AN EVALUATION OF THE OXIDATIVE STRESS INDEX IN SERA OF ACUTE MYELOID LEUKEMIA AND ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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ABSTRACT

The aim of the current study is to check the variation in serum TOS, TAC and OSI of leukemic patients of type AML & ALL. The study included 60 patients with leukemia, who were divided into two groups according to the type of leukemia: Acute Myeloid Leukemia (AML, n=30) and Acute Myeloid Leukemia (ALL, n=30). Age & gender matched healthy individuals, (C, n=70) were also included as control. Serum total oxidant status (TOS) and total antioxidant capacity (TAC) were measured using Erel's methods. Serum total Oxidant Status (TOS) and Oxidative Stress (OSI) levels were higher in AML and ALL patients (p-value <0.000) than in controls with a reduced levels of antioxidants in these patients. And with no difference in the all measured parameters (TOS, TAC, OSI) between both patients groups AML & ALL (P>0.05). The result in the current support the idea that there is a persistence of oxidative stress in both acute myeloid leukemia (AML) & acute lymphoblastic leukemia (ALL).

Keywords: Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Oxidative stress index, Total oxidant status, Total antioxidants capacity

INTRODUCTION

Generally cells belonging to both myeloid and lymphoid lineage are produced from hematopoietic stem cells during the process of haematopoiesis. Abnormal growing leukocytes that formed from stem cells in the bone marrow leads to a tumor condition commonly referred to as leukemia. This type of tumor is classified based on the type of blood cell influenced into two types: acute and chronic which may be classified according to the location of progression. According to the French-American-British model (FAB) of classification, acute leukemia is classified into two subtypes: acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), which affect groups of leukocytes called lymphocytes and myeloid respectively.

Acute myeloid leukemia (AML) is identified by an increase in the number of myeloid cells in the bone marrow and accumulation of the blast and uncontrolled proliferation factors which really affects...
differentiation. On the other hand, acutely lymphoblastic leukemia (ALL) is an ailment identified by uncontrolled diffusion and maturation arrest of lymphoid precursor cells in bone marrow resulting in an increase of malignant cells. It had been reported that Acute lymphoblastic leukemia ALL occurs approximately five times more frequently than AML and accounts for about 78% of all diagnosed childhood leukemias.

Reactive oxygen species (ROS) are free radicals derived from diatomic oxygen, with a wide spectrum of reactivity. They are known to play a double role in biological systems, where they may be either advantageous, or disadvantaged for living systems. Advantageous roles of ROS involve antimicrobial role during phagocytosis by cells of the innate immune system and also ROS which are generated by the mitochondria, or nicotinamide adenine dinucleotide phosphate (NADPH) oxidases demonstrated influence on cell-cycle progression, cell movement, and growth factor signaling in a diversity of ordinary cell types. Many pathologic states are accompanied by intolerable cellular ROS production and/or an inadequacy in antioxidant defenses, leading to a situation known as oxidative stress. Such stress may prevent, or enhance apoptosis and necrosis, depending on the intensity of their actions. It is well known that overproduction of ROS such as superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl anion (OH) beyond the ability of antioxidant defense systems to scavenge them may cause oxidative stress. These radical species have ability to peroxidize unsaturated bonds of membrane lipids, denature proteins and attack nucleic acids. These states may form the molecular baseline of many diseases including cancer.

Evidence for the presence of chronic oxidative stress has been reported in many cancers diseases, both in solid tumors such as prostate carcinoma, melanoma and in several hematopoietic malignancies including acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML) and acute myeloid leukemia (AML). Most of these studies carried on by measuring the changes in individual oxidants or antioxidants parameters. Free radical formation is naturally controlled by antioxidants, which are capable of deactivating, or stabilizing free radicals before they attack the different components of the cells. Since the measurement of all known antioxidants separately is time consuming and many antioxidants may remain undiscovered, in addition to that the total activity (TAC) may be greater than the sum of the individual antioxidants because of their cooperative interactions, therefore it was recommended that measurement of total antioxidant activity is better than measuring the individual antioxidant.

