CHANGES IN THE SERUM IRON, AMINOTRANSFERASES AND BLOOD PROFILE OF THE PRE-TREATMENT LEUKEMIA PATIENTS.

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ABSTRACT: The objective of the current study was to investigate the changes in the serum iron levels, transaminases activity and demonstrate the changes in the complete blood profile of pre-treatment leukemia patients. 2ml of blood samples from the patients and Control subjects were collected in EDTA containing vacutainers to study the blood profile by an automated hematology analyzer and 6ml of the blood was transferred to vacutainers (without any clotting factor), left at room temperature for 1h, centrifuged at 4000 rpm for 10 minutes and serum was isolated and stored at -20°C until further use. A significant increase in the WBC count ($P<0.0001$), lymphocytes percentage ($P<0.0001$), ALT ($P=0.0076$) and AST activity ($P=0.0381$) was found. The platelets were significantly decreased in the leukemia patients ($P<0.0001$). Serum iron levels were decreased in the early ($P=0.2380$) and older age groups ($P=0.0027$) and other groups had an increased serum iron levels. From these findings we can conclude that different hematological parameters changes significantly in the pre-treatment leukemia patients including the WBCs, platelets, serum iron, and amino transferases in different age groups which could be of medical importance for therapeutic purpose.

Key words; *Leukemia, Iron, Transaminases*

INTRODUCTION

Leukemia, a type of cancer starts in the blood forming tissues, is characterized by the over-production of WBCs. The most important factors associated with the leukemia are radiations, increasing use of marrow depressing drugs and the growing contamination of the atmosphere with chemical pollutants (Schwartz and Upton, 1958). Leukemia has been a target of immunological studies in human cancer because viable tumor cells are available for study and normal hematopoietic cells from the same individual are often available as normal counterparts (Naito *et. al.*, 1983).

Leukemia can be chronic or acute. It is categorized into four types based on the nature and type of affected white blood cells; Chronic lymphocytic leukemia (CLL), Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL) and Acute myeloid leukemia (AML).

CLL, a slow-growing type of leukemia occurs in middle-aged and elderly individuals, is characterized by the morphologically mature but immunologically less mature lymphocytes (Anonymus, 1989; Catovsky *et. al.*, 1989).

CML, a myeloproliferative disorder of pleuripotent hematopoietic progenitor cells; usually occurs in adults and is characterized by excessive proliferation and accumulation of granulocytes and occasionally red blood cells and platelets (Ghalaut *et. al.*, 2006).

ALL, a malignant disorder of lymphoid progenitor cells affecting both children and adults; is manifested by the formation of unformed blasts that are abnormal and unable to develop and fight infections (Pui *et. al.*, 2008;Ravindranath, 2003;Ries *et. al.*, 1999). The Primary accepted nongenetic risk factors for ALL are prenatal exposure to x-rays and postnatal exposure to high doses of radiation (Ross *et. al.*, 1994;Pui *et. al.*, 2004).

AML is characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation frequently resulting in hematopoietic insufficiency i.e. granulocytopenia, thrombocytopenia or anemia with or without leukocytosis with a tendency to grow as the age increases (Menzin *et. al.*, 2002). Sometimes in AML too many stem cells develop into abnormal red blood cells or platelets (Lowenberg *et. al.*, 1999).

Iron is thought to be a risk factor for cancer development in epidemiological studies in humans. Excessive body iron stores and inappropriate iron administration are known to interfere with natural body defenses. Increased body stores of iron manifest the increased growth rate of cancer cells (Kang, 2001;Walker and Walker, 2000).

Hepatic enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are synthesized in the liver. In case of liver injury the liver cells spill the enzymes into blood, raising the enzyme levels in the blood and signaling the liver damage. These enzymes are very important in the diagnosis of leukemia. Since, leukemia patients have elevated level of these enzymes in the blood indicating liver damage (Lee *et. al.*, 2008).

The complete blood count, a useful diagnostic tool for the evaluation of leukemia includes the hemoglobin (Hgb), hematocrit (Hct), white blood cells (WBCs), platelets (PLT) and red cell indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

The present study was designed to; estimate iron levels and transaminases activity in pre-treatment leukemia patients and demonstrate the changes in the complete blood profile of the leukemia patients to define the cause of this pathology.

MATERIALS AND METHODS

Blood samples of pre-treatment leukemia patients were collected from Institute of Nuclear Medicine and Oncology while blood samples of healthy persons were collected from Punjab University New Campus Lahore. The study involves the Blood profile comparison, serum transaminase activity and iron levels of the pre-treatment leukemia patients with control group. A total of 22 confirmed patients (14 males and 8 females) with four types of leukemia and control samples of 19 healthy subjects were obtained for study. The patients were divided into four groups on the basis of the ages. Group I includes patients from 1-15 years, Group II from 16-30 years, Group III from 31-45 years and Group IV from 46-60 years.

