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Prospective Biochemical Analysis of Chronic Myeloid Leukemia Patients in Response to Tyrosine Kinase Inhibitors

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ABSTRACT

Chronic myeloid leukemia (CML) is a chronic proliferating cancer of bone marrow presently treated with BCR-ABL tyrosine kinase inhibitors (TKI). Within interacellular system, increased reactive oxygen species (ROS) production in response to antioxidant (AOX) defense systems lead to oxidative stress (OS). Our study aimed at biochemical profiling of CML patients in response to imatinib or nilotinib therapeutic drugs. Fresh venous blood sample (10 mL) of 170 CML diagnosed patients and 10 healthy individuals was collected in heparin vial from oncology department, Mayo hospital and Jinnah hospital Lahore, Pakistan. Biochemical profiling of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), nitric oxide (NO), advanced oxidation protein product (AOPP), advanced glycation end products (AGE's) and micronutrients (retinol, ascorbic acid, alpha tocopherol), complete blood count, liver profile, renal profile, lipid profile, serum electrolytes were evaluated in CML and control groups. Our study reported that no significant difference in biochemical profile among treated with Nilotinib or Imatinib tretated groups while in comparision to control group, a marked difference was observed. Antioxidant biomarkers i.e. MDA, SOD and CAT were augmented in control group as compared to CML treated groups. Decreased GSH level was reporetd in the CML group while increased in the nilotinib treated group compared to the Imatinib treated group. Other stress markers i.e. NO, AGEs and AOPP were also found to be high in level in control group compared to CML treated group. Micronutrient i.e. retinol, ascorbic acid, alpha tocopherol were increased in treated groups as compared to the control group. Our study concluded that oxidative stress that is responsible for the progression of CML is manageable with the use of chemotherapeutic drugs.

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HMA conducted all the research trials and prepared the manuscript. AA and MKAK supervised the research work and performed statistical analysis.

Key words
CML, ROS, Oxidative stress, GSH, Imatinib, MDA, Nilotinib, SOD, Vitamins

INTRODUCTION

Leukemia is a group of blood cancers that initiates in the bone marrow and causes uncontrolled multiplication of blood cells. These uncontrolled proliferated blood cells are known as blast or leukemia cells (Ahmad *et al*., 2019). White blood cells (WBCs) determine the progression and development of leukemia. Myeloid, also known as myelogenous leukemia can initiate in the WBCs and platelets other than lymphocytes (Ahmad *et al*., 2019). Acute myeloid leukemia (AML) is a rapidly growing form of blood cancerous cells and bone marrow (Recher, 2021). Chronic myeloid leukemia (CML), also called chronic myelogenous leukemia, initiates in the bone marrow blood forming cells, later spreads to the blood over the time. Eventually, the cancer spreads to other parts of the body (Zuo *et al*., 2021).

Clonal hematopoiesis (CH) explains the expansion of the clonal population of large blood cells with one or more somatic variations. CML is referred as clonal hematopoietic stem cell disorder that reported for around 30% of the cases of fully developed leukemia (Miranda-Filho *et al*., 2018). With the emerging use of tyrosine kinase inhibitors (TKIs), the life expectancy of CML patients has improved significantly (Apperley, 2015). Analogous epidemiological variables i.e. age standard rate (ASR) is mandatory to measure the liability of CML globally.

Currently, accessible record reveals that the distribution of CML events varies with different factors i.e. age, gender, and region (Miranda-Filho *et al*., 2018). Incidence of CML differs from 0.5/100,000 to 1.65/100,000 people in different countries annually (Hoglund *et al*., 2015; Chang *et al*., 2011; Chen *et al*., 2013). CML is more communal in men than in women with a men/

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women ratio of 1.2-1.7 (Berger *et al*., 2005; Rohrbacher and Hasford, 2019).