The aim of the current study is to check the variation in serum TOS, TAC and OSI of leukemic patients of type AML & ALL.

MATERIAL AND METHODS
CHEMICALS: All chemicals used in this study were of the highly analytical grade.

Studied Groups
Two groups of leukemia patients were included in the present project, 60 of them were leukemia (AML & ALL) patients of both genders with age ranged from (14 - 55 years), attending Hereditary
Hematology Center in Baghdad/Iraq, and 70 age and gender matched healthy individual to be used as a control group. All patients included in the present study were under treatments for different periods of time. They were receiving approximately 4 doses/month from chemical drugs (Hyper CVAD, &Cytosar).

The patients who were alcohol drinker, smoker, and also presented acute or chronic diseases such as diabetes, parasitosis or any immune dysfunction were excluded from the study.

The study protocol was proved by the Ethics Committee of College of Science / University of Baghdad.

**Measurement of total oxidant status [TOS]**

Erbel's method was used to determine total oxidant status value\(^{19}\). The assay was calibrated with hydrogen peroxide as standard solution. The absorbance's of the hydrogen peroxide standard solutions was measured at a wave length \(\lambda=560\) nm plotted against their concentration to construct the standard plot. The straight line equation derived from their standard curve was used to quantify TOS in the studied samples.

**Determination of total antioxidant capacity [TAC]**

The total antioxidant status value was determined using Erbel's method\(^{20}\). The assay was calibrated with vitamin C standard solution. The measured absorbance's at a wave length \(\lambda=444\) nm was plotted against their concentrations to construct the standard plot. The straight line equation derived from this standard curve was used to quantify TAC in the studied samples.

**Calculation of oxidative stress index (OSI)**

Oxidative stress index value was calculated from the below equation \(^{21}\). OSI (arbitrary unit) = TOS (\(\mu\text{mol H}_2\text{O}_2\text{ Eq/L}\)) / TAC (\(\mu\text{mol Vitamin C Eq/L}\)).

**Data analysis**

The data throughout this work was reported in the form of (mean value ± the standard deviation). The data were compared by SPSS version 20 (One-Way ANOVA), where the difference is considered as highly significant when \((P < 0.001)\), significant when \((P < 0.05)\) and no significant when \((P > 0.05)\).

**RESULTS**

The general characteristic of the current study groups are shown in Table (1).
Table 1: The mean value and standard deviation (mean ± SD) of different studied groups with distribution of their ages and gender.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Ages (year)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>30</td>
<td>32.466±11.12</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Control(C)</td>
<td>70</td>
<td>28.557±14.55</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>ALL</td>
<td>40</td>
<td>26.4±14.301</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Total Oxidant Status (TOS) {which can be defined as the *in vivo* marker of a shift developing in an oxidative/anti oxidative ratio in favor of the oxidative side}, was measured in serum of the different studied patients groups as aforementioned in materials & methods section and the results are presented in (Fig. 1).

**Fig. 1:** Comparison between levels of TOS among the different studied groups.

The above results show presence of a highly significant increase (p<0.000) in TOS in both studied patients groups compared with controls while no significant difference is obvious in this parameters between AML and ALL groups (p>0.05).

Total Antioxidant Capacity (TAC) {which is a biomarker for measuring the antioxidant potential of body fluids can be defined as the moles of oxidants neutralized by one liter of solution}, was measured in the studied groups as mentioned in methods & materials section and the results are shown in (Fig. 2).
Fig. 2: Comparison between levels of TAC among the different studied groups.

The results in (Fig. 2) show presence of a highly significant decrease (P<0.000) in TAC in the patients groups compared with that of control group while no significant (p>0.05) in TAC is found upon comparison of this parameters between AML and ALL.

The Oxidative Stress Index (OSI) which is the most accurate method to express OS$^{24}$ was calculated in serum samples of both patients groups, as mentioned above in the materials and methods section and (Fig. 3) shows the obtained results.

Fig. 3: Comparison between OSI among the different studied groups.

