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Evaluation of tumor necrosis factor-alpha and interferon-alpha with some biochemical parameters in different groups of chronic myeloid leukemia patients in Baghdad

A Thesis

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By

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Dedication

To the men who raised me as a seed in life and irrigated me by her kindness … to her dear and noble soul…….. my Mother

To those whom I live for their sake, and I owe them my happiness……… myBrothers and Sister

To every loving and loyal heart … I dedicate my work.

Mohammed

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Thanks to all participants who cooperated with me and gave me their precious blood samples.

Mohammed

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Summary

Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by the BCR/ABL oncogene and characterized by clonal expansion of the hematopoietic progenitor cells and myeloid cells resulting from the (9:22) translocation. The aim of this study is to investigate the extent to which tumor necrosis factor-α and interferon- α and some biochemical parameters are involved in chronic myeloid leukemia in blood samples of patients with newly diagnosed patients. The effect of pharmacological interventions (imatintb) on theses parameters were also investigated in treated CML patients. Blood samples were obtained from 30 male and female CML patients (with either newly diagnosed and treated) referred to the national center of hematology, AL-Mustansiriya university, and the office of teaching laboratories-medical city, Baghdad. The CML patients included 15 newly diagnosed patients (7 men and 8 women) with a mean age of 49.7 ± 10.6 year and an age range of 32-69 year. Another 15 treated CML patients (8 men and 7 women) with a mean age of 55.13 ± 13.6 year and an age range of 32-75 year were involved in this study. The range of duration of CML was between 3 months to 7 year. Fifteen apparently healthy controls (8 men and 7 women) with a mean age of 57.6 ± 14.2 year and an age range of 30-72 year are also involved in this study.

The plasma levels of tumor necrosis factor-alpha (TNF-α) and interferon-alpha (IFN-α) were measured. To investigate the role of oxidative stress in the pathophysiology of CML patients, plasma glutathione (GSH) levels were estimated. Erythrocytes were also examined for their susceptibility to *in vitro* oxidative stress-induced by H_2O_2. Criteria studied in this regard was malondialdehyde (MDA) production (an index of lipid peroxidation).

The results obtained showed a significant increase in plasma TNF-α in newly diagnosed CML (but not in the treated CML) patients. Plasma GSH levels were significantly decreased in newly diagnosed CML patients. Furthermore, red cells from newly diagnosed CML patients showed an increased levels of both basal and H_2O_2-induced MDA production.

In conclusion, the present results revealed the presence of alterations in the immune system of the CML state. The decline in plasma GSH levels, together with the elevation in both basal and H_2O_2-induced MDA levels in erythrocytes of newly diagnosed CML subjects suggest the presence of increased susceptibility to oxidative stress in CML. The ability of the anti-leukemic imatinib or linotinib to normalize most of the alterations are suggestive of a possible, among others, antioxidant mechanism of activity of these drugs.

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List of Abbreviations

ABL	Ablsen gene
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BCR	Break point cluster gene
CCyR	Complete cytogenetic response
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CML-CP	Chronic myeloid leukemia-chronic phase
CMR	Complete molecular response
CP	Chronic phase
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EDTA	Ethylenediaminetetraacetic acid
GC-MS/MS	Gas chromatography-mass spectrometry
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte macrophage-colony stimulating factor
GSH	Glutathione
GPx	Glutathione peroxide
HCL	Hydrochloric acid
H_2O_2	Hydrogen peroxide
HOCL	Hypochlorous acid
IAP	Inhibitor of apoptosis protein
IFN-α	Interferon-α
ISGs	Interferon stimulated gene
LC-MS/MS	Liquid chromatography-mass spectrometry
LOOH	Lipid hydroperoxides
MHC	Major histocompatibility complex
mRNA	Messenger RNA
Ph+	Philadelphia chromosome-positive
PUFAs	Polyunsaturated fatty acids
PH	Power of hydrogen

RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RBCs	Red blood cells
STI	Signal transduction inhibitor
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NaN_3	Sodium nitrate
Ph	The philadelphia
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reacting substances test
TCA	Trichloroacetic acid
TKI	Tyrosine kinase inhibitors
4-HNE	4-hydroxynonenal

1.1 Introduction

Leukemia is a progressive, malignant disease of the blood forming organs characterized by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow, and can be classified clinically on the basis of duration and character of the disease into acute and chronic leukemia (Hoffbrand et al., 2006). Basically, leukemia is a cancer of the blood and bone marrow cells, leukemia is categorized according to the primary type of cell affected and the disease course. (Howlader *et al*, 2012). There are four main types of leukemia: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML), as well as a number of less common types (World Cancer Report, 2014).

One of the important types of leukemia is chronic myeloid leukemia (CML), which is the stem cell disorder with acquired chromosomal abnormality, Philadelphia chromosome (ph) which arises from the reciprocal translocation of part of the long arm of chromosome 9 in which proto oncogene ablson gene (ABL) is located to long arm of chromosome 22, in which break point cluster region BCR is located. (Mughal and Goldman, 2007).The BCR-ABL fusion gene can be detected using several molecular methods.

1.2 Aims of Study

This study was designed to:

1- Measure the levels of Tumor necrosis factor-α (TNF-α) and Interferon-α (IFN-α) in plasma and biochemical (parameters GSH, erythrocyte MDA and) associated with newly diagnosed chronic myeloid leukemia (CML) in Iraqi patients.

2- Investigate the effect of imatinib or nilotinib therapy on these parameters in treated CML patients.

1.2 Literature Review

1.2.1 Chronic myeloid Leukemia

Chronic myeloid leukemia is a malignant clonal disorder resulting from the cancerous transformation of a very primitive hematopoietic stem cell (Goldman and Melo, 2003; Sawyers, 1999). Chronic myeloid leukemia is characterized by the reciprocal chromosomal translocation (9; 22), leading to the formation of the Philadelphia chromosome. This encodes the constitutively active Bcr-Abl tyrosine kinase, which profoundly affects proliferation, apoptosis, and cell adhesion signaling pathways (Goldman and Melo, 2003). The resulting BCR-ABL fusion on the Philadelphia chromosome is transcribed to chimerical RNA and then translated into a fusion tyrosine kinase protein of varying size P190 KD to P210 KD (Galli *et al*, 2005).

1.2.1.1 Role of the Philadelphia chromosome

The "Philadelphia chromosome" (Ph) is seen in >90 percent of cases of CML (Kurzrock, Gutterman and Talpaz, 1988). Ph is a balanced reciprocal translocation between chromosomes 9 and 22 (Goldman and Melo, 2003). ABL is transferred from chromosome 9 to 22. DNA from chromosome 22 is shifted to 9 to take ABL's place. This translocation leads to the fusion of two parts of normal genes, the ABL gene on a portion chromosome 9 with a section on chromosome 22 called BCR. ABL is hooked with a breakpoint promoter region (BCR); this promoter area provides a continuous signal to the cell to transcribe the gene for the tyrosine kinase protein coded in ABL. The BCR-ABL gene is transcribed into messenger RNA (mRNA) and the mRNA is subsequently translated into the tyrosine kinase protein. The tyrosine kinase fusion protein that is produced is continuously active irrespective of regulatory influences within the cell (i.e., constitutively active). This uncontrolled enzymatic activity then usurps the normal physiologic processes of the cell (Goldman and Melo, 2003). The formation of the BCR-ABL fusion gene within a pluripotential hematopoetic stem cell is the first step in developing CML (Goldman and Melo, 2003).

1.2.1.2 Diagnosis

Most patients are diagnosed asymptomatically based on laboratory abnormalities in level of white blood cell count (leukocytosis), anemia, and elevated platelets (thrombocytosis). Diagnosis and staging require a peripheral complete blood count with a white blood cell differential analysis, bone marrow examination with quantification of the percentage of blasts and basophils, and cytogenetic studies for the Philadelphia chromosome or its variants. Histopathologic examination the bone marrow aspirate demonstrates excessive numbers of cells (hypercelluar marrow) with a shift in the myeloid series to immature forms; the number of immature cells increases as patients progress from chronic to blastic phases of the disease (National Cancer Institute, 2005). White blood cell differential counts of both peripheral blood and bone marrow demonstrate a spectrum of mature and immature granulocytes similar to that found in normal marrow. Increased numbers of eosinophils, basophils or monocytes may be present, and a megakaryocytosis may be noted in the marrow. Lymphocyte counts are usually suppressed, and the myeloid/erythroid ratio in the marrow is usually markedly elevated (Savage, Szydlo and Goldman, 1997).

1.2.1.3 Staging

The information provided in the physical exam, peripheral white blood count, bone marrow examination, and cytogenetic studies, FISH or RT-PCR are used to determine the patient's stage of illness and predict their course. The staging in CML is usually described in terms of "phases". CML historically has had a triphasic course, presenting in an initial chronic phase with a median duration of 3–5 years, invariably progressing over time to an accelerated phase with a median duration of 6–18 months and finally to blastic phase lasting 3–6 months. (Sawyers, 1999 ; Bhatia *et al*, 2003). Blast crisis is a period within blast phase that resembles acute leukemia, with two-thirds of patients having an acute myeloblastic or undifferentiated type of leukemia and the other one-

third having an acute lymphoblastic leukemia (Faderl *et al*, 1999). In Blast crisis, patients have fever, malaise and an enlarging spleen in addition to the increasing number of blasts in their blood or bone marrow. In up to one-fourth of patients, blast crisis develops without an intervening accelerated phase (Kantarjian *et al*, 1988).