The subjects were sampled for blood with sterilized disposable syringes (Becton Dickinson, Private Ltd.), 2ml of blood was transferred to EDTA containing vacutainers (Becton Dickinson, Private Ltd.) and blood profile was carried on the automated hematology analyzer (Model MEK-6318 K, Power Input 190 VA, 220-240V, Nihon Kohen Corp). 6ml of blood was transferred to vacutainers (without any clotting factor) and were left at room temperature for 1h till further processing. The blood in the serum collecting vacutainers was centrifuged at 4000 rpm for 10 minutes. The separated serum samples were collected in labeled eppendorfs and were stored at -20°C. The analysis of transaminases and serum iron levels were performed using Randox AST kit (EC 2.6.1.1; AS 1204), ALT kit (EC 2.6.1.2; AL 1205) and serum Iron kit (SI 257). The results were analyzed by one-way ANOVA and Student's t-test using GraphPad Prism 4.0 software.

RESULTS AND DISCUSSION

Biochemical analysis

Serum Iron: An increase in iron level was observed in the group II and III, however, in the group I and IV, the iron level was declined when compared with control. These changes were significant when analyzed using one-way ANOVA (P=0.0233; Figure 1A).

Aminotransferase levels: Serum activity of AST and ALT levels was found increased in pre-treatment leukemia patients. These changes in the serum transaminase activity was statistically significant (AST; P=0.0381 and ALT; P=0.0076) when analyzed statistically by one-way ANOVA (Figure 1B, C).

Blood Analysis: Hemoglobin, Red blood cells and Hematocrit Hgb, RBCs and Hct %age in different age groups of leukemia patients was decreased with the pattern of increasing level sequentially from group I to group IV. This decline was highly significant when analyzed by one-way ANOVA (P<0.0001; Figure 1D, E, F).

White blood cells: The increase in the WBC count was highly significant in different age groups of leukemia patients when analyzed with one-way ANOVA (P<0.0001; Figure 2A).

Mean corpuscular hemoglobin: Comparison of MCH between control group and different age groups of leukemia patients shows decline in group II and IV. The level of MCH was slightly increased in group I and III in leukemia patients however; these changes in the MCH level were not significant when analyzed by one-way ANOVA. (P=0.5915; Figure 2B).

Mean corpuscular hemoglobin concentration MCHC in the leukemia patients was decreased when compared with control. However, there was slightly increased level of MCHC in group II. This change in the level was significant when analyzed by one-way ANOVA (P=0.0041; Figure 2C).