Last few years ago, high level of improvement has been qualified for the therapy of chronic myeloid leukemia. Still interferon alpha and stem cell transplantation were only the remedial choices for the treatment of chronic myeloid leukemia disease persons. Stem cell transplantation until a suitable healthful remedial method and react as effective choice especially for those patients that do not react properly for tyrosine kinase inhibitors. Although in present era, the molecularly targeted tyrosine kinase inhibitor's field burst that run by imatinib mesylate drug, change the ordinary past and remedial access to chronic myeloid leukemia disease (Olavaria *et al*., 2002).

Among TKI, Imatinib mesylate (IM) was first TKI approved for the treatment of CML (Jabbour and Kantarjian, 2018). Imatinib is a phenylamino pyrimidine whose primary target is tyrosine kinase (TK) activity in BCR-ABL1 and progressively inhibits the ATP binding site of the BCR-ABL protein, making it effective for BCR-ABL signal transduction, which results in the loss of downstream pathways in BCR-ABL transduction activity (Hoglund *et al*., 2013). Nevertheless, it is projected that nearly 20-30% of patients will ultimately develop resistance to treatment. Imatinib drug has developed the common treatment for chronic myeloid leukemia due to its noticeable activity lesser toxicity outline. In first stage of dosage finding study of disease persons that were biased to or failed to interferon alpha treatment, dosages of twenty-five to one thousand mille grams were examined at daily basis (O'Brien *et al*., 2003).

Reactive oxygen species (ROS) cannot be measured in a direct way due to short survival time. ROS can be measured by indirect methods or oxidative stress (OS) markers used to measure oxidized proteins, lipid products (Cacciapuoti, 2016). Oxidation of lipids is measured as malonyl dialdehyde (MDA) and total lipid hydroperoxide. Antioxidants play a vital role in building the body's resistance to free radicals (Vinturis and Gaman, 2020).

Oxidative stress (OS) may define as it is the unbalance between high construction of ROS (reactive oxygen species) and the cellular antioxidant defense mechanism. Reactive oxygen species included many biological procedures at low meditation like cell signaling, enzyme activation, gene expression, apoptosis, antimicrobial resistance and body process, but ROS at higher level induce purposeful and physical cellular modifications (Dalle-Donne *et al*., 2006). OS is recently accepted as an outstanding aspect for acute and chronic sickness, even for carcinogenic and leukemia diseases. However, mechanism of resistance of body would perform a significant function in the form of antioxidant and it effort to overcome the loss, adjustment itself to above nerve- ranking. Antioxidants are the elements that discard, salvage and conquer the composition of reactive oxygen species, or may defend their roles so antioxidants could react as a major function for different illnesses like cancer and their medical appearance (Abdollahi *et al*., 2014).

The study aimed to highlight the biochemical analysis of chronic myeloid leukemia patients in response to tyrosine kinase inhibitors.

MATERIALS AND METHODS

Experimental work was performed in Biochemistry research lab, University of Central Punjab, Lahore, Pakistan. Whole experimental work was performed after the approval of Human Research and Ethics Committee (HREC) constituted by the UCP for FLS vide No. UCP/Reg+/Notification/2329 dated April 03, 2018. Fresh venous blood sample (10 mL) of 170 CML diagnosed patients and 10 healthy individuals was collected in heparin vial. CML patients with no any other previous medical complications/ chronic history were hospitalized in Oncology Ward, Mayo hospital and Jinnah hospital Lahore, Pakistan.

Biochemical studies

Biochemical markers employed in this study were superoxide dismutase (SOD) (Spitz and Oberley, 2001), malondialdehyde (MDA) (Battisti *et al*., 2008), catalase (CAT) (Weydert and Cullen, 2011), glutathione (GSH) and ascorbic acid (VIT C) (Gladwin and Wang, 2006), nitric oxide (NO) (Moshage *et al*.,1995), retinol (Vitamin A) (Rosenberg *et al*., 1992) and α- tocopherol (Vitamin E) (Ragino and Kashtanova, 2002). AGE's and AOPP were measured following protocol of Kalousova *et al*. (2002). Liver function tests (LFTs), renal function tests (RFTs), serum electrolytes and lipid profiles were measured by using Beckman coulter AU480 automated chemistry analyzers (Schumann *et al*., 2002; Tolman and Rej, 1999).

