Oxidative stress and total antioxidant status in acute leukemia at diagnosis and post remission induction phase

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Abstract: This study evaluated the activity of superoxide dismutase (SOD1), glutathione reductase (GR) and total antioxidant status (TAS) in the hemolysate and sera of patients with acute leukemia (AL) at diagnosis, post remission induction phase and in healthy controls. However, total antioxidant status and glutathione reductase activities normalized after remission induction phase in acute myeloid leukemia (AML) only whereas levels of SOD were reduced but not achieved the normal level in acute lymphoblastic leukemia (ALL). TAS activity showed no difference in either sex among any subtype of acute leukemia but glutathione reductase level was significantly higher in female ALL patients. Activity of SOD was elevated in T-cell ALL and acute myelomonocytic leukemia however; no significant difference in the activity of GR and TAS was noted. Levels of antioxidant were reduced insignificantly in patients who achieved complete remission.

Keywords: Superoxide dismutase, total antioxidant status, oxidative stress in acute leukemia.

INTRODUCTION

Acute leukemia is characterized by cessation of differentiation and clonal proliferation of the hematopoietic progenitor cells. Acute leukemia is classified into myeloid (AML) and lymphoid (ALL) lineage on the basis of the type of cell involved (Sultan, 1987; Cranfield, 1995). AML is more common in adults whereas ALL more commonly found in children (American cancer society, 2006; Lowenberg *et al*., 1999). Pediatric ALL is treated conventionally with chemotherapy; however, cure rate does not exceed more than 35% in developing countries as compared to 92% cure rate reported from the West (Mostert *et al*., 2006). Chemotherapy induced toxicity, infections and hemorrhage are the major causes of treatment related mortality in acute leukemia. There are many factors for poor outcome in developing countries including delay in diagnosis, suboptimal monitoring, poor socioeconomic status, malnutrition, abandonment of therapy (Yellon and Hausenloy, 2007). Antioxidants play an important role in normal cell differentiation (Martignoni *et al*., 2003). It is a well known fact that activated oxygen species (AOS) are generated as a result of oxygen metabolism and energy production; they are also implicated in tumour progression (Valko *et al*., 2007; Navarro *et al*., 1997). On the other hand antioxidants have anti carcinogenic potential. Superoxide or Hydrogen peroxide can increase the growth, as well as cell death eventually (Oberley *et al*., 1995; Susuki *et al*., 1997). Literature provided an evidence that Superoxide anion and hydrogen peroxide have an ability to play a role of an intra and intercellular messengers increase cellular proliferation (Valko *et al*., 2007; Navarro *et al*., 1997; Burdon, 1995). An increase in ROS production and decrease in antioxidants levels may induce oxidative stress (Sarmento-Ribeiro *et al*., 2012). In acute leukemia, increased ROS formation may introduce a signal transduction pathway to induce leukemic cells to proliferate but also lead to apoptosis. Serum contains a number of many different enzymatic and non-enzymatic antioxidants such as ascorbic acid, alpha tocopherol, beta carotene, uric acid, cortisol and albumin. Enzymatic antioxidants include Glutathione peroxidase, Glutathione reductase, Catalase and Superoxide dismutase (SOD) (Sarmento-Ribeiro *et al*., 2012; Al-Tonbary *et al*., 2009). These antioxidants act as a cofactor in a number of metabolic reactions. In human SOD is found in type 1, 2 and 3 which are located in cytoplasm, mitochondria and extracellular respectively.SOD 1 and 3 contain copper and zinc, whereas SOD2 has manganese.