1.2.1.4 Imatinib

Imatinib was the first signal transduction inhibitor (STI), used in a clinical setting. It prevents a BCR-ABL protein from exerting its role in the oncogenic pathway in chronic myeloid leukemia (CML). Imatinib directly inhibits the constitutive tyrosine kinase activity, which results in the modification of the function of various genes involved in the control of the cell cycle, cell adhesion, cytoskeleton organization and finally in the apoptotic death of Ph(+) cells (Deininger, 2008). Imatinib binds to BCR-ABL kinase domain, which is in an inactive conformation in a pocket reserved for the ATP binding site, thus preventing the transfer of a phosphate group to tyrosine on the protein substrate and the subsequent activation of phosphorylated protein. As the result, the transmission of proliferative signals to the nucleus is blocked and leukemic cell apoptosis is induced (Schindler *et al*, 2000).

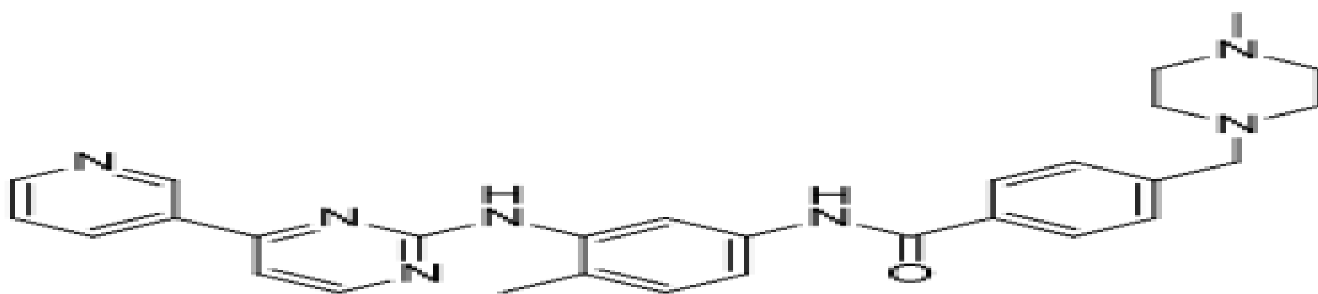

Figure (1-1) Imatinib chemical structure

1.2.2 Cytokines

Cytokines are soluble messenger molecules, eg. Lymphokines (produced by lymphocyte) and interleukines (made by other white blood cells) that facilitate communication between different compartment of the immune system. Examples include Interferons ,Tumor Necrosis Factor- alpha (TNF-a), granulocyte –colony

stimulating factor (G-CSF), granulocyte macrophage- colony stimulating factor (GM-CSF). These cause biological effects in destination cell population (e.g. activation, division or migration of destination cells) and often trigger inflammation (Gemmy, 2012).

The cytokines produced by variety of cells of the innate and adaptive immune system. There major functional activities are concerned with the regulation of the development and behavior of the immune effector cells (Swardfager, 2010). The cells regulated by cytokines must express a receptor for the factor. Thus, cells are regulated by the quantity and type of cytokines to which they are expressed and by the expression of up regulation and down regulation of cytokines receptor. Cytokines act in concernt with one another to create synergistic effects that reinforce the other actions on a given cells .The interaction of multiple cytokines generated during atypical immune response are referred to cytokine cascade (Dowlati et al., 2010).

1.2.2.1 Tumor necrosis factor-alpha (TNF-α)

Tumor necrosis factor-α is the prototypic ligand of the TNF superfamily (Idriss and Naismith, 2000). It is a proinflammatory cytokine that was originally described as antitumorigenic (Carswell, 1975; Ichinose, Tsao and Fidler, 1988). TNF-α produced by immune cells like macrophages and lymphocytes (Beutler *et al*, 1985; Ramesh and Reeves, 2004). Tumor necrosis factor-α (TNF-α) is one of the most important pro-inflammatory and tumor-related cytokines for its regulating immune response, inflammation, Th1/Th2 balance and lymphomagenesis (Sethi, Sung and Aggarwal, 2008). Increased levels of TNF-α have been detected in autoimmune disease and many malignancies (Macia *et al*, 1996; Halida, Guo and Aliya, 2011). TNF-α has increased susceptibility to many kinds of tumors and autoimmune diseass, such as hepatocellular carcinoma, myeloma, lymphoma, ulcerative colitis, and Crohn's disease (Wilson, Giovine and Duff, 1995; Ho SY, Wang YJ and Chen HL *et al*, 2004). TNF-α is expressed as a 26-kDa plasma membrane protein that is secreted into the extracellular space by the metalloproteinase TNF-α-converting enzyme (Black *et al*, 1997). Tumor necrosis factor-α (TNF-α) is a pleiotropic GF whose role in hemopoiesis is highly

dependent on cell context, its concentration, with both inhibitory and stimulatory effects reported (Snoeck *et al*, 1996). Although originally described as cytotoxic to cancer cells, given its ability to induce apoptosis (Carswell *et al*, 1975) TNF-α is often produced by malignant and immune cells present in the inflammatory reaction surrounding tumors (Grivennikov *et al*, 2009). Regardless of its source, TNF-α can contribute to tumorigenesis by creating a tumor-supportive inflammatory microenvironment and through direct effects on malignant cells (Grivennikov and Karin, 2011).

1.2.2.1.1 Tumor Necrosis Factor_alpha (TNF_α) and Chronic Myelogenous Leukemia (CML)

TNF-α's pleiotropic effects are secondary to its ability to activate both proapoptotic and prosurvival signals (Aggarwal, Gupta and Kim, 2012). On NFκB/p65 expression, TNF-α is unable to induce apoptosis because it simultaneously activates NFκB/p65, which promotes, among others, the expression of the inhibitor of apoptosis protein (IAP) family. IAPs block the proapoptotic caspase 8 activation, which is also induced by TNF-α, so that in their presence, the net output of TNF-α signaling is to promote survival and proliferation of its target cells. IAP2 in particular is directly activated by NFκB/p65 and, in turn, activates it through a positive feedback loop (Wang *et al*, 1998). CML cells express a constitutively active NFκB/p65, and treatment of CML CD34+ cells with TNF-α inhibitor which promotes subunit disassembly of the TNF-α trimer, reduced phosphorylation levels of NFκB/p65 on the activating serine 536 (He *et al*, 2005). Although to a moderate extent suggesting residual NFκB/p65 phosphorylation was present, possibly as a result of BCR-ABL kinase activity) and of its upstream inhibitor IκBα, which is degraded when phosphorylated (Aggarwal et al., 2012). TNF-α also exerts stimulatory effects on normal hemopoiesis indirectly by inducing interleukin 3 and granulocyte/macrophage colony-stimulating factor common β-chain receptor (CSF2RB) expression in normal CD34+ cells (Sato *et al*, 2013).

1.2.2.2 Interferon-α

Interferon-alpha is a pleiotropic cytokine belonging to the type I cytokine family (Yan *et al*, 2008). Type I interferons are normally produced by the innate immune system in response to viral infections (Elkon and Stone, 2011; Liu, Sanchez and Cheng, 2011). They act via type I interferon receptors (INFARs) to trigger the JAK/STAT signaling cascade, leading to induction of interferon-stimulated genes (ISGs) that amplify interferon signaling, activate the adaptive immune system, and produce factors that directly inhibit viral replication (Elkon and Stone, 2011; Liu, Sanchez and Cheng, 2011). IFN-α can influence the function and activation of most types of adaptive immune cells (Liu, Sanchez and Cheng, 2011; Fitzgerald-Bocarsly, Dai and Singh, 2008). Its effects range from upregulation of major histocompatibility complex (MHC) and costimulatory molecules to increased survival and activation of dendritic cells, B cells, and T cells (Elkon and Stone, 2011; Baccala *et al*, 2007).

1.2.2.2.1 Interferon-α (IFN-α) and CML

Until the early 1980s, CML therapy was based on busulfan or hydroxyurea, which had a negligible effect on the natural course of the disease. Talpaz *et al*, 1987 carried out the first pilot clinical trial of partially pure IFNα for the management of CML followed by a larger study. The pivotal finding was that IFNα induced cytogenetic responses, which were more durable and reproducible than those induced by chemotherapy. Both IFNα and chemotherapy (hydroxyurea or busulfan) could induce hematological responses in CML, IFNα significantly improved patient survival, with a 5-year survival rate of 50–59% compared with 29–44% for patients receiving busulfan or hydroxyurea (Hehlmann *et al*, 1994). Interferon-alpha (IFN-α) was one of the main treatment options before the discovery of TKIs. Reports of complete cytogenetic response (CCyR) and complete molecular response (CMR) associated with excellent long-term prognosis achieved after IFN-α therapy have been published (Mahon *et al*, 2002; Kantarjian *et al*, 2003). IFN-α has the potential to control progression of CML-CP and was the first non-myelotoxic drug shown to cause a marked reduction in the percentage of Philadelphia chromosome-positive (Ph+) cells in the bone marrow. Combination therapy with cytarabine and IFN-α in CML-CP patients was shown to

increase the rate of major cytogenetic responses and prolong survival compared to IFN-α alone (Guilhot *et al*, 1997). Interferon-α (IFN) was the standard front-line agent for chronic myeloid leukemia (CML) before the introduction of tyrosine kinase inhibitors (TKIs). IFN resulted in hematologic responses of 60–80% and cytogenetic responses of 30–40% in newly diagnosed chronic phase (CP) CML patients (Kantarjian *et al*, 1996).

1.2.3 Oxidative Stress

A diverse number of stimuli have been shown to induce apoptosis; many of them are also known to compromise the fine balance between intracellular oxidants and their defense systems (Kassab-Chekir et al., 2003).

Under aerobic situations, the participation of oxygen in redox reactions is unavoidable and a variety of highly reactive chemical entities are produced (Genestra, 2007). These are commonly referred to as Reactive Oxygen Species peroxides, nitric oxide and superoxide. Many of these agents have a beneficial role in the cell but, when present in excess, the cell becomes oxidatively stressed (Halliwell, 2007).

