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To evaluate serum Tryptase levels in patients of acute myeloid leukemia before and after chemotherapy.

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Abstract

Tryptase is serine protease, primarily expressed in mast cells and less abundantly in blood basophils. It was found recently that myeloblasts in patients with acute myeloid leukemia produce significant amount of tryptase. The study was planned to evaluate the status of Serum tryptase levels in patients of AML before and after chemotherapy. Twenty newly diagnosed cases of AML & 20 age and sex matched healthy controls were taken. AML patients were treated with combination chemotherapy of cytarabine and daounorubicine. Routine biochemistry, complete hemogram, serum Tryptase levels were performed in newly diagnosed patients before treatment and in controls. The investigations were repeated at first complete remission or after 5 weeks of chemotherapy (whichever is earlier). Tryptase was measured by ELISA. Fifteen Patients achieved remission after induction chemotherapy while five patients failed to achieve remission. Baseline Tryptase levels were significantly raised in AML patients (Tryptase 29.55 ± 8.31 ng/mL vs 3.52 ± 0.47 ng/mL $p<0.001$). Tryptase levels at diagnosis were significantly higher in patients who failed to achieve remission than who achieved remission ($p<0.005$). Serum tryptase levels were positively correlated with age and blast cells and correlation was significant ($p=0.001$, $p=0.001$) in cases (before chemotherapy). Serum Tryptase levels have prognostic value in AML patients. Studies are required in larger number of patients.

Keywords: AML, Tryptase, Chemotherapy

Introduction

Leukemia is a malignant neoplasm of hematopoietic stem cells, characterized by diffuse replacement of marrow by neoplastic cells. It is one of the leading cause of death due to malignancy.[1] They are classified

as acute and chronic depending upon the course of disease. The acute leukemia is characterized by rapid increase of immature blood cells (blast cells) as a result of failure of bone marrow to produce normal cellular elements of blood. It results in anemia, hemorrhagic state due to thrombocytopenia and infections due to neutropenia.[2] Acute leukemias are futher classified into acute lymphoblastic leukemia (ALL) mostly in children and acute myeloid leukemia (AML) which occurs mostly in adults.[3]

AML is characterized by a high degree of heterogeneity with respect to chromosomal abnormalities, gene mutations and changes in expression of multiple genes and micro RNAs. Cytogenetic abnormalities can be detected in approximately 50 to 60 percent of newly diagnosed AML patients.[4]

Tryptase is a serine protease, tetrameric structure of 134 kDa, primarily produced and stored in mast cells (MCs) and less abundantly in basophils.[5] The gene encoding mast cell tryptase is present on chromosome 16.[6] The enzyme is made up of four non-covalently bonded subunits. There are two main types of tryptase in mast cell α-tryptase and β-tryptase. α-tryptase are classified in to α1 and α2 tryptase and β-tryptase in to β1, β2, β3.[7,8] β2 Tryptase is stored in the secretory granules of mast cells. α-tryptase is secreted constitutively from mast cells as an pro enzyme and is the major form found in the normal healthy adults.[9]

It has been recently shown that in AML patients, serum tryptase levels correlate with poor prognostic signs before chemotherapy and return to normal or near normal values in patients achieving a hematologic clinical remission after induction chemotherapy.[10] Thus this study was designed to study serum tryptase levels in AML patients before and after chemotherapy.

Materials and Method

The present study was conducted in the Department of Biochemistry, P.G.I.M.S., Rohtak in collaboration with the Clinical Hematology Unit of the Department of Medicine. Twenty newly diagnosed patients of AML and twenty age and sex matched healthy controls were included. History, general physical examination, total and differential leukocyte count, bone marrow examination and cytogenetic studies facilitated in making diagnosis.

