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PRE AND POST TREATMENT EFFECT OF IMATINIB AND ANTI-OXIDANT STATUS IN CHRONIC MYELOID LEUKEMIA PATIENTS

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ABSTRACT

Background: Chronic myeloid leukemia (CML) is being a disorder marked by excessive proliferation of bone marrow elements and especially blood cells (leucocytes). Structural characteristics in CML, loss of leucocytes function, chromosomal translocation between chromosome 9 and chromosome 22 forming Philadelphia (Ph) chromosome and enlarged spleen result in an imbalance between free radical production and antioxidant defences. CML is generally associated with old age, hypertension, Genetic mutation, environmental factors and oxidative stress. To determine the anti-oxidant status, micronutrients level in CML patients before and after treatment with Imatinib.

Methodology: Comparative Study. Fifty (pre-treatment imatinib) diagnosed patients of CML and same fifty (post treatment imatinib) CML individuals were taken in the study. Blood sample collected from Mayo Hospital Lahore. 5.0 ml blood samples were taken and subjected to centrifuge at 3000-4000 rpm for 10-15 minutes for the separation of serum. The levels of oxidative stress biomarkers (GSH, SOD, AGES, NO, AOPP, MDA), serum electrolytes and micronutrients were estimated.

Results: NO level in CML (post treatment imatinib) patients was elevated remarkably (0.418± 0.077) as compared to CML (pre-treatment imatinib) patients (0.188± 0.244) and statistically significant (p-value <0.00). MDA level was also increased in (post treatment imatinib) CML patients (0.451 ± 0.076) as compared to (pre-treatment imatinib) CML patients (0.257 ± 0.159) and statistically significant (p-value <0.00). The level of advanced oxidation protein product (AOPP) in CML (pre-treatment imatinib) patients was decreased (0.607 ± 0.0.136) as compared to CML (post treatment imatinib) patients (1.23 ± 0.271) and also statistically significant (p-value <0.00).

Conclusion: The present study concluded that Strong association exists between Oxidative stress, Imatinib (TKI), Micronutrients and Serum electrolytes balance in CML patients. Pre- treatment imatinib patients of CML disease have increased Lipid per oxidation leads to elevated level of MDA remarkably where as Anti-oxidants decrease. Elevation in the Nitric oxide, Glutathione, superoxide dismutase and decrease level of vitamins are the cause for the progression of chronic myeloid leukemia CML disease.

Keywords: *CML, Tyrosine kinase inhibitors, oxidative stress, Lipid Peroxidation, Imatinib*

INTRODUCTION

Chronic myelogenous leukemia (CML) is a fatal myeloproliferative disease characterized by massive expansion of hematopoietic progenitor cells with the appearance of cells of the granulocyte (GR) lineage in the peripheral blood (Abdollahi *et al.,* 2014). CML depends on the phase of the disease and the amount of blasts in the bone marrow, as well as other factors like the age of the patient, blood counts, and if the spleen is enlarged (Abdullah and Maha, 2022).The disease course is triphasic, starting with a chronic phase, progressing to an accelerated phase and ultimately ending in a terminal phase (Abraham *et al.,* 2022). Triphasic of CML is chronic, accelerated, and blastic. The phase is determined by the progression of the disease and is measured by the number of blast cells. Blast cells are abnormal white blood cells that don't mature properly and crowd out healthy cells. In chronic phase the blood and bone marrow contain less than 10% blasts. Blasts are immature white blood cells (Ahmad *et al.,* 2022).

Chronic myeloid leukemia (CML) is caused by Bcr-Abl, a constitutively active tyrosine kinase. Imatinib mesylate (Gleevec, Glivec), a specific small-molecule inhibitor of Bcr-Abl, has become the standard drug therapy for CML in all phases of the disease (Arsalan *et al.,* 2023). Responses to imatinib may occur at the hematologic, cytogenetic and molecular levels. The two large multicenter studies of imatinib in patients with CML in first chronic phase, either newly diagnosed or after failure of interferon-alpha (IFN) , used a dose of 400 mg imatinib daily (Ali *et al.,* 2016). Imatinib at a dose of 400 mg daily is the current standard therapy for CML in chronic phase. Higher doses are promising, but results await confirmation in prospective studies. Discontinuation of treatment is almost invariably followed by recurrence, consistent with persistence of residual disease. Monitoring of patients on therapy should be done according to standardized guidelines, with qPCR for BCRABL emerging as the most important test (Achkar *et al.,* 2016).