A number of gross biochemical changes occur as a consequence of oxidative stress, the extent depending on the severity of the insult (Bahorun et al., 2006). Oxidative overload causes gross cellular damage resulting in alteration of redox state (e.g. depletion of nucleotide coenzymes, disturbance of sulphur containing enzymes), saturation and destruction of the defense and repair system. If the cellular balance is not restored, a number of pathological processes are elicited (Ceriello, 2008). Predominant processes resulting from oxidative stress include oxidative lipid degradation (Lipid peroxidation), the loss of intracellular calcium homeostasis and alteration of metabolic pathways (Suneerat et al., 2010).

1.2.3.1 Free Radicals, Reactive Oxygen and Nitrogen Species

A free radical may be defined as a molecule or molecular fragments containing one or more unpaired electrons in it is outermost atomic or molecular orbital and are capable of independent existence (Valko et al., 2007).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals and other non-radical reactive derivatives (Pacher et al., 2007). The reactivity of radicals is generally stronger than non-radical formed from molecules by the hemolytic cleavage of a chemical bond and via redox reactions. Once formed these highly reactive radicals can start a chain reaction (Fialkow et al., 2007). ROS and RNS include radicals such as superoxide (O2.-), hydroxyl (OH.), hydroperoxyl (HO2.), alkoxyl (RO.), peroxyl (ROO.), nitric dioxide (NO.), nitrogen dioxide (NO2.) and lipid peroxyl (LOO.); and non-radicals like hydrogen peroxide (H2O2), hopochlorous acid (HOCI), ozone (O3), singlet oxygen (1O2), peroxynitrate (ONOO-), nitrous acid (HNO2), dinitrogen trioxide) (N2O3), lipid peroxide (LOOH) (Pham-Huy et al 2008). Non radicals are also termed as oxidants and capable to lead free radical reactions in living organisms easily. Radicals derived from oxygen are characterized as the most important class of radical species generated in living systems (Valko et al., 2006).

At high concentrations, ROS can be important mediators of damage to cell structures, nucleic acid, lipids and proteins (Dalle-Donne et al., 2005). Superoxide (O2.-) radical is responsible for lipid peroxidation and also have the capability to decrease the activity of other antioxidant defense system enzyme such as glutathione peroxide (GPx); it causes damage to the ribonucleotide which is required for DNA synthesis (DeCoursey and Ligeti, 2005). The protonated form of O2- is hydroperoxyl (HO2.), which is more reactive and able to cross the membrane and causes damage to tissue. Hydroxyl (OH.) radical is most reactive chemical species. It is a potent cytotoxic agent and able to attack and damage almost every molecule found in living tissue (Leonard et al., 2004). Hydrogen peroxide (H2O2) is not a radical but it produces toxicity to cell by causing DNA damage, membrane disruption and release calcium ions within cell, resulting in calcium dependent proteolytic enzyme to be activated. Hypochlorous acid (HOCL) is produced by the enzyme myeloperoxidase in activated neutrophils and initiates the deactivation of antiproteases and activation of latent proteases leading to tissue damage (Lobo *et al*, 2010). It has ability to damage biomolecules, directly and also decomposed to liberate toxic chlorine. Metal induced generation of ROS attack

DNA and other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Waris and Ahsan, 2006).

1.2.3.2 Lipid peroxidation

Lipid peroxidation can be described generally as a process under which oxidants such as free radicals or nonradical species attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) that involve hydrogen abstraction from a carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydroperoxides as described previously (Yin, Xu and Porter, 2011). The overall process of lipid peroxidation consists of three steps: initiation, propagation, and termination (Yin, Xu and Porter, 2011). Lipid peroxidation or reaction of oxygen with unsaturated lipids produces a wide variety of oxidation products. The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH). Among the many different aldehydes which can be formed as secondary products during lipid peroxidation, malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) have been extensively studied by Esterbauer and his colleagues in the 80s (Esterbauer, Schaur and Zollner, 1991). MDA appears to be the most mutagenic product of lipid peroxidation (Esterbauer, Eckl and Ortner, 1990).

1.2.3.2.1 Malondialdehyde

Malondialdehyde is an obligate product of the oxidation of arachidonic acid by lipoxygenase pathways (Fogelman *et al*, 1980). Malondialdehyde can also react with deoxyadenosine and deoxyguanosine in DNA and form DNA adducts that are mutagenic. Thus, the formation of malondialdehyde has implications for atherogenesis and carcinogenesis (Marnett, 1999). MDA has been widely used for many years as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid (TBA) (Esterbauer and Cheeseman, 1990). The TBA test is predicated upon the reactivity of TBA toward MDA to yield an intensely colored chromogen fluorescent red adduct; this test was first used by food chemists to evaluate autoxidative degradation of fats and oils (Sinnhuber,

1958). However, the thiobarbituric acid reacting substances test (TBARS) is notoriously nonspecific which has led to substantial controversy over its use for quantification of MDA from *in vivo* samples. Several technologies for the determination of free and total MDA, such gas chromatography-mass spectrometry (GC-MS/MS), liquid chromatography-mass spectrometry (LC-MS/MS), and several derivatization-based strategies, have been developed during the last decade (Giera, Lingeman and Niessen, 2012). Because MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations (Giera, Lingeman and Niessen, 2012). Inhibition of the formation of malondialdehyde by antioxidants during the cooking of hamburger meat may result in reduced concentrations of malondialdehyde in plasma and urine as the result of inhibition of malondialdehyde formation *ex vivo* or the inhibition of its formation or absorption from the gastrointestinal tract in vivo (Gorelik *et al*, 2008; Gorelik *et al*, 2008). Such a reduction would suggest that the processes of lipid peroxidation and DNA adduct formation could be reduced (Gorelik et al, 2005, Gorelik et al, 2008).

1.2.3.3 Glutathione

Glutathione is a tripeptide of glutamate, cysteine and glycine found at high concentration in virtually all mammalian tissue. The functionality of glutathione originates from the sulfhydryl (SH) group of the cysteinyl moiety (Conklin 2004). Glutathione is involved in detoxifying reactive oxygen species (ROS) through the ascorbate-glutathione cycle (Foyer and Noctor, 2011; Noctor et al., 2012). Glutathione is also involved in the detoxification of heavy metals (Ammar et al, 2008; Tan et al, 2010) and protects proteins from oxidation through glutathionylation (Zaffagnini et al, 2012). Glutathione is a key regulator of redox signaling which controls gene expression and contributes to cell survival (Foyer et al., 2001; Maughan and Foyer, 2006). Cellular antioxidants known as ROS scavengers protect cells against toxic free radicals (wu et al, 2004). Reduced GSH is a principal constituent of thiol pool and vital intracellular scavengers of ROS (wu et al, 2004). GSH is involved in the synthesis and repair of DNA. GSH serves as a reductant mean antioxidant in oxidation reaction resulting

oxidized GSH, thereby decreased GSH levels may reflect depletion of non-enzymatic antioxidant reserve (wu et al, 2004).

1.2.3.4 Oxidative stress and antioxidant status and CML

Chronic myeloid leukemia (CML) is characterized by neoplastic proliferation of hematopoietic cells. It is the first human malignancy where a specific marker, the Philadelphia (Ph) chromosome, was associated with CML (Nowell and Hungerford, 1960). At the gene level, breaks occur in the ABL and BCR genes on chromosome 9 and 22 respectively (Kurzrock *et al*, 2003). Reactive oxygen species (ROS) or free radicals are generated as byproducts of normal cell metabolism. They can be produced and act inside the cell, or they can be generated within the cell and released to extra cellular space (Frei, 1994). Philadelphia chromosome produces a fusion protein, BCR-ABL in CML patients and the resultant protein causes a perturbation of stem cell function through unclear mechanisms involving increased tyrosine kinase activity (Kurzrock *et al*, 2003). It was demonstrated that BCR-ABL fusion protein is associated with increased levels of ROS in hematopoietic cell lines compared with their non-transformed parental cell lines (Sattler *et al*, 2000). High ROS with the increase in the activity of tyrosine kinase protein could play an important physiological role in signal transduction and induced proliferative pathway in the cell (Sattler *et al*, 2000). Oxidative stress defines as a pervasive condition of increased and/or Inadequate removal of ROS (Halliwell and Gutteridge, 1999; Irshad M and Chaudhuri, 2002). Lipid oxidation is evaluated in term of malondialdehyde (MDA) (Dalle-Donne *et al*, 2006, Ray *et al*, 2000). Oxidative stress is now recognized to be a prominent feature of many acute and chronic diseases, and even cancer and leukemias (Halliwell and Gutteridge, 1999; Singh *et al*, 2001). Elevated levels of lipid peroxidation products support the hypothesis that the cancer or malignant cells produce large numbers of ROS and that there exists a relationship between ROS activity and malignancy (Cerutti, 1994). Moreover body’s defense mechanism would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to above stressful situation (Dalle-Donne *et al*, 2006; Uzun *et al*, 2007). Cellular antioxidants known as ROS

scavengers protect cells against toxic free radicals (Wu *et al*, 2004). The most important biological function of GSH is to work as a non-enzymatic reducing agent to support in keeping cysteine thiol side in a reduced state on the surface of proteins (Wu *et al*, 2004). GSH is involved in the synthesis and repair of DNA. GSH serves as a reductant mean antioxidant in oxidation reaction resulting oxidized GSH, thereby decreased GSH levels may reflect depletion of non-enzymatic antioxidant reserve (Wu *et al*, 2004). Antioxidants are compounds that dispose, scavenge, and suppress the formation of ROS, or oppose their actions so that cellular antioxidants could play a major role in various diseases including cancer and their clinical manifestations (Dalle-Donne *et al*, 2006; Uzun *et al*, 2007).

2. Materials and Methods

2.1 Materials

2.1.1 Equipment

The used equipment and their sources are given in Table (2-1)

Table (2-1): The used equipments and their sources.