Chemotherapy consisting of cytarabine and daunorubicin (the standard 3 plus 7 regimen) is given intravenously to all the patients of AML. In this regime in induction phase daunorubicin (60 mg/m^2 body surface area/day) by I.V infusion in 30 minutes for 3 days is given and cytarabine (100 -200 mg/m^2 body surface area/day) for 7 days is given. Patient also receives supportive treatment in the form of blood, blood products, antibacterial and antifungal agent as and when required. After remission is achieved, patients receive consolidation chemotherapy in the form of high dose of cytarabine (HiDAC) that is 3g/m^2 body surface area two times per day on days 1, 3, 5 for a minimum of 2 cycles (average 1-4).[11]

Routine biochemistry parameters, complete hemogram and serum tryptase levels were analysed in healthy controls, newly diagnosed patients and the investigations were repeated either after first complete remission or after 5 weeks of chemotherapy, whichever was earlier. The results were compared between cases and controls along with before and after chemotherapy in cases. In healthy controls routine biochemistry, hemogram and serum tryptase levels were performed. Voluntary consent was taken from all subjects enrolled and ethical clearance was taken from institutional ethical committee.

Sample collection

Venous blood was withdrawn from each subject using aseptic precautions in purple EDTA vacutainer for haematological investigation (analysed same day) and 6ml blood in red plain vacutainer from which serum was separated and divided in to two parts. In one part biochemical investigation was done on the same day and the other part was stored at -20 ^{0} C for analysis of serum tryptase. Serum tryptase levels were repeated after 5th week or at remission whichever was earlier.

Estimation of serum tryptase

Serum tryptase was assayed by Enzyme Linked Immunosorbent Assay (ELISA).[12] In more than 99% of healthy individuals the serum tryptase level is below or equal to 15 ng/ml. In addition tryptase levels remain remarkably stable during viral or bacterial infection or other inflammatory disease processes.[13,14]

Statistical analysis

IBM SPSS ver. 20 was used for various statistical analysis. Student t test was applied to the data confirming to normal distribution. Correlation coefficient (r) was used to determine the relationships between different quantitative values. Statistical analysis was expressed by mean± standard deviation. For all tests a probability <0.05 was considered significant.

Results

The patients were divided as under:

Group I: (n=20) - Healthy age and sex matched controls.

Group IIa: (n=20) – AML Patients before chemotherapy.

Group IIb: (n=20) – AML Patients after chemotherapy.

Results are summarized I table 1 and 2. All twenty patients presented with pallor and body ache, while 16 presented with organomegaly and 5 patients presented with icterus. Out of total twenty AML patients 15 patients (75%) achieved remission after induction chemotherapy while 5 patients (25%) failed to achieve remission. The mean serum tryptase level in remission group was 25.9±4.87ng/mL and 21.2±7.14ng/mL in AML patients before and after chemo therapy respectively and found to be statistically significant ($p<0.05$, figure 2). The mean serum tryptase level in treatment failure patients was 40.4±6.98 ng/mL and 49 ± 7.38 ng/mL before and after chemo therapy respectively and found to be statistically feebly significant ($p<0.1$, figure 2). The mean serum tryptase level was 21.2±7.14 ng/mL and 49 ± 7.38 ng/mL in AML patients who achieved remission and who did not respond to treatment respectively and was found to be statistically extremely significant($p<0.0001$, figure 2). Serum tryptase levels were positively correlated with age ($r= 0.651$, $p= 0.001$, figure 3) and blast cells ($r=0.663$, $p=0.001$, figure 4) and correlation was significant in cases (before chemotherapy). Raised serum tryptase levels found in AML patients decrease after chemotherapy though higher levels than controls were detected.