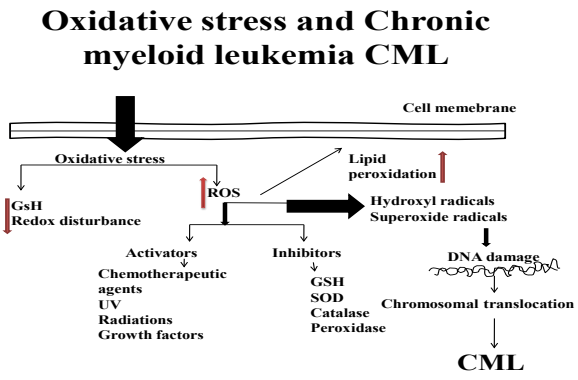

Fig. 1. CML mediated oxidative stress.

Hematological studies

For the analysis of complete blood count, blood samples were collected in EDTA vials. Blood samples were analyzed within 3 h after collection. The complete blood count (CBC) was performed using basic laboratory equipment automated hematology analyzer, which counts cells and collects information on their size and structure. The concentration of hemoglobin was measured, and the red blood cell indices were calculated from measurements of red blood cells and hemoglobin. Instrumental adjustment was evaluated regularly with commercial calibrant 5C (Beckman Coulter) (Schumann *et al*., 2002).

Statistical analysis

Mean values of each control and treated samples in triplicate were averaged and statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) (Version 23). Analysis of Variance (ANOVA) and Dunett's T3 Post Hoc test was applied to check the significance and to study comparison among groups respectively. Pearson's correlation coefficient was applied to check the relationship among variable statistically. Calculated values were expressed as Mean ± S.E.M.

RESULTS

Our findings predicted no significant difference regarding biochemical markers between Nilotinib and Imatinib treated CML groups. However, when these two treated groups compared with the control group, substantial difference was reported in our study (Table I). Data revealed that elevated level of oxidative stress biomarkers (MDA, SOD and CAT) in the CML control group compared to treated groups and healthy group. Decreased GSH level was reported in the control group in contrast to treated group (Table I). Stress related markers (NO, AGE's, and AOPP) showed augmented trend in control group as compared to treated groups. Our findings demonstrated that micronutrient (Ratinol, Ascorbic acid and tocopherols) levels was increased in healthy subjects and treated groups as compared to CML control group (Table I). Our findings related to MDA among both genders in treated group revealed no significant differences statistically (Table I).

Data presented in Table II revealed that no significant difference exist in BCR-ABL between treated groups of CML. BCR-ABL level was elevated remarkably in CML patients as compared to healthy individuals. Hematologic profile shows that Hb and RBC's level was dropped in CML patients as compared to healthy persons and treated groups. Table demonstrated that platelets and WBC's count high in CML patients as compared to healthy control and no significant difference found in treated groups but significant as compared to diseased subjects (Table II). Results predicted that serum electrolyte profile (Na^+, K^+, Cl^-) has no significant change in treated groups as compared to diseased persons while serum chloride (Cl-) level was jumped high in diseased group as compared to treated and healthy groups (Table II). Results of liver function test (LFT's) biomarkers shows that serum significant difference exist in treated groups in case of alkaline phosphate (ALP) while other biomarkers (ALT, AST, total bilirubin) were elevated in CML patients as compared to healthy subjects and in case of AST significant difference shows in treated group as compared to healthy individuals (Table II). Results of renal profile (serum urea and serum creatinine) predicted that imatinib and nilotinib decreased the level of urea while serum creatinine level was elevated in diseased group as compared to healthy subjects. Serum uric acid shows no significant change in the level among treated, healthy and

Table I. The Mean±SEM of oxidative stress, antioxidants and micronutrients of the different groups which were examined.