Name of Equipment	Company	Origin
Automatic micropipettes	Gilson P10,P100,P1000	Germany
Balance	MettlerAE240	Swiss
Blood collection plain tubes	AFMH	England
Blood collection plastic can tubes	AFMH	England
Blood collection pyrex test tubes	AFMH	England
Cooling centrifuge	Tomy Seiko company	Japan
Distillator	American	USA
EDTA containing tubes	AFMH	England
Eppendorff bench centrifuge	Netherland and Hinz Gbm 2000	Germany
Eppendorff tube	Eppendorf	Germany
Flow cytometry	Apogee	England
Magnetic stirrer with hot plate	Lassco	India
Micro ELISA system (reader)	Thermo	Germany
Multichannel micropipettes	Gilson	France
Pipette tips	Gilson	France
PH meter	Orient	USA
Plastic disposable	Meheco	China

syringes; 5ml		
Printer	Epson	UK
Refrigerator and freezer (-20°C)	Arcelik	Turkey
Sensitive balance	Sartorius	Germany
Spectrophotometer	Cintra 5-GBC	France
Volumetric cylinders	Volac	England
Volumetric Flasks	Volac	England
Auto hematology analyzer	Diagon	Hungary
Vortex	Clay Adams	Germany
Water bath	Memmert	Germany

2.1.2 Chemicals

The used chemicals and their sources are given in Table (2-2):

Table (2-2): The used chemicals and their sources.

Name of chemicals	Origin
Absolute Ethanol	Fluka
FITC Mouse Anti_Human CD4	Partec
PE Mouse Anti_Human CD8	Partec
Deionized Water	Promega
Glucose Kit (cat.EC 12249)	biolab
Tumor Necrosis Factor-Alpha (TNF-a) ELISA Kits (cat.DTA00C)	R&D system
Interferon-alpha (IFN-a) ELISA Kits (cat.41100-1)	Pbl interferon source
Lymphopreb	Sigma
Normal Saline	ADWIC
Trichloroacetic Acid (TCA)	Fluka

Thiobarbituric Acid (TBA)	Fluka
Sodium Arsenite	Fluka
Human Glutathione (GSH) ELISA Kit (cat.CSB-E09495h)	Cusabio
HCL	BDH
NaCl	BDH
NaOH	BDH
NaN_3	BDH
Hydrogen Peroxide (H_2O_2)	BDH
Tris base	BDH

2.2 Subjects

Blood samples from 71 male and female CML subjects (with either newly diagnosed or already treated patients) referred to the national center for blood diseases – Al Mustansiriya university, for evaluation and treatment during the period Nov. 2013 to Jan. 2015 were used in this study.

The CML subjects included 15 newly diagnosed (untreated) patients (7 men and 8 women) with a mean age of 49.7±10.6 yr and an age range of 32 – 69 yr. Also, another 56 already diagnosed as having cml and currently receiving treatment included 29 men and 27 women with a mean age of 54.4±13.3 yr and an age range of 31 – 75 yr. The range of duration of diseases between several months to 7 yr.

Before blood sampling, all participants were informed about the study objectives, and their consent were obtained.

Thirty seven control subjects were also used in this study. They included 21 men and 16 women with a mean age of 47.0±11.84 yr with an age range of 30 – 75 yr.

2.3 Blood sample collection

Blood samples were obtained from patients and control subjects, in fasting state by venipuncture, using a 5 ml disposable syringe. Three ml of blood were obtained and

dispensed in EDTA tubes. Then, it was centrifuged at 3000 rpm for 10 minutes to collect plasma. The plasma was divided into aliquots in Eppendorff tubes for measuring antioxidant total glutathione (GSH) and fasting blood glucose (FBG) and used for measuring the cytokines (TNF-a and IFN-a). From the same and other patients and controls, a 3ml were obtained in (EDTA) tubes for the measurement of HB A1c, oxidative stress (MDA), lymphocytes separation, complete blood picture (CBP), and CD-Markers (CD4+ and CD8+). Complete blood picture was performed by automated machine by the center of blood diseases and office of teaching laboratories.

2.4 Plasma Levels of Tumor Necrosis Factor-alpha (TNF-α) ELISA Kit, R&D system)

2.4.1 Principle of the assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNF-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TNF-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TNF-α bound in the initial step. The color development is stopped and the intensity of the color is measured.

2.4.2 Materials provided

- TNF-a Microplate
- TNF-a Conjugate
- TNF-a Standard
- Assay Diluent RD1F
- Calibrator Diluent RD6-35
- Wash Buffer Concentrate
- Color Reagent A

- Color Reagent B
- Stop Solution
- Plate Sealers

2.4.3 Samples collection and storage

Plasma: plasma was collected using EDTA, heparin, or citrate as an anticoagulant. The content was Centrifuged for 15 minutes at 1000 x g within 30 minutes of collection. Samples assay either immediately or aliquot were stored at ≤ -20 °C. Repeates freeze-thaw cycles was avoided.

2.4.4 TNF-α Reagents Preparation

All reagents were warmed to room temperature before use.

Wash Buffer - If crystals formed in the concentrate, heated to room temperature and mix gently until the crystals completely dissolved. Amount of 20 mL of Wash Buffer was diluted into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B were mixed together in equal volumes within 15 minutes of use and Protected from light. An amount of 200 µL was required per well in the resultant mixture.

TNF-α Standard – the vial label for reconstitution volume was followed. Reconstitute the TNF-α Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. The standard allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

An amount of 900 µL of Calibrator Diluent RD6-35 was transferred (for serum/plasma samples) into the 1000 pg/mL tube. Then 500 µL of the appropriate Calibrator Diluent was transferred into the remaining tubes. A series of dilutions were made. Each tube was thoroughly mixed before the next transfer. The 1000 pg/mL standard serves as the highest standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).

2.4.5 TNF-α assay procedure

All reagents and samples were brought to room temperature before use. All samples, standards, and controls were assayed in duplicate.

1. All reagents and working standards were prepared as directed in the previous sections.

2. Excess microplate strips from the plate frame were removed and returned to the foil pouch containing the desiccant pack, and reseal.

3. An amount of 50 μL of Assay Diluent was added to each well. Diluent RD1F which have a precipitate present was mixed well before and during assay.

4. An amount of 200 μL of Standard, control, or sample were added to each well. Covered with the adhesive strip, and incubated at room temperature for 2 hours.

5. Each well and wash were aspirated, the process was repeated 3 times for a total of 4 washes. Each well was washed by filling with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. For good performance, a complete removal of liquid at each step is essential was removed. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate and blot were inverted it against clean paper towels.

6. An amount of 200 μL of TNF-α Conjugate was added to each well. Then Covered with a new adhesive strip.

For Serum/Plasma Samples: Incubated for 2 hours at room temperature. The aspirated and washed 4 times.

7. An amount of 200 μL Substrate Solution was added to each well and incubated at room temperature for 20 minutes and Protected from light.

8. An amount of 50 μL of Stop Solution was added to each well. The color in the wells should be changed from blue to yellow .If the color in the wells is green or if the color change does not appear uniform, gently the plate was taped to ensure through mixing.

9. The amount of the TNF-α in the samples were assayed spectrophotometrically at 450 nm within 30 minutes.

2.4.6 Calculation of results

Create a standard curve by reducing the data using computer software capable of generating a four parametric logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the clog of the TNF-α concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

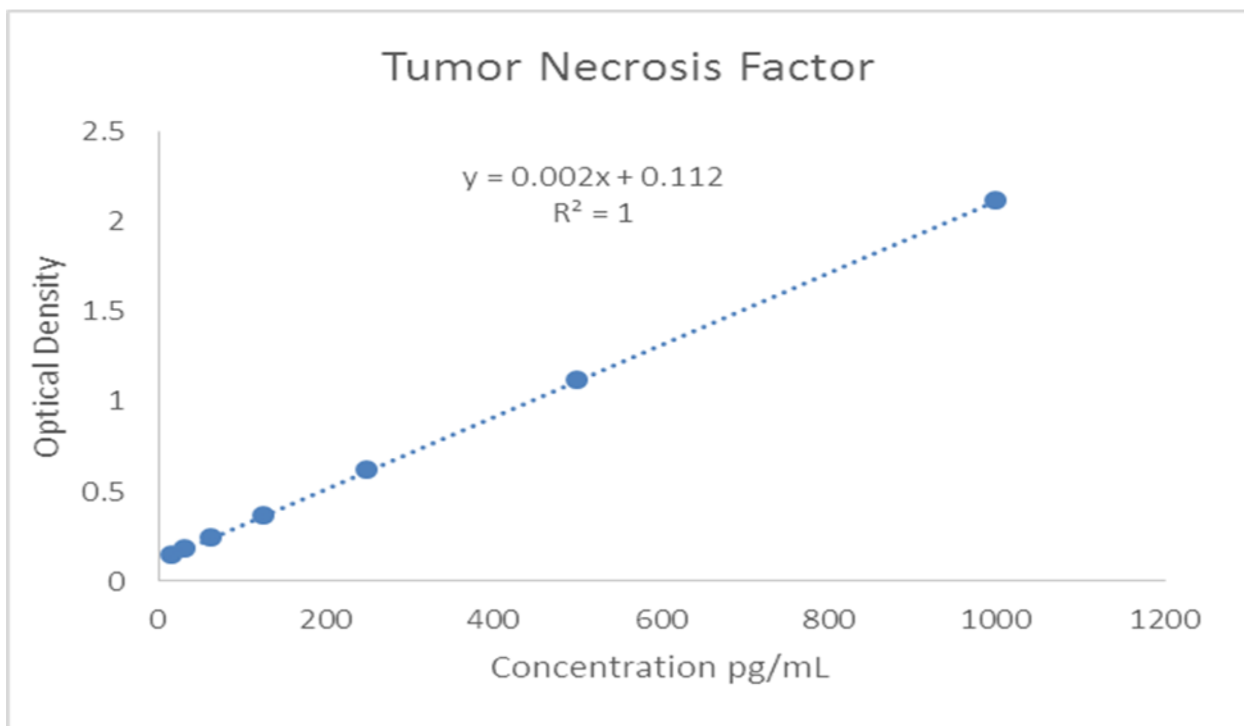

Figure (2.1) Standard curve of TNF-α

2.5 Plasma Levels of Interferon-alpha (IFN-α) (ELISA Kit, pbl interferon source)

2.5.1 Materials provided

• Pre-coated microtiter plate(s)

