Original Article (II)

Blood Cell Types and Cytokine Patterns in Solid Tumour Patients

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ABSTRACT

Introduction: We studied absolute blood cell counts and cytokine patterns in patients with solid tumours and in normal controls.

Methods: 36 patients and 36 normal controls were included in the study. All were above 15 years of age. Cancer group patients had either cytological or histopathological confirmation of malignancy. Routine staging investigations were done. TNM stage I and II were classified as early stage; stage III and IV as advanced stage. Control group subjects were required to have normal clinical examination as well as normal kidney and liver function on laboratory assessment. Persons on steroids, history of atopy or HIV infection were ineligible. Blood cell types were estimated by SYSMEX, SF3000 (KOBE, Japan). Sandwich ELISA method was used to estimate cytokine levels (IL-10 and IL-2). Non-Parametric test, Mann-Whitney test was used to compare the two groups. Univariate Anova was used to correct for gender imbalance between the groups.

Results: Median age was 53.5 years (range 24-87) for cancer group and 47.5 years (range 19-75) for normal group. Smoking and alcohol in take was equally distributed in both groups. In cancer group, 15 patients had early-stage and 21 patients had advanced stage cancer. The cancer group had significantly reduced haemoglobin (P=0.0001), triglycerides (P=0.049), total cholesterol (P=0.0003), Albumin (P=0.0008) and elevated ESR (P=0.001), absolute neutrophil count (P=0.001) and neutrophil/ lymphocyte (N/L) ratio (P=0.009). Plasma IL-10 in cancer group was 3.2 pg/ml, while in normal group it was 2.6 pg/ml (P=0.11). Plasma IL-2 in cancer was 2.6 pg/ml, while in normal group, it was 3.1 pg/ml (P=0.91).

Conclusions: Cancer patients have significantly elevated ANC and N/L ratio. Hemoglobin, cholesterol, albumin and triglycerides were decreased in cancer patients.

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INTRODUCTION

Neutrophils and neutrophil to lymphocyte (N/L) ratio were shown to be elevated in some solid tumours.[1] Further, higher N/L ratio was correlated with survival in the early gastric cancer.[2] Certain cytokine patterns in solid tumours, suggesting altered immune balance were shown. Th1 cytokine pattern correlated with the absence of the cancer disease and Th2 pattern was correlated with the presence of

cancer.[3] In clinical situations such as metastatic renal cell carcinoma and metastatic melanoma, IL-2 (Th1 cytokine) is having a therapeutic effect.[4,5] Similar Th1 and Th2 cytokine patterns correlating with the remission and disease status were demonstrated in various infectious diseases as well.[6-8]

In this study, we studied the absolute levels of blood cell types, plasma levels of IL-2 (for Th1) and IL-10 (for Th2) in solid tumour patients and compared with the normal individuals.

METHODS

36 patients with solid tumours and 36 normal controls were included prospectively from 1st december 2002 to 20th feb 2003. Inclusion criteria were (i) all patients aged 15 years or above (ii) patients with non-hematological malignancies(solid tumours) (iii) patients with performance status PS-I to PS-III (ECOG). Exclusion criteria were (i) patients aged less than 15 years. (ii) patients with haematological malignancies. (iii) patients diagnosed to have prior autoimmune diseases. (iv) patients receiving immunosuppressive drugs. (v) patients who are on steroids by any route, including inhalations, for prolonged period (>2 weeks prior to presentation). (vi) patients with active tuberculosis or other infectious diseases. (vii) patients with HIV infection, irrespective of treatment status. (viii) persons having fever (>98.6° F). (ix) patients with known history of atopy. Additionally, control group subjects were required to have normal liver and kidney functions tests, normal chest-x-ray, normal electrocardiogram, normal urine routine examination, and normal clinical examination.

CELL TYPE AND CYTOKINE ESTIMATION

Fasting sample of blood (7ml) was drawn from each subject and was distributed into two separate EDTA containing tubes in the volumes of 5ml and 2ml.The 2ml anticoagulated blood was subjected immediately for absolute blood cell counts estimation by the SYSMEX, SF-3000(KOBE, JAPAN). The remaining 5ml of anti coagulated blood was centrifuged and plasma was separated from it within one hour of collection, aliquoted and stored at -50^0 C till analysis. This aliquot was used for estimation of albumin, cholesterol, triglycerides, IL-2 and IL-10. Albumin, cholesterol and triglycerides were estimated on Synchron CX9 random Access analyzer (Beckman instruments Inc. California, USA) by standard methods using commercial kits. The cytokines, IL-2 and IL-10, were estimated by using commercial kits from Immunotech, Beckman-Coulter, France. The assay involved Sandwich ELISA, wherein the cytokine in the sample was captured by the specific monoclonal antibody bound to the wells of a micro titer plate followed by the binding of second biotinylated monoclonal antibody. In the second step, addition of the streptavidin – peroxidase followed by chromogen was used to quantitate the assay.