Parameters	Healthy control	CML Patients (without treatment)	Nilotinib treatment	Imatinib treatment
MDA (mmol/ml)	0.93±0.06^{a}	3.99±0.19^{b}	0.85±0.09^{a}	0.87±0.08^{a}
SOD (µM/mL)	0.76±0.04^{a}	2.58±0.13^{b}	0.67±0.07^{a}	0.58±0.04^{a}
CAT (mmol/ml)	1.12±0.13^{bc}	2.24±0.15^{bd}	0.39±0.06^{a}	0.51±0.10^{a}
GSH (mg/dl)	3.73±0.58^{a}	0.48±0.04^{b}	0.51±0.03^{b}	0.45±0.03^{b}
NO (µm/L)	3.29±0.32^{bc}	5.30±0.16^{bd}	0.78±0.09^{a}	0.69±0.07^{a}
AGEs (U/ml)	2.70±0.21^{bc}	1.39±0.26^{bd}	0.89±0.09^{a}	0.88±0.08^{a}
AOPP (ng/ml)	2.13±0.35^{b}	1.56±0.19^{a}	1.35±0.07^{a}	1.29±0.07^{a}
Ratinol (µg/ml)	1.24±0.07	0.55±0.09^{b}	1.24±0.29	1.11±0.06^{a}
Ascorbic acid (µg/ml)	1.87±0.11^{b}	0.63±0.06^{b}	2.26±0.05^{a}	2.11±0.06^{a}
Tocopherol (µg/ml)	6.07±0.45^{a}	0.95±0.08^{b}	1.13±0.05^{b}	0.97±0.05^{b}

a, b, c, d = Different superscript show the statistical difference between the groups. Level of significance: P<0.05

Table II. Hematologic and biochemical profile of the different groups which were examined. (The values are Mean±SEM).

Parameters	Healthy control	CML Patients (without treatment)	Nilotinib treatment	Imatinib treatment
BCR-ABL (%)	4.26±0.69[b]	77.33±6.39[a]	61.68±4.07[a]	68.57±3.70[a]
Haemoglobin (g/dl)	14.30±0.30[b]	10.12±0.30[a]	10.84±0.77[a]	11.02±0.26[a]
RBC (×10)6/ul	5.30±0.15[b]	3.40±0.13[a]	3.52±0.092[a]	3.66±0.07[a]
Platelets $(\times 10)^3$/ul	266.80±10.34	344.54±37.62	299.74±29.92	243.26±14.25
WBC $(\times 10)^3$/ul	5.89±0.27[b]	124.70±31.05[a]	48.40±9.74[a]	53.74±11.04[a]
Neutrophils (%)	49.10±1.64[b]	54.54±3.14	55.39±1.49[a]	54.78±1.46
Lymphocytes (%)	30.20±1.55	29.50±3.63	31.05±1.99	34.26±1.53
Na^+ (mmol/L)	139.60±0.84	138.6±0.86	138.28±0.48	137.84±0.42
K^+ (mmol/L)	4.25±0.14	3.92±0.09	4.10±0.06	4.07±0.07
CL^- (mmol/L)	81.10±4.59[b]	101.04±2.13[a]	99.61±1.14[a]	100.71±1.00[a]
ALT (SGPT) (U/L)	27.30±1.93	28.63±1.86	28.98±1.80	27.81±3.06
AST (SGOT) (U/L)	20.70±1.36[b]	27.40±1.36	30.10±1.67[a]	29.96±1.54[a]
ALP (IU/L)	83.20±3.86[b]	108.54±5.15[b]	108.64±4.10[a]	115.55±5.86[b]
Total bilirubin (mg/dL)	0.74±0.05	4.61±3.87	2.06±1.27	0.80±0.05
Urea (mg/dL)	22.00±1.43	21.77±0.93	20.95±0.56	19.34±0.58
Creatinine (mg/dL)	0.65±0.03	0.79±0.05	0.73±0.02	0.85±0.08
Uric acid (mg/dL)	4.57±0.19	4.92±0.23	4.82±0.13	4.73±0.09
Cholesterol (mg/dl)	162.60±3.80	161.04±2.08	159.54±1.37	156.22±1.24

a, b = Different superscript show the statistical difference between the groups. Level of significance: $P<0.05$

Table III. Pearson's correlation coefficient between various parameters of CML patients.