A major role of reduced glutathione (GSH) in erythrocytes is the prevention of hemoglobin denaturation, provide erythrocyte's membrane integrity and detoxification of reactive oxygen species in red blood cells. GSH is the product of reaction catalyzed by Glutathione Reductase (GR). Decreased level of Glutathione has been reported in many diseases. Many antineoplastic agents such as Methotrexate are known to produce adverse effects on GR enzyme activity (Akkemik *et al*., 2011). The enzymatic levels of SOD are altered to a considerable extent in various diseases. In previous studies in all types of leukemia, SOD enzyme level in leucocytes was reported to be significantly higher and decreased to normal when patients achieve remission. No relationship was reported between SOD level and the type of leukemias (Kokoglu *et al*., 1989; Nishiura *et al*., 1992). Increased generation of $O_2^{\cdot}$ by leukocytes and higher

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activities of SOD and glutathione peroxidase in erythrocytes were unique features of AML (Er *et al*., 2007).

To our knowledge and through literature survey there is no published data on antioxidants in leukemia from Pakistan. This prompted us to plan this study to evaluate the impact of antioxidants in healthy individuals, acute myeloid leukemia and acute lymphoblastic leukemia patients at time of diagnosis and post- remission induction chemotherapy.

MATERIALS AND METHODS

Setting: This study was carried out in the department of biochemistry, University of Karachi, Pakistan and Department of Hematology, National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan during May 2011 to March 2012. Study protocol was approved by institutional review board. Written informed consent was obtained from adult patients/ controls and from legal guardians of minors.

Study population

Sample Size: Total 126 subjects were enrolled including patients and controls.

Subjects: Study individuals were divided in the following manners

C: Age matched Control (n=40)

NDM: Newly diagnosed cases of acute myeloid leukemia (n=20)

RIM: Remission Induction in acute myeloid leukemia (n=16)

NDL: Newly diagnosed cases of acute lymphoblastic leukemia (n=25)

RIL: Remission induction in acute lymphoblastic leukemia (n=25)

Peripheral blood samples were analyzed for the estimation of superoxide dismutase (SOD1) in erythrocytes while glutathione reductase (GR) & total antioxidant status (TAS) measured in serum using Randox-Ransod, Glut-Red and TAS (Randox laboratories, Crumlin, Antrim, UK) respectively. Diagnosis of acute leukaemia was made by a hematopathologist and verified by a panel of hematologists who also classified each case according to the WHO classification (Vardiman *et al*., 2008). Remission induction chemotherapy [Hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, and Dexamethasone (Hyper-CVAD)] regimen is the standard of care for acute lymphoblastic leukemia (ALL) in adults (18-64 years) (Ito *et al*., 1996). For paediatric age group (1-18 years) UKALL XI protocol for remission induction phase (1-28 days) (Hann *et al*., 1998) is the standard of care at our centre. For AML, Daunorubicin and Cytosine Arabinoside (3+7) is the standard remission induction therapy for AML patients.

Sample collection and storage

10cc venous blood was drawn from controls and all patients at the time of diagnosis and at day 28 post chemotherapy. Whole blood was distributed into following manner (5cc blood in a tube with no additive and 4cc in vacuette containing lithium heparin from BD). All tubes were kept in cold box at 4^{o}C. Serum and heparinized erythrocytes were separated by centrifugation at 600 x g for 10 minutes at 4^{o}C within 1 hour of blood collection.

Sample preparation

Serum were stored in red coded cryovials at –80^{o}C and used for TAS and GR analysis. Packed red cells were then washed for 3 times with 0.9% normal saline. The packed erythrocytes were hemolysed with 0.5 ml of distilled water. Then, 0.25 ml of cold 95% ethanol plus 0.15 ml of cold chloroform were added to the hemolysate under vigorous stirring (20 min at 0^{o}C), and then centrifuged at 850 ×g for 5 min at 4^{o}C. The clear supernatant was saved in blue coded cryovials at -80^{o}C (McCord and Fridovich, 1969). All samples were run in batches of 30.

Analytical methods

Hematological Determinations: Complete blood count (CBC) was performed on automated hematology analyzer; Sysmex XE-2100 (Sysmex Kobe, Japan).