Imatinib (GlivecR, GleevecTM), is orally administered, and has dramatically diminished the use of allogeneic stem cell transplantation (Alkan *et al.,* 2023). Cytogenetic abnormalities produce a constitutively active BCR-ABL tyrosine kinase, and this has been implicated in the pathogenesis of CML. BCR-ABL TKIs can inhibit the BCR-ABL pathway and significantly reduce the proliferation of BCR-ABL positive CML cells (Ashariati and Ugroseno, 2013). Imatinib, a 2-phenylamino pyridine-based ATP competitive inhibitor, binds to the inactive conformation of the ABL protein tyrosine kinase and competitively blocks the ATP binding site and prevents its conformational switch to the active form (Baccarani *et al.,* 2019). Imatinib inhibits cellular proliferation and tumor formation without the induction of apoptosis.The BCR-ABL protein is considered an ideal target for imatinib, since the BCR- ABL mutation is present in almost all patients with CML. Imatinib specifically inhibited or killed proliferating myeloid cell lines containing BCR-ABL but was minimally harmful to normal cells. In addition to BCR-ABL, imatinib also inhibits the c-KIT ad PDGFR tyrosine kinases (Balatzenko *et al.,* 2011).

CML patients treated with imatinib as first-line therapy will have disease progression. Major causes of imatinib resistance include the emergence of leukemic clones with mutations in the kinase domain of BCR-ABL and clonal evolution (Berger *et al.,* 2022). Because of the three known targets of Glivec, many potential cancers can be speculated to be good candidates for clinical testing of this new drug. However, in most cancers, tumor-genesis is complex and involves the disruption of multiple genes and signaling pathways. By contrast, CML can be considered as one of the few

examples of a malignancy in which a single signaling pathway defect is thought to cause the disease. In addition, in contrast to most of the solid tumors, for which the measurement of tumor response is complex, pharmacodynamic response in CML can be measured easily using blood leukocyte count as the end point (Bhatti *et al.*, 2012).

Oxidative stress, a pervasive condition of an increased number of reactive oxygen species, is now recognized to be prominent feature of various diseases and their progression. Thus antioxidants, which control the oxidative stress state, represent a major line of defense regulating overall true state of health (Bidikian *et al.*, 2023). The relationship between antioxidants status and levels of well-known markers of oxidative stress that are measured as lipid peroxides and oxidized proteins reflect better health indices and postures. Antioxidant is a broad term that refers to a myriad of different compounds. Because of the disparity in the biological actions and targets of antioxidants, there is no simple paradigm for advising patients of their safe use during conventional chemotherapy and radiotherapy (Bilajac *et al.*, 2022).

Radiation therapy is directed at specific areas or tumors, reducing the damage to normal cells or tissue. Radiation treatment also can be used to abolish the cancerous cells that remain after surgery. In Chemotherapy treatment uses drugs to stop the growth of cancer cells. Generally chemotherapy kills or stops cells from dividing throughout the body. Surgery is sometimes essential to remove a distended spleen. This type of surgery is known as splenectomy. Monoclonal antibody therapy treats the cancer with antibodies which are prepared in vitro from immune system cells (Bourgeais *et al.*, 2017).

