Evaluation of Advanced Glycation End Products and other Biochemical Parameters in Patients with Leukemia

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Abstract:

This study's objective is to assess the Advanced glycation end products (AGEs), oxidative stress and antioxidant parameters in sera of leukemia patients and control group. In the present study we measured the concentration of serum levels of carboxymethyl-lysine (CML), pyrraline, Nitric Oxide (NO), Malondialdehyde (MDA), superoxide dismutase (SOD), catalase and glutathione (GSH) in leukemia patients and control group. This study was performed on 80 individuals, 50 children, men and women with leukemia patients (20 females and 30 males) and 30 healthy individuals (children, women and men, 12 females and 18 males) as control group who had no leukemia. The results show that the median and mean serum level of each CML, MDA and GSH are significantly, but CAT and SOD are insignificantly higher in leukemia patients than in control group (CML= 479.3 (411.1, 545.4) ng/ml (p value <0.0001), (MDA= 175.7 (160.8, 191.8) ng/ml (p value= 0.0357), (GSH= 5.772 ± 0.3615) μg/ ml (p value= 0.0194), (CAT= 6.857 (6.281, 7.425) ng/ml (p value= 0.8193) and (SOD= 1.111 (0.8918, 1.478) ng/ml (p value= 0.0891). The median serum level of Pyrraline and NO are insignificantly lower in leukemia patients compared to control group (Pyrraline= 156.9 (137.3, 179.0) pg/ml (p value = 0.8995) and (NO= 119.3 (96.66,192.6) μmol/L (p value= 0.2289). The correlation analysis demonstrates that there are significantly positive correlations between serum CML and MDA (r= 0.3487) (p value= 0.0152) and between serum pyrraline with MDA (r= 0.3838) (p value = 0.0093) and GSH (r= 0.3371) (p value= 0.0236).

Keywords: Advanced Glycation End products (AGEs), Leukemia, Oxidative Stress, Antioxidants, Cancer
Introduction

Cancer is responsible for almost 13 percent among wholly humanoid global deaths [1]. Beside heart problems, cancer is the major biggest cause of mortality globally, as well as the number of cancer-related deaths has been rising in previous decades [2]. Cancer is the term for the unchecked growth the presence of aberrant cells everywhere in the body. These abnormal cells are referred to as cancer cells, malignant cells, or tumour cells. These cells have the capacity to enter healthy live cells. Many tumors and the abnormal cells that make up cancer tissue can be identified by the identity of the tissue where the mutant cells originate (for instance, breast cancer, lung cancer, colorectal cancer) [3]. Leukemia is among the top ten cancers that affect people from all races. Leukemia is still a public health matter. Even though overall fatality rate has decreased in comparison to previous centuries, 4 distinct kinds of leukemia, acute and chronic myelogenous leukemia, acute and chronic lymphocytic leukemia [4-6]. The term leukemia was coined by combining two Greek words, leukos (white) and haima (blood), to describe a form of blood or bone marrow malignancy indicated due to an unusual rise in white blood cells [7]. Acute lymphoblastic leukemia (ALL) is by far the most widely used kind of leukemia when it comes to infants over 80 percent in those diagnosed with the disease. These aberrant cells have ceased normal cell development and could be inhibiting spontaneous erythropoiesis [8].

Advanced glycation end products (AGEs) are a broad category of non-enzymatic results of aldose sugar interactions involving proteins or lipids. The browning in food products is caused by the interaction of the protein with the sugar Though glycation is a broad term to the adding of sugar to proteins, it alludes about the non-enzymatic addition of sugar. The term "glycosylation" is frequently used to describe the enzymatic addition of sugars [9]. The Maillard reaction, which begins with protein glycation and progresses to the creation of AGEs, has been linked to the growth of diabetes problems, such as the etiology diseases of the heart, kidneys, and nervous system and malignancies of various types. RAGE is an advanced glycation end product receptor plays a crucial
role in cancer genesis and pathology. Soluble RAGE inhibits pathological impacts mediated by RAGE in a natural way. Despite the fact that aging is a significant contributor to the development of major diseases like cardiovascular disease, neurodegeneration, and cancer, the molecular pathways behind age-related disorders remain unclear due to the complicated and multidimensional structure of the aging process [10]. The way AGEs interact with cell surface receptors is supported by an increasing amount of research. RAGE induces oxidative stress, which in turn triggers proliferative, angiogenic, and inflammatory responses, implicating AGEs from genesis and progression of a variety of cancers. These findings recommend that AGE accumulation and subsequent activation of the RAGE signaling pathway may contribute to an elevated cancer risk in diabetics and the elderly [11, 12]. AGE buildup is associated to a number of cancer disparity risk factors, comprising poor diet, lack of activity, and obesity. While several research has looked into the role of RAGE in carcinogenesis, few have looked into the contributing to function of AGEs to cancer development and progression, as well as their potential significance as a lifestyle-linked biological component leading to the discrepancy in cancer mortality [12]. Endogenous and exogenous AGE accumulation can impair biological macromolecule function in a variety of ways. AGEs accumulate in both the intracellular and extracellular compartments, contributing and progression for a wide range of illnesses. An increase in the AGE accumulation pool is connected to increased protein dysfunction [13].