Estimation of superoxide dismutase activity in erythrocytes

Commercial Randox-Ransod kit (Randox Laboratories, Crumlin, and Antrim, UK) was used for evaluation of Cu, ZnSOD activity based on the method developed by McCord and Fridovich (Mccord and Fridovich, 1969). Manufacturer's instructions were followed to determine the activity. Xanthine produces superoxide radicals in the presence of xanthine oxidase which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. One unit of SOD caused 50% inhibition of the rate of reduction of INT under the conditions of the assay.

Absorbance of standard and test samples was measured at 510 nm with different time interval. Difference of absorbance per minute of standard (std), sample and sample diluent (S_1) and then % inhibition after conversion of individual rates into % sample diluent rate and subtraction 100% was calculated. Multiplication of dilution factor to SOD units per ml from standard curve and divided this value by gm hemoglobin per ml to get SOD unit/gram of hemoglobin which was calculated by given step by step calculation.

$100 - (\Delta A_{\text{std OR sample /min x 100}}) \div \Delta A_{\text{S1/min}} = \%$ inhibition

SOD units /ml from standard curve x dilution factor = $SOD_{\text{units/ml}}$ of whole blood

SOD units/ml ÷ gram Haemoglobin/ml = SOD units/g Haemoglobin

Table 1: Demographic and hematological feature

Study population					
Variable	Healthy individuals	AML		ALL	
Groups N	C 40	NDM 20	RIM 16	NDL 25	RIL 25
Age (yrs)	29.5±4.5 (24-38)	30.8±13 (15-65)	29.0±18 (4-50)	21.6±11.1 (1–38)	19.9±13.3 (1–59)
Male	20 (50%)	10 (50%)	09 (56.2%)	19 (76%)	18 (72%)
Female	20 (50%)	10 (50%)	07 (43.7%)	06 (24%)	07 (28%)
Hb (g/dl)	14.6±0.8 (12.4-16.7)	9.1±1.7 (6.2-12)	10.3±0.8 (9.2-11.9)	9.2±1.7 (5.9-2.7)	10.7±2.2 (7.6-6.1)
White Cells $x10^9$/L	7.2±1.35 (4.5-11.8)	48.6±40.6 (0.38-121.7)	2.3±4.4 (0.2±16.8)	48.6±40.6 (0.3-121.7)	6.9±7.6 (0.08-7.0)
Platelet $x10^9$/L	255.7±43.1 (142-360)	24.5±15.8 (3-59)	76.2±72.7 (10-253)	33.9±38.9 (5-183)	181±156 (7-573)
Blast%	NP	72.5±32.6 (7-100)	1.2±2.6 (0-07)	51.2±29.6 (8–96)	1.2±2.6 (0-07)

General characteristics including age, gender and hematological feature including leukemic cells are documented in acute leukemia patients. All quantitative variables are expressed as mean ± SD, minimum and maximum value. Qualitative variables are mentioned in number and frequency in percentage. Number of participants is labeled with 'n'. NP (not Possible), C; control, ND; newly diagnosed acute leukemia, NDL; newly diagnosed ALL, NDM; newly diagnosed AML, RI; remission-induction acute leukemia; RIL; remission-induction ALL, RIM; remission-induction AML.

Estimation of glutathione reductase activity in serum

Measurement of GR activity was evaluated by using the commercial Randox-Glut Red kit (Randox Laboratories, Crumlin, Antrim, UK). Manufacturer's instructions were followed to determine the activity. This method is based on the methods of Goldberg and Spooner (1983). The GR and NADPH, the oxidized glutathione (GSSG) were converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The change in absorbance at 340 nm was measured. The results were expressed in unit /liter (U/L).

Estimation of total antioxidant status in serum

TAS levels were measured by the commercial kit Randox-TAS (Randox Laboratories, Crumlin, Antrim, UK) based on the method of Miller (1993). Manufacturer's instructions were followed to determine the activity. In this method, ABTS® (2,2′-azino-di-3-ethylbenzothiazoline-6-sulphonate) was incubated with peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTSU+. This product has a relatively stable blue-green color, which is measured at 600 nm. The antioxidants in the plasma sample suppress color production to a degree proportional to their concentration. Millimole per liter (mmol/l) unit was using for expression of results. All antioxidants were measured on X series instrument Rx Daytona after running control and calibration.