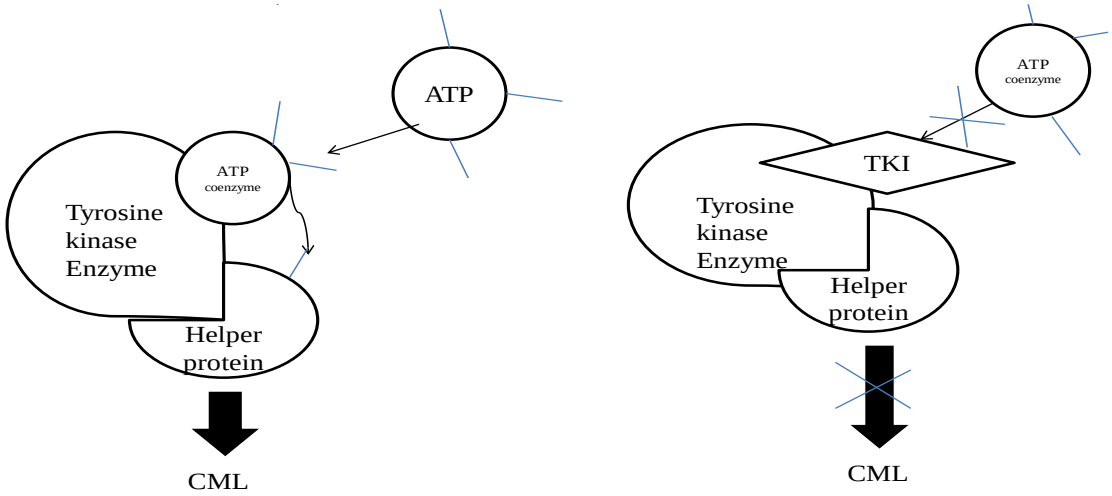

Figure 1: Mechanism of Action Tyrosine Kinase Inhibitors (Imatinib) in CML

MATERIALS AND METHODS

Place of Work

The whole experimental work was done in the Biochemistry Lab, School of Biochemistry and Medical Lab Technology, Faculty of Allied Health Sciences Minhaj University Lahore after the approval of Ethical and Research Committee, Minhaj University Lahore.

Study Design

Whole study was divided into two groups i.e. 1st group A consist of Diseased Persons (Pre-treatment) and 2nd group B Consist of post treatment (Post Group).

Sr.No	Group	Sample Size (n)
A	CML Pre-Treatment (Non-Imatinib)	50
B	CML Post-Treatment (Imatinib)	50

Blood/Data Collection

Venous blood sample (5.0 mL) of fifty (50) (pre-treatment imatinib) diagnosed patients of CML and same fifty (post treatment imatinib) CML individuals were taken in clotted gel vial from oncology department, Mayo Hospital Lahore. Blood was further processed for the estimation of Reduce Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Estimation of Nitric oxide (NO), Estimation of micronutrients (Vitamin A, Vitamin C and Vitamin E), and Electrolytes concentration by flame photometer (Na^+ and K^+) by kit method.

Blood/Sample Analysis

Blood was centrifuged at 4000 rpm for 10 minutes and serum was separated. Blood sample was collected into EDTA tubes or gel clotted vials.

Estimation of Superoxide Dismutase (SOD)

SOD was measured by spectrophotometric method of Kakkar *et al.,* (1984).

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

MDA was measured by spectrophotometric method of Ohkawa *et al.,* (1979). MDA is the test used for the oxidative stress that occurs in the cell. The peroxidation of lipids is basically damaging because the formation of lipid peroxidation products leads to spread of free radical reactions.

Estimation of Catalase (CAT)

CAT was measured by spectrophotometric method of Aebi, (1984). Catalase is a common antioxidative enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen per second.

Determination of Glutathione (GSH)

Estimation of Glutathione was measured by Moron *et al.,* (1979).

Determination of Nitric Oxide (NO):

NO is produced by various cell types in Pico molar to Nano molar range and has a very short half-life (t1/2 <5s) in biological fluids; therefore, a direct measurement of its production is difficult and the analysis of $NO2^-$ and $NO3^-$, the stable products of NO oxidation, is often performed to estimate NO level in biological fluids and cell culture medium. Nitrite concentration is typically measured by a well-known method such as colorimetric Griess assay (Moshage *et al.,* 1995).

Estimation of Vitamin C (VIT C)

Ascorbic acid (VIT C) was analyzed by the method described by Roe and Keuther (1943) by spectrophotometrically. Ascorbate is converted into dehydroascorbate on treatment with activated charcoal, which react with 2,4-dinitrophenyle hydrazine to form osazones. These osazones produce an orange coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540 nm.

Estimation of Vitamin A (VIT A)

Vitamin A (Tocopherol) was estimated in the plant samples by the Emmutir-Engel reaction as reported by Rosenberg (1992). The Emmerie-engel reaction is based on the reduction of ferric to ferrous ions by tocopherols, which with 2,2-dipyridyl, forms a red colour. Tocopherols and carotenes are first extracted with xylene and take absorbance at 460nm to measure carotenes. A correction is made for this after adding ferric chloride and read at 520nm.

Extraction of Tocopherol (VIT E)

The plant sample (2.5g) was homogenized in 50 ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through whatman No 1 filter paper. Aliquots of the filtrate were used for the estimation [128].