The above resultssindicate the percense of a highly significant increase (p<0.000) in OSI in both studied patients groups compared to that of controls. While no significant change in this pararmeter upon comparsion its level between AML and ALL groups is observed (p >0.05).
DISCUSSION

The uncontrolled growing of hematopoietic stem cells in the bone marrow lead to a common malignancy in individual which is known as Leukemia. This type of disease is originated from loosing the capacity of these stem cells to differentiate normally into mature blood cells. Several clinical studies reported that free radicals are involved in the molecular mechanism that underlie hematologic disorders. Disturbances of oxidative stress metabolism is reported to be one of the properties that characterize the tumor cells.

The highly significant increase (p<0.00) in TOS observed in both AML & ALL patients groups agrees with Battisti et al. study in which they reported the presence of a persistence oxidative stress in Brazilian children patients with acute lymphoblastic leukemia (ALL). In their study they measured plasmatic thiobarbituric acid (TBARS) reactive substance, serum protein carbonylation and other oxidative stress parameters and reported the presence of a high level of TBARS and protein carbonylation which indicated increased levels of cells alteration that is due to the presence oxidative stress.

The cumulation production of free radical is common for many types of cancer cell and is linked with alteration in the redox regulations of cellular signaling pathways. Leukemic cells are under repeated state of oxidative state blocked, which is due to their production of high amount of oxygen reactive species (ROS) compared with non leukemia cells.

As it is known and mentioned in the introduction section the effect of reactive oxygen and nitrogen species is balanced by presence of antioxidants which may be enzymatic (such as glutathion reductase, superoxide dismutase, catalase and glutathion oxidase), or non enzymatic chain breaker such as vitamin E.

Throughout the presente study, a highly significant decrease in TAC (p<0.00) is observed in both type of patients. This agree with Mahmood et al. study result, who measured the activity of many antioxidant enzymes, that including the above mentioned enzymes as well as vitamin E level in serum of Pakistanin patients with acute leukemia (AML & ALL). They reported a significant decrease in these enzymes activities in their patients studies group.

Furthermore our results agree with that Mate et al. result who studied the levels of antioxidant enzymes (superoxide dismutase and glutathion oxidase) in ALL compared with healthy control in Spain. As well as our results agree with Zhou et al. resultin which they showed that the activity of catalase and glutathion peroxidase is reduced in case of acute leukemia in comparsion to that of healthy control in a study carried on in China.

The measured impaired antioxidants support the accumulation of free radicals. Alternatively it is possible the antioxidant system is impaired as a consequence of an abnormality in the antioxidants metabolism due to the presence of cancer. This effect could be supported by the characteristic increase in hydrogen peroxide by the cancer cells.

The presence of the oxidative stress in our patients is clear from the result in (Fig.1&3). Such result is in a agreement with the result of a study carried on ALL patient in India by Ahmad et al. who reported that a high concentration of malondialdhyde (MDA&protein carbonylation in their studied patients group such increase in these parameters indicated the presence of high concentration of ROS with impaired antioxidant system. The accumulated ROS will attack the different biomolecules and damage the cells.
It is worth to mention, that our studied patients were under treatment for different period of time and the effect of this on the measured parameters was examined, the result showed there is no effect of this period up to one year on all measured (TOS, TAC and OSI) (results no shown). This agree with the result of Battisti et al. who also found that the period of treatment has no effect, suggesting that the increase of oxidative lesions seems not to be a result of the treatment with chemotherapeutic agents but may be involved with the pathogenesis of leukemia.

This oxidative stress may also be a result of the malnutrition in these patients due to under-nourished which result in that their bodies have low levels of vitamin A and vitamin E which act as antioxidant such decrease in both vitamins lead to impaired immune hematopoietic system.

CONCLUSION

According to the results of this study, oxidative stress is present in Iraq (AML&ALL) patients as a result of increase in the preduction of free radical species and decrease in the antioxidant (TAC) system. Furthermore no significant difference was observed in TOS, TAC and OSI between AML & ALL patient groups.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