Table 1 **:** Comparison of demographics, hemogram and tryptase levels in group I and II (a & b)

Parameters		Group I (n=20)	Group IIa (n=20)	Group IIb (n=20)	P value between Group I and Group IIa	P value between Group IIa and Group IIb
Age		44.40± 16.93	36.75 ± 13.62		NS	
Gender		12 M 8 F	12 M 8 F		NS	
Hemoglobin (g/dL)	Mean ±SD	13.3± 0.92	7.6± 1.75	8.8±1.61	<0.001	0.297
	Median (IQR)	13.2 (5.2-11.2)	8.25	8.35		
Total Leucocyte count(TLC) (per µL)	Mean ±SD	13575± 9591.7	31100±12760	12070±8212	<0.001	<0.001
	Median (IQR)	7450	45000	9000		
Platelets (per µL)	Mean ±SD	351500 ± 46822.3	44450±19429	89950±39757	<0.001	<0.001
	Median (IQR)	35000	39000	57500		
Tryptase (ng/mL)	Mean ± SD	3.52 ± 0.47	29.55 ± 8.31	28.2 ± 14.18	<0.001	0.710
	Median	4.2 (3.6- 8.1)	28.0 (20.0- 49.0)	21.5 (16.5- 57.0)		

Table 2: Comparison of tryptase levels before and after chemotherapy

Tryptase (ng/mL)	Before chemotherapy (n=20)	After chemotherapy (n=20)	p value
Remission group (n=15)	25.9±4.87	21.2±7.14	0.045
Non Remission group (n=5)	40.4 ± 6.98	49 ± 7.38	0.095

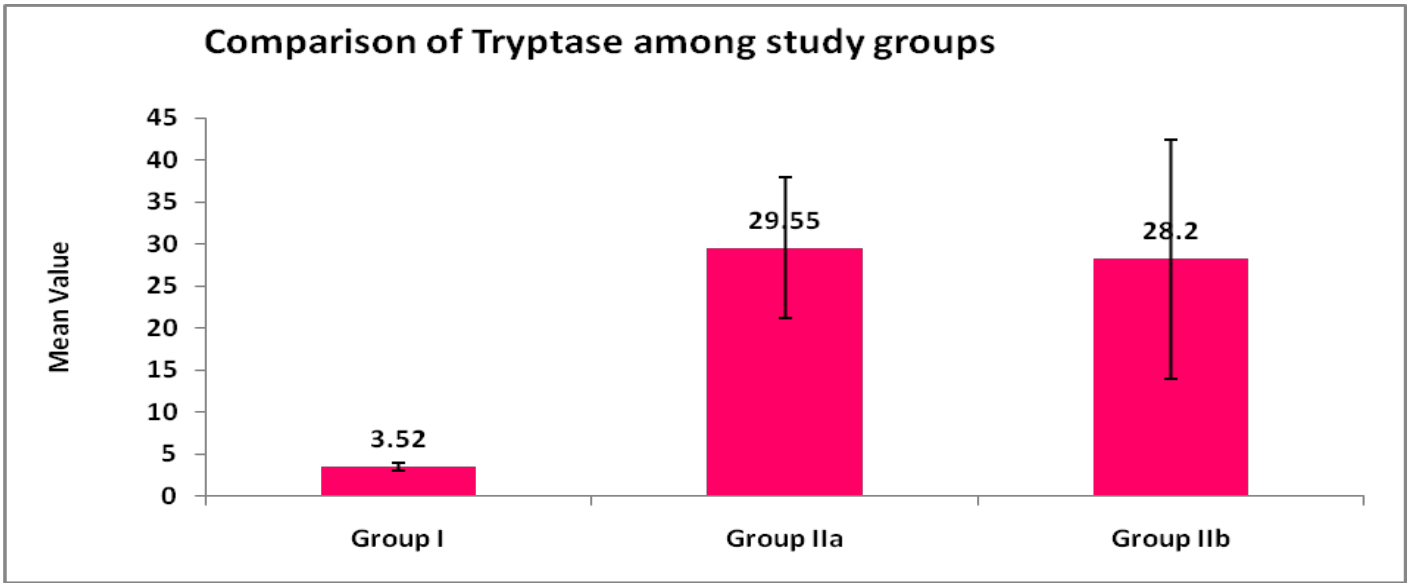

Figure 1 : Comparison of serum tryptase levels in study groups.

