Original Article

EXPRESSION OF EXTRAPOLATIVE FACTORS OF MEDICAL IMPORTANCE AND THEIR POTENTIAL ROLE IN THE DEVELOPMENT OF LEUKEMIA

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ABSTRACT:

BACKGROUND: Leukemia defined as the cancer of bone marrow i.e. leading to wild proliferation of blood forming cells. The WBCs are granulocytes, which avert bacterial infection and T and B lymphocytes. Prognostic measures for the disease are still not clear whereas, in children prognosis rate is better as compared to adults.

MATERIALS AND METHODS: Fifty (n=50) patients of leukemia and fifty (n=50) were obtained and screened at Ganga Ram Hospital Lahore. Serum was separated and estimated for their 8-hydroxy -2-deoxy guanosine, Isoprostanes, myeloperoxidase and cytokines such as TNF-α and several interleukins with their commercially available ELISA kits respectively. While neutrophils were estimated by the help of hematology analyzer and malondialdehyde (MDA) was estimatedby the method of Ohkawa.

RESULTS: The results of the present study shows that variables of oxidative stressi.e., malondialdehyde (MDA), isoprostanes (IsoP-F2a), 8-hydroxy-2-deoxy guanosine (8-OHdG), neutrophils and cytokines including interleukin-6 (IL-6), tumor necrosis factor (TNF) and myeloperoxidase (MPO)differed significantly. The recorded levels of MDA (5.26±1.26 nmoles/ml), Isoprostanes (81.26±5.26 pg/ml), 8-OHdG (1.22±0.016 pg/ml), MPO (2.16±0.16 pg/ml) were significantly increased as compared to controls. Levels of cytokines i.e., IL-6 and TNF-α remained (6.59±2.16pg/ml) and (56.26±2.26 pg/ml) in diseased group while in controls they were(0.965±0.16pg/ml) and (0.15±0.015pg/ml)respectively.

CONCLUSION: Performed research work concludes that leukemic patients have positive relation among disease progression and oxidative stress. It is characterized by increased the levels of oxidative stress markers significantly in serum concentration. Increased level of MDA signifies increased lipid peroxidation in the subjects of leukemic patients.

KEYWORDS: Oxidative stress, acute myeloblastic leukemia, lipid peroxidation

INTRODUCTION:

Leukemia is a Greek word meaning 'leukos=white' + 'haima=blood'. As the name indicates leukemia refers to the cancer of bone marrow i.e. leading to wild proliferation of blood forming cells. The WBCs are granulocytes, which avert bacterial infection or T and B lymphocytes which also fight against infection[1]. There are four major types of leukemia

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characterized by disease duration, blood cell count and specific type of blood cell participation and these types are; acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL). Hematopoietic stem cells are maintained by multipotent stem cells (MSCs). In all its types a bone marrow problem leads to excessive circulating blood cells in blood stream by favoring leukemic stem cells and bone marrow fibrosis.

Most common type of leukemia diagnosed is acute lymphocytic leukemia and include 78% of all detected children leukemias[2].The prevalence of acute lymphocytic leukemia in elder patients in every 100,000 patients is 1.0 to 1.6 which is higher as compared to patients aged 25-54 (0.6 to 0.7) as reported by surveillance epidemiology and end result study. In all patients above than 6o year of age prognosis rate is very poor due to some other correlated factors like deprived bone marrow function but in children prognosis rate is better because of introduction of CNS treatment, HSC transplant, intense post remission therapy and molecular therapies[3]. Acute myeloblastic leukemia (AML) is about 20% of pediatric leukemia[4]. ROS (Reactive oxygen species) plays role in signaling process either intracellular or extracellular, exogenously or endogenously. Antioxidants provide a basic shield against oxidative stress[5]. Oxidative stress which is caused by ROS is responsible to cause DNA damage, because in normal conditions DNA-repair mechanism is functional to repair the DNA. This irreparable DNA damage leads to mutations[6].Free radicals account for defective signaling mechanisms which are in control of normal growth, proliferation and differentiation[7]. ROS if gathered at greater levels in the cells cause DNA impairment and cell senescence i.e. a level of irretrievable growth arrest, which can be originated by the extreme proliferation and oncogenic initiation and cell destruction through the oxidative stress[8,9]. Glutathione levels have grasping effect on OS. In case of low GSH levels elevated OS, enhanced aging process and pathogenesis of disease is observed[10].