Sodium (Na^{+1}) / Potassium (K^{+1}) / Chloride (Cl^{-1})

Electrolytes maintain regulation of appropriate heart and proper body pH and muscle functions and osmotic pressure and hydration of body fluid. The ISE module use crown ether membrane electrodes for Na+, K+, and Cl-. PVC membrane electrode is used for chloride. An electrical potential is compared to the Internal Reference Solution, electrical potential is translated into voltage and ion concentration of the sample.

RESULTS

Variable	Gender	N	Minimum	Maximum	Mean	Std. Deviation
AGE	Male	28	20.00	65.00	37.8214	13.10655
	Female	22	27.00	60.00	42.4545	8.37643

Table 1: Descriptive Statistics of Age in Both gender between Pre and Post treatment with Imatinib in CML Patients

There were two group of study, one group patients without treatment of imatinib and other group patients were treated with imatinib. This study has the condition of pre and post treatment effect. There were Fifty (50) patients of CML. Data were collected from both gender. The mean age of the male participants was (37.82±13.10) years where as female participants has (42.45±8.37) years. The minimum age (males) was 20 years and the maximum age (males) was 65 years. In case of female, the minimum age was 27 years and the maximum age was 60 years.

Table 2: Gender frequency between Pre and Post treatment with Imatinib in CML Patients.

Variables		Frequency (%) (non-imatinib)	Frequency (%) (with imatinib)	Total	P-Value
Gender	Male	28(56%)	28(56%)	56	P<0.05(0.00)
	Female	22(44%)	22(44%)	44	
	Total	50(100%)	50(100%)	100	

Out of 100 participants, 50(100%) people were not treated with imatinib and 50(100%) people treated with imatinib. Out of 50 CML (non-imatinib) patients, 28(56%) were males and 22(44%) females. Same as 50 CML (with imatinib) patients, 28(56%) were males and 22(44%) females.

Table 3	Mean ± SD and p-values of Oxidative Stress Biomarkers between Pre and Post treatment with Imatinib in **CML Patients**			
VARIABLES	**Pre-Treatment (Non-Imatinib) (n =50)**	**Post-Treatment (With-Imatinib) (n = 50)**	**t-Value**	**P-VALUE (P<0.05)**
MDA (mmol/ml)	0.257±0.159	0.451±0.076	-7.972	0.000
GSH (mg/dl)	0.346±0.0881	0.735±0.1149	-16.737	0.000
CATALASE (mmol/ml)	0.451±0.076	0.224±1.0305	1.559	0.125
SOD (µM/mL)	0.296±0.0971	0.528±0.5571	-2.916	0.005
NO	0.188±0.244	0.418±0.0770	-6.471	0.000
AGE's	0.175±0.029	0.517±0.159	-14.982	0.000
AOPP	0.607±0.136	1.239±0.271	-14.227	0.000
MDA Normal Range = 1.0-3.0mmol/mL , GSH Normal Range =8.0-12.0mg/dl , Catalase Normal Range =1.0-5.0 mmol/mL ,SOD Normal Range= 0.1-0.6 mmol/ml				

The data presented in table 3 shows clear image of oxidative stress biomarker estimated in the patient suffering from CML. The study was divided into two groups, Pre-treatment (imatinib) CML patients and Post treatment (imatinib) CM Patients. When oxidative stress biomarkers were estimated, the level of GSH and MDA in CML (non imatinib) patients was (0.34±0.08) and (0.25±0.15) while in CML (with imatinib) patients was (0.73±0.11) and (0.45±0.07).The data shows that values elevated in CML (with imatinib) patients and it was statistically significant as (0.000).The level of Catalase in CML (non imatinib) patients was (0.45±0.07) while in CML (with imatinib) patients was (0.22±1.03).It was noticed that the data was statistically non significant (0.125). The level of SOD in CML (non imatinib) patients was measured as (0.29±0.09) while in CML (with imatinib) patients was (0.52±0.55).The result shows that values increased in CML (with imatinib) and also noticed that it was statically significant as (0.005). The level of NO in the CML (non imatinib) patients was (0.18±0.24) while in CML patients (with imatinib) were (0.41±0.07). The result shows that NO level was increased in patients who were treated with imatinib. Data reveals that NO has significant statistically (0.000).AGES in CML (non imatinib) patients were estimated as (0.17±0.29) while in CML (with imatinib) patients was (0.51±0.15).It has been noticed that AGES level were increased in CML (with imatinib) patient as compared to CML (non imatinib) patients. The result was statistically significant (0.000). The level of AOPP measured in CML (Non imatinib) patients was (0.60±0.13) while in CML patients (with Imatinib) were (1.23±0.27). The value was observed greater as compared to (non imatinib) CML patient and result was statistically significant (0.000).