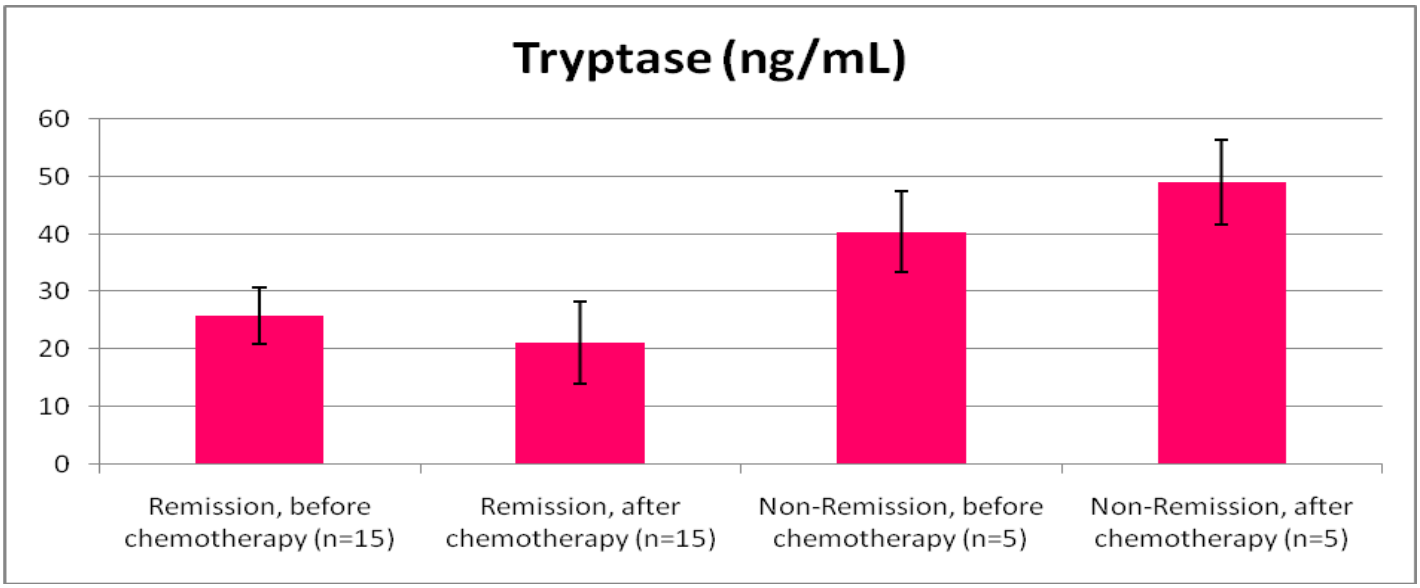

Figure 2: Comparison of tryptase levels in before and after chemotherapy in remission and non-remission groups