Leukemia depends on the small populace of LSCs (leukemic stem cells) for its progression and metastasis[8]. Biological aging is a significant limitation for the initiation and survival of cancer cells[11]. The gathering of DNA damage through mis-repair may result in mutagenesis which subsequently results in the transformation in combined defective apoptosis. LIF (Leukemia inhibitory factor) is an interleukin 6 cytokine class that effect cell growth by hindering differentiation. Low levels of LIF leads to cell differentiation[12]. Different factors are responsible to create imbalance in the normal homeostatic balance and leads to disease[13]. A study by[14] inspected PI3K inhibitor because of its stated action in contradiction of myeloid leukemic cell lines. Promotion of programmed cell death in chronic lymphoid leukemic cells and no effect on normal T and B cells has been observed.

MATERIALS AND METHODS:

For the current research work fifty (n=50) leukemic patients and fifty (n=50) age-sex matched controls were screened at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. All the patients were provided with signed consent form. All protocols were according to the Ethical committee of University of Lahore. 5ml blood was taken in the vial and serum was separated and stored at -80°C for the future assays.

INCLUSION CRITERIA

Patients with diagnosed leukemia of age 20-70 years were added in the current study

EXCLUSION CRITERIA

Individuals with the history of drugs, alcohol, smoking or any disease such as, HCV, HIV and Diabetes were excluded out of the study.

BIOCHEMICAL ASSAY

DETERMINATION OF MALONDIALDEHYDE (MDA)

Malondialdehyde (MDA) was estimated calorimetrically by estimating Thiobfarbituric acid reactive substances (TBARS). 200µl of sample, 0.2ml of 8.1% Sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA will be added. After centrifugation it at the speed of 3000 rpm for 10 min layers were

separated and absorbance was measured at 532 nm against an appropriate blank result was expressed in milimoles.

ESTIMATION OF ISOPROSTANES

Isoprostanes were determined by the help of commercially available ELIZA kits (Caymen Chemicals).

DETERMINATION OF 8-HYDROXY-2-DEOXYGUANOSINE (8-OHdG)

Estimation of 8-hydroxy-2-deoxyguanosine with the help of commercially available ELIZA kits (Enzo-USA).

ESTIMATION OF MYELOPEROXIDASE (MPO)

Myeloperoxidase (MPO) was determined by the help of ELISA kit (by Caymen Chemicals).

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 16+. Results were subjected to independent T test. Significant P value was taken as $p<0.05$.

RESULTS:

Results of the present study execute increased levels of malondialdehyde (MDA) inpatients (5.26±1.26nmoles/ml) as compared to healthy individuals as compared to healthy individuals (1.26±0.05nmoles/ml) as shown in table 01, figure 01 (A). Along with the increased levels of MDA, levels of Isoprostanes and 8-OHdG were also increased in the diseased group (81.26±5.26pg/ml, 1.22±0.016pg/ml) as compared to their controls respectively (4.26±1.49pg/ml, 0.06±0.001pg/ml) table 01, figure 01 (B,C). Similarly in the current study levels of Interleukin-6 were also increased in the diseased group (6.59±2.16pg/ml) in contrast with controls (4.26±1.06pg/ml) table 01, figure 01 (D). Moreover, levels of TNF-α and MPO were also significantly increased in diseased groups (56.26±2.26pg/ml) and (2.16±0.16mmol/l) as compared to controls(26.25±3.26pg/ml) and (1.56±0.052mmol/l) table 01, figure 01 (E,F). As shown in table 01, figure 01 (G) levels of neutrophils also remained higher in the leukemic patients (88.16±3.26 %) as compared to controls (60.31±3.06 %).

