Determination of Selenium in Blood Serum of Children with Acute Leukemia and Effect of Chemotherapy on Serum Selenium Level

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Summary

The concentration of selenium in serum was measured by the neutron activation method in three groups of children: 30 healthy children, 20 children with Acute Myeloblastic Leukemia (AML) and 40 with Acute Lymphoblastic Leukemia (ALL) (L1; n=20, L2; n=20). The samples were taken before and after induction chemotherapy. Age, sex, FAB, initial WBC, BUN, creatinine and urinary analysis did not show a significant change in the amount of selenium in serum. Selenium concentration in serum samples of ALL children before chemotherapy showed no significant differences as compared with that of normal individuals, but there were significant differences between children with AML and normal individuals (76.46 ± 24.59 μg/L vs 102.38 ± 19.25 μg/L, with p<0.02). In conclusion, the question of whether these deficiencies are responsible for the disease or are the result of a secondary effect of the cancer remain to be answered. Immediately after induction chemotherapy, the selenium concentration in the serum of ALL children decreased significantly (80.14± 15.48 μg/L vs 110.72± 28.3 μg/L, p<0.001), but this was not the case for AML children. These findings may be due to the difference in the drugs administered in induction chemotherapy of ALL and AML children.

Keywords: Acute Lymphoblastic Leukemia (ALL), Acute Myeloblastic Leukemia (AML), chemotherapy, human blood serum, Neutron Activation Analysis (NAA), selenium.

Introduction

Selenium in appropriate chemical form and concentration exhibits anticarcinogenic properties and is anti-mutagenic, acting as an antiproliferative and cytotoxic agent. Other properties stimulate immune functions that influence lymphocyte proliferation, increase cellular respiration and reduce the pathogenicity or tumorigenicity of viral agents. Accordingly, selenium is receiving increasing attention as a nutritional cancer protective factor and therapeutic agent (1). Selenium is a trace element and an essential part of the enzyme glutathione peroxidase, which protects cells from oxidative damage. It has been shown to have antiproliferative, anti-inflammatory, antiviral and immune altering effects. It has also been shown that selenium at physiologic concentrations can effectively inhibit the over-all proliferation of human lymphocyte populations in response to various immune stimuli in vitro. The anti-proliferative effects of selenium may be specific for a certain lymphocyte subset (2). Some investiga-
tions have provided evidence for an association between a low selenium concentration in the body and an increase in risk of gastrointestinal, urinary and mammary cancer (3). Epidemiological studies have shown an inverse correlation between selenium intake and incidence of the majority of human cancers, including that of the lung and liver (4,5). Beguin and his group (6) have concluded from their investigations that a low selenium level does not enhance the risk of developing acute non-lymphatic leukemia (ANLL). They also indicated that the serum selenium level in patients with acute leukemia is mostly dependent on tumor activity.

As with many naturally occurring elements in the human body, selenium plays a vital role in biological phenomena. Accumulation of elements such as arsenic, cadmium and selenium in the human body as a result of daily nutrition and exposure to occupational and environmental pollution is an increasing risk to health. Therefore, there is a great need for fast and reliable analytical methods for the determination of trace elements at the nano gram levels in various tissues and biological fluids (7-8). Neutron activation analysis (NAA) has been recognized as a powerful tool in trace element measurement in a variety of samples including biological and human tissues. The advantage of NAA is mainly due to high sensitivity, precision and accuracy.

Leukemia is one of the most frequently observed types of cancer in children in the world. Statistics for the USA show that, only for children out of a hundred thousand suffer from this disease (9). In Iran, the statistic is not well established but it is estimated at 20 in 100000 (10).

In spite of intensive and extensive studies the main cause(s) of leukemia is (are) still unknown. Genetic, environmental and nutritional factors are in all probability responsible for this disease (11). It is hoped that the ever-increasing research on different aspects of the disease and administration of a variety of drugs will lead to an effective treatment of this type of blood cancer in near future.

Selenium is one of the main mineral elements with an important role in human metabolism. It is one of the vital constituents of important substances such as the enzyme glutathione peroxidase where it plays an important role in its biological behavior. This enzyme is involved in the dissociation of $\text{H}_2\text{O}_2$ and organically bound hydroperoxides, such as hydroperoxide in fat. It also contributes to the elimination of free radicals that have a destructive effect on the cell membrane and on DNA. By this means it protects the cells from carcinogens’ effects, therefore preventing the development of different cancers in the organism (12-15). Some of the selenium compounds have a cytotoxic effect on cancer cells (15) and cause macrophages to become more active as well as increase cell respiration (16-17).

