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Role of Interleukin-6 and C-reactive protein in chronic myelogenous leukemia

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Abstract

Introduction: Role of Interleukin-6(IL-6) is well established in hematopoiesis and C-reactive protein (CRP) level is a well recognised marker of inflammation. Elevated serum CRP and IL-6 levels have been detected in a variety of human malignancies which have been correlated with tumor progression, relapse and remission.

Aim: To study the role of Interleukin -6 and CRP in Chronic Myelogenous Leukemia (CML).

Method: The study was conducted to estimate the levels of IL-6 and CRP markers in patients of CML at the time of diagnosis (before any definitive treatment was instituted) and post chemotherapy. Study was conducted over duration of one year at a tertiary care centre and included 48 cases of CML (at the time of diagnosis before receiving any definitive chemotherapy), follow up of the same newly diagnosed patients of CML after three months of receiving chemotherapy (Imatinib/Hydroxyurea) which included 37 cases due to compliance issue, and 30 apparently healthy controls. Haematological parameters were estimated by automated Analyzer MS9-3H. Estimation of serum IL-6 and CRP level was done by sandwich enzyme linked immunosorbent assay method (ELISA).

Result: There was a significant difference ($p<0.05$) between the interleukin-6 and CRP levels of healthy controls and CML patients, as well as between untreated and treated patients of CML ($p<0.05$).

Conclusion: The study reveals that both IL-6 and CRP levels can be useful for monitoring progression of disease and determining efficacy of treatment in patients with CML.

Keywords: Chronic myelogenous leukemia, C-reactive protein,Chemotherapy, ELISA, Interleukin-6

Introduction

Chronic myelogenous leukemia (CML) is the commonest adult leukaemia in India with an annual incidence of 0.8–2.2/100,000 population in males and 0.6–1.6/100,000 population in females.[1] It is a stem cell disorder characterized by the presence of Philadelphia chromosome which produces an oncogenic BCR/ABL fusion protein leading to the proliferation of granulocytic elements at different stages of maturation. In its natural untreated course , CML characteristically has three phases: i) Chronic phase(CP) followed by ii) transformation to an Accelerated phase(AP) and iii) terminal Blast phase.[2,3]

Chronic myelogenous leukemia (CML) is the first leukemia in which a specific abnormality of the karyotype was identified i.e, the Philadelphia (Ph) chromosome. [4] Later on, it was shown that the Ph chromosome is characterized by balanced reciprocal translocation between the long arms of chromosomes 9 and 22, leading to formation of the BCR-ABL fusion oncogene.[5]

It was among the first neoplastic diseases treated with a specific molecular target therapy with Imatinib mesylate (STI571, Imatinib, Glivec or Gleevec). The remarkable therapeutic efficacy of Imatinib led treatment of CML into a new era. Presently the response to chemotherapy is monitored by using various molecular and cytogenetic studies. [6] These tests though are expensive. Therefore is a need for easily accessible and reproducible biomarkers to assess the progression of CML in different phases and for monitoring its response to Imatinib and other drugs in order to choose the best and individualized therapy in a particular patient.C reactive protein (CRP) and Interleukin-6 (IL-6) which are acute phase protein and pro inflammatory cytokine respectively, have been evaluated in different studies as potential biomarkers for various malignancies including leukemias. Elevated serum levels of CRP and IL-6 have been detected in a variety of human cancers and have been found to correlate with tumour progression, relapse and remission.[7] However, only a few studies have investigated the role of CRP and IL-6 in patients with CML . The present study aimed at studying the role of IL-6 and CRP as prognostic biomarkers to assess disease progression, remission and relapse in CML.

We also made an attempt to correlate values of IL-6 and CRP with serum Lactate dehydrogenase (LDH) and Uric Acid (UA) values which are typically increased in most of the malignancies (solid as well as hematological) and are proportionate to tumour burden. [8,9]

Material and methods

The present study was a prospective observational study conducted over a period of one year. The study was approved by the institutional review board and informed consent was taken from all the patients and controls included in the study. The study include 48 newly diagnosed cases of CML(pre chemotherapy), 30 apparently healthy subjects and 37 follow up cases of CML(post chemotherapy) presenting to the Lymphoma Leukemia Laboratory and Hemato-Oncology OPD.