• Plate sealers

• Wash Solution Concentrate

• Human Interferon Alpha Standard, 10,000 pg/ml

• Dilution Buffer

• Antibody Concentrate

• HRP Conjugate Concentrate

• Concentrate Diluent

• TMB Substrate

• Stop Solution

2.5.2 Specifications

This VeriKine™ kit quantitates human interferon alpha in media using a sandwich immunoassay.1,2 The kit is based on an ELISA with anti-detection antibody conjugated to horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. The assay is based on the international reference standard for human interferon alpha (Hu-IFN-α) provided by the National Institutes of Health.3

2.5.3 Preparation of reagents

All components were kept on ice (4°C) throughout the assay, except for the Wash Solution Concentrate and Stop Solution, which were brought to room temperature (RT),

22-25°C. The TMB Substrate was equilibrated to RT (22-25°C) during step 3 of the Assay Procedure.

Wash Buffer: The Wash Solution Concentrate may contain crystals. The bottle was placed in a warm water bath and gently mix until completely dissolved. A 1:20 working Wash Buffer was prepared by adding 50 ml of Wash Solution Concentrate to 950 ml of distilled or deionized water. Then thoroughly mixed before use. The diluted Wash Buffer was stored at RT (22-25°C) until use.

Human Interferon Alpha Solution: The Human IFN Alpha Standard, provided at 10,000 pg/ml was diluted, in Dilution Buffer as indicated. In certain situations "test" samples could contain substances that could interfere with assay results. Therefore, if so, the IFN standard curve diluted was run in sample matrix.

2.5.4 Standard Curve Preparation:

A High Sensitivity standard curve 12.5 - 500 pg/ml or Extended Range standard curve 156 - 5000 pg/ml were constructed.

a) Six polypropylene tubes (S1-S6) were labled.

b) Tubes were filled with Dilution Buffer.

c) Using polypropylene tips, the Human IFN-α Standard was added to S6 and mixed gently. Change tips between each dilution.

d) Indicated amount was removed from S6 and add to S5. The step was repeared to complete series to S1. Then the solutions were refrigerated.

Sample Preparation: Test samples of unknown interferon concentration to be tested were prepared using Dilution Buffer as required. Measurements in duplicate were made and refrigerated until use in step 1 of the assay procedure.

Antibody Solution: Antibody Concentrate was diluted in Dilution Buffer. As recommended, the lot specific Certificate of Analysis (COA) for the correct volumes to

use and left 15 minutes prior to use in step 2 of the assay procedure and kept at RT (22-25°C).

HRP Solution: HRP Conjugate Concentrate was diluted in Concentrate Diluent. As indivated, the lot specific Certificate of Analysis (COA) for the correct volumes to use and left 15 minutes prior to use in step 3 of the Assay Procedure and keep at RT (22-25°C).

2.5.4 Assay procedure

All incubations were performed at room temperature (RT), 22-25°C, keeping the plate away from drafts and other temperature fluctuations. The plate were covered using plate scales as directed. During all wash steps, contents of plate were removed by inverting and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate. All wells were filled with a minimum of 250 µl of diluted Wash Buffer at each wash step. Refer to Preparation of Reagents for dilution of concentrated solutions.

1. Standards and Test Samples: The number of microplate strips required to test the desired number of samples plus the appropriate number of wells needed to run blanks and standards were determined. Each standard, blank and sample were run in duplicate. Strips 1 and 2, rows A-H, for serially diluted standards and blanks were used. Extra microplate strips from the frame, seal in the foil bag provided and store at 2-8°C were removed. Unused strips were used in later assays. An amount of 100 µl of the interferon standard, blank or sample to each well were added and covered with plate sealer and incubate for 1 hour. After 1 hour, the contents of the plate was removed and the wells were washed one time only with diluted Wash Buffer (refer to Preparation of Reagents).

2. Antibody Solution: An amount of 100 µl of diluted Antibody Solution (refer to Preparation of Reagents) was added to each well and covered with plate sealer and incubate for 1 hour. After 1 hour, the contents of the plate was removed and the wells were washed three times with diluted Wash Buffer.

3. HRP: An amount of 100 µl of diluted HRP Solution (refer to Preparation of Reagents was added to each well and covered with plate sealer and incubate for 1 hour. During this incubation period, the TMB Substrate Solution was warmed to RT (22-25°C). After 1 hour, the contents of the plate was removed and the wells were washed four times with diluted Wash Buffer.

4. TMB Substrate: An amount of 100 µl of the TMB Substrate Solution was added to each well. And incubated in the dark, at RT (22-25°C), for 15 minutes. Do not use a plate sealer during the incubation.

5. Stop Solution: After the 15 minute incubation of TMB, no washing was done. An amount of 100 µl of Stop Solution to each well was added.

6. The concentration was measured using a microplate reader at 450 nm within 5 min after addition the stop solution.

2.5.5 Calculation of results

By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Blank OD should be subtracted from the standards and sample OD to eliminate background. Because the interferon samples are titrated against the international standard, the values from the curves can be determined in units/ml as well as pg/ml. the conversion factor of about 3-5 pg/unit is applicable for human IFN-α. Nevertheless, this conversion factor is only an approximation. A shift in optical densities is typical between users and kit lots. The back fit concentration extrapolated from the standard curve is a more accurate determination of the sample titer and performance of the kit. Variations, from the typical curve provided can be a result of operator technique, altered incubation time, fluctuations in temperature, and kit age. Results of typical standard curve using 4-parameter fit are provided for demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.

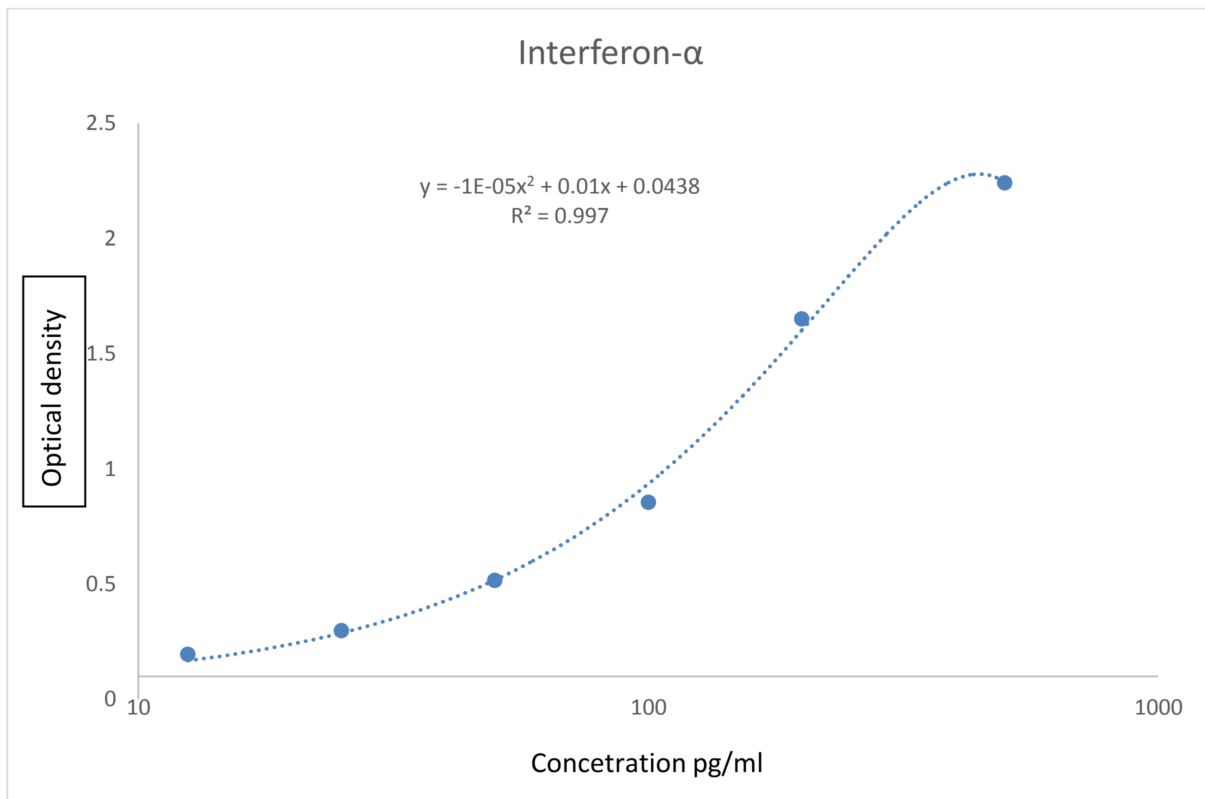

Figure (2.2) Standard curve of Interferon-α

2.6 Isolation of human lymphocytes

Lymphocytes were isolated according to Boyum method using lymphoprep solution. Lymphoprep an already made, sterile and endotoxin tested solution for the isolation of pure lymphocyte suspension. The solution contains sodium diatrizoate and polysaccharide.

Principle of the separation procedure: the most common technique for separating leucocytes increasing their sedimentation rate. The sedimentation of leucocytes is only slightly affected and can be collected from the upper part of the tube when the erythrocytes have settled.