Parameters	CML	Overall	Imatinib treatment	Nilotinib treatment
MDA × GSH	0.393^{NS}	-0.120^{NS}	-0.296^{**}	-0.324^{**}
MDA × NO	-0.038^{NS}	0.902^{**}	0.811^{**}	0.840^{**}
MDA × AGEs	-0.425^{*}	0.531^{**}	0.945^{**}	0.955^{**}
MDA × Vit. C	-0.348^{NS}	-0.718^{**}	-0.418^{**}	-0.298^{*}
MDA × Vit. E	0.013^{NS}	0.160^{*}	0.459^{**}	0.388^{**}
SOD × NO	0.583^{**}	0.853^{**}	0.714^{**}	0.459^{**}
SOD × AGEs	0.040^{NS}	0.444^{**}	0.850^{**}	0.484^{**}
SOD × Vit. E	0.090^{NS}	0.181^{*}	0.516^{**}	0.373^{**}
CAT × NO	0.032^{NS}	0.720^{**}	0.455^{**}	0.836^{**}
CAT × AGEs	0.585^{**}	0.552^{**}	0.466^{**}	0.910^{**}
CAT × AOPP	0.300^{NS}	0.492^{**}	0.544^{**}	0.772^{**}
GSH × AGEs	-0.521^{*}	-0.257^{**}	-0.216^{**}	-0.253^{*}
GSH × Vit. E	0.535^{*}	0.295^{**}	0.243^{**}	0.285^{*}
NO × AGEs	-0.220^{NS}	0.433^{**}	0.830^{**}	0.831^{**}
AGEs × AOPP	0.797^{**}	0.854^{**}	0.889^{**}	0.848^{**}
AGEs × Vit. C	0.443^{*}	-0.322^{**}	-0.409^{**}	-0.327^{**}
AGEs × Vit. E	-0.547^{**}	0.291^{**}	0.549^{**}	0.418^{**}
AOPP × Vit. C	0.292^{NS}	-0.250^{**}	-0.320^{**}	-0.294^{*}
AOPP × Vit. E	-0.216^{NS}	0.437^{**}	0.586^{**}	0.544^{**}

diseased groups. Serum cholesterol level has no significant change between diseased and healthy groups while imatinib slightly decreased the level of cholesterol (Table II).

Our results displayed the significant positive correlation of MDA with NO and AGEs in both treated groups while it showed negative correlation with GSH and Vit C (Table III). Highly significant and positive correlation of SOD with NO, AGEs and Vit E was reported in treated group (Table III). Highly significant positive correlation of CAT with NO, AGEs, and AOPP was noticed in both treated groups. Similarly, highly positive correlation of GSH × Vit E and NO × AGEs was reported in our studies (Table III). AGEs × AOPP, AGEs × Vit E and AOPP × Vit E showed significant positive correlation in both treated groups while AGEs × Vit C, AOPP × Vit C displayed the significantly negative correlation in both treated groups (Table III). As a whole, our study revealed the highly significant positive correlation among all except MDA × Vit C, GSH × AGEs and MDA × GSH which showed significant negative correlation among treated groups and overall (Table III).