**Oxidative stress** is a term for instability ratio of reactive oxygen species (ROS) to antioxidant mechanisms activity [14]. ROS are molecules that have a high reactivity due to their chemical makeup and can be formed by oxygen or nitrogen metabolism. ROS and RNS are produced by free radicals such as superoxide anion radical (O₂⁻), hydroxyl radical (OH•), nitric oxide radical (NO•), alkoxyradical (RO•) and peroxyl radical (ROO•). Other non-free radicals that can be detected include hydrogen peroxide (H₂O₂), peroxynitrite (ONOO•⁻), ozone (O₃), singlet oxygen (¹O₂), nitrous acid (HNO₂), hypochlorous acid (HOCl), organic peroxides (ROOH), and aldehydes (HCOR). Reactive oxygen species in the mitochondria cause metabolic activities that result in
oxygen reduction via the electron transport chain [15]. ROS and RNS are organic or inorganic molecules with unpaired electrons. Although in oxidative metabolism, oxygen is a key source which can also be partially depleted in vivo to create ROS via redox reactions [16]. Several pathophysiological processes, including atherogenesis, immune system alteration, diabetes mellitus, ageing, cognitive impairment, chronic inflammation, and carcinogenesis, are influenced by oxidative stress, which is characterized a disparity between an overproduction of reactive oxygen species (ROS) and a deficiency in the organism's antioxidant status [17-20].

**Antioxidants** are substances which possess the capability for donate an electron to a free radical while remaining stable. As a consequence, the free radical loses its reactivity and becomes more stable. Antioxidants can donate an electron to a free radical without getting destabilized, effectively stopping a free radical chain reaction. Antioxidants play a range of physiological roles in the body by slowing the oxidation process, even at low doses. Plant compounds act as radical scavengers, helping to convert radicals into less reactive forms. Fruits, vegetables, and tea all contain antioxidants that scavenge free radicals [21]. Antioxidants are found in abundance within internal organs and help to counteract the effects of oxidants. Antioxidants protect cells from damage caused by oxidative stress [22]. Antioxidants are classified into enzymatic and non-enzymatic groups [23]. The enzymatic group includes catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (GPse), glutathione reductase (G-reductase). The non-enzymatic antioxidants are glutathione (GSH), bilirubine, uric acid, beta-carotene, vitamins A, C, and E, as well as additional antioxidants that are both natural and man-made substances [23].

**Materials and Methods**

This research was done at surgical Specialty Nanakali Hospital from Erbil City. Blood samples were collected from eighty subjects, fifty from patients (20 females and 30 males) children, men and women who had leukemia as a patient group. The additional samples came from thirty individuals, children, men and women (12 females
and 18 males) as control group who had no leukemia. The Victims ranged in age from (4-75) years of age and the Control groups ranged in age from (7-49) aged years. Details about the individual was obtained from a patient's documentation leukemia patients. Blood sample collection started in October 2021 and finished in November 2021. The blood sample were left for a while without anticoagulant to allow bloods to clot. Serum samples were obtained by centrifugation for ten minutes to analysis and held till evaluation at -40 °C. The biochemical tests (CML, Pyrraline, MDA, NO, catalase, SOD, Glutathione) were performed by using ELISA kits (ZellBio GmbH assay kit) respectively, to the entire automated Enzyme-Linked Immunosorbent Assay (ELISA) of Germany origin. The experiments have been done at Research Center of Koya University.

Statistical analysis

A software program Graph Pad-Prism (version 9) was used for data analysis. The results of the data are either median with interquartile ranges or mean with standard error. The Pearson correlation was used to ascertain the association between the two groups after the two samples student's t-test was performed to assess differences in median values between the two groups. Mann-Whitney U test and independent student's t-test (unpaired t-test) were used in the comparison of nonparametric and parametric values between two groups, respectively. P< 0.05 was used as the significant level. The area under the curve (AUC) for the diagnostic accuracy in leukemia patients was calculated using ROC curve (Receiver operating characteristic) analysis.