2.6.1 Separating procedure: (Marive and Joe, 1974)

• Blood was collected into a tube containing anticoagulant (EDTA). The blood was diluted by addition of equal volume of 0.9% NaCl. Carefully 6 ml of the diluted blood

was layered over 3 ml lymphoprep in a 15 mm centrifuge tube. Alternatively, lymphoprep was underlayered. Mixing of blood and separation fluid was avoided. The tube was capped to prevent the formation of aerosols.

• The tubes were centrifuged at 800* g for 20 minutes at room temperature. If the blood stored for more than 2 hours, the centrifuge time was increased to 30 minutes.

• After centrifugation the mononuclear cells from a distinct band at the sample medium interface was separated and the cells were removed from the interface using a Pasteur pipette without removing the upper layer.

• The harvested fraction was diluted with 0.9% Nacl to reduce the density of the solution and the cells was pelleted by centrifuge for 10 minutes at 250x g.

• Leukocyte lysate was prepared by freezing /thawing method. Cleaned cells (250 µl) were lysed through 15 minutes in cold water (1750 µl) at 0 °C. Cell debris was removed by centrifugation at 1000 g for 15 minutes at 4°C. Supernatants were stored until analyzed at - 20 °C.

• Cell viability, as judged by ability to exclude trypan blue, was determined greater than 98%.

2.7 Analysis of T-Lymphocyte Subset by Flow cytometry

2.7.1 Description

Cylyse stands for an erythrocyte lysing reagent kit for wash and no wash procedure with a complete preservation of the surface proteins and practically no loss of cells.

Cylyse is particularly suitable for absolute cell counting and for assays, demanding a minimum loss of leukocytes. Residual debris does not need to be removed by centrifugation due to the properties of the lysing reagent buffer.

Fixative reagent A fixes and stabilizes the leukocytes. The fixed samples can be stored for up to 24 hours at 2-8°C before analysis.

2.7.2 Assay procedure

Antibody labelling: an amount of 100 µl of whole blood or isolate leukocytes was mixed with conjugated antibodies (e.g. 10 µl or as recommended by the antibody supplier) in a test tube (code No. 04-2000). Then mixed thoroughly incubated for 15 minutes in the dark at room temperature.

Leukocyte fixation: an amount of 100 µl of reagent A was added, then mixed thoroughly and incubated for 10 minutes in the dark.

Erythrocyte lysis: an amount of 2.5 ml of reagent B was added, then shaked gentle and incubated for 20 minutes in the dark.

The samples were analyzed using flow cytometer. Fixed samples were stored at 2-8°C, protected from light, up to 24 hours until analysis.

2.7.3 Storage

Samples were stored at room temperature. Temperatures below 17°C could lead to a white precipitation in lysing reagent B. The precipitate could be re-dissolved at 35°C (for e.g. in a water bath).

For measuring CD4 count: the following formula was used

Lymphocyte count = $\frac{percentage\ of\ lymphocyte}{100}$ X wbc count

Number of cells containing CD4+ = $\frac{percentage\ of\ CD4+}{100}$ X lymphocyte count

For measuring CD8 count: the following formula was used

Lymphocyte count = $\frac{percentage\ of\ lymphocyte}{100}$ X wbc count

Number of cells containing CD8+ = $\frac{percentage\ of\ CD8+}{100}$ X lymphocyte count

2.8 Plasma levels Total Glutathione (GSH) (ELISA Kit, Cusabio)

2.8.1 Principle of the assay

The assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for GSH has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any GSH present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for GSH is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of GSH bound in the initial step. The color development is stopped and the intensity of the color is measured.

2.8.2 Sample preparation

It is recommend to dilute the serum or plasma samples with Sample Diluent (1:200) before test. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

2.8.3 Reagent preparation

1. **Biotin-antibody (1x)** - the vial was centrifuged before opening. Biotin-antibody was diluted to a 100-fold dilution. A suggested 100-fold dilution is 10 µl of Biotin-antibody + 990 µl of Biotin-antibody Diluent.

2. **HRP-avidin (1x)** - the vial was centrifuged before opening. HRP-avidin was diluted to a 100-fold dilution. Which is 10 µl of HRP-avidin + 990 µl of HRP-avidin Diluent.

3. **Wash Buffer(1x)** - If crystals have formed in the concentrate, it was warmed up to room temperature and mixed gently until the crystals completely dissolved. A 20 ml of Wash Buffer Concentrate (25 x) was diluted into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

4. **Standard**

The standard was centrifuged vial at 6000-10000rpm for 30s.The Standard was reconstituted with 1.0 ml of Sample Diluent. This reconstitution produces a stock solution of 50 µg/ml. The standard was mixed to ensure complete reconstitution and allow the standard was allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. A 250 µl of Sample Diluent was pipetted into each tube. The stock solution was used to produce a 2-fold dilution series (below). Each tube was mixed thoroughly before the next transfer. The undiluted Standard served as the high standard (50µg/ml). Sample Diluent served as the zero standard (0 µg/ml).

2.8.4 GSH Assay Procedure

All reagents and samples were brought to room temperature before use. The sample again after thawing was centrifuged before the assay. All samples and standards were assayed in duplicate.

1. All reagents, working standards, and samples were prepared as directed in the previous sections.

2. The Assay was done according to Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.

3. An amount of 100µl of standard and sample was added per well. Then covered with the adhesive strip provided and incubated for 2 hours at 37°C. A plate lay out was provided to record standards and samples assayed.

4. The liquid of each well was removed with no wash.

5. An amount of 100µl of Biotin-antibody (1x) was added to each well. Covered with a new adhesive strip. Then incubated for 1 hour at 37°C. (Biotin-antibody (1x) may

appear cloudy. It was warmed up to room temperature and mixed gently until solution appears uniform.)

6. Each well and wash were aspirated, the process was repeated two times for a total of three washes and washed by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and left to stand for 2 minutes, complete removal of liquid at each step was done to good performance. After the last wash, any remaining wash Buffer was removed by aspirating ordecanting. The plate was inverted and blotted it against clean paper towels.

7. An amount of 100µl of HRP-avidin (1x) was added to each well and covered the microtiter plate with a new adhesive strip and incubated for 1 hour at 37°C.

8. The aspiration/wash process was repeated for five times as in step 6.

9. An amount of 90µl of TMB Substrate was added to each well. Then incubated for 15-30 minutes at 37°C and protected from light.

10. An amount of 50µl of Stop Solution was added to each well, the plate was gently tapped to ensure thorough mixing.

2.8.5 Calculation of results

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the X-axis against the concentration on the Y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the GSH concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

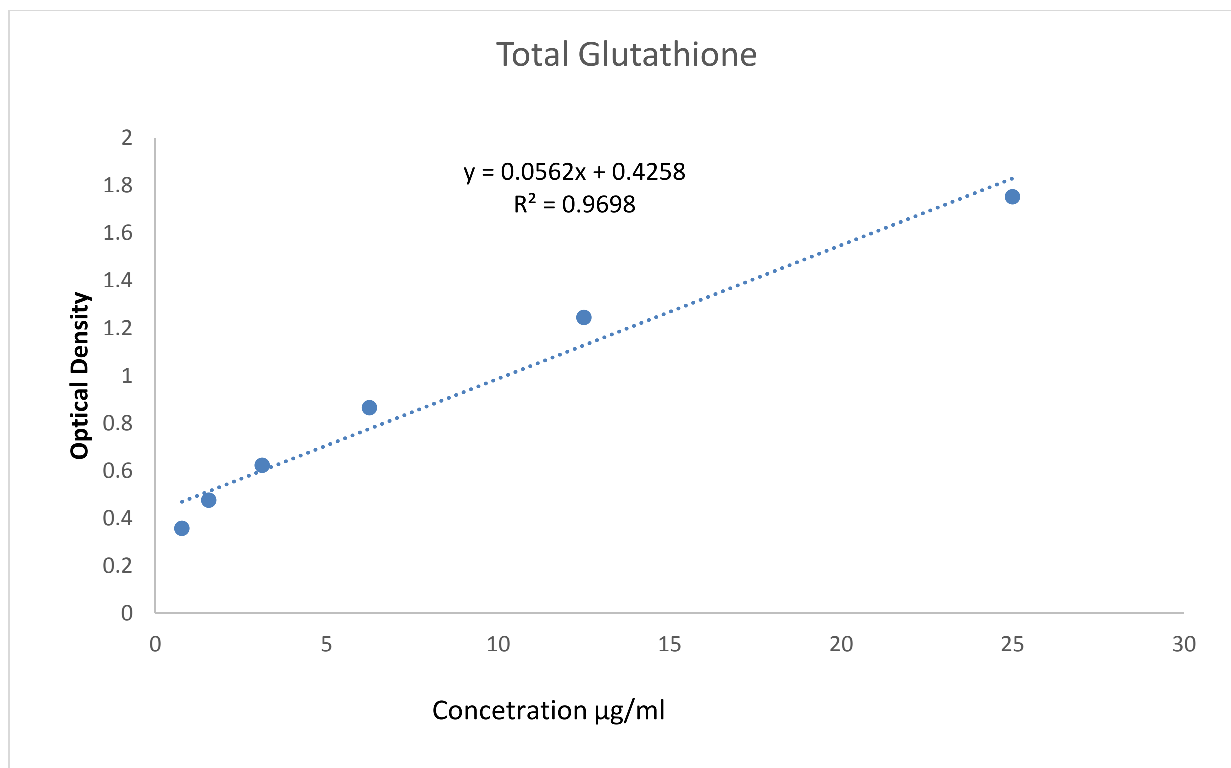

Figure (2.3) Standard curve of total glutathione (GSH)

2.9 Determination the levels of Malondialdehyde (MDA) in Red Blood Cells (RBC)

A slight modification of the method and stocks and Dormandy (1971) as modified by Gillbert *et al* (1984) was used to measure MDA production.

