Original Article

ROLE OF PROGNOSTIC VARIABLES OF MEDICAL IMPORTANCE AND THEIR INTERPLAY IN LEUKEMIA: A STUDY FROM LOCAL POPULATION

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ABSTRACT:

Introduction: Leukemia is defined as the cancer of blood-forming tissues. It is equally common in children and adults. It involves abnormal production of white blood cells (WBCs) which are primarily responsible for the defense in the human body thus, abnormality in the production of WBCs leads to the failure in combating the infection. Aim of the current study is to rule out the significant markers of prognostic importance that play an important role in the development of leukemia in the local population

Material and Methods: Thirty (n=30) patients of leukemia and thirty (n=30) healthy controls were enrolled for the current study by random sampling. This cross sectional study was approved by the Departmental Research Committee (DRC), Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore. Blood and Saliva samples were collected and subjected for the analysis of the MDA, isoprostanes, Interleukin, MPO, and Neutrophils levels with the help of their respective protocols.

Results: Results of this study showed that the levels of oxidative stress markers and interleukins were significantly increased in patients with leukemic disorders as compared with the healthy subjects. It showed that levels of MDA, isoprostanes, 8-OHdG, TNF-α and interleukin-6 were significantly higher (p-value = 0.019, 0.001, 0.041, 0.008 and 0.016 respectively) in the serum and saliva samples of patients as compared to that in the healthy subjects. Levels of MPO and Neutrophils presented significantly (p-value= 0.043, 0.007) higher levels in the blood samples whereas, these were not detected in the saliva samples of the patients.

Conclusion: The current study suggests the significant role of oxidative stress markers in the initiation and progression of leukemia. It shows levels of interleukin and markers of DNA damage remained elevated in the patients with leukemia as compared to that of healthy individuals. Therefore, therapy with significant antioxidants can improve the status of individuals suffering from leukemia in the local population.

Key Words: Leukemia, Neutrophils, Interleukin-6

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 a wild proliferation of blood-forming cells. Bone marrow cells include white blood cells (WBCs) which combat infection, red blood cells (RBCs) which carry oxygenated blood and platelets which aid blood clotting.[1]

Major types of leukemia are acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphoblastic leukemia (CLL). In all its types bone marrow problem leads to excessive blood cells in the bloodstream by favoring leukemic stem cells and bone marrow fibrosis. The most common type of

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leukemia diagnosed is acute lymphocytic leukemia, which includes 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.[3] While acute myeloblastic leukemia (AML) is about 20% of pediatric leukemia.[4] Reactive oxygen species (ROS) are diverse compounds produced by the mature myeloid cell lines in an innate response. They play a role in the signaling process either intracellular or extracellular, exogenously or endogenously.[5] Oxidative stress due to ROS is responsible for DNA damage.[6] Oxidative stress may be held accountable for defective signaling mechanisms that alter the efficacy of drugs and programmed cell death of malignant cells.[7] Thus, antioxidants play their pivotal role in altering the anomalies that may be caused by the production of reactive oxygen species i.e., elevated levels of Superoxide dismutase (SOD), Glutathione (GSH) and Catalase (CAT) have reported grasping effect on the oxidative stress, in case of lower levels of these anti-oxidants enhanced progress of diseases pathogenesis and aging are reported.[8] Extensive literature signifies the interactions with the bone marrow microenvironment that is responsible for the hematopoiesis and morphology of bone marrow. Elevated levels of transforming growth factor beta-1 are important to control cell proliferation, survival and apoptosis.[9] Literature reports the role of various markers such as interleukins, isoprostanes (Iso-P), 8-hydroxy-2-deoxyguanosine (8-OHdG), Tumor Growth Factor-beta (TGF-β) have a significant role in the disease progression.[10] Markers like 8-OHdG and Iso-P signifies increased lipid peroxidation and DNA damage in the cells of the infectious patients. As reported by the number of studies lipid peroxidation by-products i.e., MDA is involved in the formation of DNA adducts leading to DNA damage and cell death.[11]

MATERIAL AND METHODS:

Thirty (n=30) patients of Leukemia and thirty (n=30) healthy age-sex matched controls were enrolled in the current study. After getting informed consent blood and saliva samples were obtained and stored for their future analysis. All of the protocols were approved by the Departmental Research Committee (DRC) of the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. Samples were subjected to the determination of Malondialdehyde (MDA), isoprostanes (IsoP-F2α), 8-hydroxy-2-deoxyguanosine (8-OHdG), Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Myeloperoxidase (MPO) and Neutrophils with the help of their respective ELISA and spectrophotometric methods. Results of the findings were subjected to Independent T-test with the help of SPSS v.21 and were expressed in the form of Mean±S.D. where $p<0.05$ remained significant.

