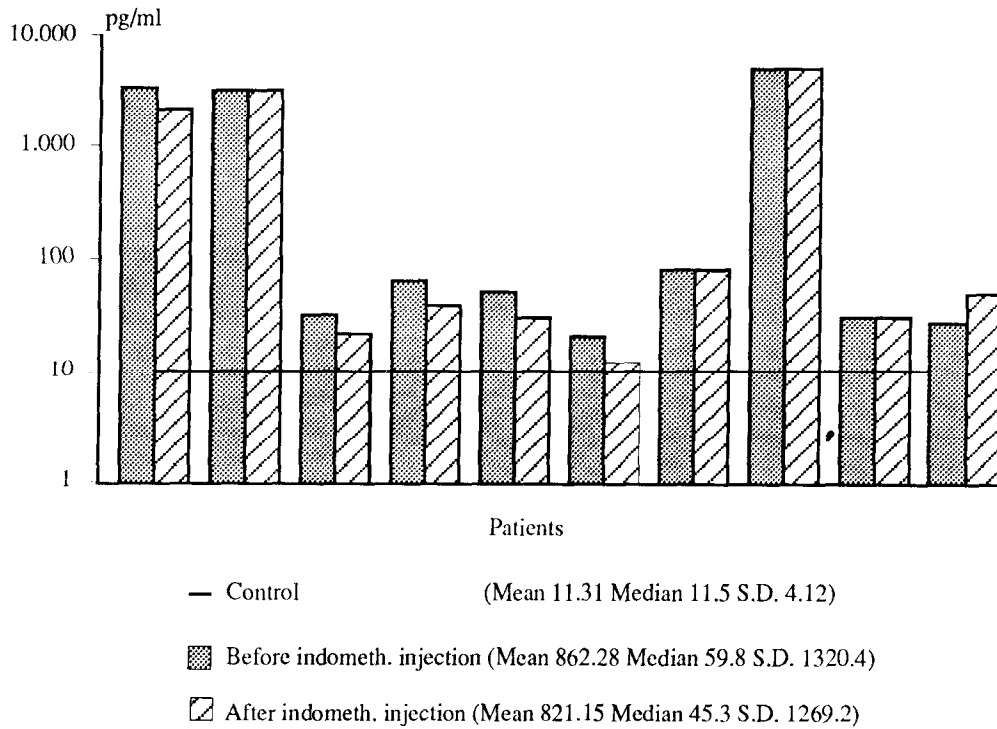


Fig. (4): TNF level in control and septicemic patients before and after indomethacin.

### Discussion

Tumour necrosis factor was first described by Carswell et al. in 1975 [3] as a protein in the serum obtained from animals which had been injected with viable *Bacillus Calmette-Guerin* (BCG) and which, 14 to 21 days later, were injected with endotoxin. This factor, which reached maximal levels 2 hours after endotoxin administration, caused regression of some transplanted tumours *in vivo* and was cytostatic or cytotoxic to some tumour cells in culture.

This work compared the level of TNF estimated in the patients of breast and bladder cancer preoperatively and postoperatively with the control level. The preoperative level of TNF in breast and bladder cancer patients was found to be higher than that of the control group. This difference is statistically significant ( $p < 0.05$ ). These data may suggest that TNF can be used as tumour marker. More studies and higher number of cases are needed to locate the exact time of that elevation and to verify its relation to the type and stage of the tumour.

The postoperative level of TNF was found also to be higher than the control group in both breast and bladder cancer patients. Longer follow up for TNF level in those patients was needed to know if this elevation is related to a residual tumour or a metastatic lesion. Also we want to know if the level reaches to the range of that of the control group later on or not.

The postoperative level of TNF decreased in six patients of bladder cancer and increased in three patients. This increased level might be explained by the postoperative complications as two patients were subjected to wound infection and one case suffered from leakage in the

anastomotic line. Incomplete excision of tumours and the incidence of infections were proved to stimulate TNF production [4]. TNF level pre and post-operatively may be used to assess the radicality of the operative procedure if the post operative sample was taken after a larger duration in the absence of surgical complications. The postoperative level of TNF can be used to predict the prognosis of the cancer patients. More studies and larger group of patients are needed to determine its specificity and to define the value of this postoperative variation either alone or in association with other parameters to evaluate the postoperative follow up.

TNF, a protein produced in large quantities by endotoxin activated macrophages has been implicated as an important mediator of the lethal effect of endotoxin [5]. The level of TNF increased in human volunteers by the injection of *E-coli* endotoxin. The increase was accompanied by fever and tachycardia. Pre-treatment with ibuprofen did not prevent rise in TNF level but the rise was not accompanied by fever or tachycardia [6].

The level of TNF in the ten patients in this study that received indomethacin was found higher than the control group before and after indomethacin. The difference was highly significant ( $p < 0.005$ ). Indomethacin also caused a significant drop of pulse rate, rise of blood pressure, no significant change in temperature and mild decrease in respiratory rate. These changes occurred immediately after indomethacin injection. This improvement in the vital signs was temporary lasting for about 2-1 hours and returned to the pretreatment values. These results are similar to that obtained by Mozses et al. [7]. So indomethacin may have a beneficial effect in treating septicemia but

it has to be scheduled with a plasma level necessary to maintain the vital signs to tide the patients over the critical stage of de-compensated septic shock.

Therefore, TNF level has to be estimated in wider scale in malignant patients with different stages preoperatively as index marker of malignant tumour and after a longer duration postoperatively in the absence of wound infection or other surgical complications as a prognostic factor to assess radicality of resection of these tumours. Indomethacin has to be reassessed and used to maintain the vital signs of septicemic patients without inducing damage to other organs until actual control of infection is attained.

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