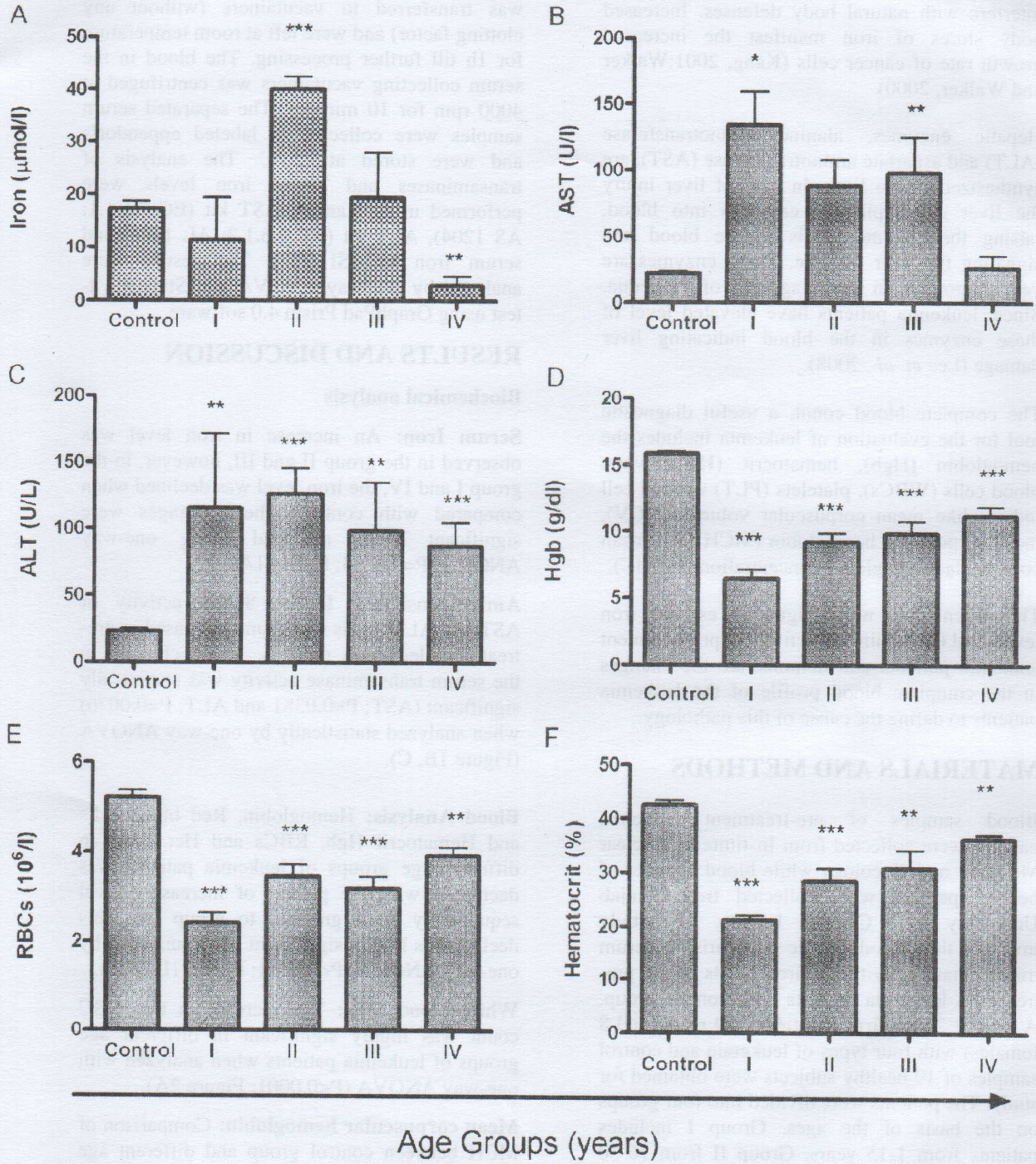

Fig. 1: Serum iron concentrations (A), AST (B) and ALT (C) activity were increased which were statistically significant when analyzed by using one-way ANOVA. A significant decrease in Hb (D), RBCs (E) and HCT (F) was observed using one-way ANOVA. Results indicate mean ± S.E.M. (*P<0.005, **P<0.001, ***P<0.0001).