Five ml of venous blood was collected from each subject from antecubital vein out of which 3ml was immediately in Haemochek K_3 EDTA tubes for haematological parameters and 2 ml was collected in serum tubes for analyzing IL-6 and CRP levels. Following collection, blood in the serum tubes was

centrifuged for 20 minutes at 3000 rpm and sera was separated and stored at -80^0 C.

A 3 part cell counter analyzer MS9-3H was used for White Blood Cell (WBC) count, Platelet count and haemoglobin estimation. For differential leucocyte count (DLC) Leishman stained slides were examined under microscope.

For the determination of Interleukin-6 the AviBion Human IL-6 ELISA Kit was used (REF: IL06001) which is based on the principle of sandwich enzyme immunoassay technique. The referential range of assay was 7.8-500 pg/ml.

To estimate CRP values Calbiotech high sensitivity C-Reactive Protein ELISA Kit was used (Calbiotech Inc. USA) which employs solid phase direct sandwich technique. The standard laboratory referential range is 0.2 to 10 mg/L.

We also determined the serum Lactate Dehydrogensae (LDH) levels and uric acid levels in all the patients and controls. Serum LDH levels were determined using the Infinite LDH by Accurex biomedical reagents. Merck semiautomation analyzer was used for reading the values. The expected normal range of LDH using this kit was161-322 IU/L.

Serum Uric acid levels were estimated using the Infinite Uric acid by Accurex biomedical reagents. Merck semiautomation analyzer was used for reading the values. The expected normal range of uric acid using this kit were 3.4-7.0 mg/dl in males and 2.4-5.7mg/dl in females.

Statistical analysis

The baseline characteristics of patients were described using the median, range and 95% confidence interval, and were compared by Student's paired t-Test, chi-square analysis, bivariate analysis, and Pearson's correlation as appropriate. Data analysis was performed using Statistical Package for Social Sciences (SPSS) Version 15.0 statistical Analysis Software with statistical significance defined as P value <.05.

Results

A two-staged research design was adopted in the present study. In the first stage, assessment was done as an observational study by comparing newly diagnosed cases of CML with controls, while the second stage was carried out as an impact assessment following a chemotherapeutic intervention for a period of 3 months amongst diagnosed cases of CML.

Part I of study: Out of the 78 subjects enrolled in first stage of the study, a total of 48 (61.5%) were newly diagnosed cases of CML while remaining 30 (38.5%) subjects were the control group of the study. Irrespective of the group, majority of subjects were males. There were 19 (39.6%) females among CML cases and 9 (30%) amongst controls. This gender difference between the two groups was not statistically significant (p=0.391).

The mean levels of IL-6, CRP, LDH, uric acid, WBC and platelet count were higher in CML cases as compared to controls while the mean haemoglobin level of CML cases was lower as compared to controls. For all the parameters, a significant difference among groups was observed [Table 1].

IL-6 had a strong correlation (r>0.7) with CRP levels, WBC and Hb levels. Except for inverse correlation with Hb, with all the other parameters, the correlation of IL-6 levels was direct. A moderate correlation (r=0.5 to 0.7) was observed with all the other parameters [Table 2].CRP and WBC too showed similar pattern of relationship with other parameters. Haemoglobin had inverse correlation with all the other variables. Platelet count had a moderate correlation with all the variables.

Part II of study: Impact of chemotherapeutic intervention.

A statistically significant change in post treatment levels of all the biomarker and hematological parameters was observed ($p<0.001$). Before treatment, the mean IL-6, CRP, WBC and platelet count was higher in cases of CML as compared to controls. However, all the markers showed a declining trend towards normal range after receiving chemotherapy for three months. [Table 3 and 4]

Even in second part of the study IL-6 levels showed a strong correlation ($r>0.7$) with CRP levels and WBC count. Haemoglobin had an inverse correlation with most of the variables. Platelet count had a moderate correlation with WBC count. [Table 4]

Discussion

Interleukin-6 (IL-6) is a glycoprotein composed of 184 amino acids with a molecular weight of 26 kDa. It is a cytokine with pleiotropic properties and a variety of biological activities such as immune regulation, haematopoiesis, inflammation and oncogenesis. [10]

Interleukin-6 along with Interleukin-3 stimulates the proliferation and differentiation of early progenitor cells of myeloid series like erythrocytic, granulocytic, monocytic and megakaryocytic precursors. [11]