Table 4	Mean ± SD and p-values of Micro nutrients between Pre and Post treatment with Imatinib in CML Patients			
VARIABLES	**Pre-Treatment (Non-Imatinib) (n = 50)**	**Post-Treatment (With-Imatinib) (n = 50)**	**t-Value**	**P-VALUE (P<0.05)**
Vitamin A	1.791±2.728	0.382±0.099	3.636	0.001
Vitamin C	2.345±0.567	2.300±0.273	0.527	0.601
Vitamin E	0.526±0.164	1.331±0.205	-21.822	0.000

The data presented in table 4 shows clear image of different micro nutrients estimated in the patient suffering from CML. The study was divided into two groups, Pre treatment (imatinib) CML patients and Post treatment (imatinib) CML Patients. When the level of serum micronutrients was measured, it was observed that vitamin A level in CML (non imatinib) patients was increased (1.7±2.7) as compared to CML (with imatinib) patients was (0.3±0.09) and statistically significant (0.001).The level of vitamin C in CML (non imatinib) patients measured as (2.3±0.56) while in CML (with imatinib) patients was (2.3±0.27) and also noticed that it was not statistically significant (0.601).The vitamin E level in CML (non imatinib) patients was (0.52±0.16) while in CML (with imatinib) patients was (1.3±0.20).The result shows that the data was statistically significant (0.000).

Table 5	Mean ± SD and p-values of Serum Electrolytes profile between Pre and Post treatment with Imatinib in **CML Patients**			
VARIABLES	**Pre-Treatment (Non-Imatinib) (n = 50)**	**Post-Treatment (With-Imatinib) (n = 50)**	**t-Value**	**P-VALUE (P<0.05)**
K^+	3.916±0.615	3.820±0.302	0.922	0.361

Na^+	139.72±8.748	136.22±2.794	2.628	0.011
Cl^-	104.54±2.111	103.82±3.101	1.355	0.182

The data presented in table 5 presenting clear image of serum electrolytes estimated in the patient suffering from CML. The study was divided into two groups, Pre-treatment (imatinib) CML patients and Post treatment (imatinib) CM Patienst. When the serum electrolytes balance (Na, K, Cl) were estimated that serum Na level in CML (non imatinib) patients was (139±8.7) decreased as compared to CML (with imatinib) patients as (136±2.7).So the data was statistically significant (0.01).But the other electrolytes K, Cl level were observed in CML (non imatinib) patients as (3.9±0.61) and (104±2.1) while in CML (with imatinib) patients as (3.8±0.30) and (103±3.1).The values almost same in both groups. So, the data was not statistically significant as (0.361) and (0.182).

Table 6 Evaluation of Blood Profile and BCR-ABL in Patients of CML Receiving Imatinib

Parameters	**N**	**Mean**	**Std. Deviation**	**Sig. (2-tailed)**
BCRABL	50	79.476	21.934	0.000
Heamoglobin	50	10.3064	2.94674	0.000
RBCs	50	4.118	0.6191	0.000
Platelets	50	259.4738	165.7146	0.000
WBCs	50	32.3926	120.1728	0.063
Neutrophils	50	56.396	13.06492	0.000
Lymphocytes	50	34.4282	13.55468	0.000
Eosinophils	50	2.7626	0.97862	0.000
Monocytes	50	4.668	5.5967	0.000
Basophils	50	0.3354	0.44051	0.000

The data presented in table 6 shows the clear image of BCR-ABL and Complete blood profile of CML patients treated with imatinib. The level of BCR-ABL in CML imatinib treated patients was (79.4±21.9) and data shows statistically significant as (0.000).

The values of complete blood profile in CML imatinib treated patients were as Hbs(10.3±2.9) , RBCs (4.1±0.61) , platelets (259.4±165.7) , Neutrophils (56.3±13.0) , Lymphocytes (34.4±13.5) , Eosinophils (2.7±0.97) , Monocytes (4.6±5.5) and Basophils (0.3±0.4) and all parameters data shows statistically significant as (0.000). But the level of WBCs in CML imatinib treated patients was (32.9±120.1).The value of WBCs was not statistically significant as (0.063).