DISCUSSION

In CML patients a main treatment option shows that tyrosine kinase inhibitors adenosine triphosphate have

competitive effect with TKI's optimum responders have close to existing probability (Kantarjian *et al*., 2003). After many trials, it is recommended that life time cure must be suggested for disease persons with such a so called molecular undetectable disease (MUD). The long time response of TKI's may not remain as effective in the course of 10 years and the chances for the development of resistance may occur. TKI's disruption may be predicted prospectively (Hochhaus *et al*., 2000). Imatinib, nilotinib and dasatinib may be clogged for long time in disease persons had DMR's (deep molecular responses). Deep molecular responses score of assessable RT-qPCR (reverse transcriptase polymerase chain reaction) sensitivity known as MR4, MR4.5, and MR5 (molecular response) (Molldrem *et al*., 2000). Recently diagnosed chronic myeloid leukemia patients in chronic phase with positive philadelphia chromosomes have initial cure with imatinib, dasatinib, nilotinib and bosutinib drugs. After usage of interferon alpha treatment, imatinib is also successful for the treatment of chronic myeloid leukemia patients of positive Philadelphia chromosomes in acute phase or chronic phase (Molldrem *et al*., 2000; Shaker *et al*., 2011).

MDA is the end product of lipid peroxidation and involve in the regulation of neoplasm related gene aspects (Donne *et al*., 2006). The serum MDA level considered major biomarker for the estimation of lipid peroxidation. Donne *et al*. (2006) concluded in their study that serum MDA level was elevated in CML patients as compared to control subject significantly. It was hypothesized that higher level of lipid peroxidation may cause malignancy or these malignant cells originate a large amount of reactive oxygen species activity (Yilmaz *et al*., 2003; Morabito *et al*., 2004). Ciarcia *et al*. (2016) evaluated the formation of MDA, a biomarker of oxidative stress. The results achieved in healthy subjects and in CML-peripheral blood mononuclear) cells before or after treatment on MDA levels explore that the MDA value in basal CML were remarkably higher with respect to control cells similar to present study.

Jadeski and Chakraborty (2002) described that the mean serum SOD levels were elevated in CML patients as compared to control group. SOD is a higher cellular defense mechanism against superoxides in the cells. The serum SOD level may be exalted due to change in gene aspects in hematopoietic cells (Jorgensen and Holyoake, 2007). SOD considered a dependable biomarker of oxidative stress and their activity is performed to validate a probable correlation between inductions of oxidative stress. CML-PBM cells dropped the SOD activity. The addiction of Li in CML-PBM cells also dropped the SOD potential from 34.63 to 26.12 U/mg proteins ($P<0.05$ vs. basal CML). The in vivo treatment with DAS, IM, and NIL remarkably declined the SOD activity in CML-PBM cells (Ciarcia *et al*., 2016).

Lala and Orucevic (1998), described that elevated serum catalase level was observed than healthy volunteers. The high accumulation of ROS leads to the increase in the production of free radicals due to which the antioxidant level decreases in the body. The antioxidant enzyme i.e. CAT is decreased in lymphocytes of CML patients as compare to healthy subjects. Declined catalase expression produced due to increased formation of H_2O_2 which is responsible for the activation of signaling pathways which induce multiplication, relocation and invasion in cancer cells.

Wu *et al*. (2004) reported that the serum GSH level was significantly declined as compared control one. Due to the elevation of ROS in hematopoietic cells the serum GSH level was decreased. They reported decrease level of GSH with respect to control subjects. The present study was in agreement with the study conducted by Wu *et al*. (2004) which explore the serum GSH level was statistically significantly decreased in CML patients as compared to treated groups.

Meeta *et al*. (2001) evaluated that the serum NO level was elevated in chronic myeloid leukemia patients as compared to healthy volunteers. Serum NO level decreases by using imatinib and statistically significant. After imatinib therapy, a significant decrease was observed in serum level of NO in chronic myeloid leukemia patients. In present study the serum NO level increased in CML patients as compared to healthy volunteers and statistically significant.

The present study reports that AGEs had significantly high value of cancer control which is similar to study conducted by Zdenek *et al*. (2014).

Mayes described that in all leukemic patients, it is observed that ascorbic acid level significantly decreased in CML patients than drugs treated group. The white blood cells engulf ascorbic acid that is available in serum or plasma (Mayes, 2000). Low amount of ascorbic acid in serum may cause aggregation of tocopheroxyl radical that is not converted into tocopherol again (Wang *et al*., 2001). Present study reports that ascorbic acid level was declined in CML patients while treatment with imatinib and nilotinib helps to improve the ascorbic acid level in CML patients.