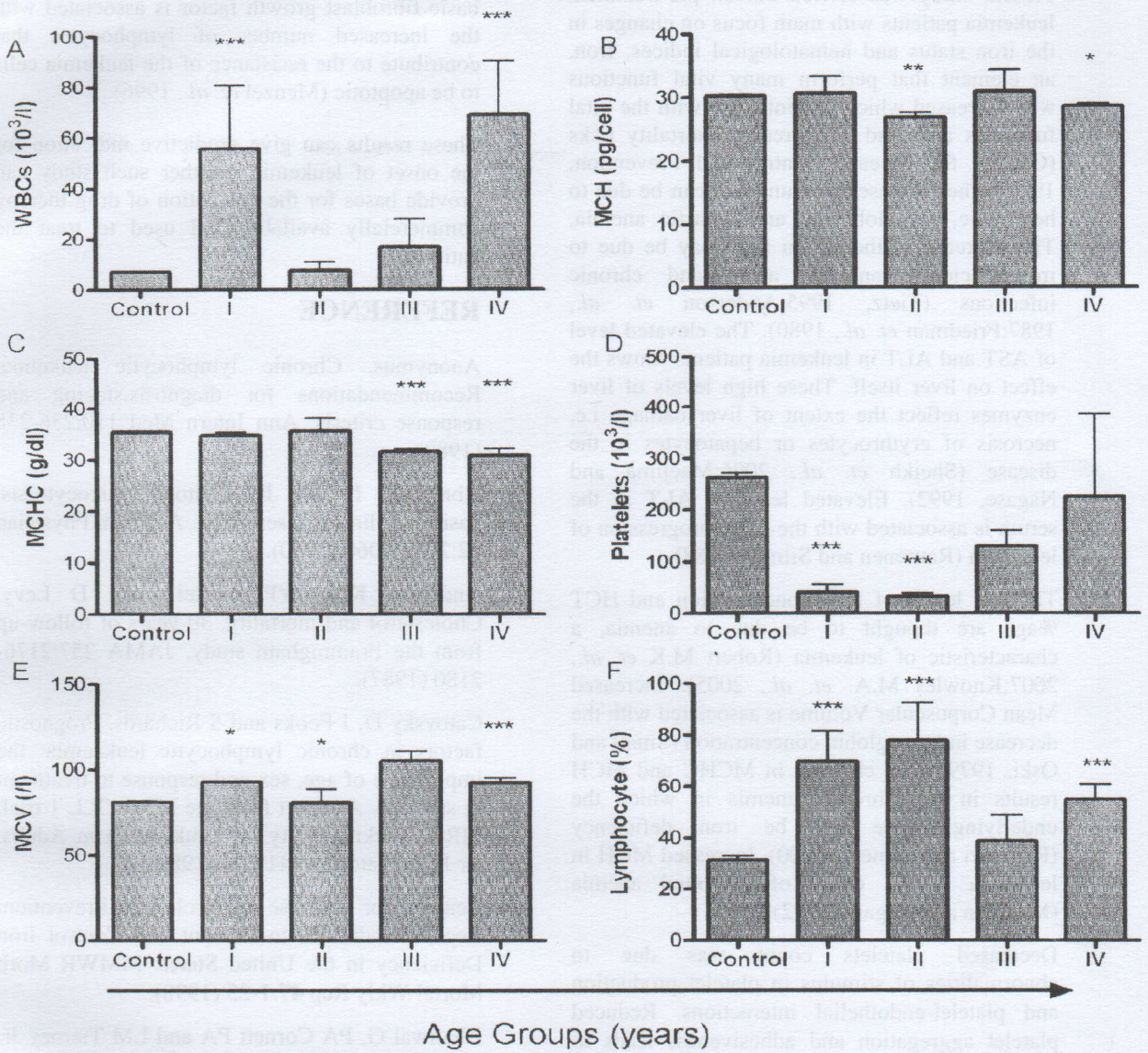

Fig. 2: A significant increase in WBCs (A) and LYM %age (F) and a significant decrease in PLTs (D) were observed when analyzed by using one-way ANOVA. MCH (B), MCHC (C) and MCV (E) show variations in different age groups. Results indicate mean ± S.E.M. (*P<0.005, **P<0.001, ***P<0.0001).

Platelets: The PLT count in leukemia patients was also decreased in comparison with control group. This decrease in PLT count was highly significant when analyzed by one-way ANOVA (P<0.0001; Figure 2D).

Mean corpuscular volume: MCV in different groups of leukemia patients was increased in comparison with control group except in group II. These changes were statistically highly significant when analyzed by one-way ANOVA (P=0.0002; Figure 2E).

Lymphocyte percentage: LYM %age was increased in leukemia patients when compared with control values. This increase in the level

was highly significant by using one-way ANOVA ($P<0.0001$; Figure 2F).

Present study was carried out on pre-treatment leukemia patients with main focus on changes in the iron status and hematological indices. Iron, an element that perform many vital functions was decreased which can interfere with the vital functions and lead to increased mortality risks (Centers for Disease Control and Prevention, 1998). The increase in serum iron can be due to hemolytic, megaloblastic and aplastic anemia. The decrease in the serum iron may be due to iron-deficiency anemia, acute and chronic infections (Tietz, 1995;Anderson *et. al.*, 1987;Friedman *et. al.*, 1980). The elevated level of AST and ALT in leukemia patients shows the effect on liver itself. These high levels of liver enzymes reflect the extent of liver damage i.e. necrosis of erythrocytes or hepatocytes in the disease (Sheikh *et. al.*, 2006;Maejima and Nagase, 1992). Elevated level of ALT in the serum is associated with the rapid progression of leukemia (Rautonen and Siimes, 1988).

The low levels of Hgb concentration and HCT %age are thought to be due to anemia, a characteristic of leukemia (Robert M.K *et. al.*, 2007;Knowles M.A. *et. al.*, 2005). Increased Mean Corpuscular Volume is associated with the decrease in hemoglobin concentration (Small and Oski, 1979). The changes in MCHC and MCH results in hypochromic anemia in which the underlying cause can be iron deficiency (Remuzzi and Minetti, 2000). Increased MCH in leukemia is the cause of profound anemia (Maejima and Nagase, 1992).

Decreased platelets count was due to abnormalities of stimulus in platelet production and platelet-endothelial interactions. Reduced platelet aggregation and adhesiveness leads to defective blood clotting, which ultimately results in increased bleeding (Nasri and Baradaran, 2006;Rizvi *et. al.*, 1999). The decreased level of RBC in leukemia can be counted due to hemolytic anemia, which is a common cause of lymphoproliferative disorders (Dhaliwal *et. al.*, 2004). Leukemia patients have shown a significant increase in the number of white blood cells. Increased WBC count is the most important parameter that suspects the leukemia (Walker *et. al.*, 1990). WBC count >30,000/mm^3 is an indication of marrow abnormality (Abramson and Melton, 2000).

The elevated levels of lymphocytes in leukemia patients i.e. lymphocytosis may be due to increased non-activated lymphocytes. Elevated basic fibroblast growth factor is associated with the increased number of lymphocytes that contribute to the resistance of the leukemia cells to be apoptotic (Menzel *et. al.*, 1996).

These results can give predictive indication for the onset of leukemia. Further such study can provide bases for the evaluation of drug therapy commercially available and used to treat the patients.

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