C - reactive protein was first identified in the plasma of patients during the acute phase of pneumococcal pneumonia. CRP has a high affinity for the C-polysaccharide of Streptococcus pneumonia. [12] It is one of the first acute phase proteins to be studied and is found in the serum in cases of infection and inflammation. [13,14]. CRP is a sensitive but not a specific serum biomarker for inflammation and tissue damage, as it is synthesized by hepatocytes as an inflammatory response to infection, trauma, and malignant diseases. [15, 16]

Role of IL-6 and CRP as a marker for prognosis and survival outcomes has been studied in numerous malignancies for example, multiple myeloma, melanoma, lymphoma, ovarian, renal, pancreatic, and gastrointestinal tumours.[17,18] However there have been very few studies focussing on the role of these biomarkers in CML.

This study was therefore undertaken to assess the role of IL-6 and CRP in CML patients, to correlate their values with the course of disease and to assess their role in prognosis and follow up of these patients.

In the present study mean serum IL-6(41.56±14.38 pg/ml), CRP (8.75±4.04mg/L), LDH (1442±622 IU/L) and uric acid (8.60±1.58mg/dl) levels in the study group of untreated CML cases were significantly higher as compared to the levels in normal healthy controls (4.48±1.59pg/ml, 1.27±0.61mg/L,185±25 IU/L, 5.51±2.43mg/dl for IL-6, CRP, LDH and Uric acid respectively) as well as against the average value ($p < 0.001$) [Table 1]. Results obtained in the present study are coherent with previous studies done on patients of CML, which demonstrated higher levels of IL-6 and CRP in newly diagnosed cases of CML. [19, 20, 21,22,23]

The derangement in IL-6 levels in newly diagnosed patients of CML is indicative of its probable role in maintaining the clonal proliferation in leukemic cells. [24,25,26]

In the present study CRP level were found to be elevated in the pre-chemotherapy state which is in agreement with the findings of Fang et al., and Takamura et al., an observation which they attributed to chronic inflammation .[27,28]

In the present study IL-6 had a strong correlation ($r>0.7$) with CRP and WBC count [Table 2]. This finding is in accordance with previous study of Katrrina et al., Praveen et al., and Le Coutre et al., mentioning

about orrelation of IL-6 and CRP with WBC counts . [29,30] However results from the present study are not in agreement with the study conducted by Z.Humlova et al., in which they analyzed correlation amongst the immunological parameters such as IL-6 and CRP in CML patients. According to Mahmud et al., there was a lack of direct correlation between IL-6 and CRP against the known fact that IL-6 is an inducer of CRP. [22,17] In the study of Iwona et al., based on the evaluation of immunological cells in CML patients treated with tyrosine kinase inhibitors no statistically significant difference between the concentration of IL-6 in untreated CML patients and in the healthy controls was noted which is again discordant with the results of present study. However in their study they found a correlation between serum IL-6 levels and peripheral blood WBC count(r=0.492; p=0.04) which was also noted in the present study. [31]

Pre chemotherapy values of serum LDH and uric acid were significantly raised in untreated cases of CML (1442±622IU/L and 8.6±1.58mg/dl respectively) as compared to controls. This was in agreement with other studies done on serum LDH and uric acid in hematological malignancies. [32,8,9]

A statistically significant change in post chemotherapy treatment levels of all the biomarker and hematological parameters was observed in the present study ($p<0.001$) [Table 3].

The post chemotherapy levels of parameters were 7.86±8.92pg/ml for IL-6, 1.77±2.11mg/L for CRP, 184±46.5IU/L for LDH and 3.99±1.35mg/dl uric acid which were close to normal healthy controls value [Table 4]. These findings are in agreement with the study of Singer MK et al., who worked on the validity of IL-6 and CRP as prognostic markers in CML patients. [19]

The significant reduction in IL-6 levels post chemotherapy explains its role in cell cycle which is to inhibit p53-induced apoptosis and suppression of phosphorylation of the retinoblastoma protein. Declining IL-6 levels indicates a favourable course of the disease. [33] Hence, the findings of present study are in accordance with the published literature, which emphasise that the IL-6 level parallels the progression of the disease. [34] The reduction in serum values of biochemical markers like LDH and uric acid also depicts the decrease in leukemic cell burden. [35]

A more consistent follow up investigation and in vitro treatment of leukemic cells with chemotherapeutic agents might help in exploring the therapeutic implications of IL-6.Further studies on expression of IL-6 are required to elucidate the mechanisms involved in myeloid cell leukemias, in order to identify potential targets for the treatment of myeloid neoplasms and to evaluate the chemotherapeutic agents modulating IL-6 and CRP paracrine loop pathway to explore its therapeutic potential.