Result and Discussion

The findings of the current investigation show that the median levels of serum CML in leukemia patients and control group are 479.3 (411.1, 545.4) and 355.1 (322.1, 402.6) (ng/ml) respectively, as shown in (Table 1 and Figure 1). These data exhibit that the median levels of serum CML was noticeably higher in leukemia patients as matched with control group (P˂ 0.0001). Similar result was obtained from previous study that demonstrated the level of CML in leukemia patients is significantly increased compared
with control group [24-28]. This may be a result of persistent inflammation and elevated oxidative stress. The use of various AGE tissue levels as indicators for the emergence of certain cancers is debatable, even while cancer patients do actually exhibit generally elevated CML levels in both their plasma and tumour tissue. This is brought on by the systemic effects of AGE accumulation on a number of different metabolic conditions and chronic illnesses, including type-II diabetes. Furthermore, elevated AGE levels are inextricably linked to lifestyle choices made by an individual, serving as a marker of their oxidative status and risk for developing metabolic illnesses. [24, 29, 30].

The findings of this research show that the median pyrraline levels in leukemia patients and control group are 155.5 (145.4, 182.3) (pg/ml), 156.9 (137.3, 179.0) (ng/ml) respectively, as shown in (Table 1 and Figure 1). These data exhibit that the median level of serum pyrraline was more or less similar to control group (P <0.8995). This is in disagreement with previous study that demonstrated the level of pyrraline in leukemia patients is increased compared with control group [11, 28, 31, 32]. The current study is consistent with the earlier study that demonstrated the serum pyrraline is insignificantly reduced in patients with leukemia compared with control group. This could be because of the lifestyle and medication therapies that reduce AGE levels pyrraline [25].

The outcomes of the extant study demonstrate that the median serum levels nitric oxide in leukemia patients and control group are 105.7 (91.18, 147.40) (ng/ml), 119.3 (96.66, 192.60) (μ mol/ L) respectively, as shown in (Table 1 and Figure 1). These data exhibit that there is no discernible difference of median level of serum NO between leukemia patients and control group. According to a prior study, this is in accordance that demonstrated NO level in leukemia patients is insignificantly decreased compared with control group [33-35].

The median level of serum MDA in leukemia patients and control group are 175.7 (160.8, 191.8) (ng/ml), 162.7 (145.1, 185.8) (ng/ml) respectively. These data exhibit that the median levels of serum MDA are significantly higher in leukemia patients compared
to the control group (P< 0.0357) showing in (Table 1 and Figure 1). This concurs with previous study that demonstrated the level of MDA in leukemia patients is significantly increased compared with control group [36-39]. These data are in disagreement with previous study that demonstrated the level of MDA in leukemia patients is significantly increased when compared to control group [40].

The median levels of serum SOD in leukemia patients and control group are 1.111 (0.8918, 1.478) (ng/ml), 0.9749 (0.7927,1.258) (ng/ml) respectively, as shown in (Table 1 and Figure 1). These data exhibit that no discernible differences exist in the median levels of serum SOD between leukemia patients and control group. Similar result was recorded that demonstrated the level of SOD in leukemia patients is insignificantly increased compared with control group [41, 42]. This is in disagreement with previous study that demonstrated the level of SOD in leukemia patients is significantly increased compared with control group [37, 43].

The median levels of CAT in leukemia patients and control group are 6.857 (6.281, 7.425) (ng/ml), 6.697 (6.088, 8.206) (ng/ml) respectively, as shown in (Table 1 and Figure 1). These data exhibit that there are no significant differences in the median levels of serum CAT between leukemia patients and control group. Previous study demonstrated that the level of CAT in leukemia patients is insignificantly increased compared with control group [44]. Numerous prior research demonstrated the levels of SOD in leukemia patients are insignificantly increased compared with control group [43, 45, 46].

The mean levels of serum GSH in leukemia patients and control group are 5.772 ± 0.3615 (μg/ml), 4.195 ± 0.5923 (μg/ml) respectively, as shown in (Table 1 and Figure 1). These data exhibit that the median levels of serum GSH are significantly high in leukemia patients compared to control group (P< 0.0194). This is in accordance to previous study that demonstrated the level of GSH in leukemia patients is significantly increased compared to control group [44]. The result is disagreement with existing
research that demonstrated the levels of GSH in leukemia patients are significantly increased compared with control group [36, 37, 39, 45, 47].

