

## RESEARCH ARTICLE

# Correlation of Inhibin and Several Antioxidants in Children with Acute Lymphoblastic Leukemia

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### Abstract

**Background:** Acute lymphoblastic leukemia (ALL) is most common in childhood. Inhibin (a non-steroidal glycoprotein hormone of gonadal origin) can be used as marker of fertility. The current study was conducted to evaluate inhibin levels in ALL patients and to estimate its correlation with some antioxidants in these in comparison with control subjects. **Materials and Methods:** This study was conducted on sixty patients with ALL and thirty children as controls. Fasting blood samples were taken from each subject and analyzed for haemoglobin, serum protein, vitamin E and C, in addition to glutathione and inhibin. **Results:** The results of the study showed highly significant decreases ( $p < 0.001$ ) in haemoglobin, glutathione and inhibin levels with significant decreases ( $p < 0.05$ ) in serum protein and vitamin E levels for patients group in comparison with controls while there was no significant differences in vitamin C. Moreover, there were significant correlations between inhibin levels and serum protein, glutathione and both vitamins (E and C) in the ALL patient group ( $r = 0.81, 0.80, 0.77$  and  $0.69$ , respectively). **Conclusions:** The present results indicated infertility in patients with ALL demonstrated by low inhibin level as a consequence of abnormality in anti-oxidative metabolism due to the cancer process. So, it can be suggested the need for routine measurement of inhibin for leukemic patients to estimate the action of hormones of gonadal origin.

**Keywords:** Acute lymphoblastic leukemia - infertility - inhibin - glutathione - vitamin e - vitamin c

*Asian Pac J Cancer Prev*, 15 (12), 4843-4846

### Introduction

Leukemia is the most regular form of cancer in children less than fourteen years (Wiemels et al., 2010) Accounting for nearly one-third of all childhood neoplasms in developed countries (Rudant et al., 2010; Wiemels et al., 2012). Two main types characterize leukemia, namely acute lymphoblastic leukemia (ALL), which is the most common type, and acute myeloblastic leukemia (AML) (Diamantaras et al., 2013) ,it represented 80% of leukemia at this period of life, whilst lymphoblastic lymphoma reports for 30% of pediatric lymphomas (Bhardwaj et al., 2012). Breastfeeding was not associated with risk of childhood leukemia and there may be some other environmental and genetic factors that might be responsible for the occurrence of this disease and must be explored further (Waly et al., 2011), while residence near very high voltage overhead power lines, in distances  $\leq 500$  meters, and Magnetic Fields  $> 0.45 \mu\text{T}$ , should be considered a risk factor for the pathogenesis of acute leukaemias in children (Feizi and Arabi, 2007), meanwhile secreted frizzled-related protein gene methylation may be involved in AL progression (Shen et al., 2011). The rate of being relapse-free in ALL children treated under the Thai

national protocol at Srinagarind Hospital was better than with former protocols; however, it is still not as good as in developed countries. (Tharnprisan et al., 2013).

Inhibin is a non-steroidal glycoprotein hormone of gonadal origin with chief action as negative feedback control of the production of Follicle Stimulating Hormone (FSH) by anterior pituitary gland which in order modulates male and female reproductive functions (Chand et al., 2010), it is a marker of sertoli cell (Cavallini, 2006) and the granulosa cell functions (Seifer et al., 1997).

Any species have unpaired electrons are called free radicals, they are usually unstable and highly reactive excessive generation free radicals can result in An imbalance between oxidants and antioxidants, the two terms of the equation that defines oxidative stress, the basic tenet of several pathophysiological states, such as neurodegeneration, cancer, and aging (Yoshikawa and Naito, 2002). The most common antioxidant in the brain, glutathione, is found in mill molar concentrations in most cells (Chava et al., 2012 ). Glutathione (GSH) is an important scavenger of Reactive Oxygen Species (ROS), precursor of metal chelating phytochelatin, xenobiotic defense compound and controller of cell proliferation (Pasternaka et al., 2014). Vitamin C (L-ascorbic acid, AA)