RESULTS:

TABLE- 01: Levels of different variables in leukemia

Variables	Control (n=30)	Serum (n=30)	Saliva (n=30)	p- value
MDA (nmol/ml)	0.95± 0.001	5.26± 1.26	1.26± 0.05	0.019
IsoP- F2α (ng/ml)	0.99± 0.0056	81.26± 5.26	4.26± 1.49	0.001
8-OHdG (pg/ml)	0.02± 0.0011	1.22± 0.016	0.06± 0.001	0.041
IL-6 (pg/ml)	4.26± 1.06	6.59± 2.16	0.965± 0.16	0.016
TNF-α (pg/ml)	26.25± 3.26	56.26± 2.26	0.15± 0.015	0.008
MPO (mmol/L)	1.56± 0.052	2.16± 0.16	0.00± 0.00	0.043
Neutrophils (%)	60.31± 3.06	88.16± 3.26	0.00± 0.00	0.007

The current study showed that serum MDA levels were increased significantly (p=0.019) in patient as compared to controls. Whereas an insignificant increase was observed in saliva of patients (Fig. 1)
Serum Isoprostanes was significantly higher (p=0.001) in patient as compared to controls. In saliva, its level was slightly increased. (Fig. 2)
Levels of serum 8-OHdG were signifincatly higher (p=0.041) in patients as compared to values of controls. While it was slightly detectable in saliva. (Fig. 3)
Levels of IL-6 were not detectable in saliva but were significantly higher (p=0.016) in the serum of patients as compared to controls. (Fig. 4)
Levels of serum TNF-α were significantly elevated (p=0.008) in patients as compared to controls. There was no effect on slivery TNF-α. (Fig. 5)
Serum MPO levels were significantly higher (p=0.043) as compared to controls. There was no effect on salivary MPO. (Fig. 6)
Neutrophils percent was significantly higher (p=0.007) as compared to controls. No neutrophil was detected in saliva. (Fig. 7)

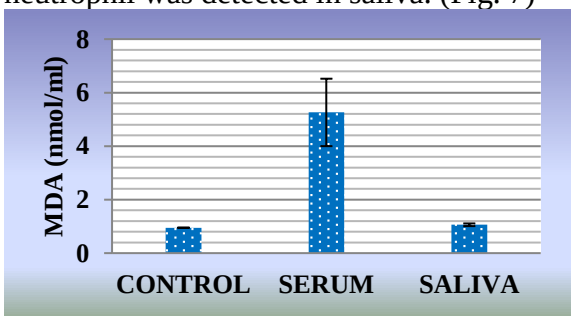

Fig. 1 MDA levels in serum and saliva of patients.

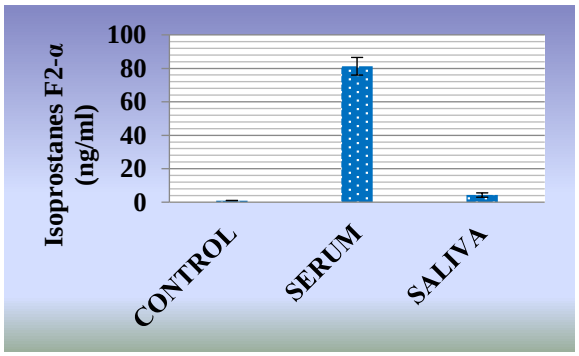

Fig. 2. Isoprostanes levels in serum and saliva.

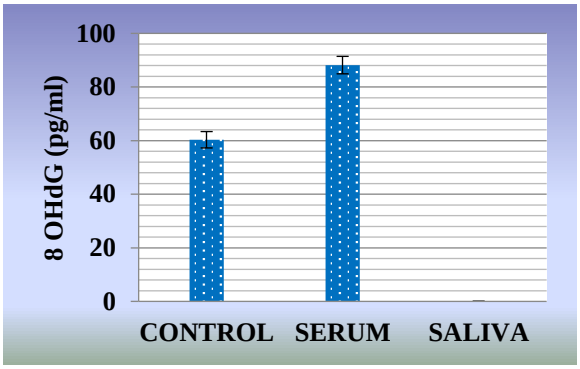

Fig. 3. 8OHdG levels in serum and saliva.

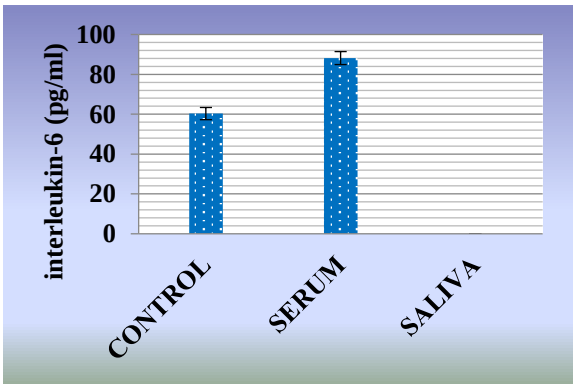

Fig. 4. Serum interleukin-6 levels in serum and saliva.

