RESEARCH ARTICLE

Determination of Serum Myeloperoxidase (MPO) and lactate dehydrogenase (LDH) as a tumour Marker in Chronic Myeloid Leukaemia (CML)

Khitam Abdul Wahhab Ali[1], Abeer Anwer Ahmed[2], Suhad Taha Mohammed[3]

Abstract

Objective: To evaluate the efficacy of serum myeloperoxidase and lactate dehydrogenase levels as tumour markers in chronic myeloid leukaemia patients after one-year treatment with tyrosine kinase inhibitors.

Method: The case-control study was conducted at the College of Medicine, Mustansiriyah University, Baghdad, Iraq, in collaboration with the National Centre of Haematology, Baghdad, from December 2019 to April 2020. The cases comprised chronic myeloid leukaemia patients aged ≥18 years who had completed one-year treatment with tyrosine kinase inhibitor. They were divided into two groups on the basis of major molecular response. Group 1 patients had major molecular response >0.1%, while group 2 patients had major molecular response <0.1%. Group 3 had healthy controls matched for age and gender. Serum myeloperoxidase and lactate dehydrogenase concentrations were measured using enzyme-linked immunosorbent assay. Data was analysed using SPSS 25.

Results: Of the 88 subjects, 32(36.4%) were in group A with mean age 44.9±12.6 years, 26(29.5%) were in group B with mean age 48.23±10.6 years, and 30(34%) were in group C with mean age 43.1±9.3 years. There was a significant increase in myeloperoxidase and lactate dehydrogenase levels in patient groups compared group 3 controls ($p<0.05$). Between the patient groups 1 and 2, the difference was significant for myeloperoxidase ($p<0.05$), but not for lactate dehydrogenase ($p>0.05$).

Conclusion: There was higher oxidative stress in chronic myeloid leukaemia patients.

Key Words: Tyrosine Kinase, Oxidative, Peroxidase, Immunosorbent, Leukemia, Myelogenous, Chronic, BCR-ABL, Lactate Dehydrogenases, Tumour

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Introduction

Myeloperoxidase (MPO) is one of the myeloproliferative disorders in which granulocytic cells proliferate, but fail to achieve full maturation, leading to increased number of granulocytes and the presence of immature cells in peripheral blood with characteristic cytogenetic abnormality, or Philadelphia (Ph) chromosome.[1]

The development of inhibitors, particularly of breakpoint cluster region-Abelson (BCR-ABL) tyrosine kinase activities, as one of the therapeutic agents revolutionised the treatment of the chronic myeloid leukaemia (CML)[2]. Imatinib mesylate (IM) can be defined as the first generation of tyrosine kinase inhibitors (TKIs), inhibiting the BCR-ABL tyrosine kinase activity through the blocking of Adenosine Triphosphate- binding site (ATP-binding site), leading to apoptosis of leukemic cells[3].

MPO is a homodimer peroxidase enzyme that is encoded by theMPO gene on chromosome 17 in humans. MPO catalyses the formation of hypochlorous (HOCl) acidfrom hydrogen peroxide (H2O2) and chloride anion (Cl-). Immune cells may employ basic levels of MPO-derived HOCl to kill bacteria and other pathogens under normal settings. As an oxidant, HOCl can operate as a toxin, killing cells by causing oxidative damage to proteins and deoxyribonucleic acid (DNA), or as a signal molecule, causing cellular apoptosis by activating certain signalling pathways.[4,5].

Plasma MPO levels are increased in CML and may be beneficial in predicting response to IM treatment in patients in the accelerated / blastic crisis (ACC/BC) phase.The MPO gene is found on chromosome 17's long arm section q12–24, and its major transcriptional product consists of 11 introns and 12 exons. In the endoplasmic reticulum, this protein product is enzymatically inactive and forms complexes with chaperons, such as calreticulin and calnexin. The insertion of a home moiety into apropos MPO results in the formation of pro MPO, which is enzyme-inactive. Three MPO isoforms have been identified based on the size of heavy chains: MPO I, MPO II, and MPO III. MPO also has a calcium-binding region, which is critical for active site structure and function[6].

Antioxidants prevent the formation of reactive oxygen

[1,3]Department of Chemistry and Biochemistry, Mustansiriyah University. Baghdad, Iraq.[2]Department of Pathology and Forensic Medicine, Mustansiriyah University. Baghdad, Iraq.