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was isolated and proven to have anti-scorbutic properties by King and Waugh (King and Waugh, 1932), it is a powerful water soluble antioxidant which guards low density lipoproteins from oxidation, decreases harmful oxidants in the stomach and promotes iron absorption (Darias et al., 2011a). Vitamin C is a water soluble vitamin required for the formation of collagen and cartilage, as well as bone formation and remodeling (Mehdi et al., 2013; Darias et al., 2011b). Meanwhile Vitamin E is the mutual name for a group of fat soluble compounds with distinctive antioxidant activities (Salama, 2013), it is found naturally in some foods, added to others, and available as a dietary complement. As a free radical scavenger and lipophilic molecule, vitamin E may protect the membranes from oxidative damage by reacting with fatty acid peroxides by electron transfer (Traber and Atkinson, 2007; Ernst et al., 2013), also, it may regulate gene expression (Azzi, 2007; Rimbach et al., 2010 )

The aim of this study was to evaluate the correlation between inhibin (as the indices of infertility) and some antioxidant in the blood of ALL patients.

### Materials and Methods

The sampling procedure was done in sixty patients (7.23±3.24years )with Acute lymphoblastic leukemia (ALL) as they were succumbed to the Protection of Children Hospital Medical City in Baghdad, Iraq .The diagnosis for ALL based on the subsequent findings: leukocyte count, involvement of tissues other than bone marrow. None of these patients received antioxidant medicines or foods. Patients were compared with 30 healthy control subjects (6.92±4.10year). Five milliliters were collected from each subject by vein puncture, centrifuged at 3000 rpm for 5 min after allowing the blood to clot at room temperature. The total serum protein was measured by spectrophotometric methods supplied by Giese Diagnostic. Glutathione was estimated by the method of Beutler’s method (Beutler et al., 1963). Ascorbic acid levels were estimated by the method of Tietz (Tietz, 1986), and vitamin E levels were determined according to a modified of Hashim and Schuttringer (Hashim, and Schuttringer, 1966).The serum inhibin was measured by Enzyme Linked Immunosorbent Assay (ELISA)(Cusabio Biotech Com), and then protein electrophoresis have been done by polyacrylamide gel 7.5%. Protein was stained with coomassie brilliant blue G-250.

All statistical analyses in studies were performed

**Table 1. Some Hematological and Biochemical Parameters Levels in the Control and Patients Groups Included in this Study (Mean±SD)**

Characteristic	Patients group (n=60)	Control group (n=30)	p value
Age [year]	7.23±3.24	6.92±4.10	>0.05
Hb[g/dl]	8.63±1.47	11.73±0.67	<0.001
S. Protein[g/dl]	6.50±0.99	7.33±0.89	<0.05
Vitamin E[μmol/l]	15.34±4.99	23.23±4.99	<0.05
Vitamin C [mg/dl]	2.06±0.14	2.14±0.14	>0.05
GSH [mg/dl]	27.34±10.49	56.56±9.79	<0.001
Inhbin[pg/ml]	32.77±12.00	118.33±68.55	<0.001

using SPSS version 18.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Expressive analysis was used to display 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 alterations between variables of differentiated groups.

### Results

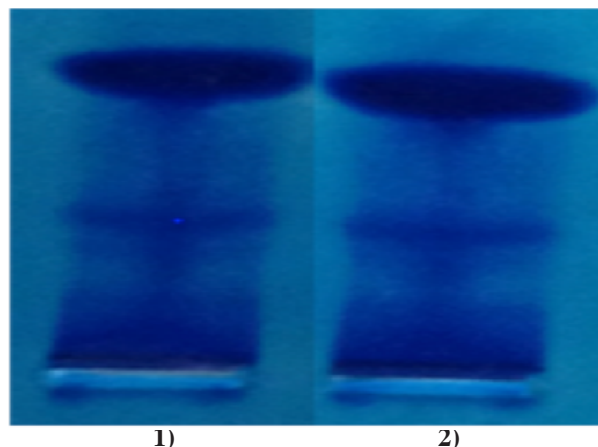
Ninety subjects comprising of sixty ALL patients and thirty control were included in the present study. Table 1 shows mean and standard deviation of age, haemoglobin (Hb), serum protein, both vitamins (E and C), in addition of glutathione and inhibin levels for the control and ALL patients groups. The mean age of the patients was (7.23±3.24) years, and for the control was (6.92±4.10) years.