Conclusion

In the present study we found that serum IL-6 and CRP levels are significantly high in newly diagnosed cases of CML, as compared to normal healthy subjects and these levels rapidly decline after chemotherapy, in comparison to the normal subjects. This supports the possible role of IL-6 in leukemogenesis in myeloid cells and highlights the role of IL-6 and CRP in tumour progression.

However larger focussed studies are needed to provide further concrete evidence for the utility of these serological biomarkers in monitoring the progression and remission of disease in patients with CML.

Table 1: Comparison of biomarkers and hematological parameters of controls with cases before chemotherapy

SN	Parameter	Cases (n=48)		Controls (n=30)		Significance of difference	
		Mean	SD	Mean	SD	"t"	"p"
1.	IL-6 (pg/ml)	41.56	14.38	4.48	1.59	14.032	<0.001
2.	CRP (mg/L)	8.75	4.04	1.27	0.61	10.047	<0.001
3.	LDH (IU/L)	1442	622	185	25	11.037	<0.001
4.	Uric Acid (mg/dl)	8.60	1.58	5.51	2.43	6.816	<0.001
5.	WBC (10^5/µl)	1.24	0.53	0.59	0.01	12.029	<0.001
6.	Hb (gm/dl)	9.37	1.30	14.29	1.48	-15.414	<0.001
7.	Platelet count (10^5/µl)	5.13	0.84	2.67	0.49	14.466	<0.001

Table 2: Interrelationship of different biomarkers and hematological parameters (Pearson Correlation coefficient) (n=78)

	IL-6	CRP	LDH	UA	WBC	Hb	Platelet
IL-6	1	0.906(**)	0.681(**)	0.559(**)	0.871(**)	-0.820(**)	0.695(**)
CRP		1	0.603(**)	0.520(**))	0.853(**)	-0.725(**)	0.608(**)
LDH			1	0.720(**)	0.590(**)	-0.678(**)	0.806(**)
UA				1	0.508(**)	-0.506(**)	0.581(**)
WBC					1	-0.778(**)	0.635(**)
Hb						1	-0.714(**)
PLT							1

Table 3: Evaluation of change in different biomarker and hematological parameters in case group patients post chemotherapy

SN	Parameter	Before intervention		After intervention		Change		Significance of difference	
		Mean	SD	Mean	SD			"t"	"p"
1.	IL-6 (pg/ml) (n=37)	39.94	13.85	7.86	8.92	32.08	14.25	13.69	<0.001
2.	CRP (mg/L) (n=37)	8.13	4.11	1.77	2.11	6.37	3.54	10.79	<0.001
3.	LDH (IU/L) (n=37)	1434	619	184	46.5	1250	586	12.97	<0.001
4.	Uric acid (mg/dl) (n=37)	8.66	1.59	3.99	1.35	4.67	1.34	21.17	<0.001
5.	WBC (10^5/µl) (n=37)	1.19	0.50	0.07	0.05	1.12	0.50	13.37	<0.001
6.	Hb (gm/dl) (n=37)	9.62	1.13	11.69	1.39	-2.07	1.21	10.30	<0.001
7.	Platelet count (n=37) (10^5/µl)	5.17	0.90	2.68	1.44	2.49	1.64	9.11	<0.001

Table 4: Comparison of biomarkers and other hematological parameters of controls with cases after chemotherapy.

SN	Parameter	Cases (n=37)		Controls (n=30)		Significance of difference	
		Mean	SD	Mean	SD	"t"	"p"
1.	IL-6 (pg/ml)	7.86	8.92	4.48	1.59	2.051	0.044
2.	CRP (mg/L)	1.77	2.11	1.27	0.61	1.255	0.214
3.	LDH (IU/L)	184	46.5	190	30	-0.090	0.928
4.	Uric acid (mg/dl)	3.99	1.35	5.51	2.43	-3.250	0.002
5.	WBC (10^5/µl)	0.07	0.05	0.06	0.01	2.149	0.035
6.	Hb (gm/dl)	11.69	1.39	`14.29	1.48	-7.323	<0.001
7.	Platelet count (10^5/µl)	2.68	1.44	2.67	0.49	0.015	0.988

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