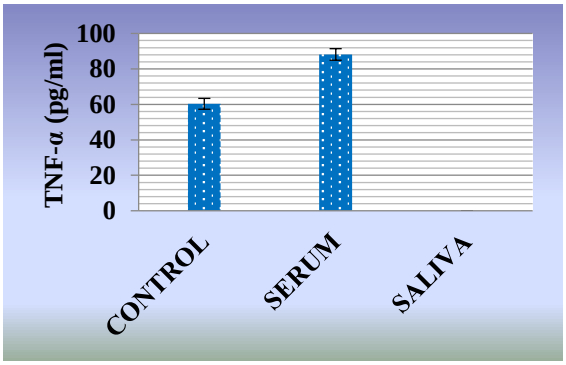

Fig. 5. Serum TNF-α levels.

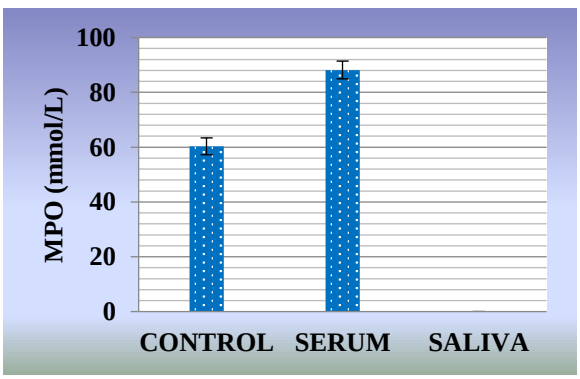

Fig. 6. MPO levels in serum and saliva.

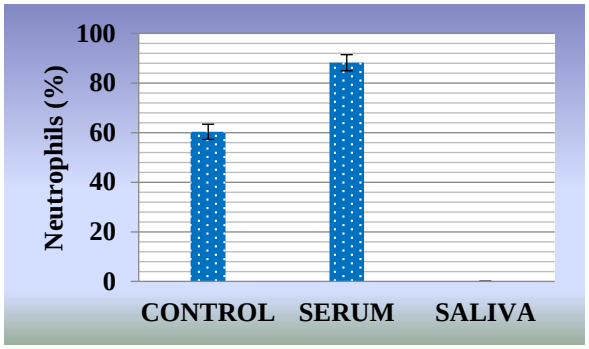

Fig. 7. Neutrophils % in serum and saliva.

DISCUSSION:

The variables performed, showed a significant difference in leukemic patients and controls hence proving the link of these variables to the occurrence and prevalence of leukemia. The study was performed on serum and saliva samples and the parameters included MDA, Isoprostanes, 8-hydroxydeoxyguanosine, tumor necrosis factor-alpha, myeloperoxidase, neutrophils, and interleukin 6. All of these variables were observed to be influenced by leukemic conditions as compared to control. An increase in the levels of MDA and other reported inflammatory markers in the serum samples signifies their importance, it shows MDA levels were increased with the elevation in the disease condition and led to increased DNA damage which was observed in the terms of increased levels of isoprostanes and 8-hydroxydeoxyguanosine.[12,13] And uptake of interleukin-6, a pro-inflammatory cytokine along with its regulatory actions in metabolism, regeneration, and neural processes. It provokes the immune and hematopoietic actions. Tumor necrosis factor is a multifunctional cytokine involved in many physiological processes that control inflammation, antitumor response, and homeostasis through its receptors. These receptors mediate cytotoxicity, T cell proliferation, and conflict with infection. Inflammatory cytokines play an important role in the onset and progress of hematological malignancies.[14]

Literature shows the 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 the 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. These results are accordance with results of our study, showing high TNF-alpha and IL-6 levels were non-significantly higher showing a 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 leukocytes 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 in accordance with our results.[15] According to a study by Kim *et al.*,[16] Myeloperoxidase serves as an important factor in distinguishing leukemic patients from the ones that need a 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:

The findings of the current study conclude the role of oxidative stress and reactive oxygen species in the initiation of infection and leading to the development of leukemia in patients. Increased levels of MDA, IsoP, and 8-OHdG signifies alleviated DNA damage and increased oxidative stress in the patients. Thus, it may be stated that the treatment of the subjects with the antioxidants can have a significant effect on leukemic patients than in healthier subjects.

CONFLICT OF INTEREST:

Authors declare no conflict of interests

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AUTHOR'S CONTRIBUTION:

RA: Conceived and presented idea
MSQ: Collection of data, carried out experiment, writing
ZK: Collection of data, editing
SW: Writing, developed theory and performed computation
SI: Writing, performed analytical calculations,
NF: Editing
AM: Data analysis

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