2.9.1 Reagents

1- 0.9% NaCl – 2 mM NaN_3 (store in fridge)

2- 28% TCA – 0.1 M Na arsenite (store in fridge)

3- 1% thiobarbituric acid (TBA) in 0.05 M NaOH (prepare fresh-requires heating)

4- 50Mm Tris – 0.1 Mm EDTA pH 7.6 (store in fridge)

5- Peroxidizing agent (H_2O_2), freshly prepared in saline/azide (11µl 30% H_2O_2+ 10 ml = 10 mM. Add 0.5 ml of this solution to 0.5 ml of saline azide solution = 5 mM.

2.9.2 Procedure

Centrifuge blood, remove plasma and buffy coat, wash twice with cold saline (or saline/azide if oxidative enzymes do not need to be assayed). Weigh 2 separate aliquots of 0.1 ml packed red cells. Add 0.9 ml cold saline to each aliquot. The two tubes of a 10% suspension of red cells in saline azide were pre-incubated for 5 min at 37°C. Peroxidative challenge was induced by the addition of an equal volume (1.0 ml) of 5 mM H_2O_2 in isotonic saline azide solution to one tube (final concentration of H_2O_2 2.5 mM). Following a 30 min incubation at 37°C, the reaction was terminated by addition of 1.0 ml of 0.5% of 28% (TCA)–0.1 M sodium arsenite. The mixture was centrifuged, and combined with 1.0 ml of 0.5% TBA in 0.05 M sodium hydroxide. Color development was achieved by boiling for 15 min. The tubes were cooled under tap water and the absorbance was estimated at 532 and 453 nm. The extent of lipid peroxidation by product (MDA) was estimated using the following equation and by quantification of the TBA–reactive substances based upon the molar extinction coefficient of MDA (1.56 x 10^5 M^{-1} cm^{-1}):

$$(abs_{532} - blk_{532}) - 20\% \, (abs_{453} - blk_{453})$$

For Hb measurement, weigh 0.1 ml packed red cells. Lyse in 9.9 ml H_2O, centrifuge, and assay the supernatant.

2.10 Determination of Fasting Blood Glucose (FBG) (Glucose Kit, Biolab)

a. An aliquot (10µl) of the plasma was added to tubes containing 1ml of Reagent R, then it were mixed well and incubated at 37°C for 10 min.

b. An aliquot (10µl) of the plasma was added to tubes containing 1ml of Standard, then it were mixed well and incubated at 37°C for 10 min (Standard)

c. An aliquot 1 ml of reagent R was added to the tubes and incubated at 37°C for 10 min (Blank).

d. The samples and standard, were read at (505 nm) wavelength against the Blank. The color is stable for at least 30 minutes.

e. The Glucose concentration (mg/ml) was calculated in the samples as follow:

$$\text{Glucose mg/ml} = \frac{(A)sample\ x\ 100(standard\ conc.)}{(A)standard}$$

2.11 Determination the levels of Glycosylated hemoglobin (Glycohemoglobin Kits, Stanbio)

2.11.1 Reagents

1-Glycohemoglobin Ion-Exchange Resin. Cat. No. P351- (Tubes): each tube contains 3.0 mL cation-exchange resin, 8 mg/dl., buffered at pH 6.9 was used.

2- Glycohemoglobin Lysing Reagent. Cat. No. 0352: contains potassium cyanide, 10 mmol/L and surfactants was added.

3- Glycohemoglobin Standard (Lyophilized). Cat. No. 0353-(1 vial):

2.11.2 Reagent preparation

To reconstitute standard, aluminum seal and rubber stopper were removed carefully to avoid loss of contents. Using a volumetric pipet. A 1.0 ml distilled/deionized water was added to the vial. Rubber stopper was replaced and allow to stand for 10 minutes at room temperature. Contents was swirled gently while observing for presence undissolved material until the solution is completed. Reconstitution was hastened by mechanically shaking the vial gently. The standard was used exactly as the patient sample in the procedure.

2.11.3 Storage and Stability

Ion-Exchange Resin and Lysing Reagent are stable at room temperature (15-30°C) until their respective expiration dates. Turbidity and discoloration indicates deterioration and reagents should be discarded. Standard (lyophilized) is stable at (2-8°C) until the

expiration date. Reconstituted standard should be used within 30 minutes or stored in 0.1 ml aliquots at -20°C. Refrigerated (2-8°C) standard is stable up to 30 days.

2.11.4 Material provided

Resin Separators. Cat. No. 0355

2.11.5 Specimen Collection and Preparation

Whole blood collected with EDTA is preferred. but heparin can be used.

2.11.6 Sample Stability: Glycohemoglobin in blood collected with EDTA is reportedly stable for 1 week at 2-8°C.

2.11.7 Procedure

2.11.7.1 Hemolysate Preparation

1- An amount of 0-5 mL (500µL) Lysing reagent was pipetted into tubes as labeled Standard (s), Unknown (u), and Control (c).

2- An amount of 0.1 mL (100 µL) of each well-mixed blood sample was pipetted into appropriately labeled tube and mix.

3- The solution was allowed to stand for 5 minutes at room temperature (15-30°C) to complete hemolysis.

2.11.7.2 Glycohemoglobin Separation and Assay

1- Pre-Fil* resin tubes were labeled as Standard (S), Unknown (U), and Control (C).

2- An amount of 0.1 mL (100 µL) of the prepared hemolysate into was pipetted appropriately labeled resin tube.

3- Resin separator was positioned in the Pre-Fil* tube so rubber sleeve is approximately 1-2 cm above liquid level.

4- Tubes was mixed on a hematology rocker for 5 minutes. Alternatively, tubes may be mixed by hand if held above the resin.

5- At the end of the 5 minute mixing, resin separator was pushed into tube until resin is firmly packed in bottom of the 13 mm tube.

6- Each supernate was poured directly into separate cuvettes for absorbance measurements.

7- Absorbance (A_{gly}) of Standard, Unknown and Control $_{vs.}$ water were read at 415nm within 60 minutes.

2.11.7.3 Total Hemoglobin Assay

1- An amount of 5.0 ml deionized water was pipetted into tubes labeled as Standard (S), Unknown (U), and Control (C).

2- An amount of 0.02 mL (20 µL) of hemolysate was pipetted into appropriately labeled tube.

3- Mixed well and transferred to cuvette for absorbance reading.

4- Absorbance (A_{tot}) of Standard, Unknown and Control $_{vs.}$ water were read at 415nm within 60 minutes.

2.11.8 Results

For each standard and unknown, the ratio were aclculated (R) of the glycohemoglobin absorbance to the hemoglobin absorbance as follows:

$$R = \frac{\text{A gly}}{\text{A tot}}$$

$$\text{Glycohemoglobin (\%)} = \frac{\text{R (Unknown)}}{\text{R (Standard)}} \text{ x concentration of glycohemoglobin}$$

standard (%)

2.12 Statistical analysis:

Each value represents mean ± standard deviation (SD). Statistical analyses were performed using student´s t-test (for two group analysis). Multiple mean comparisons were made with the one–way analysis of variance (ANOVA) using a significant level of $P < 0.05$. Specific group differences were determined using Tukey´s test (Snedecor and Cochran, 1976).

3. Results and Discussion

Complete blood picture showed significantly increase of WBC, Neutrophile%, Lymphocytes %, Basophils %, Eosinophils%, HCT, Hb, RBCs, PLT in newly diagnosed CML patients when compared with apparently healthy controls. Furthermore, treatment of patients significantly lowered of WBC, Neutrophile%, Lymphocytes %, Basophils %, Eosinophils%, HCT, Hb, RBCs, PLT levels to a value comparable to that of apparently healthy controls. (Table 3-1).

Table (3-1) Complete blood picture in apparently healthy controls and CML patients**.**

		CML patients	
parameters	Apparently healthy controls (n=15)	Newly diagnosed (n=15)	Treated (n=15)
WBC (10^9/L)	6.73 ± 4.85(a)	197.56 ± 92.37(b)	5.54 ± 1.88(a)
Neutrophile %	58.58 ± 7.72(a)	79.3 ± 5.7(b)	57.6 ± 6.9(a)
Lymphocytes %	33.5 ± 6.83(a)	77 ± 5(b)	31.1 ± 7.75(a)
Basophils %	0.67 ± 0.38(a)	3.88 ± 1.46(b)	0.37 ± 0.17(a)
Eosinophils%	1.44 ± 1.02(a)	2.7 ± 1.13(b)	1.5 ± 1.05(a)
HCT %	39.7 ± 2.7(a)	28.3±3.88(b)	34.9 ± 4.9(a)
HB (g/dL)	12.7 ± 2.9(a)	9.58±1.45(b)	11.5 ± 1.78(a)
RBCs (10^{12}/L)	4.33 ± 0.65(a)	3.32±0.51(b)	3.9 ± 0.58(a)
PLT (10^9/L)	289 ± 52.2(a)	279.6 ± 225.9(b)	216.8 ± 59.8(a)

Values are expressed as mean ± standard deviation (SD)

Values with different letters are significantly different ($P<0.05$)

n = number of subjects

b = referred to significantly different

3.1 Plasma Levels of Tumor Necrosis Factor_alpha (TNF_α) and Interferon_alpha (IFN_α)

The plasma levels of (TNF_α) showed a significant increase in newly diagnosed CML patients compared to apparently healthy controls. Furthermore, treatment of patients significantly lowered (TNF_α) levels at a value comparable to that of apparently healthy control (Table 3-2).

On the other hand, the plasma levels of (IFN_α) revealed no significant change among the three groups (Table 3-2).