Statistical Methods: Mann-Whitney test was used to compare the two groups and checked with student's t test. Univariate ANOVA was used to know the effect gender imbalance between the two groups. Chi-square test was used to find out the associations between the two categorical variables.

RESULTS

Clinical features: Subject characteristics of both groups are provided in the Table-1. Patients in 'cancer group' were older than the normal group (P= 0.07). The mean weight of the cancer group was lower (P= 0.0001). The gender distribution in the cancer group was 1.1:1 for male: female, while in normal group it was 5:1. Clinical cachexia was evident in 16 (44.4%) of cancer patients, while none had among control group. The groups were similar in smoking and alcohol and other co-morbid conditions. Frequencies of

Table-1: Clinical Characteristics

Parameter	Cancer group n=36	Control group n=36
Median Age (range)	53.5 (24-87)	47.5 (19-75)
Sex, male: female	19:17	30:6
Smokers	16.7%	27.8%
Alcohol Users	16.7%	27.8%
NIDDM	8.3%	2.8%
Advanced Stage of cancer	58.3%	Not applicable
Weight-Kg,	53.5 (12.85)	66.8 (14.3)
Mean (Std.Dev)		
Cachexia	44.4%	none

NIDDM: Non- Insulin dependent diabetes mellitus
Std.Dev: Standard Deviation

various tumour types are provided in the table-2.

Laboratory data: The data on laboratory parameters in both groups is presented in the table-3.As the data was not of Gaussian distribution; the Mann-Whiteny test was used to compare the two groups. The two groups differed significantly in haemoglobin (Hb), erythrocyte sedimentation rate (ESR), absolute neutrophil count (ANC), N/L ratio, serum albumin, cholesterol and triglycerides. The cytokine levels did not show statistically significant difference between the groups, although the IL-2 was higher in the normal group and IL-10 was higher in cancer group.

The effect of gender and group was examined by univariate ANOVA model, keeping the 'group' variable as main factor and 'sex' variable as covariate. It was observed that sex had no effect on the ANC ($F = 3.11 \times 10^{-7}$, $P = 0.9995$), N/L ratio ($F = 1.691$, $P = 0.198$), Cholesterol ($F = 0.672$, $P = 0.415$) and albumin ($F = 0.002$, $P = 0.965$).

Table-2: Tumor site and their frequencies

ORGAN	Frequency (%)
Uterine cervix	7 (19.4)
lung	6 (16.7)
breast	4 (11.1)
liver	4 (11.1)
colo-rectal	3 (8.3)
Primary Unknown	3 (8.3)
Brain	1 (2.8)
Esophagus	1 (2.8)
Choriocarcinoma	1 (2.8)
head&neck	1 (2.8)
ovary	1 (2.8)
pancreas	1 (2.8)
stomach	2 (5.6)
testis	1 (2.8)

In the same model, the group was found to have significant effect on ANC ($F = 9.963$, $P = 0.002$), with estimated marginal difference in mean ANC values between cancer and normal groups was 1994 (95% CI 732, 3255); N/L ratio ($F = 14.28$, $P = 0.0003$), the estimated marginal difference between two groups was 2.188 (95%CI 1.03, 3.34); cholesterol ($F = 16.231$, $P = 0.0001$), the estimated marginal difference was 41.2 (95% 20.8, 61.6); albumin ($F = 15.101$, $P = 0.0002$), the estimated marginal difference was

Table-3: Laboratory Parameters.

Parameter	Group	N	Mean	Std. Deviation	MW TEST	P value
Hb	normal	36	14.14	1.93	232	0.0001
	cancer	36	11.49	2.32		
ANC	normal	35	4595.7	1359.2	305.5	0.01
	cancer	28	5627.8	2012.3		
ALC	normal	35	2057.1	532.6	507.5	0.26
	cancer	28	1859.8	847.8		
AEC	normal	35	408.5	197.4	622.5	0.47
	cancer	28	468.3	221.9		
ABC	normal	35	50.8	23.0	611	0.34
	cancer	28	44.2	20.6		
AMC	normal	35	459.7	167.9	567.5	0.60
	cancer	28	485.4	226.7		
Platelates	normal	36	2.92	0.84	524	0.1625
	cancer	36	3.48	1.64		
ESR	normal	30	11.7	5.1	111.5	0.001
	cancer	30	42.63	20.8		
N / L ratio	normal	35	2.3	0.7	329	0.009
	cancer	28	3.4	2.3		
Albumin	normal	36	3.59	0.36	353	0.0008
	cancer	36	3.1	0.61		
Cholesterol	normal	36	176.23	35.99	313.5	0.0003
	cancer	36	138.11	43.41		
Triglycerides	normal	33	109.3	43.6	454	0.0429
	cancer	32	86.2	39.8		
FBS	normal	33	89.52	20.88	544.5	0.5519
	cancer	36	97.08	34.67		
IL-10	normal	33	2.6	1.3	509.5	0.11
	cancer	32	3.2	1.6		
IL-2	normal	28	3.1	1.5	639	0.96
	cancer	28	2.6	1.1		

