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Serum total sialic acid as a marker for prognosis of chemotherapy in patients of acute leukemia

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ABSTRACT

Leukaemia is clonal neoplastic proliferation of immature cells of hematopoietic system which are characterized by aberrant or arrested differentiation. A tumor marker can be produced by the tumor itself or by the host in response to a tumor. Measurement of tumor markers can be used to monitor cancer and predict the therapeutic response. N-Acetylneuraminic acid, usually called sialic acid is naturally widespread carbohydrate with numerous biological functions. It has been reported that human serum contains no free sialic acid and 90% of this serum sialic acid is bound to alpha and beta globulins. Aberrant glycosylation processes in tumour cells may contribute to the biosynthesis of the carbohydrate structures so that malignant or transformed cells contain increased levels of sialic acid on their surfaces. To study total serum sialic acid levels were measured in patients of Acute Leukemia and age and sex matched healthy controls. Total serum sialic acid was estimated by the method given by Warren's TBA method. Malignant or transformed cells contain increased levels of sialic acid on their surfaces.Mean total serum sialic acid value observed in 25 healthy controls in the present study was 36.08± 7.48 mg/dl. In the present study mean total serum sialic acid level in all acute Leukemia patients was 85.20±7.64 mg/dl. Total serum sialic acid was estimated in acute Leukemia patients after 6-8 weeks of chemotherapy or after remission (if achieved earlier). Mean total serum sialic acid level after chemotherapy was 48.24±9.55 mg/dl. Thus, there was a decrease in value as compared to 85.20±7.64 mg/dl before chemotherapy. The decrease is statistically significant (p<.001).The study concludes that sialic acid rise in leukemia patients and decreases after the response to chemotherapy.

Keywords: Sialic acid, acute Leukemia, biological marker, chemotherapy

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INTRODUCTION

Leukaemia is clonal neoplastic proliferation of immature cells of hematopoietic system which are characterized by aberrant or arrested differentiation. These leukemia cells accumulate in the bone marrow cavity resulting in signs and symptoms of the disease. Leukemia is divided into acute and chronic depending upon the clinical course. Further, they are divided into myeloid and lymphoid series depending upon cell of origin [1]. Tumor markers are naturally occurring or modified molecules that can be measured in serum, plasma, or other body fluids and their concentration changes in the presence of cancer [2]. Measurement of tumor markers can be used to monitor cancer, predict the therapeutic response. Although the existence of sialic acids (SA) has been known for over 50 years [3]. It is only in recent years that progressive improvements in analytical techniques have enabled detailed mapping of the structural diversity of sialic acids unraveling different modifications. Sialic Acid was named from the Greek 'Sialos' for Saliva. Sialic acid is widely distributed in tissues and body fluids of animals. In plasma, it is found in orosomucoid, α1-antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, complement proteins and transferrin. Some of these sialyated glycoproteins are also called acute phase reactants [4]. There is only a minute amount of free sialic acid in tissues and body fluids, and no direct biologic role has been identified for this unbound sialic acid. On the contrary, bound sialic acid is of major importance in cell biology because of the external position of SA on glycoproteins and glycolipids, and on the outer cell membranes [5].

Aberrant glycosylation processes in tumour cells may contribute to the biosynthesis of the carbohydrate structures so that malignant or transformed cells contain increased levels of sialic acid on their surfaces. Cell shape, anchorage and growth rate have been shown to influence the sialic acid content of the cell [6]. In some studies, the total sialic acid content has been observed to increase in highly metastatic cells compared with nonmetastatic cells.[7] So, the aim of study was to estimate total serum sialic acid in patients of acute leukemia before and after chemotherapy and compare them with age and sex matched healthy controls.