Data analyses

Statistical package for social sciences (SPSS) version 17 was used for data compilation and analysis. Descriptive statistics were applied and Mean ± SD, minimum and maximum range, 95% confidence were calculated for quantitative while frequency and percentages were calculated for qualitative variables. Numbers of participants are denoted in parenthesis. Patients were divided into two groups i.e. control and cases further, the patients were grouped as ALL and AML. Independent sample t-test was used to evaluate mean differences between control and cases of both types of acute leukemia as well as in male and female groups. One way ANOVA was applied to test the mean differences in between stratified variables of defined groups or in the subtypes of AML and ALL patients. P-value <0.05 was taken as significant.

RESULTS

Antioxidant levels were evaluated in AML and ALL patients prior to and after chemotherapy. Table 1 describes the demographic and hematological features of acute leukemia patients in newly diagnosed, after remission induction phase of chemotherapy and in control subjects. Of 126 study subjects, 86 were AL patients including 60 males and 26 females. Mean age was 30.8±13. Forty-eight patients were newly diagnosed while 38 received remission induction. Mean leukemic cell and white blood

Table 2: Association between antioxidant level and gender, blast count, age respectively in ALL and AML

Variables	N (ALL/AML)	SOD	GR	TAS
Female	13/17	14293/10238	147.7/141	1.5/1.7
Male	37/19	18217/16229	107/119	1.5/1.8
P Value		0.3/0.1	0.03/0.4	0.9/0.5
Blast Stratification P Value	50/30	0.1/0.2	0.7/0.5	0.2/0.1
Age Group P Value	50/30	0.5/0.3	0.9/0.3	0.7/0.9

Impact of Superoxide dismutase (SOD), Total antioxidant Status (TAS) and Glutathione Reductase (GR) are expressed in terms of mean. Independent sample T-test was used to evaluate mean differences of antioxidants in between male and female of AML and ALL groups. One way ANOVA was applied to test mean differences of antioxidants in stratified age group (<10, 11-20, 21-30, 31-40, >50 years) and blast count (1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100%) respectively. N indicates number of patients of acute lymphoblastic leukemia/acute myeloid leukemia. P-value <0.05 was taken as significant.

Table 3: Antioxidant levels in subtypes of acute leukemia

Subtypes	SOD	TAS	GR
ALL			
CALLA +ve (Navarro et al., 1997)	13307.1 ±1786	1.54±0.1	38.4±17
T- cell(Sarmento-Ribeiro et al.,2012)	29424 ± 835	1.4 ±0.2	98.7±49.5
B - cell (Mceligot et al., 2005)	14920 ± 78	1.7 ±0.2	14.3±3.3
P value	0.02	0.35	0.46
AML			
Acute leukemia without maturation (Ito et al.,1998)	13851±2914	1.61±0.0	74.9±22.6
Acute leukemia with maturation (Martignoni et al.,2003)	16531±1914	1.7±0.1	33±24.5
Acute myelo monocytic leukemia (American cancer society, 2006)	23708±42	2.2±0.6	50.7± 43.3
APML (American cancer society, 2006)	10417±4123	1.6±0.2	100.5±42.6
Acute erythroid leukemia (Cranfield, 1995)	12953±1214	1.6±0.3	48±32.7
P Value	0.02	0.27	0.20

Antioxidant levels including superoxide dismutase (SOD), glutathione reductase (GR) and total antioxidant status (TAS) are denoted as mean±SE, minimum and maximum value in subtypes of acute lymphoblastic leukemia including Precursor B CALLA positive, B-Cell, T-Cell and acute myeloid leukemia including acute leukemia without maturation, acute leukemia with maturation, acute myelomonocytic leukemia and acute erythroid leukemia. n= number of patients in subtypes of acute leukemia. One way ANOVA was applied to test the mean differences of defined antioxidant enzymes in between different subtypes of ALL or AML ($p<0.05$).