Pujari *et al*. (2007) investigated that level of retinol in CML patients decreased significantly as compared to patients treated with drugs. Deficiency of retinol may cause inadequate defense mechanism against free radicals as a result lipid peroxidation may increase. In Present study serum retinol level also decreased in CML patients due to increased production of reactive oxygen species,

on the other hand nilotinib and imatinib elevate the retinol level in patients and this was the agreement with the study conducted by Pujari *et al*. (2007).

Pujari *et al*. (2012) demonstrated that tocopherol level significantly decreased in CML patients than treated groups with drugs which was highly significant. To lower down level tocopherol the expected mechanism is that there may be higher level of untreated lipid peroxidation (Burke and Carroll, 2010; Bourgeais *et al*., 2017). Due to increase in lipid peroxidation, it may increase free radical formation with insufficient defense mechanism for free radicals and increased lipid peroxidation (Steegmann *et al*., 2016).

The present study shows that mean ± S.E.M. values of cholesterol had no significant difference between groups which was in agreement with the study conducted by Zdenek *et al*. (2014) while not in agreement with the study which shows the cholesterol statistically significant elevated in nilotinib-treated patients. The difference in results due to the difference in protocol and quantity of medicine (Kim *et al*., 2013; Petrikova *et al*., 2021).

The present study showed that the elevation of LFTs in the groups which were treated by imatinib and nilotinib which was agreement with the study of Tian (2016). At the starting level, elevation of transaminases were very common and it triggered because of damage of chronic leukemia cell penetration (Khaleel, 2017).

In the present study the mean±SEM values of RFTs had no significant difference. Furthermore, the treatment with imatinib and nilotinib had no significant difference in patients groups, which was in agreement with the study conducted by Khaleel (2017).

Shah (2007) investigated that hematologic parameters (RBC's, WBC's, Platelets, hemoglobin concentration) showed significance differences in CML patients as compared to healthy subjects and patients treated with imatinib and nilotinib. They reported that leucocyte count was raised remarkably in CML patients. Median of lymphocytes is lower in chronic phase CML. Hayran *et al.* (2006), reported that ideal level of peripheral blood count to diagnose the CML is based on leucocytes and neutrophils counts. They observed that white blood count elevated remarkably in CML patients and this elevation might be associated with and physical and pathological finding i.e. organomegaly or splenomegaly. In present study, white blood count was also elevated in CML patients and decline in treated patients. The present study was in agreement with the studies conducted by Shah (2007) and Hayran *et al*. (2006).

Li *et al*. (2021) in their study reported that red blood cell distribution width (RDW) play a crucial role in the prognosis and TKIs treatment effects in CML patients. They concluded in their study that RBCs level dropped dramatically in CML patients at the time of diagnosis which also leads to decline in the concentration of Hb. This is due to the increase production of infective/defective RBCs which leads to the excessive destruction of RBCs and this destruction is responsible for the elevation of RDW. In present study, the erythrocytes count was also declined in CML patients and this study was in agreement with the study conducted by Li *et al*. (2021).

CONCLUSION

In present study, there is growing evidence that oxidative stress is key component in the pathophysiology of CML. The extremely increase per oxidation of lipid membrane along with fall off activity of antioxidant enzyme was observed in CML patients. Present study concluded that nilotinib has great potential to lower the lipid peroxidation level and to enhance the defense activity of micronutrients as compared to imatinib in CML patients. Nilotinib also proven to reduce the production of leukocytes in comparison with imatinib. While imatinib shows higher potential to decline the oxidative stress markers (NO, AGE's and AOPP) with contrast to nilotinib. Present study may also be recommended that antioxidant supplementation support the enzymatic defense system to reduce oxidative stress. Further studies will also be required to investigate that which antioxidant, at which dosage and in which combination with chemotherapeutic drugs give positive result with least risk.

Statement of conflict of interest

The authors have declared no conflict of interest.

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