Study the activity of Acid, Alkaline RNase and 5′-nucleotidases in sera of Child with Acute lymphoblastic Leukemia

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Abstract: Introduction: Acute lymphoblastic leukemia (ALL) is a cancer of the blood and bone marrow (spongy tissue in the center of bone). Childhood acute leukemia is the most common cancer in children, accounting for approximately one-third of all childhood neoplasms in developed countries. Materials and Methods: Laboratory investigations including serum total protein, acid and alkaline RNAs and 5′-nucleotidases enzymes. Blood samples were collected from 60 patients diagnosed to Acute lymphoblastic leukemia (ALL) after one month treatment with induction therapy. Age and sex matched 30 healthy persons selected as control. Results: serum total protein showed a significant decrease in patients group when compared to control group (P<0.01), Activities and Specific Activities of Serum Alkaline RNase, acid RNase and 5′-nucleotidases showed a significant increase in patients group when compared to control group (P<0.001). Conclusions, The activity of these enzymes in serum give the idea to be suitable in predicting treatment response in the long term follow up of patients. It has been suggested that the measurement of acid RNase, ALK RNase, and 5′-Nucleotidase enzymes could be measured as malignant disease markers

Key word: Acute lymphoblastic leukemia (ALL), 5′-nucleotidases, alkaline RNase and Acid RNase

INTRODUCTION

The leukemia defined as a group of malignant diseases in which progeny of the cells have a growth advantage over normal cellular elements owing to an increased rate of proliferation with decreased rate of normal marrow function and ultimately marrow failure. The clinical features, laboratory findings, and responses to therapy vary depending on the type of leukemia (David and Archie, 2004). Acute lymphoblastic Leukemia [ALL] is the most common cancer found in the pediatric population (Healy et al., 2007). Although, the exact cause of leukaemia is still unknown, scientists suspected that viral, genetic, environmental or immunological factors may be involved (Hassanzadeh et al., 2011, Olaniyi et al., 2011). There are two main categories of leukaemia: acute and chronic. Chronic leukaemia is primarily the disease of adults, with the exception of chronic myelogenous leukaemia which sparingly occur in children. In acute leukaemia, about 80% of Acute Lymphoblastic Leukaemia (ALL) occurs in children and Acute Myeloblastic Leukaemia (AML) is far more common in adults (Olan iyi et al., 2011).

The 5′-nucleotidases constitute a ubiquitous family of enzymes that catalyze either the hydrolysis or the transfer of esterified phosphate at the 5′ position of nucleoside monophosphates. These enzymes are responsible for the regulation of nucleotide and nucleoside levels in the cell and can interfere with the phosphorylation-dependent activation of nucleoside analogs used in therapies targeting solid tumors and viral infections (Santos et al., 2013). The 5′-nucleotidases have been found in bacteria, animals, and plants (Norbert, 2006). In the animals, there are cytosolic and extracellular enzymes, which are structurally distinct (Miron et al., 2007).

Ribonucleases (RNases) are a group of enzymes that cleave RNAs at phosphodiester bonds resulting in remarkably diverse biological consequences (Wan-Cheol and Chow, 2009). Its catalyze the degradation of RNA into smaller components. With the use of chromatographic techniques, a large number of RNases in prokaryotes and eukaryotes have been purified. Knowledge of the activities, sequences and structures of RNases facilitates their classification into endoribonucleases and exoribonucleases (Evandro and Tzi, 2011).

The aim of the current study was to determine the activity of 5′-nucleotidases and Acid and alkaline RNAs in sera of children with ALL after one month treatment with induction therapy, and study the correlation between these biochemical parameters. Further the reliability of serum 5′-nucleotidases and Acid and alkaline RNase measurement as an aid to monitoring the response to chemotherapy of Acute lymphoblastic Leukemia.

MATERIALS AND METHODS

Samples:

Blood samples of 60 patients diagnosed to Acute lymphoblastic leukemia (ALL) as they were submitted to the Protection of Children Hospital Medical City in Baghdad, were collected. The diagnosis for ALL founded on the following findings: leukocyte count, age, involvement of tissues other than bone marrow. Patients were compared with 30 healthy control subjects were included (mean age 39.27±5.53), who are devoid of conditions

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like psychiatric disorders, diabetes mellitus, or history of any drug intake are selected as control. Five ml of venous blood was drawn from (60) patients of ALL ranging between (1-16) years old, after one month induction therapy treatment and normal control.

**Methods:**

The blood was allowed to clot for 10-15 min. at room temperature, centrifuged for (10) min. at (3000xg). Serum was removed and was divided into two parts the first to measure the biochemical parameters and the other part was stored at -18 °C until the time of assay. Alkaline RNase activity was determined in serum by a method of Umeda (Umeda et al., 1969). Acid RNase activity was determined in serum by a method of Smith (Smith et al., 1974). Serum total protein was determined by Lowry et. al. (Lowry et al., 1951) using bovine serum albumin (BSA) as a standard protein. Protein electrophoresis has been done by cellulose acetate paper.

**Statistical Analysis:**

All statistical analyses in studies were performed using SPSS version 17.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability P< 0.05 = significant, P> 0.05 = non-significant. Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the differences between variables of differentiated groups.

**Results:**

The patients group were divided for age 1-16 years [50% (1-8 year), 50% (9-16 year)] and sex was divided into [31 male, 51.67%] and [29 female, 48.33%]. All patients were matched for age 5-11 years and sex was divided into 50% male and 50% female.

A significant decrease in sera protein of patients group when compared to control group (P<0.01) as shown in table 1 and figure 1, while Activities and Specific activities of serum Acid and alkaline RNase showed a significant increase in patients group in comparison to control group (p<0.001) as shown in table 1.