The elevation of serum SOD, CAT, and GSH activity in leukemia patients compared to controls could be because of the acceptance of medicines that the patients receive during therapy, which may obstruct the biologic sites' preservation and the immediate eradication of radicals. Patients with leukemia also produce more reactive oxygen species than healthy people do, which is a significant change in the antioxidant defense [44]. This finding confirms that abnormal or cancerous cells create a lot more ROS so this cancer and ROS activities are related, including hematological malignancies in a variety of human cancers. However, there is significant debate over the function of oxidative stress in the emergence of leukemia malignancy and the efficacy of chemotherapy agents that work biologically by causing oxidative stress in the cells they are administered to [48].

Table (1) Comparison the serum levels of carboxymethyl-lysine (CML), pyrraline, Nitric Oxide (NO) and Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase, glutathione (GSH) in patients with leukemia and control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>479.3 (411.1, 545.4)</td>
<td>355.1 (322.1, 402.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pyrraline (pg/ml) (Median (IQR), 25%, 75 %)</td>
<td>155.5 (145.4, 182.3)</td>
<td>156.9 (137.3, 179.0)</td>
<td>0.8995</td>
</tr>
<tr>
<td>NO (μ mol/L) (Median (IQR), 25%, 75 %)</td>
<td>105.7 (91.2, 147.4)</td>
<td>119.3 (96.7, 192.6)</td>
<td>0.2289</td>
</tr>
<tr>
<td>MDA (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>175.7 (160.8, 191.8)</td>
<td>162.7 (145.1, 185.8)</td>
<td>0.0357</td>
</tr>
<tr>
<td>SOD (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>1.11 (0.89, 1.78)</td>
<td>0.97 (0.79, 1.26)</td>
<td>0.0891</td>
</tr>
<tr>
<td>CAT (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>6.857 (6.281, 7.425)</td>
<td>6.697 (6.088, 8.206)</td>
<td>0.8193</td>
</tr>
<tr>
<td>GSH (μg/ml) (Mean ± SE)</td>
<td>5.772 ± 0.3615</td>
<td>4.195 ± 0.5923</td>
<td>0.0194</td>
</tr>
</tbody>
</table>
The correlation analysis reveals that there is a significantly positive relation between serum CML and MDA ($r = 0.3487$) as shown in (Table 2 and Figure 2). Whereas, there are negative (but no significant) correlations between serum CML and
NO and CAT whose correlation coefficients (r) values are (r=0.1246), (r= -0.01338). There are positive (but not significant) correlations between serum CML and SOD, GSH, their correlation coefficients (r) values are (r= 0.1508, r= 0.2302) respectively, shown in (Table 2).

Table (2) Correlations analysis between CML with MDA, NO, SOD, CAT, GSH in patients with leukemia

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient (r) (Pearson correlation)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML and MDA</td>
<td>0.3487</td>
<td>0.0152</td>
</tr>
<tr>
<td>CML and NO</td>
<td>-0.1246</td>
<td>0.3989</td>
</tr>
<tr>
<td>CML and CAT</td>
<td>-0.01338</td>
<td>0.9289</td>
</tr>
<tr>
<td>CML and SOD</td>
<td>0.1508</td>
<td>0.3062</td>
</tr>
<tr>
<td>CML and GSH</td>
<td>0.2302</td>
<td>0.1154</td>
</tr>
</tbody>
</table>

Figure 2: Correlation analysis between serum CML and oxidative stress parameter (MDA) in patients with leukemia.

There was significantly positive correlation between serum pyrraline with MDA and GSH which the correlation coefficient (r) values are (r= 0.3838), (r= 0.3371) as shown in (table 3 and fig.3). There are positive (but no significant) correlations between
serum pyrraline with NO, CAT and SOD, the correlation coefficient (r) values for serum pyrraline with NO, CAT and SOD are (r = 0.1722, r= 0.02543, r= 0.001126) respectively shown in (Table 3).

Table (3) Correlations analysis between Pyrraline with MDA, NO, CAT, SOD, GSH in patients with Leukemia

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient (r) (Pearson correlation)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrraline and MDA</td>
<td>0.3838</td>
<td>0.0093</td>
</tr>
<tr>
<td>Pyrraline and NO</td>
<td>0.1722</td>
<td>0.2580</td>
</tr>
<tr>
<td>Pyrraline and CAT</td>
<td>0.02543</td>
<td>0.8698</td>
</tr>
<tr>
<td>Pyrraline and SOD</td>
<td>0.001126</td>
<td>0.9941</td>
</tr>
<tr>
<td>Pyrraline and GSH</td>
<td>0.3371</td>
<td>0.0236</td>
</tr>
</tbody>
</table>