MATERIAL AND METHODS

The study was conducted in total 50 cases in which 25 patients were of acute lymphoblastic and acute myeloid leukemia which were admitted to ward or attending Clinical Hematology Clinic of Pt. B.D. Sharma PGIMS, Rohtak. These patients were compared to age sex matched 25 healthy control subjects. An only diagnosed case (in which diagnosis was made by clinical examination, complete hemogram, bone marrow examination and cytochemistry) was taken for study. Patients were monitored both clinically and haematologically. Five ml of venous blood was collected aseptically from antecubital vein when diagnosis was made, i.e. before the start of chemotherapy. Next sample was taken after completion of chemotherapy. If the patient does not go into remission a second sample was taken after 6-8 weeks of chemotherapy. Serum was separated and analyzed on the same day. Haemolyzed and lipaemic samples were not analyzed. The detail of the patient's clinical data was entered in a specialized proforma made for the purpose of the study.

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Remission criteria [8] Patients were given standard chemotherapy for AMLand ALL. When leukemia is no longer detected in peripheral blood and bone marrow, patient is said to be in complete remission.

Remission was considerd if:

1. Less than 5% blast in bone marrow and absence of leukemic cells in blood
2. Restoration of normal peripheral blood count.
3. Absence of physical findings attributable to extra medullary involvement of leukemia. Serum total sialic acid was estimated by Warren's TBA method [9]

OBSERVATIONS AND DISCUSSION

The present study included 25 patients of Acute Leukemia (Group I) out of which 13 were of AML and 12 were of ALL and 25 age and sex matched healthy controls(Group II).In the present study mean age of Acute Leukemia patients (Group I) at presentation was 30.52 years (range 11-58yr). The mean age for healthy controls was 28.4 years (range 11-53yr). (Table I). The age for both groups was comparable.Mean age for AML patients was 33.46 years. The mean age for ALL patients (n=12) was 27.33 years. Age and Sex distribution of healthy controls (group II) was comparable to Acute Leukemia patients (group I) (Table II). There were 13 males and 12 females i.e. male to female ratio of 1:1 which is comparable to Acute Leukemia patients. The most common symptoms with which patient presented included generalized weakness and swelling where as most common signs were pallor and fever. 20 (80%) patients presented with generalized weakness out of which majority were of AML (55%), and 9 were of ALL (45%).Total 4 (16%) patients presented with bleeding all were of ALL. 23 (92%) had pallor out of which 11 (47%) were of AML and 12 (53%) were of ALL. The hemoglobin levels were low in all cases. .6 AML patients (54%) out of 11 (44%) had fever while 5 (46%) patients of ALL suffered from it. 23 (92%) patients had hepatomegaly 13 patients were of AML (57%) whereas 10 (43%) of all. In total 17 (68%) had splenomegaly among them 5 (29%) were of AML while 12 (71%) were of ALL. In this series 15 (60%) patients had lymphadenopathy in which 13 (87%) were of AML and only 2 patients were of ALL (13%) (TableIII).

Table 1 AGE AND SEX DISTRIBUTION OF ACUTE LEUKEMIA PATIENTS

S. No.	Age groups in years	Male	Female	No. of patient	Percentage
1.	11-20	5	0	5	20
2.	21-30	5	5	10	40
3.	31-40	0	4	4	16
4.	41-50	2	1	3	12
5.	51-60	1	2	3	12
6.	Total	13	12	25	

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Table 2 AGE AND SEX DISTRIBUTION OF NORMAL PATIENTS

S. No.	Age groups in years	Male	Female	No. of patient	Percentage
1.	11-20	3	1	4	16
2.	21-30	8	6	14	56
3.	31-40	0	5	5	20
4.	41-50	1	0	1	4
5.	51-60	1	0	1	4
6.	Total	13	12	25	

Table 3 Patients' Signs and Symptoms at Presentation

	Symptoms	No. of patient	Percentage
1.	Generalised Weakness	20 (9 ALL,11 AML)	80
3.	Bleeding	4 (4 ALL, 0AML)	16
	Signs		
1.	Pallor	23 (12 ALL, 11 AML)	92
2	Fever	11(5 ALL, 6 AML)	44
3	Hepatomegaly	23 (10 ALL, 13 AML)	92
4	Splenomegaly	17 (12 ALL, 5 AML)	68
5	Lymphadenopathy	15 (2 ALL, 13 AML)	60

*Total 25 patients of which 12 ALL and 13 AML.