Correspondence: Khitam Abdul Wahhab Ali

Email: drkhitamalthamer@gmail.com

species (ROS) and the scavenging of free radicals. Antioxidants reduce or stop chain processes by eliminating free radicals or suppressing other oxidation reactions by getting oxidised. As a result, antioxidants are frequently reducing agents, like polyphenols or thiols.[7]

Lactate dehydrogenase (LDH) is an enzyme that dehydrates lactate. It is an H transfer (oxidoreductase) enzyme that catalyses the reversible conversion of pyruvate to lactate usingnicotinamide adenine dinucleotide hydrogen(NADH) as a cofactor[8].Serum LDH is higher in many types of cancer, but not specific to a certain type because of its varied distribution, but it might indicate tissue damage, and can be used as a prognostic factor of cancer development, disease features, tumour size as well as metastasis and organomegaly. Higher LDH levels might indicate tissue damage or the presence of leukaemia cells in the blood[9].

BCR-ABL transcript was also identified in the peripheral blood in association with high LDH levels. In this respect, it is likely that measuring LDH and BCR-ABL transcript throughout the follow-up of CML patients might be used as a therapeutic follow-up marker[10].

The current study was planned to evaluate the efficacy of serum MPO and LDH levels as tumour markers in CML patients after one-year treatment with TKIs.

Patients and Methods

The case-control study was conducted at the College of Medicine, Mustansiriyah University, Baghdad, Iraq, in collaboration with the National Centre of Haematology (NCH), Baghdad, from December 2019 to April 2020.

After approval form the ethics review committee of Mustansiriyah University, the sample was raised from among those attending NCH.

Sampling technique: The samples were from adults by Simple random sampling technique, randomly distributed regarding age and sex , they were CML patients aged ≥18 years who had completed at least one year of treatment with IM, and had no history of solid tumour, other malignant diseases or infectious diseases. Those excluded were patients with another type of haematology and solid cancer, non-compliant with treatment protocol, and having comorbid that could affect normal value of the study markers, like inflammatory and infectious diseases, cardiovascular diseases and atherosclerosis, obesity neurodegenerative diseases, liver diseases and cystic fibrosis.

After taking informed consent from those who were enrolled, data was recorded using a questionnaire that explored medical history, age, gender, CML duration and treatment, and other illnesses, while their medical records were also checked. The patients were then divided into two groups on the basis of major molecular response (MMR). Group 1 had non-MMR patients with BCR-ABL >0.1%, while group 2 patients had MMR patients with with BCR-ABL<0.1% as per the European Leukaemia Netguidelines.[11]Group 3 had healthy controls matched for age and gender.

Ten ml of blood had been collected from each patient, the serum was separated from blood samples in gel tubes, and then incubated at a temperature of (- 20°C) until the time of biochemical test. Serum MPO and LDH concentrations were measured by spectrophotometer using enzyme-linked immunosorbent assay (ELIZA) (MyBioSource Company, United States).Blood for Complete blood count was placed in EDTA tube for which was done using a haematology analyser (Hemolyzer 5NG analyticon, Germany).

Data was analysed using SPSS25. Data was expressed as frequencies and percentages, or as mean ± standard deviation, as appropriate. Students' t-test was used to compare the 2 values, and analysis for variance to compare >2 values. The significance of difference was tested using chi-square test. P<0.05 was taken as statisticallysignificant.

Results

Of the 88 subjects, 32(36.4%) were in group A with mean age 44.9±12.6 years, 26(29.5%) were in group B with mean age 48.23±10.6 years, and 30(34%) were in group C with mean age 43.1±9.3 years. Demographic and clinical characteristics, including biochemical markers, were noted (Table 1).

Table-1: Demographic and data

Parameter*	not in major molecular response CML(n=32)	in major molecular response CML (n=26)	Control group (n=30)	P Value
Age (years)	44.9±12.6	48.25±10.6	43.1±9.3	0.230
Duration (years)	8.4±4.9	8.2±5.0	----	0.889
WBC (x103)	12.53±14.22	6.17±2.47	8.07±2.73	0.021
Neutrophil	8.78±11.69	3.52±1.76	4.67±1.25	0.014
Haemoglobin (g/dL)	13.49±1.73	12.71±1.27	13.22±1.25	0.127
Platelets (x103)	289.48±111.2	222.31±44.52	218.70±51.18	0.001
LDH (IU/L)	288.97±128.3	254.97±61.39	178.67±42.63	0.0001
Calcium (mg/dL)	9.14±0.49	8.97±0.70	9.78±0.39	0.0001
Serum uric acid (mg/dL)	4.93±1.34	4.82±1.59	4.70±1.45	0.827

Table-2: Mean levels of serum myeloperoxidase (MPO) and lactic dehydrogenase (LDH) in the study groups.