The acid RNase / alkaline RNase activity ratio was calculated for the enzyme in patients and control group, the mean of acid RNase / alkaline RNase activity ratio in the sera show a significant decrease (p <0.01) in patients group compared to control as shown in figure 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>S. Protein[g/dl] (mean ±SD)</th>
<th>Alk RNase Activities (U/L) (mean ±SD)</th>
<th>Specific Activities [U/mg] (mean ±SD)</th>
<th>Acid RNase Activities (U/L) (mean ±SD)</th>
<th>Specific Activities [U/mg] (mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>[n=60]</td>
<td>6.29 ± 1.07</td>
<td>93.74±33.00</td>
<td>1.55±0.67</td>
<td>71.76±20.29</td>
<td>1.20±0.49</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[n=30]</td>
<td>7.00 ± 0.66</td>
<td>37.88±12.49</td>
<td>0.57±0.19</td>
<td>39.00±10.86</td>
<td>0.64±0.20</td>
</tr>
<tr>
<td>P Value</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Significant at 0.05 level of significance

**Fig. 1:** Cellulose acetate profile of serum protein.

1. serum control.
2. serum ALL patients.
3. serum ALL patients
The mean levels of serum 5'-Nucleotidase showed a significant increase in patients group in comparison to control group (P<0.001) as shown in table 2 and figure 3.

**Table 2: Activities and Specific Activities of Serum 5'-Nucleotidase of ALL group and control Group.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients group [n=60]</th>
<th>Control group [n=30]</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activities [U/L] (mean value ±SD)</td>
<td>82.08±26.98</td>
<td>11.80 ±3.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Specific Activities [U/mg] (mean value ±SD)</td>
<td>1.35±0.51</td>
<td>0.17±0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Significant at 0.05 level of significance

Fig. 2: Mean and standard deviation of Acid RNase / Alk RNase ratio in patients and control Group.

Fig. 2: Mean distribution of serum 5’-Nucleotidase in patients group and control group
**Discussion:**

Serum proteins are useful indicators for initial screening of any abnormal function, inflammation and diseased condition. The expression of different proteins can vary depending on the age of the person (N. Sheikh et al., 2012).

In these studies, results indicate a decrease in serum protein and this is consistent with other studies (Jacqueline et al., 1998, Atta et al., 2006). Acute protein loss is generally due to reduced protein intake coupled with a hypermetabolic state resulting in rapid depletion of visceral proteins. This combined mechanism may explain the reduced levels of albumin observed in 1/3 children at diagnosis (Jacqueline et al., 1998).

The present study showed high level of the two enzymes in patients group when compared to control group. Many authors has reported level of serum RNase activities have been noticed to increase in several diseases such as malignant neoplasia (De Lorenzo and D'Alessio, 2008, Ilinskaya et al., 2001) renal insufficiencies (Ita M et al., 2008) pancreas disorder an leukemia (Majied et al., 2012). Alterations in nucleic acid metabolism have been demonstrated in a variety of malignant conditions in addition to the hyperuricemia that often observed in some malignant conditions, which may reflect in part the increased synthesis of RNA in the tumor cells, and consequently increases in the enzyme levels (J.W Naskalaki, 1978, E.Fernandez et al., 2000). Evidence was presented by some investigators, showed that serum content of RNase was a resultant of the elaboration of the enzyme by white cells. Other possibilities are:- its secretion by tumor cells or the surrounding tissues, and its excessive entry into serum rather than to diminish its urinary excretion (Hathama and Shatha, 2007).

The 5′-nucleotidases are a family of enzymes that catalyze the dephosphorylation of nucleoside monophosphates and regulate cellular nucleotide and nucleoside levels (Hunsucker et al., 2005). The results in the present study showed high level of this enzyme the increase effectiveness of the enzyme is possible to increased synthesis of DNA and RNA is one of the biochemical changes often observed in cancer cells (Van, 1999), and because 5′-nucleotidases are key enzymes for redistribution of nucleotides and are necessary for reversible transit of nucleosides and nucleobases across cell membranes. Maintaining proper nucleotide ratios is critical for maintaining accuracy in DNA replication and repair. In addition, alterations in 5′-nucleotidases activities have implications in spherocytic hemolytic anemia, immunodeficiency and the efficacy of nucleoside analog chemotherapies (Katrina and Charles, 2010). Also the present result may be due to effect of leukemia on liver, and Serum 5′NT increases in diseases of liver as shown in Previous study (Kataria et al., 2011). or may be due to the role of adenosine in physiology of several organs, Recent studies have reported that adenosine is a significant mediator of regulatory lymphocyte function. Numerous data indicates that adenosine affects T lymphocyte activation, proliferation and lymphocyte-mediated cytolysis (Sakowicz and Pawelczyk, 2011), so increase enzyme lead to increase adenosine. One of the well known effects of adenosine is its differential regulation of pro- and anti-inflammatory cytokines and free radicals production (Haskó et al., 2000).

As our knowledge no previous studies have showed to that increased in activity of acid RNase, ALK RNase, and 5′-Nucleotidase in child with ALL after one month induction therapy treatment. The activity of these enzymes in serum give the idea to be suitable in predicting treatment response in the long term follow up of patients. It has been suggested that the measurement of acid RNase, ALK RNase, and 5′-Nucleotidase could be measured as malignant disease markers. Its use in clinics may be very helpful. More comprehensive studies are needed if this situation is important to clarify in the pathogenesis of the acute leukemia. Enzyme levels may be a useful indicator of response to chemotherapy, so more and more test to use this hypothesis will be the basis of the future work.

**REFERENCE**