Figure 3: Correlation analysis between serum pyrraline and oxidative stress parameter (MDA) and antioxidant parameter GSH in patients with leukemia
The ROC curve analysis is applied for serum CML and Pyrraline with the intent of define a potential biomarker for leukemia. The AUC value in serum CML 0.8390 that has a broad range that demonstrated greater sensitivity than specificity. The S.E value is 0.04894 and the 95% CI value is 0.7431 to 0.9349, (p <0.0001). While The AUC value in serum Pyrraline 0.5101. The S.E value is 0.08011 and the 95% CI value is 0.3530 to 0.6671. These data show that the serum CML is definitely a potential biomarker for leukemia as a result of the significant range of AUC. While, this study shows that pyrraline is not a good biomarker for leukemia as a compared with CML, because the AUC value is 0.5101. The ROC curve analysis is accomplished for serum NO and MDA with the intent of define a potential biomarker for leukemia. The AUC value in serum NO was 0.5843. The S.E value is 0.07192 and the 95% CI value are 0.4433 to 0.7252. While the AUC value in serum is MDA 0.6492, it that has a broad range, demonstrating greater sensitivity than specificity. The S.E and 95%CI values are 0.06910 and 0.5138 to 0.7846 (p<0.0361). These data show that the serum NO and MDA could not be good potential biomarkers for leukemia. The ROC curve analysis is implemented for serum SOD, CAT and GSH so as to determine a potential biomarker for leukemia. The AUC value in serum SOD 0.6168The S.E value is 0.06949 and the 95% CI value is 0.4806 to 0.7530. The AUC value in serum CAT 0.5156. The S.E value is 0.07138 and the 95% CI value is 0.3757 to 0.6556 The AUC value in serum GSH 0.6837 that exhibited a wide range and great sensitivity rather than specificity. The S.E value is 0.07071 and the 95% CI value is 0.5451 to 0.8223, (p<0.0101). These data found that the serum GSH is certainly a potential biomarker for leukemia because to the high range of AUC. Shown in table (Table 4).
Table (4) ROC curve study to assess clinical precision of CML, Pyrraline, MDA, NO, SOD, CAT, CH, GSH in patients with Leukemia.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC</th>
<th>S.E</th>
<th>95%CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>0.8390</td>
<td>0.04894</td>
<td>0.7431 to 0.9349</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pyrraline(pg/ml) (Median (IQR), 25%, 75 %)</td>
<td>0.5101</td>
<td>0.08011</td>
<td>0.3530 to 0.6671</td>
<td>0.8959</td>
</tr>
<tr>
<td>MDA (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>0.6492</td>
<td>0.06910</td>
<td>0.5138 to 0.7846</td>
<td>0.0361</td>
</tr>
<tr>
<td>NO (μmol/L) (Median (IQR), 25%, 75 %)</td>
<td>0.5843</td>
<td>0.07192</td>
<td>0.4433 to 0.7252</td>
<td>0.2262</td>
</tr>
<tr>
<td>SOD (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>0.6168</td>
<td>0.06949</td>
<td>0.4806 to 0.7530</td>
<td>0.0886</td>
</tr>
<tr>
<td>CAT (ng/ml) (Median (IQR), 25%, 75 %)</td>
<td>0.5156</td>
<td>0.07138</td>
<td>0.3757 to 0.6556</td>
<td>0.8163</td>
</tr>
<tr>
<td>GSH (μ g/ ml) (Mean ± S.E.M)</td>
<td>0.6837</td>
<td>0.07071</td>
<td>0.5451 to 0.8223</td>
<td>0.0101</td>
</tr>
</tbody>
</table>

Conclusion

The present study shows that the serum advanced glycation end product CML is significantly increased in leukemia patients compared to healthy group, while serum pyrraline level was more or less similar to control group. It is found that the serum oxidative stress parameter (MDA) is considerably higher in leukemia patients compared to control group, whereas the serum level of NO insignificantly decreased in comparing leukemia patients to the healthy controls. The results of this study show that the antioxidant parameter (GSH) is significantly increased in leukemia patients compared to control group, whereas, the median serum levels of SOD and CAT is insignificantly elevated in patient’s leukemia compared to control group. These data show that the serum CML is definitely a potential biomarker for leukemia, while the advanced glycation end product is a biomarker for development some specific cancers due to direct relation with increase inflammation.
References


alter response to therapy, and can be targeted by lifestyle intervention, *Breast cancer research and treatment*. 173, 559-571.