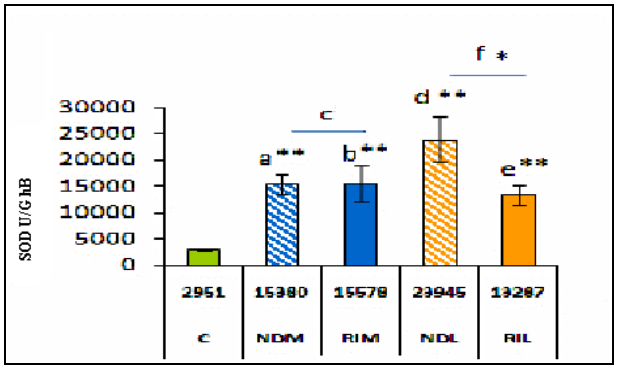
cell counts (WBC's) were high in ND patients (60.5±32.6) as compared to RI (4.3±4.45) patients while hemoglobin and platelet counts were reduced in ND as compared to C group. However, leukemic cells and WBC's were decreased, hemoglobin and platelet count were increased in RI patients as compared to ND.

Association between antioxidant levels with age, gender and blast count is documented in table 2 according to the subtypes (ALL and AML) of acute leukemia. Mean levels of SOD were significantly higher in T-lineage acute lymphoblastic leukemia (T-ALL) and in acute myelomonocytic leukemia ($p<0.05$). There was no statistically significant difference in GR levels in any type of acute leukemia but higher levels were found in female patients of ALL ($p<0.05$). Mean levels of SOD, GR and TAS showed no significant impact with respect to stratified age group (<10, 11-20, 21-30, 31-40, >50 years) and blast cell counts (1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100%) in ALL and AML($p>0.05$). Expression of antioxidant enzymes (SOD, GR and TAS were higher in those patients who achieved complete remission (<5% blast in bone marrow) relatively who had insufficient remission but statistically was not significant (table 4).

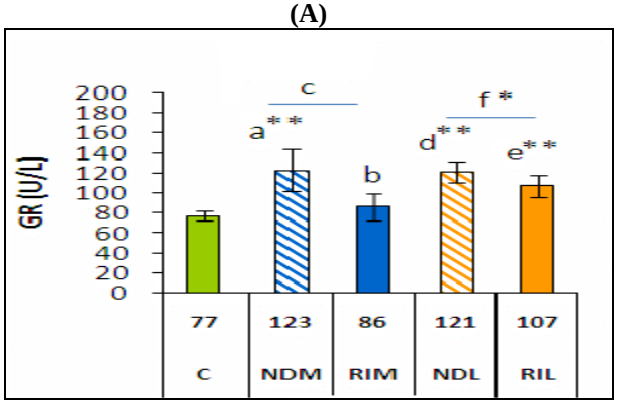
Significantly increased SOD activity was noted in ND and RI patients of both types of acute leukemia as compared to C group (<0.05) (fig. 1A). The levels of SOD were decreased in RIL patients but did not reach the normal levels. However, activity of SOD were mildly reduced in NDM versus RIM group but no significant difference was observed ($p>0.05$). Increased levels of glutathione reductase were noticed in NDM and NDL in comparison to C group ($p<0.05$) whereas decreased in RIM, RIL and compete the normal level in RIM only but not achieved in RIL patients. Statistically significant difference was observed in activity of glutathione reductase between ND and RI patients of acute leukemia ($p<0.05$) relatively in case of AML no difference was found.