Table-01: Different expression of prophetic variables involved of medical importent and their interplay in the leukemic pateints

VARIABLES	CONTROL (n=50)	PATIENTS (n=50)	*P*- VALUE
MDA (nmol/ml)	1.26±0.05	5.26±0.26	0.019
IsoP- F2α (pg/ml)	4.26±1.49	81.26±2.89	0.000
8-OHdG (pg/ml)	0.087±0.018	1.22±0.025	0.041
IL-6 (pg/ml)	4.26±0.32	6.59±0.41	0.016
TNF-α (pg/ml)	26.25±2.26	56.26±3.26	0.008
MPO (mmol/L)	1.56±0.064	2.16±0.098	0.043
Neutrophils (%)	60.31±3.06	88.16±3.26	0.007

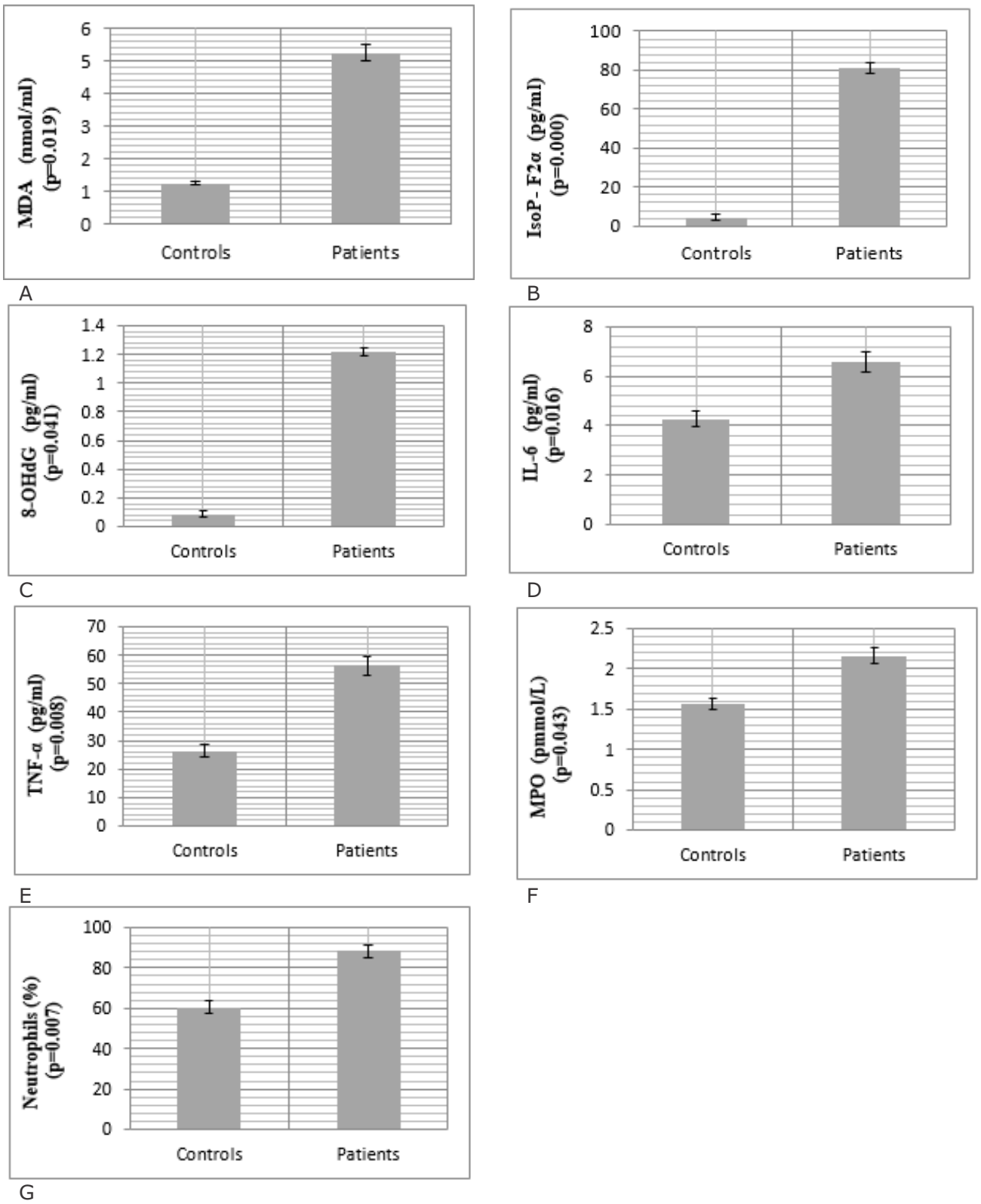

Figure 01: different expression of prophetic variables involved of medical importent and their interplay in the leukemic pateints