Given this significant contribution of selenium in the human body the possibility exists that it could be used in the treatment of blood cancer. The action of selenium depends on the compound in which selenium is present. Basti and his group (16) have shown that selenosistin and sodium selenite have an anti-carcinogenic effect (16,18). Serum selenium has been accepted as a suitable indicator in the human body (19-24).

The main concern in Iran is the diagnosis and treatment of acute lymphoblastic leukemia (ALL) in children. There are three types of ALL, L1, L2 and L3. This classification is based on the cellular morphology as elucidated by a group French-American-British (FAB) investigators. It should be borne in mind that L3 is very rare.

In this paper the results of our measurements of selenium in the blood serum of children with different type of acute leukemia, are presented.

There are many commonly-used methods for the determination of selenium in blood samples, including fluorometry, hydride-generation atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry, gas chromatography, neutron activation analysis and X-ray fluorometry (34). We measured selenium in blood serum by neutron activation.

Our investigation shows that there are grounds for further study of the use of special selenium compounds both for treatment as well as for preventive measures.

**Materials and Methods**

**Neutron Activation Analysis**

Selenium has six isotopes in nature: $^{74}\text{Se}$ (%9.0), $^{76}\text{Se}$ (%2.25), $^{77}\text{Se}$ (%7.6), $^{78}\text{Se}$ (%23.5), $^{78}\text{O}\text{Se}$ (%49.6) and $^{82}\text{Se}$ (%9.4). $^{75}\text{Se}$ is produced as a result of neutron absorption by $^{74}\text{Se}$. $^{75}\text{Se}$ has a 120-day half-life decaying to unstable As75 by beta emission and becomes to stable As75 while emitting several gamma rays.

From twenty children with Acute Lymphoblastic Leukemia (ALL) 20 blood serum samples (10 L1 samples and 10 L2 samples) were prepared prior to chemotherapy and 20 after induction chemotherapy. As a control 30 blood samples from 30 healthy children were pre-
Table 1. Interfering reactions in serum irradiation

<table>
<thead>
<tr>
<th>Serum Element</th>
<th>Produced Radioisotope</th>
<th>Reaction</th>
<th>Half-life (y)</th>
<th>γ-Ray Energy (KeV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr-54</td>
<td>Ti-51</td>
<td>Cr-54 (n,α) Ti-51</td>
<td>5.8m</td>
<td>320,928</td>
</tr>
<tr>
<td>Cr-52</td>
<td>V-52</td>
<td>Cr-52 (n, p) V-52</td>
<td>3.75m</td>
<td>1434</td>
</tr>
<tr>
<td>Fe-56</td>
<td>Mn-56</td>
<td>Fe-56 (n, p) Mn-56</td>
<td>2.58h</td>
<td>847,1811</td>
</tr>
<tr>
<td>Fe-64</td>
<td>Cr-51</td>
<td>Fe-54 (n,α) Cr-51</td>
<td>27.7d</td>
<td>320,511</td>
</tr>
</tbody>
</table>

pared. All the samples both from the healthy children and the children with ALL were taken in the morning before breakfast. Centrifugation at the rate of 3500 rpm (equivalent to 20.8 g) for 15 min separated off the serum. Then the serum was kept in a vial at temperatures ranging between -5° and -10°C. The serum sample was placed in an incubator for 72 h at 50°C to dry. A pestle was used to grind the dried serum sample in a porcelain mortar. The ground serum sample was sealed in a polyethylene capsul and irradiated in a neutron flux of $10^{12}$n/cm²·s. The irradiation time was 6 hours and the cooling time one month. The gamma-ray energy spectrum of each sample was obtained by using a solid-state, highly pure germanium (HPGe) detector connected to a computerized multi-channel analyzer (MCA). Table 1 shows the radioisotopes produced during serum irradiation along with the respective reactions. The measurements were carried out in Nuclear Research Center, Atomic Energy Organization of Iran in the summer of 1997 and serum samples were prepared at the Ali-Asghar Hospital.

Figure 1 shows the gamma-ray energy spectrum of Se-75 including many distinct photopeaks, at 121.1166 Kev (17.144 %), 136.25 Kev (58.3 %), 264.66Kev (58.5%), 279.54 Kev (24.79%), and 400.66Kev (11.37%). The first number indicates the gamma-ray energy and the number in brackets shows the branching ratio or abundance of the respective gamma ray in total count rate.

The accuracy of the applied methodology was checked by means of simultaneously irradiated Standard Reference Materials of dried animal blood (A-13) (IAEA). The selenium background in each polyethylene capsule was also checked.