There was mild increment in TAS level noticed in untreated cases NDM and NDL as compared to control group while in RIL patients the levels were almost revert to normal significantly ($p<0.0$) (fig. 1A). Notably, no difference was observed in RIM patients and in between

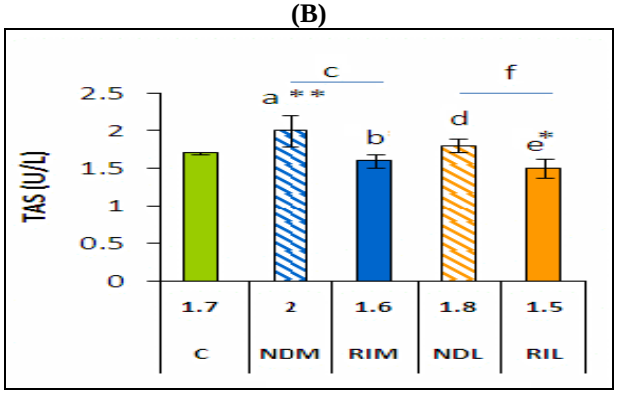
NDM, versus RIM, NDL versus RIL patients (p>0.05) (fig. 1C).



(A)



(B)



(C)

Fig. 1: Numerical values of Superoxide dismutase (SOD) in erythrocytes, glutathione reductase (GR) and Total antioxidant status (TAS) in sera of the patients with acute leukemia are shown as mean ± S.E. Alphabetical notation indicates comparison between two groups, * shows level of significance p<0.05 and ** p<0.01.

Independent sample t-test was used to see the significant mean differences of antioxidant enzymes in between control and cases as well as in AML and ALL groups. [a]NDM vs C (Newly diagnosed acute myeloid leukemia versus control), [b]RIM vs C (Remission induction cases of acute myeloid leukemia versus control), [c]NDM vs RIM, [d]NDL vs C (Newly diagnosed acute lymphoblastic leukemia versus control), [e]RIL vs C(Remission induction cases of acute lymphoblastic leukemia versus control), [f]NDL vs RIL.

DISCUSSION

Acute leukemia is a hyper catabolic state in which excess generation of oxygen derived radicals compromise the antioxidant defense system thereby leads to oxidative stress (Valko *et al*., 2007; Toyokuni *et al*., 1995). The function of phagocytes is impaired in leukemic patients who are already vulnerable to various infections (Iacobini *et al*., 1995). Many authors have published altered levels of antioxidants such as SOD, catalase, vitamin E and thiols in different types of human cancer (McEligot *et al*., 2005). Published reports have contradictory findings about the levels of antioxidants in hematological malignancies before and after exposure to antineoplastic agents and unable to find out the exact mechanism. For this reason we evaluated SOD, GR and TAS activity in acute lymphoblastic and myeloid leukemia patients at presentation and the impact of chemotherapy on their activities (fig. 1).The antioxidant defense system is classified as primary which prevent oxidant formation, secondary that Scavenges ROS and tertiary which produces it's effect by removing or repairing oxidatively modified molecules (Gutteridge and Halliwell, 2000). The primary antioxidant includes SOD and glutathione peroxidase which inhibit the production of reactive oxygen species (ROS). Secondary antioxidants include vitamin E, vitamin C, beta carotene, uric acid, bilirubin and albumin. Tertiary antioxidants are responsible for maintenance of damaged biomolecules by free radicals (Jacob, 1995). Many studies indicate erythrocytes as an ideal cell for the determination of the oxidant – antioxidant imbalance because of defined life span lacking the ability to synthesize the protein de novo and is more prone to oxidation induced damage (Navarro *et al*., 1997; Glass and Gershon, 1984). We found increased activity of SOD in erythrocytes in untreated and treated patients of AML and ALL as compared to control population (fig. 1A). However, in AML patients prior to chemotherapy, levels were less in comparison to post-remission induction phase. Similarly a research group reported increased activity of SOD in erythrocytes of patients of AML before treatment (Er *et al*., 2007). In contrast, elevated level of SOD in the sera of acute leukemia patients was reported by another group which was reduced after chemotherapy (Nishiura *et al*., 1992). Contradictory to this, SOD activities were shown to be decreased in ALL patients prior to chemotherapy and after remission induction as compared to control (Battisti *et al*., 2008). It is also important to mention here that the method, we have used for SOD determination only determines Cu/Zn SOD. Furthermore SOD was not measured in the leukemic cells. And also we haven`t done the levels of Reactive Oxygen Species and rely on the Total antioxidant levels and the enzymatic antioxidants to prove the oxidative stress in the light of our results.