Parameter	Non-MMR CML N=32	MR CML N=26	Controls N=30	P value ANOVA	Non-MMR x Control	MMR x Control	Non-MMR x MMR
Myeloperoxidase (MPO) (ng/ml) Mean ±SD	30.02±16.93	21.20±14.71	8.66±4.42	0.000^	0.0001#	0.0001#	0.041
Range	10.330-91.95	1.971-63.462	3.077-23.80				
LDH(IU/L) Mean ±SD	288.97±128.31	254.97±61.39	178.67±42.63	0.000^	0.0001#	0.0001#	0.220
Range	(200-889.4)	(185.7-424)	(129-281)				

#significant difference between two independent means using Students-t-test at 0.05 level.
^Significant difference among more than two independent means using ANOVA-test at 0.05
CML: Chronic myeloid leukaemia, MMR: major molecular response, LDHL Lactic dehydrogenase. ANOVA: Analysis of variance, SD: Standard deviation.

Table-3: Area under the curve (AUC), sensitivity and specificity of group 2 patients against group 3 controls for MPO.

Test Result Variables	AUC	Cut of value	Sensitivity%	Specificity%
Myeloperoxidase (MPO) (ng/ml)	0.875	10.95	85.4	85.48

Table-4: Correlations of serum MPO level with study variables.

		Myeloperoxidase (MPO) (ng/ml) Non-MMR CML	MMR CML	Control
Age (years)	r	-0.093	0.113	-0.041
	P	0.612	0.583	0.830
Duration (years)	r	-0.224	-0.280	-
	P	0.217	0.166	-
LDH (IU/L)	r	-0.193	-0.063	0.122
	P	0.289	0.759	0.521
Calcium (mg/dL)	r	0.504**	0.137	0.001
	P	0.003	0.504	0.999
Serum uric acid (mg/dL)	r	0.367*	0.056	0.136
	P	0.039	0.786	0.473
WBC (x103)	r	-0.223	0.170	-0.039
	P	0.220	0.408	0.839
Neutrophil	r	-0.222	-0.037	0.087
	P	0.223	0.859	0.646
Lymphocyte	r	-0.231	0.439	0.277
	P	0.204	0.062	0.139
Monocyte	r	-0.108	0.326	0.139
	P	0.558	0.104	0.464
Eosinophil	r	-0.307	0.288	-0.140
	P	0.088	0.153	0.459
Basophil	r	0.013	-0.108	-0.052
	P	0.942	0.599	0.785
Haemoglobin (g/dL)	r	-0.221	-0.075	0.177
	P	0.223	0.715	0.350
Platelets (x103)	r	-0.001	-0.005	-0.179
	P	0.996	0.983	0.343
Malondialdehyde (ng/ml)	r	0.267	-0.083	-0.017
	P	0.139	0.688	0.927

Correlation is significant at the 0.05 level. **Correlation is highly significant at the 0.01 level. MMR: Major molecular response, CML: Chronic myeloid leukaemia, LDHL Lactic dehydrogenase, WBC: White blood cell.

There was a significant increase in MPO and LDH levels in patient groups compared group 3 controls ($p<0.05$). Between the patient groups 1 and 2, the difference was significant for MPO ($p<0.05$), but not for LDH ($p>0.05$) (Table 2).

Area under the curve (AUC), sensitivity and specificity of group 2 patients compared to group 3 controls were noted for MPO (Table 3).The correlation of MPO levels with related biochemical variables were noted separately (Table 4).

Discussion

The present study showed that MPO levels for all CML patients were elevated significantly compared to the healthy controls, which is in line with an earlier study[4]. Plasma MPO levels may be useful in predicting response to IM therapy. Studies have indicated that increased MPO levels was associated with oxidant-antioxidant imbalance.[6]

In addition, serum LDH levels in the current study were significantly increased in the two patient groups compared to the control group, indicating that there was tissue damage or leukaemia cells in the blood, which has been shown by previous results.[12]

Habte et al.reported a high level of LDH in CML patients compared to the controls at diagnosis.[13]One study mentioned that the significant elevation in LDH levels in CML patients could be due to other causes, such as chemotherapy treatment alterations.[14]

In the current study, the mean serum calcium (Ca+2) levels showed significantly positive correlation with mean serum MPO levels in CML non-MMR patients. Gorudkoet al.[15] showed that MPO levels induced Ca2+mobilisation from the intracellular calcium stores of neutrophils and influx of extracellular Ca2+.

Limitations: The current study has limitations as the sample size was not calculated which could have affected the power of the study.

Conclusion

Serum MPO and LDH levels were significantly increased in CML patients compared to healthy controls, indicating the presence of higher oxidative stress in CML patients.

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Conflict of Interest: None.

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