**Population group and Sampling**

The patients in this study were all children aged 3 to 8 years. Sampling for each patient was done twice, first before the start of chemotherapy and then after completion of chemotherapy. It should be mentioned that chemotherapy was done in three separate steps: (1) induction (2) consolidation (3) maintenance. Second sampling in the case of induction was normally done after 28 days if the patient had shown a good marrow recovery. The drugs administered were hormones, anti-metabolites, antibiotics and vegetable alkaloids.
Determination of serum selenium of children with acute leukemia

Results and Conclusions

In our study the healthy children mean selenium concentration in serum was 102.38 ± 19.25 μg/L. Table 2 shows that serum selenium level in Iranian children is not much higher than other countries ignoring age. However, the Se level in children blood serum in Iran is not meaningfully different from other reported values in other countries. In this case the number of samples were 30 that is quite enough for this type of measurements.

Even though the regression calculations showed a gradual increase in the selenium level with age but the change was not significant (Figure 2). We also could not detect any significant difference in the amount of serum selenium in boys and girls. In the serum of children with acute Lymphoblastic leukemia (ALL) there was no distinct relations between serum selenium and blood urea nitrogen (BUN), creatinine, the initial number of white blood cells or the results of the analysis of urine. In ALL groups L1 and L2 similar to healthy children we could not detect any significant differences of selenium in the serum between boys and girls, pre- and post induction chemotherapy. On the other hand, we observed significant differences in the serum selenium level pre- and post chemotherapy in the groups L1 and L2. The serum selenium concentration of the L1 group pre- and post chemotherapy were 118.45±21.48 μg/L and 76.22±8.76 μg/L (P<0.001), respectively, and in group L2 103.00±33.12 μg/L and 86.09±19.89 μg/L (P< 0.005), respectively. The serum selenium in children with ALL pre- and post chemotherapy was 110.72±28.3 μg/L and 80.14±15.48 μg/L respectively (Table 3). The interesting point is that, as Table 4 shows, the serum selenium level (SSeL) of group L1, L2 and children with ALL prior to induction chemotherapy was not much different from that of the normal healthy children. After completion of induction chemotherapy, in the complete remission (CR) phase, the level of serum selenium decreased. Then the SSeL in group L1 was 80.39 ± 14.47 μg/L (P < 0.001) in group L2 80.75 ± 21.83 μg/L (P<0.03) and in ALL 80.14±14.48 μg/L (P<0.001) (see Table 5).

In the present study we observed that was is no significant difference of SSeL between boys and girls. We also observed that there was not significant in serum selenium levels of boys and girls suffering from ALL between the pre- and post-induction chemotherapy stages. These findings are quite in agreement with those of (21,29,34-36). It should be added that in children with leukemia the SSeL is slightly lower in boys than in girls, but this is not the case for healthy children. This point is important for consideration in treating boys with leukemia (29).

Our investigation shows that there is no significant difference between serum selenium in healthy children and children with ALL prior to chemotherapy. This finding agrees with the reported measurements of (29). On the other hand, the decrease of serum selenium in children with ALL after reaching CR may be due to administration of drugs used in chemotherapy.

Table 3. Serum selenium level according to sex before and after chemotherapy in different types of ALL (mean ±SD in μg/L)

<table>
<thead>
<tr>
<th>Sex</th>
<th>L1</th>
<th>L2</th>
<th>ALL</th>
<th>Normal</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td>122.90 ± 10.85</td>
<td>92.06 ± 45.57</td>
<td>95.32 ± 37.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>114.00 ± 24.38</td>
<td>113.93 ± 9.62</td>
<td>118.23 ± 17.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Comparison between the serum selenium level in healthy children and children with ALL before chemotherapy (mean ± SD in μg/L)

<table>
<thead>
<tr>
<th></th>
<th>L1</th>
<th>L2</th>
<th>ALL</th>
<th>Normal</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>118.45 ± 21.48</td>
<td>103.00 ± 33.12</td>
<td>110.7 ± 28.3</td>
<td>102.38 ± 19.25</td>
<td>76.46 ± 24.59</td>
</tr>
</tbody>
</table>

Table 5. Comparison between the serum selenium level in healthy children and children with ALL after achieving complete remission (mean ± SD in μg/L)

<table>
<thead>
<tr>
<th></th>
<th>L1</th>
<th>L2</th>
<th>ALL</th>
<th>Normal</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80.39 ± 14.47</td>
<td>80.75 ± 21.83</td>
<td>80.14 ± 15.48</td>
<td>102.38 ± 19.25</td>
<td>76.24 ± 27.44</td>
</tr>
</tbody>
</table>
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