Elevated levels of SOD in untreated AML and ALL patients can be explained as a physiological response to

Table 4: Impact of antioxidants with respect to status of disease in Post remission induction phase

Remission Status	N	SOD	GR	TAS
Complete Remission	28	14413±7118	89.0±45.3	1.5±0.4
Partial Remission	10	16916±9462	100.3±35.7	1.6±0.3
P Value		0.99	0.74	0.26

Less than 5% blast cells in marrow indicate complete remission (Larson, 2012). Antioxidant enzyme activities are mentioned in terms of mean ± SD. Independent sample t - test was used to see the significant mean difference of antioxidant enzyme in between the complete and partial remission status of the disease in post induction phase of chemotherapy in patients of acute leukemia ($p<0.05$).

oxidative stress due to the leukemic cells. Increased superoxide anion leaks out of the leukemic lymphocytes and enter erythrocytes where it is involved in the activation of SOD (Nishiura *et al*., 1992; Er *et al*., 2007). Intracellular SOD have been shown to have pivotal role in leukemic and cancer cells against $O2^{-}$- and chemotherapeutic agent (Iacobini *et al*., 1995) . Moreover decreased in SOD levels in treated ALL patients might be the reason for increased generation of free radicals and hence apoptosis of leukemic cells as free radicals are known to induced apoptosis in human leukemic cells due to inhibition of SOD by antileukemic regimens (Battisti *et al*., 2008; Huang *et al*., 2000). Elevated levels of SOD in treated AML patients can be explained as a protective response of antineoplastic agents via reducing oxidative stress (Poongothai *et al*., 2004). Glutathione reductase belongs to non enzymatic defense system which converts glutathione disulfide to the sulfhydryl form using NADPH that is required to reduce GSSG to GSH. It is an important cellular antioxidant. GSH is responsible for synthesis and repairing of DNA (Sattler *et al*., 2000). There is limited data available on serum glutathione reductase levels in acute leukemia. We found increase level of GR in untreated ALL and AML patients as compared to control group (fig. 1b). These results are in agreement with the findings of some researcher, indicates increased activities of glutathione were noticed in patients of acute leukemia (Oltra *et al*., 2001; Ferraris *et al*., 1994; Beutler and Gelbart, 1985). Many authors documented decreased or no change in the activity of glutathione in untreated patients of acute and chronic leukemia (Sarmento-Ribeiro *et al*., 2012; Bakan *et al*., 2003). The antioxidant system was suppressed due to larger amounts of free radicals in cancer cells. Glutathione reductase significantly reduced in post remission induction therapy and reached to normal levels in ALL patients but did not show the similar findings in AML. We can suggest that the GR level were reduced by chemotherapy as oxidant activities of leukemic cells limited by given treatment. Depletion of intracellular glutathione is the prime hallmark of the progression of apoptosis (Pujari *et al*., 2011). Redox state of glutathione normalized by the activity of depleting (GPx) and replenishing enzymes (GR) may be altered by AOS (Bakan *et al*., 2003). Variation of glutathione in malignancy has not been well established. TAS measures total antioxidant effect of defense system in circulation. It provides a tool between antioxidant capacity and risk of disease as well as for monitoring antioxidant therapy (Miller *et al*., 1993).