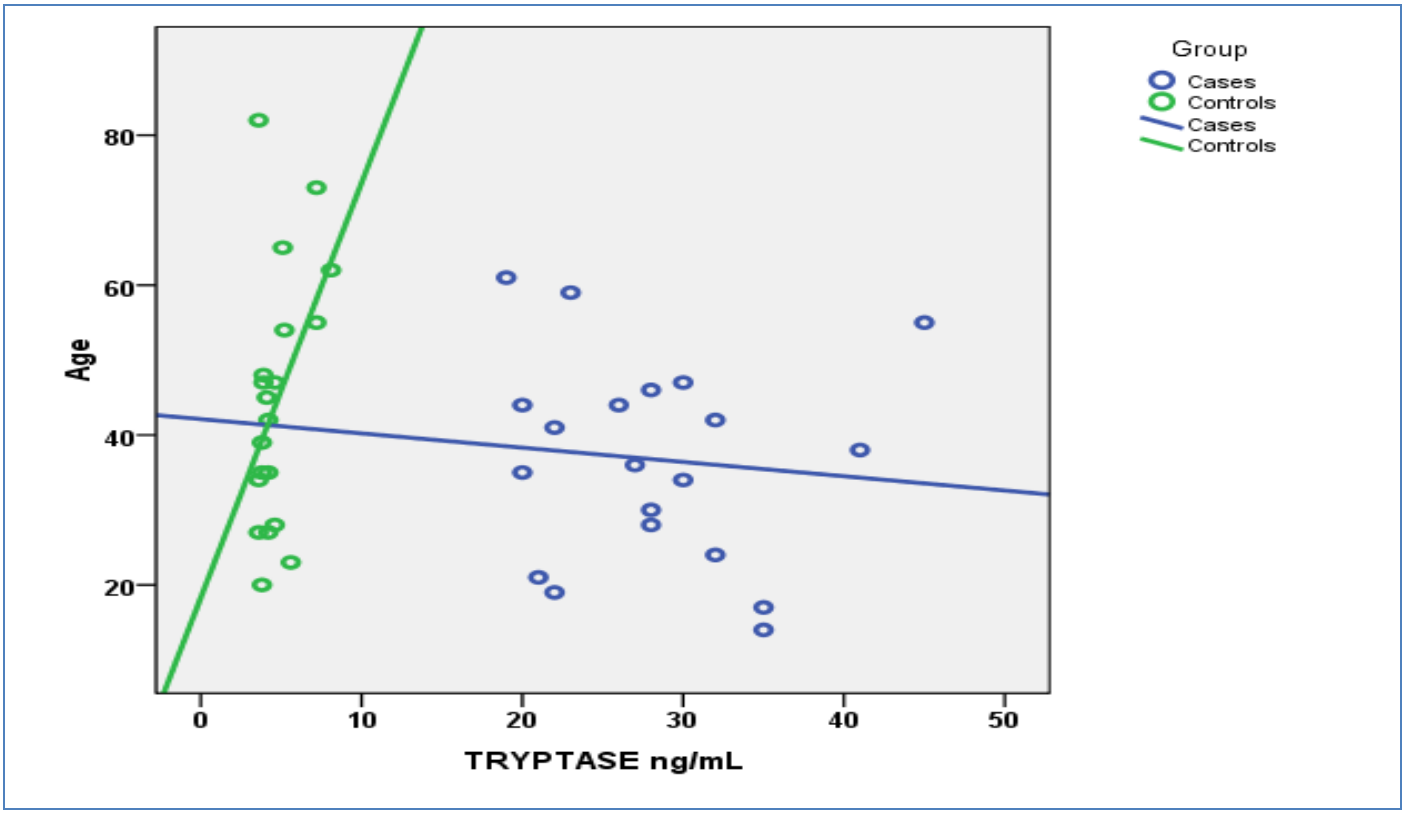

Figure 3: Correlation of serum tryptase with age

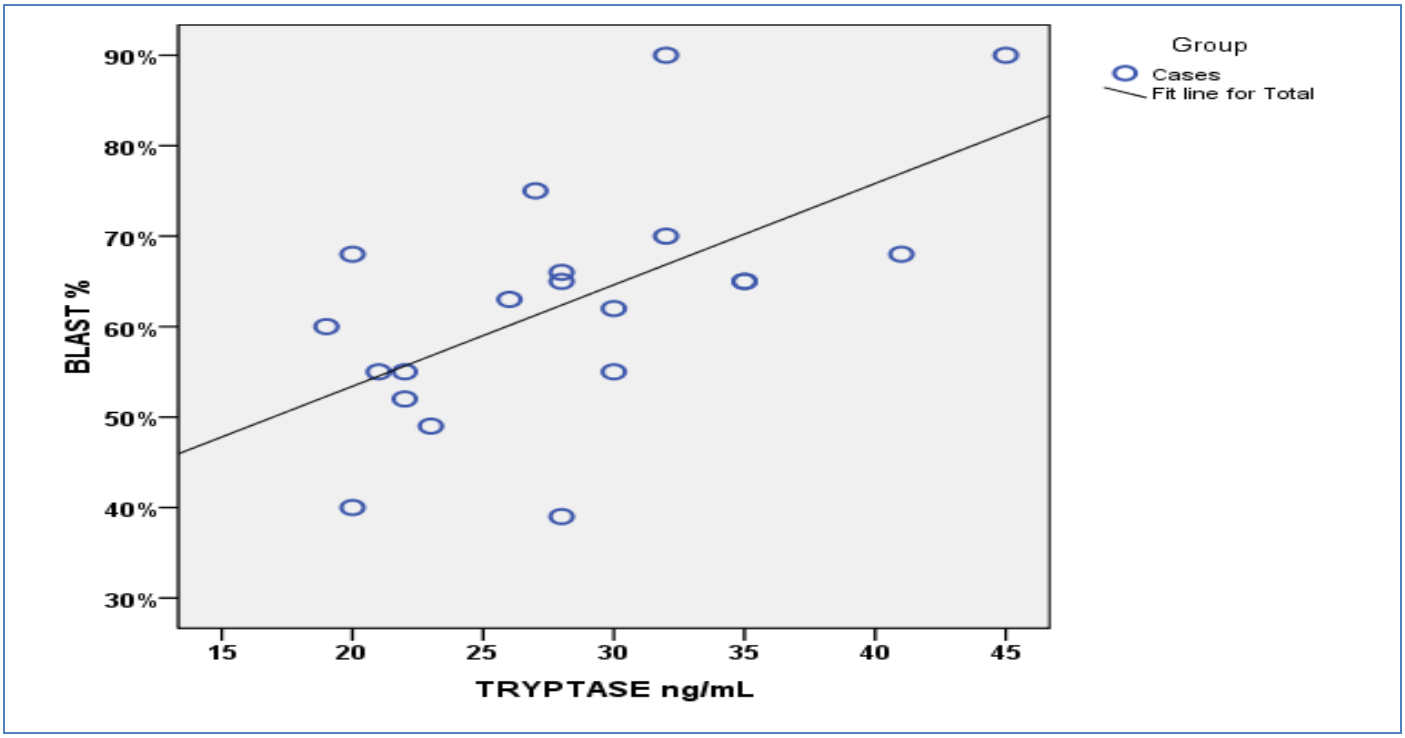

Figure 4: Correlation of serum tryptase with blast cells

Discussion

The mean age of AML patients was 36.75 ± 13.62 years and among controls was 44.40 ± 16.93 years. The controls and cases had sex ratio of 12:8. All twenty patients presented with pallor and body ache, while 16 presented with organomegaly and 5 patients presented

with icterus. The clinical symptoms usually appears ≤ 3 months before the diagnosis of leukemia. In about fifty percent cases, fatigue is the first symptom. Other symptoms include bone pain, bleeding, easy bruising, lymphadenopathy, headache, nonspecific cough and diaphoresis.

Clinical manifestations of AML result either from the proliferation of leukemic cells or from bone marrow failure that leads to decrease in normal cells. Leukemic cells can infiltrate tissues leading to hepatomegaly, splenomegaly, skin infiltrates and swollen gums.[15] The mean hemoglobin level in group I was 13.3±0.92 g/dL, in group IIa was 7.6± 1.75g/dl and in group IIb was 8.8±1.61 g/dl. The p value between group I and group IIa was found to be highly significant (p< .001) and it was not so significant between group IIa and group IIb (p<0.5). Anemia is usually present at diagnosis and can be severe. The degree varies considerably, irrespective of other hematologic findings, splenomegaly, or duration of symptoms. The median presenting leukocyte count is about 15,000/cu.mm. Between 25 to 40% of patients have counts <5000/cu.mm and 20% have counts >100,000/L.[9]he mean TLC level in group I was 13575±9591 per µL, in group IIa was 31100±12760 per µL and in group IIb was 12070±8212 per µL. The p value between group I and group IIa and between group IIa and group IIb was found highly significant (p<0.001).