DISCUSSION:

Leukemia, lymphoma and myeloma are types of cancers that can affect bone marrow, blood cells, lymph nodes and other organs of lymphatic system. Leukemia is cancer of marrow and blood, characterized by uncontrolled proliferation of immature hematopoietic white blood cells which leads to anemic and other hematologic malignancies. Study performed on leukemia, covering a range of 7 parameters. That was measured in both leukemic patients and controls. The parameters included MDA, Isoprostanes, 8 hydroxydeoxyguanosine, tumor necrosis factor alpha, myeloperoxidase, neutrophils and interleukin 6. These all variables were observed to be influenced by leukemic condition as compared to control. MDA is a byproduct of lipid per oxidation and is found to be elevated in disease conditions. Similar thing was observed in our study. It is highly potent compound that causes toxic stress in cells and forms adducts with DNA which are highly mutagenic in nature. Elevated levels of MDA were observed in serum samples of leukemic patients. Isoprostanes are prostaglandin like compounds and are known as reliable markers of oxidative stress. Elevated Isoprostane and oxidative stress are directly linked. Highly elevated levels were observed in serum samples. 8-hydroxydeoxyguanosine is the most common marker of oxidative stress induced DNA damage. It is regulated by antioxidant capacity and DNA repair enzyme activity. It serves as a prognostic marker for leukemia. In present study elevated levels were observed in leukemia serum samples.

Interleukin-6 is a pro-inflammatory cytokine along with its regulatory actions in metabolism, regeneration and neural processes. It plays vital part in host defense due to its immune and hematopoietic actions. In the study high IL-6 values were observed in leukemic serum samples. Tumor necrosis factor is a multifunctional cytokine involved in many physiological processes that control inflammation, antitumor response and homeostasis through its 2 receptors. These receptors mediate cytotoxicity, T cell proliferation, and conflict with infection. Inflammatory cytokine play an important role in onset and progress of hematological malignancies. TNF alpha values were observed to be elevated in serum samples as compared to normal controls. Neutrophils are WBCs that play key role in our innate immune system. During their circulation in bloodstream they judge infection and readily migrate to site of infection and kill invaders. Neutrophils levels increased in leukemia as observed in serum samples. Myeloperoxidase is a peroxidase enzyme that is expressed in neutrophil granulocyte. Levels of MPO were recorded to be high in leukemic serum samples.[5] The study explored the possible role of MDA and protein carbonyl as a marker of disease activity in chronic myeloid leukemia and significantly high levels of MDA and PC were reported as inpresent study. Olaniyi[15] and Zhou[16] also detected elevated MDA levels in leukemic patients. Oltra[17] examined functioning of MDA, 8-hydroxydeoxy guanosine and other oxidative stress markers in chronic myeloid leukemia and detected higher MDA and 8-hydroxydeoxy guanosine levels in leukemic subjects. Their concentration also increased as the duration of disease. The results depicted a high oxidative stress condition in chronic lymphoid leukemia. Study by Hockenberry suggests that chemotherapy in leukemic children involving Methotrexate causes increase in oxidative stress as detected by F2 isoprostanes in post induction phase. This result proves isoprostanes an important marker for detection of OS in leukemic patients and higher isoprostanes levels are in accordance with our study. Erve[18] conducted a meta-analysis to prove F2 isoprostanes as a representative marker of oxidative stress in various pathologies.