There were highly significant differences (p < 0.001) in mean value of Hb, glutathione and inhibin with significant differences(p < 0.05) in mean values of both of serum protein and vitamin E, for patients group in comparison with control group, while there was non-significant differences(p>0.05) in vitamin C values between the two groups include in the present study, Table 1. As shown in the same Table, the patients group had an approximate similar vitamin C concentration that of control (2.06±0.13 vs. 2.08±0.20, p>0.05).

The results in Table 2 indicated high correlation between inhibin with: serum protein (r=0.81, p<0.01),

**Table 2. Correlation between Inhbin with Serum Protein and Several Antioxidants in Patients with All.**

Characteristic	Inhbin [pg/ml]	
	Pearson correlation	Sig. (2-tailed)
S. Protein [g/dl]	0.81	0.01
GSH [mg/dl]	0.80	0.01
Vitamin E [μmol/l]	0.77	0.01
Vitamin C [mg/dl]	0.69	0.01



**Figure 1. Protein Electrophoresis by Polyacrylamide gel 7.5%.** Protein was stained with coomassie brilliant blue G-250 for 1. serum ALL patients.2. serum control

glutathione ( $r=0.80$ ,  $p<0.01$ ) and vitamin E ( $r=0.77$ ,  $p<0.01$ ) levels, moreover medium correlation between inhibin and vitamin C ( $r=0.69$ ,  $p<0.01$ ) levels in patients with ALL was observed.

In order to detect the differences in total protein present in the studied groups, conventional polyacrylamide gel electrophoresis was carried out on crude sera samples of control and ALL patients groups; Figure (1).

## Discussion

Pathogenic mechanisms of anemia in cancer patients is hemolysis in erythrocytes, so the results in this study indicated that the Hb levels decreased significantly in the patients of as compared to the healthy ( $p<0.001$ ), Table 1. Reactive oxygen species (ROS) has long been known to be involved in the pathophysiology of cancer (Valko et al., 2006). The most common cancers in children are leukemia and the oxidative stress is a crucial feature of ALL (Battisti et al., 2008; Mazor et al., 2008; Saadaoui et al., 2013). Meanwhile recent advances in the field of reproductive medicine have focused the attention of many researchers to consider ROS as one of the mediators of infertility causing sperm dysfunction (Agarwal and Prabakaran, 2005; Singh, 2012). Oxidative stress has been postulated as an important mediator of thermal damage to testicular tissue (Barbieri et al., 1999) and decrease in the antioxidant defense system in seminal plasma observed in varicocele (Lenzi et al., 1994; Chen et al., 2001). Un important antioxidant molecule (glutathione) led to significant improvement in the sperm quality (Yousef et al., 2003; Lenzi et al., 2004). Second line of defense against free radical attack is constituted by small antioxidant molecules such as vitamin E, vitamin C, It is reported that vitamin C and E improved male fertility by increasing sperm concentration and total motile sperm and decreasing abnormal and dead sperm in rabbits (Pitteloud et al., 2008; Najjar et al., 2009). The present study indicated that reflection of significantly reduced vitamin E concentration in ALL patients when compared with the control. It is believed that vitamin E protects molecular and morphology of the cell, primarily through destruction of cell damaging ROS (Manzoor et al., 2012).

Accordingly, we undertook this study to explore whether oxidative stress may be the mechanism underlying decreased inhibin production in ALL patients and to explore whether circulating levels of inhibin were correlated with circulating levels of some antioxidant levels. Furthermore, since it has been suggested that serum inhibin B in relation to male and female reproductive health have been published internationally and the risk of leukemia that may spread to other organs like ovary (Ahmed et al., 2001; Wen et al., 2006; Lee et al., 2008; Moos et al., 2009; Taflan et al., 2011; Dillon and Gracia, 2012), the present study attempt to clarify the correlation between inhibin and serum protein, glutathione and the two vitamins E and C in patients with ALL, the results were reanalyzed by using linear regression analysis, Table 2.

In order to detect the differences in total protein present in the studied groups, conventional polyacrylamide gel

electrophoresis was carried out on crude sera samples of control and ALL patients groups; Figure 1. Deep look at the electrozomogram indicated that there is clear difference in proteins band intensity, which reflects the significant variation in proteins concentration among the studied groups, Table 1.

The current study indicated a possible link between decreased inhibin levels and oxidative stress, so it emphasizes the importance of routine measurement of inhibin during the leukemic processes to estimate the activity of gonadal origin.

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