TAS measures total antioxidant effect of defense system in circulation. It provides a tool between antioxidant capacity and risk of disease as well as for monitoring antioxidant therapy (Miller *et al*., 1993). Gerova *et al.,* in 2006 found normal TAS level in the pre-treatment phase as well as in complete remission in patients of AML, while we found significant increased level of TAS in untreated patients of AML and mild increment in treated cases of ALL as compared to control population. No significant difference was noted in between untreated and treated cases of ALL and AML (fig. 1C). Er *et al*., in 2007 also found non-significant TAS level in AML patients compared with healthy individual. In patients of ALL, the data on variation in TAS activities is scanty. One reason may be that the method of TAS determination used measures all the primary, secondary and tertiary antioxidants in the sample and gives us a generalized picture of antioxidants; we therefore cannot predict that which antioxidant component amongst all is increased or decreased to produce an effect on overall antioxidants level in treated and untreated cases. We were not able to find any significant correlation between antioxidant enzymes and age, this cannot be well generalized and commented safely because we do not have equal number of patients in each age group e.g. for ALL about 11 patients of age group less than 10yrs, 12 of 11-20yrs, 18 of 21-30yrs, 7 of 31-40yrs and 1 for 41-50yrs and above 50yrs whereas in case of AML 4 patients of less than 10yrs, 5 of 11-20yrs, 6 of 21-30yrs, 6 of 31-40 yrs 8 of 41-50yrs and 1 of greater than 50yrs were recruited. Though we observed variation in antioxidants enzyme levels especially in SOD and GR for ALL in age group 11-20yrs where these enzymes were least of all and prominent mean difference was observed in but overall the difference was statistically insignificant. Poongothai *et al* showed similar findings (Poongothai *et al*., 2004).

Increase in oxidative stress is also mentioned to be an important factors in aging process and in progression of various diseases like cancers. Significant difference of GR is observed in females as compared to males but not significant in case of SOD and TAS levels it may be due

to the difference in sample size because less sample size was used in case of females i.e. n=13 whereas almost three times more samples of males were used n=37. Pujari *et al* reported significant differences in SOD levels in both sexes in chronic leukemia but not in acute leukemia patients (Pujari *et al*., 2011). Antioxidant levels were not found significant with difference of blast count. Increased levels of SOD were found in T-cell ALL and acute myelomonocytic leukemia among subtypes of acute leukemia in our study. The increase in the concentration of the SOD in erythrocytes in these malignant conditions may be because the concentration of the SOD levels is increased in case of Lymphocytes and Monocytes as compared to other blood cells Kato *et al*, as the number of the premature cells of this lineage increases in these leukemia`s so the levels of these enzymes also increases significantly. Similarly, increased levels of SOD were documented in ALL subgroups by Poongothai *et al* (2008) which was consistent to our findings but did not find any variation in the AML; the author indicated inefficient antioxidant mechanism to combat the severity of the disease. In the study Level of antioxidants was not found significant with difference of remission status while levels of all documented antioxidants were decreased in those who had complete remission as compared to insufficient remission status ($p<0.05$) (table 3). Mazzone *et al.* reported that under an unstimulated condition, the generation of $O2^{-}$ in blast cells were also increased (Mazzone *et al*., 1986).

CONCLUSION

Though the sample size is not sufficient to generalize our findings still on the basis of the results we suggests that there is an association between alteration of enzyme levels and AML and ALL in pre-chemotherapy and post-remission induction phase. The current findings provide evidence that impaired antioxidant system may support the accumulation of free radicals and an abnormality in the oxidative metabolism in patients with acute leukemia. Anti neoplastic agents decrease the levels of antioxidant enzyme SOD, GR and TAS. This may be a protective effect of chemotherapy against ROS system. Free radicals generate antioxidant damage in leukemic cells. Our study may give an opportunity to produce new therapeutic strategies for the inhibition of leukemic cells and increase the chemosensitivity by modulating antioxidant levels. Furthermore more studies with sufficient sample size is needed for true representation of the diseased population.

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