Singer et al., 2011 studied role of TNF-alpha, IL-6, IL-8 and CRP as survival prognostic markers in chronic lymphocytic leukemia. These pro inflammatory markers play an important role in pathogenesis of chronic leukemia. In hematological malignancy TNF-alpha, IL-6 and IL-8 were recorded to be higher while CRP levels were significantly reduced. The results of this study were in accordance with our study, showing high TNF-alpha and IL-6 levels were non-significantly higher showing high burden of disease. Therefore proving TNF-alpha a persistence analytical marker in chronic lymphoid leukemia. Tumor necrosis factor is involved in interactions between a leukemic cell

and normal BM cell which provide a suitable environment for LC to survive. TNF can be produced by macrophages, NK cells, neutrophils etc. There are conflicting reviews of TNF roles as it is supposed to be helping in tumor growth and according to some studies it initiates apoptosis of tumor cells. TNF was higher in patients of acute myeloid leukemia as in accordance with present results[19]. In another study by Aguayo[20] the analysis of acute myeloid leukemic patients at diagnosis or relapse condition the TNF levels were highly elevated in CL and ALL but not in acute myeloid leukemia. They concluded that inhibition of TNF during therapy must be elucidated more. Foa[21] concluded that tumor necrosis factor play an important part in B-CLL and hairy cell lymphoma. B cells in neoplastic condition have more tendency to produce TNF, hence it plays role in disease propagation. Their role is different in case of early and mature BCL. A study conducted by Tian[22] expressed that levels of tumor necrosis factor are notably elevated in serum of acute myeloid leukemic patients, therefore showing that this variable is profoundly expressed in myeloid leukemia. Release of premature LS is supposed to be linked with tumor necrosis factor-alpha provoked expression of matrix metalloproteinase 9. Elevated levels of TNF-alpha were associated with increased fatigue.

Reynauad[23] studied IL-6 as a major player in chronic myeloid leukemia and found elevated IL-6 levels as in our study. Interleukin-6 is supposed to have growth promoting effect on acute myeloid leukemic cells[24]. A study by burger[25] proved that overexpression of interleukin-6 is involved in B-cell and plasma cell derived tumors. Myeloperoxidase is a marker of myeloid cell line and transcription of myeloperoxidase gene is only initiated during late myeloblast maturation stage. MPO is highly expressed in immature myeloid cell and neutrophils also. Matsuo[26] studied that increased concentration of myeloperoxidase in leukemic blast cells serves as an important prognostic marker in AML. In a study by Catovsky[27] they detected myeloperoxidase deficient polymorphonuclearneutrophils in 43% of the cases of acute myeloid leukemia studied. According to a study by Kim[28], Myeloperoxidase serves as an important factor in distinguishing leukemic patients from the ones that need transplant. Hence, all of the above stated studies were in accordance with our results showing elevated levels of MDA, Isoprostanes, 8-hydroxydeoxy guanosine, IL-6, MPO, neutrophils and TNF-alpha.

CONCLUSION:

Performed research work concludes that leukemic patients have positive relation among disease progression and oxidative stress. It is characterized by increased levels of oxidative stress markers significantly in serum concentration. Increased level of MDA signifies increased lipid peroxidation in the subjects of leukemic patients.

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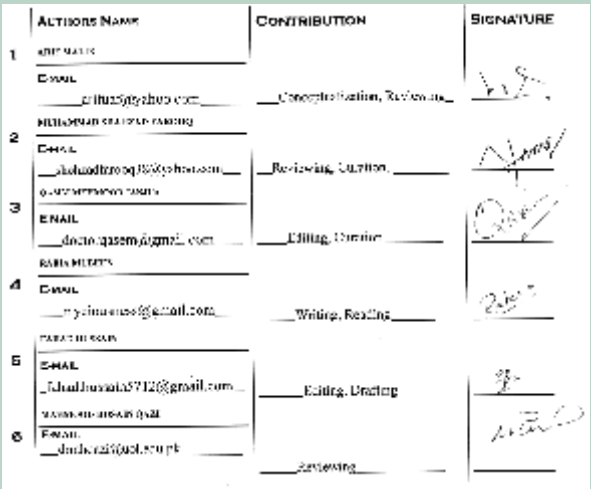

Submitted for publication: 21.06.2017

Accepted for publication: 20.12.2017
After Revision

O son of Adam, when you see that your Lord, the Glorified, bestows His Favors on you while you disobey Him, you should fear Him (take warning that His Wrath may not turn those very blessings into misfortunes).

Hazrat Ali (Karmulha Wajhay)