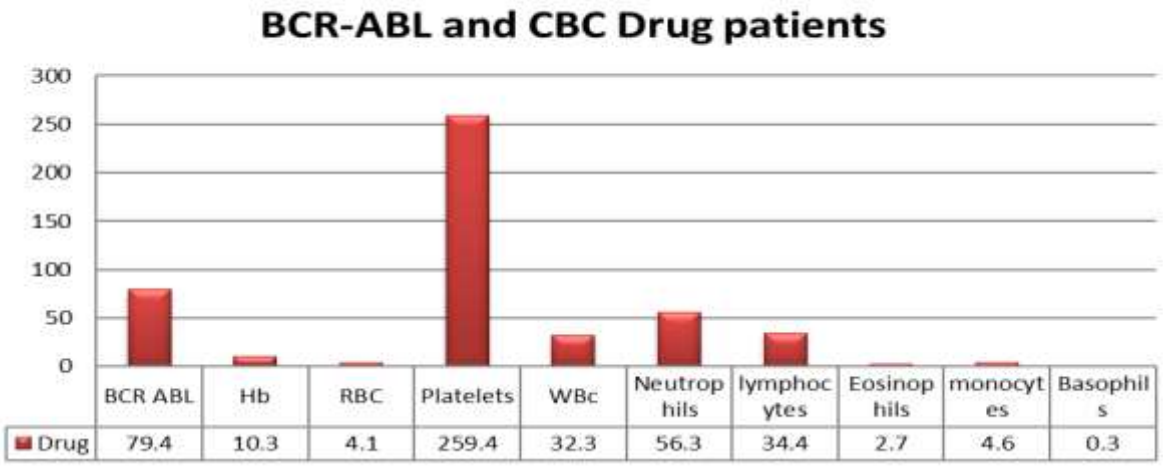

Figure 2	Graphical representation of Blood Profile and BCR-ABL in CML **Patients** Receiving Imatinib

Table 7	Paired Samples Correlation of oxidative stress Biomarkers, Micro nutrients and serum electrolytes in CML Patients (With Imatinib and Without Imatinib)

Variables		**N**	**Correlation r**	**Sig.**
Pair 1	NO & NO1	50	.063	.664
Pair 2	AGES & AGES1	50	.020	.888
Pair 3	SOD & SOD1	50	.035	.808
Pair 4	AOPP & AOPP1	50	-.088	.546
Pair 5	GSH & GSH1	50	-.298	.036
Pair 6	MDA & MDA1	50	.068	.639
Pair 7	Catalase & Catalase1	50	.086	.552
Pair 8	VIT.A & VIT.A1	50	-.093	.521
Pair 9	VIT.C & VIT.C1	50	.125	.385
Pair 10	VIT.E & VIT.E1	50	.017	.906
Pair 11	Na & Na1	50	-.088	.541
Pair 12	K & K1	50	-.194	.178
Pair 13	CL & CL1	50	-.004	.980

NO without Imatinib , NO1 with Imatinib

DISCUSSION

Chronic myelogenous leukemia (CML) is a fatal myeloproliferative disease characterized by massive expansion of hematopoietic progenitor cells with the appearance of cells of the granulocyte (GR) lineage in the peripheral blood. In 1973, Janet Rowley recognized that the Ph chromosome was indeed the product of a reciprocal translocation between the long arms of chromosomes 9 and 22, the t (9; 22) (q34; q11) (Yeung *et al.*, 2022). The *ABL* gene from chromosome 9 had been known as the human homolog of a murine leukemia virus; the translocation partner from chromosome 22 was termed *BCR* for breakpoint cluster region, since DNA breaks occurred in a relatively small genomic region.The bcr-abl gene is formed on chromosome 22 where the piece of chromosome 9 attaches. The changed chromosome 22 is called the Philadelphia chromosome (Yuda

et al., 2023). Because of the damage to the DNA, the Philadelphia chromosome results in the production of an abnormal enzyme called a tyrosine kinase. Along with other abnormalities, this enzyme causes the cancer cell to grow uncontrollably.

A CML is developed by the exposure to very high doses of radiation. A minor increase in risk also occurs in some individuals treated with high-dose Radiation therapy of other cancers like lymphoma. Factors that increase the risk of chronic myelogenous leukemia (Zdenek *et al.,* 2014): Older age and radiation exposure, such as radiation therapy for certain types of cancer . The disease course is triphasic, starting with a chronic phase, progressing to an accelerated phase and ultimately ending in a terminal phase. Triphasic of CML is chronic, accelerated, and blastic.