Total Sialic Acid In Healthy Controls And Acute Leukemia Patients

TABLE 4 Comparison of total sialic acid Levels in healthy controls and Acute Leukemia patients at presentation

	Controls (n=25) (mg/dl)	Acute Leukemia Patients at presentation (n=25) (mg/dl)	p value
Total sialic acid	**36.08±7.48**	**85.20±7.64**	**<.001**

Normal value given by Warren's TBA method is 56.2 -13.5mg/dl. Our results are comparable with the earlier studies. As shown in table IV, the mean Total sialic acid in 25 healthy controls was 36.08±7.42 mg/dl and 85.20±7.64mg/dl for patients of Acute Leukemia at the time of presentation. This increase was statistically highly significant (p<.001). Present study was conducted in 25 patients of Acute Leukemia (Group Ia&1b) and 25 age and sex matched healthy controls (Group II). There were 12 patients of Acute Lymphoid Leukemia (Ia) and 13 patients of Acute Myeloid Leukemia (1b) (Table I).

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Following observations were made during study

Table 5 Comparison Of total sialic acid Levels In Acute Leukemia Patients Before And After Chemotherapy

	Before Chemotherapy (mg/dl)	After Chemotherapy (mg/dl)	P value
Total patients (n=25)	85.20±7.64	48.24±9.55	<. 001
In remission (n=22)	84.99±7.73	44.91±2.49	<. 001
Not in remission (n=3)(small sample size)	86.74±6.75	72.72±2.41	Not significant

Total sialic acid Levels in Acute Leukemia patients before and after chemotherapy:
For 12 ALL patients mean was 84.58±5.30 mg/dl. For 13 AML patients it was 85.77±9.01 mg/dl. After chemotherapy, SA levels showed a decrease with a mean of 48.24±9.35 mg/dl. This decrease was statistically highly significant (p<.005) (Table V). ALL subgroup (n=12) had mean 49.92±11.20 mg/dl whereas AML subgroup (n=13) had mean 46.69±6.87 mg/dl. Again statistical difference was of no significance.

Comparison of total sialic acid in patients with remission and without remission

Out of 25 patients of Acute Leukemia 22 achieved complete remission whereas 3 could not attain remission. Mean SA values for the remission group were 80.45±16.04 mg/dl before chemotherapy and 44.91±2.41 mg/dl after chemotherapy. The mean SA values for non-remission group before and after chemotherapy were 86.74±6.75 mg/dl and 72.72±2.41 mg/dl respectively. There was a statistically insignificant decrease in this subgroup after chemotherapy. There was a highly significant difference (p<.001) between the two subgroups at start of chemotherapy. However, the non-remission group is too small for a valid comparison.

CONCLUSIONS

In the present study Acute Leukemia patients were mostly in there middle age. There was no preponderance of either sex as male to female ratio was 1:1.Generalized weakness and swelling was common complaint where as most common signs were pallor and fever.

In growing cells, the rate of carbohydrate synthesis is significantly higher compared with nongrowing cells, but in cancer cells shedding is a continuous and rapidly on-going phenomenon [10]. Cell shape, anchorage and growth rate have been shown to influence the sialic acid content of the cell[6]. Aberrant glycosylation processes in tumor cells may contribute to the biosynthesis of the carbohydrate structures so that malignant or transformed cells contain increased levels of sialic acid on their surfaces. Cell activation, transformation, and malignant growth increase the spontaneous shedding of cell surface components [11].

Carbohydrate portions of glycoproteins and glycolipids undergo neoplastic alterations, and the changes in glycoprotein carbohydrates include an increase in branched asparagine-linked and polylactosamine sugar chains as well as in sialylation. In particular, alteration of sialic acids is associated with cancer cell behavior, such as invasiveness and metastasis. Altered glycosylation of functionally important membrane glycoproteins may affect tumor cell adhesion or motility, resulting in invasion and metastasis [12].

The high concentration of sialic acid at the onset of the disease, although decreasing during treatment, as there is restoration of normal peripheral blood count. There was significant rise in concentration of sialic acid in the patients who were not in remission but the sample size was small and further more studies are needed. From this study it seems that determination of sialic acid concentration may be useful in monitoring the treatment and it can be used as another prognostic marker of acute leukemia.

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