Hb: hemoglobin, ANC: Absolute neutrophil count ALC: Absolute lymphocyte count, ABC: Absolute basophil count, AMC: Absolute monocyte count, AEC: Absolute eosinophil count, ESR: Erythrocyte sedimentation rate, FBS: Fasting blood glucose, MW: Mann-Whitney test

0.494 (95% CI 0.24, 0.74).

However, in case of haemoglobin, both sex (F = 6.493, P = 0.013) and group (F = 18.057, P = 0.001.) were found to have an effect.

The N/L ratio was not affected by the cachexia (Chi-square=0.544, P=0.461). Advanced stage were associated with higher N/L ratio (Chi-square =3.797, P=0.034).

DISCUSSION

In this study we found in cancer patients that the absolute neutrophil count, N/L ratio, ESR were elevated, while haemoglobin, albumin, cholesterol and triglycerides levels were decreased. N/L ratio in patients with advanced stage was higher than those with early stage disease. The study groups were well matched in age, smoking and alcohol status, while gender was not matched. However, gender mismatch did not influence the study results.

Although the role played by the neutrophils in carcinogenesis is not clear, various hypotheses have been put forth. It has been suggested that neutrophils may have anti tumour effect.[9] The possible mechanisms of such anti tumour function include the ability to produce oxygen, N/L ratio and Immuno acidic protein (IAP).[1] Additionally, the possibility of inflammation and specific anti tumour immunology being related has been reported.[3] It was shown in a mouse model of non Hodgkins lymphoma that the neutrophils were essential for Rituximab (anti CD-20 monoclonal antibody) function.[11] Subsequently it was shown that the granulocyte stimulating factor in combination with Rituximab resulted in longer duration of response than the rituximab monotherapy in patients with relapsed NHL.[12] Antibody-dependent cell mediated cytotoxicity (ADCC) of neutrophils is believed to be responsible for this. Could this become a predictive factor for rituximab function?

Clinically, neutrophils were shown to be elevated in advanced colorectal carcinoma[1] and N/L ratio was correlated with the survival in early gastric cancer[2]. Further N/L ratio of >2 was associated with poor outcome. In the present study, the cancer group had higher N/L ratio to normal group. Advanced stage cancer patients had higher N/L ratio to early stage disease. The N/L ratio was unaffected by the gender and cachexia.

Many population based prospective studies have demonstrated an inverse association between the cholesterol level and the cancer incidence and mortality.

The mechanisms remain unclear. One study showed that the cancer mortality was 66% higher in the group with the lowest plasma cholesterol than in the group with the highest plasma cholesterol.[13]

Another large study in Japan showed the inverse association of serum cholesterol with total and cancer mortality for men, which remained significant after controlling for age, smoking, alcohol and weight.[14] The inverse relationship between the cholesterol and cancer mortality was demonstrated in Dutch civil servants, after making adjustments for age, BMI and smoking.[15] Further, the inverse cholesterol-cancer relationship in men was present for cholesterol determinations done 6 or more years before the diagnosis of cancer.[16] A case- control study in patients with head and neck cancer and pre-cancerous conditions, showed lower serum cholesterol and triglycerides in cancer patients.[17] In the present study, cancer patients were found to have significantly lower weight, haemoglobin, cholesterol, triglycerides and albumin. All these are possibly indicative of the cancer-cachexia syndrome, as 44.4% of the cancer group had clinical cachexia.

Specific cytokine patterns have been shown to be associated with cancer. The host cytokine profile was shown to be of Th2 pattern in patients with colorectal cancer, while it was Th1 in normal individuals.[3] Soluble IL-2 receptor level was found to be elevated in gastric cancer patients and found to be independent

prognostic factor for stage III and IV disease.[18] serum soluble IL-2 R was increased in advanced breast cancer.[19] All these, are suggestive of impaired cell mediated immunity in patients with active cancer. In the present study, the plasma levels of IL-2 (for Th1) and IL-10 (for Th2) were not significantly different between the cancer and normal groups.

The possible reason for this result is that these two cytokines together do not represent the totality of T cell function and intratumoural levels of the cytokine milieu may be different from that of peripheral blood.

In summary, this study showed significantly higher ANC and N/L ratio in solid tumour patients. N/L ratio correlated with the stage. This will require further validation in organ specific tumours. The role of neutrophil in cancer biology is intriguing and will be focus of further research. The role of cholesterol in cancer biology, other than as a component of cachexia also needs to be studied further.

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