Imatinib mesylate (Gleevec, Glivec), a specific small-molecule inhibitor of Bcr-Abl, has become the standard drug therapy for CML in all phases of the disease. Responses to imatinib may occur at the hematologic, cytogenetic and molecular levels. Imatinib (GlivecR, GleevecTM), is orally administered, and has dramatically diminished the use of allogeneic stem cell transplantation. Ninotinib is a type of biological therapy called a tyrosine kinase inhibitor (TKI). Tyrosine kinases are proteins that act as chemical messengers (enzymes) (Zhang *et al.,* 2022). They can stimulate cancer cells to grow. Nilotinib blocks a tyrosine kinase protein called Bcr-Abl. The protein is made by chronic myeloid leukaemia cells that have an abnormal chromosome called the Philadelphia chromosome.

Oxidative stress, a pervasive condition of an increased number of reactive oxygen species, is now recognized to be prominent feature of various diseases and their progression. Thus antioxidants, which control the oxidative stress state, represent a major line of defense regulating overall true state of health. The relationship between antioxidants status and levels of well-known markers of oxidative stress that are measured as lipid peroxides and oxidized proteins reflect better health indices and postures. Evaluate the role of oxidative stress in pathophysiology of Chronic myeloid leukemia by measuring the circulating plasma lipid peroxide levels in terms of malonyldialdehyde, total lipid hydroperoxide and oxidized proteins as protein carbonyl whereas antioxidant status were estimated in terms of reduced glutathione and total thiol in plasma of Chronic myeloid leukemia patients (Zhang *et al.,* 2022).

Table 7 demonstrated the paired sample correlation between CML patients before and after treatment with imatinib. Data predicted that positive correlation exist between CML patients before and after treatment evaluation. Correlation between nitric oxide CML patients was ($r = 0.063$). NO level was high in CML (non imatinib) patients but increased after treatment with imatinib. As imatinib have cytotoxic nature which increase the NO level in CML patients receiving imatinib. AGEs level in CML patients was ($r = 0.020$). AGEs level increased in CML patients after treatment with imatinib as AGEs function protein glycation. SOD level in CML patients was ($r = 0.035$). SOD level increased after treatment with imatinib because SOD function as lowering the level of superoxides. GSH level in CML patients was ($r = -0.298$). GSH level before treatment was low but after treatment increased in CML patients receiving imatinib. GSH helps to prevent the cellular damage by ROS.MDA level in CML patients was ($r = 0.068$). MDA level increased after treatment with iamtinib due to ROS level. Catalase level in CML patients was ($r = 0.086$). Catalase level was high before treatment. Catalase provides a site of decomposition of H_2O_2 into water and oxygen.

The level of micro nutrients (vit.A and vit.C) was ($r = -0.093$ and $r = 0.125$).There were no change in micro nutrients level in CML patients after treatment with imatinib. But the level of Vitamin E in CML patients was ($r = 0.017$). Vitamin E level increased after treatment with imatinib as its act anti-oxidant. Serum electrolytes (Na, K, Cl) level in CML patients was ($r = -0.088$, $r = -0.194$ and $r = -0.004$).There was no change in serum electrolytes level before and after treatment with imatinib.

CONCLUSION

Present study was divided into two groups, group A i.e. CML patients without treatment of imatinib and group B i.e. CML patients treated with imatinib. This study has the condition of pre and post treatment effect. There were 100 patients of CML. Data were collected from both gender (male and female). In this study, there is growing evidence that oxidative stress is key component involve in

the pathophysiology of Chronic myeloid leukemia. It is predominantly assume that oxidative stress may represent a central point. The present study has obviously shown elevated production of free radicals and reduced defense activity of antioxidants that support the oxidative stress hypothesis in both male and female CML patients. The extremely increase per oxidation of lipid membrane along with fall off activity of antioxidant enzyme was observed in CML patients. After these findings, the relation between antioxidants deficiencies and CML give new dimension for both gender in addition to available Chemotherapy treatment. In this study, no gender association was found in CML patients. It also proves the importance of antioxidant supplementation to support enzymatic defense system and reduce oxidative stress. Further studies demand which antioxidant, at which dosage and in which combination give positive result with least risk.

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