Tryptase

The mean serum tryptase level in group I was 3.52 ± 0.47 ng/mL and in group IIa was 29.55 ± 8.31 ng/mL. A statistically highly significant difference was found between group I and group IIa (p<0.001, figure 1). Out of total twenty AML patients 15 patients (75%) achieved remission after induction chemotherapy while 5 patients (25%) failed to achieve remission. The mean serum tryptase level in remission group was 25.9±4.87ng/mL and 21.2±7.14ng/mL in AML patients before and after chemo therapy respectively and found to be statistically significant (p<0.05, figure 2). The mean serum tryptase level in treatment failure patients was 40.4±6.98 ng/mL and 49 ± 7.38 ng/mL before and after chemo therapy respectively and found to be statistically feebly significant (p<0.1, figure 2). The mean serum tryptase level was 21.2±7.14 ng/mL and 49 ± 7.38 ng/mL in AML patients who achieved remission and who did not respond to treatment respectively and was found to be statistically extremely significant(p<0.0001, figure 3). Serum tryptase levels were positively correlated with age (r= 0.651, p= 0.001, figure 3) and blast cells (r=0.663, p=0.001, figure 4) and correlation was significant in cases (before chemotherapy). These findings are compared with the findings in previous studies in table 3

Table 3: comparison of findings of current study with other studies in literature

Authors	Finding	Conclusion
Valent et al[16]	Reported that serum tryptase levels returned to normal or near normal values in de novo patients who acheived complete haematological remission after induction chemotherapy.	Can be used as a Prognostic markers
Sperr et al[17]	Serum tryptase levels increased in 39% of de novo	It acts as proinflammatory markers ,

	AML patients, 44% of secondary AML patients, and in patients with myelodysplastic and myeloproliferative diseases. Approximately 5% of AML cases had serum tryptase levels >200 ng/ml.	increases with progression of disease.
Cairoli et al[18]	Reported elevated serum tryptase levels (>15 ng/ml) also in patients with myelodysplastic syndrome, myeloproliferativeneoplasia, AML, CML and chronic eosinophilic leukemia.	It acts as proinflammatory markers , increases with progression of disease.
Present study	Significantly higher in mortality group, correlated with TLC and blast count, decreased after remission	Poor prognostic signs with progression of disease

In one of the other study that was aimed to determine the serum total tryptase levels of 108 patients with *de novo* AML(AML refers to a spontaneous presentation not relating to previous disease or treatment) and 25 patients with secondary AML(AML developed after exposure to cytotoxic agents or as a progression of an antecedent disease, such as myelodysplastic Syndrome (MDS and myelofibrosis) by fluorescence immunoassay, the elevated serum tryptase levels, defined as greater than 15 ng/mL were detected in 39% of patients with *de novo* AML and 44% of those with secondary AML. In another study that aimed in determining the serum total tryptase levels in 168 *de novo* AML patients, the elevated serum tryptase levels were seen in 36.3% patients. Likewise, the serum total tryptase levels were elevated in 45% of 155 *de novo* AML patients screened for the study aimed in checking serum total tryptase levels at diagnosis of AML as a simple tool for selecting patients to KIT mutations.[19] Valent et al also reported that serum tryptase levels returned to normal or near normal values in denovo patients who entered complete haematological remission after induction chemotherapy.[16] In another study Sperr et al found that serum tryptase levels were >20 ng/mL in 39% of de novo AML patients, 44% of secondary AML patients, and in patients with myelodysplastic and myeloproliferative diseases. Approximately 5% of AML cases had serum tryptase levels >200 ng/mL.[17] Elevated serum tryptase levels (>15 ng/ml) also reported by Cairoli et al and Schwartz et al in patients with Myelodysplastic syndrome, Myeloproliferative neoplasia, AML, CML and Chronic eosinophilic leukemia. The percentage of cases with elevated tryptase varies depending on the type of disease and in about 30-40% of all AML patients.[18]

Conclusions

Thus serum Tryptase levels have prognostic value in AML patients. Elevated levels of Tryptase indicated high turnover of leukemic cells and low levels after chemotherapy may indicate the completeness of remission in terms of the leukemic cell turnover better than the absolute cell counts in blood. Further studies in larger number of patients with long term follow up are required to validate these findings.

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