Table (3-2) Plasma Levels of Tumor Necrosis Factor_alpha (TNF_α) and Interferon_alpha (IFN_α) in CML patients and apparently healyhy controls.

		CML Patients	
Parameters	Apparently Healthy Controls (n=15)	Newly diagnosed (n=15)	Treated (n=15)
TNF_α (pg/ml)	7.9 ± 1.44[a]	10.7 ± 2.72[b]	8.08 ± 1.63[a]
IFN_α (pg/ml)	4.2 ± 1.88[a]	3.97 ± 0.84[a]	3.8 ± 1.2[a]

Values are expressed as mean ± standard deviation (SD)

Values with different letters are significantly different ($P<0.05$)

n = number of subjects

b = referred to significantly different

Tumor necrosis factor_alpha (TNF_α) is a major pro-inflammatory cytokine that is released by activated macrophages, natural killer cells and T-cells (activated CD4+ and cytotoxic CD8+ T cells (CTLs)) (Riether *et al*, 2015). TNF_α is also produced by malignant and immune cells present in the inflammatory reaction surrounding tumors, and can contribute to tumorigenesis by creating a tumor-supportive inflammatory microenvironment (TME) and through direct effect on malignant cells (Grivennikov and Karin, 2011). TNF_α is a key mediator of cancer-associated chronic inflammation, and

may serve as a molecular link between chronic inflammation and cancer development (Du *et al*, 2014).

The present finding of increased plasma TNF_α levels in newly diagnosed CML patients suggests the presence of an inflammatory response by the immune system against CML. This finding agrees with that of Gallipoli *et al* (2013) who demonstrated higher TNF_α levels in plasma of CML patients compared to apparently healthy controls. They also observed higher production of TNF_α by stem/progenitor cells (SPCs) in CML patients relative to controls, suggesting that TNF_α supports a survival mechanism of SPCs in CML by promoting nuclear factor-kB (NF-kB) pathway activity. Recently, Kogoya *et al* (2014) demonstrated similar positive feedback loop between increased TNF_α and NF_kB pathway signals which, together with the TNF_α induced oxidative stress, may contribute to human myeloid leukemia progression.

Our results clearly indicated that the tyrosine kinase inhibitor (TKIs), imatinib managed to normalize most of the TNF_α and T-cells aberrations in the newly diagnosed CML patients, this finding supports the potential immunomodulatory effect of TKIs in CML state (Seggewiss *et al*, 2005 ; Biossel *et al*, 2006).b

The results demonstrated no significant change in IFN_α levels among the three groups. Type I IFNs (IFN-α and – β) and type II IFN (IFN-γ) are important cytokines released in response to infectious pathogens (Kurz *et al*, 2010) or to tumor cells (de Weerd *et al*, 2007). They also have direct effects on hematopoiesis (Schurch *et al*, 2013). Maintenance of hematopoiesis is dependent on dormant hematopoietic stem cells (HSCs). When exposed to hematopoietic stressful conditions, these dormant cells, through their self-renewal capacity, become activated and are induced to proliferate rapidly to maintain normal hematopoietic system, supposedly through interferon-alpha pathway (Trumpp *et al*, 2010).

Chronic myeloid leukemia is a clonal myeloproliferative neoplasia arising from the oncogenic BCR/ABL translocation in hematopoietic stem cells, resulting in a leukemia

stem cells (LSCs) (Kavalerchik *et al*, 2008). Curing CML depends on the elimination of LSCs (Schurch *et al*, 2013).

IFN-α have been shown to efficiently activate the proliferation of dormant HSCs, thereby could break their resistance to antiproliferative chemotherapeutics (Trumpp *et al*, 2010). However, chronic activation of the IFN-α pathway in HSCs impairs their function *in vivo* (Essers *et al*, 2009).

The exact mechanism responsible for the lack of alterations in endogenous IFN-α levels is not known at present. However, it is possible to speculate that the CML state may have induced a condition of chronic inactivation of the endogenous IFN-α signalling pathway leading to its functional impairment, thereby rendering both LSCs and HSCs un-responsive to the effect of the endogenous IFN-α. Further studies are needed to investigate this hypothesis. Before the introduction of tyrosine kinase inhibitors, pharmaceutical doses of IFN-α were used as a therapy for CML (Sawyers, 1999).

3.2 Biochemical parameters

3.2.1 Plasma Levels of Total Glutathione (GSH)

The plasma levels of (GSH) showed a significant decrease in newly diagnosed CML patients compared to apparently healthy controls. Furthermore, treatment of patients significantly increased GSH levels to a value comparable to that of the apparently healthy control. Table (3-3)

The present study demonstrated a significant decrease in plasma levels of GSH only in newly diagnosed untreated CML patients. This result agree with Ahmad *et al* (2008) who reported a significant decrease in the plasma levels total glutathione (GSH) in CML patients. Glutathione (GSH) is an important non-enzymatic antioxidant preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al*., 2003). GSH protect cells against toxic free radicals, involved in the synthesis and repair of DNA, and serves as a reductant

antioxidant in oxidation reduction resulting in the formation of oxidized GSH (Wu *et al*, 2004). Therefore, the observed decreased GSH levels in CML subjects may reflect depletion of non-enzymatic antioxidant reserve and the presence of endogenous oxidative stress. Evidence is available suggesting the presence of increased oxidative stress in CML (Hole *et al*, 2011; Aurelius *et al*, 2013).

ROS play a critical role in tumor metastasis (Ciarcia *et al*, 2010). Non-enzymatic antioxidants have been shown to limit carcinogenesis, and may be taken up by hematopoietic cells to bring down metastasis (Udensi and Tchounwou, 2014). The decreased plasma levels of reduced GSH in the present study may be due to over production of ROS in hematopoietic cells. As a signaling messenger, ROS are able to oxidize the critical target molecules such as protein kinase C (PKC) and protein tyrosine phosphates (PTPs), which are involved in tumor cell invasion (Sattler *et al*, 2000). Philadelphia chromosome produces a fusion protein, BCR-ABL in CML patients and the resultant protein causes a perturbation of stem cell function through mechanisms involving increased tyrosine kinase activity (Kurzrock *et al*., 2003). It was demonstrated that BCR-ABL fusion protein is associated with increased levels of ROS in hematopoietic cell lines compared with their non-transformed parental cell lines (Sattler *et al*., 2000). High ROS with the increase in the activity of tyrosine kinase protein could play an important physiological role in signal transduction and induced proliferative pathway in the cell (Wu, 2006). A recent report state that BCR-ABL kinase stimulates ROS, which causes oxidative DNA damage, resulting in mutations in the kinase domain. This suggested that ROS may play a major role in resistance to given therapy which could contribute to progression of CML (Koptyra *et al*., 2006; Udensi and Tchounwou, 2014).

Treatment of the present patients significantly increased GSH to a value comparable to that of the control. This finding agrees with Landry *et al* (2013) who reported that inhibition of Bcr-Abl induced ROS by either imatinib led to a significant reduction in ROS levels. This may be due to inhibition of NADPH oxidase dependent ROS production and expression of its subunit p22phox.

3.2.2 Malondialdehyde Levels in Red Blood Cells (RBCs)

Further analyses were carried out to investigate the response of red cells from apparently healthy controls and CML patients (untreated newly diagnosed and treated CML) to conditions of oxidative stress *in vitro*. One of the objectives for studying red cells was to investigate their predictive value in assessing changes observed in other tissues. In the absence of H_2O_2, erythrocytes from newly diagnosed (but not treated) CML patients showed a higher basal level of TBA–reactive material (MDA) compared to apparently healthy controls, suggesting the presence of increased susceptibility to oxidative stress. Moreover, when these cells were challenged with 2.5 mM H_2O_2, only red cells from newly diagnosed CML patients expressed significantly higher MDA levels compared to the apparently healthy control and treated CML erythrocytes. Clearly, the lack of comparable response (i.e., increased MDA production due to H_2O_2 challenge) of erythrocytes from treated CML patients, reflect a possible antioxidant effect exerted by imatinib therapy on lipid peroxidation by product, i.e., MDA production (Table 3-3).

The molecular basis of the increased basal and H_2O_2–induced MDA production is as yet unknown. However, together with the fact that plasma GSH levels were decreased in untreated CML patients, those two findings did support the possibility of the presence of increased endogenous oxidative stress in the newly diagnosed CML state. In fact, evidence is available which document the presence of increased plasma MDA levels in treated CML patients (Ahmed *et al*, 2008; Ahmed *et al*, 2010; Petrola *et al*, 2012). But, to the best of our knowledge, this study represents the first report in the available literature concerning the presence of increased basal and H_2O_2–induced MDA production in red cells of the newly diagnosed CML patients.

Regarding the use of exogenous oxidants to mimic oxidative stress experimentally, several studies are available in cancer research. Treatment of untransformed

hematopoietic cell lines MO7e with H_2O_2 *in vitro* resulted in an increase in ROS production and increased tyrosin kinasa activity of c-ABL (Sattler et al, 2010). Furthermore, Verrax *et al* (2007) and Beck *et al* (2011) implemented an experimental approach by selectively exposing human CML K562 cancer cells to an oxidant insult induced by the combination of menadione and ascorbate, which results in the formation of ROS.

Although the number of patients investigated there was too small to draw from conclusions, it is interesting to note that red cells from newly diagnosed untreated CML patients showed significantly higher levels of MDA (both basal and H_2O_2–induced) while those of CML treated subjects were indistinguishable from those of CML treated. Clearly more studies in a large number of patients are required to elucidate the possible mechanism(s) responsible for this novel and as yet unexplained finding in newly diagnosed CML patients. The possibility that the observed MDA levels in erythrocytes of newly diagnosed CML patients could resulted, at least in part, from an interaction between the duration, nature and severity of the CML state cannot be ruled out in the presence study. Whether or not this finding could be utilized as a potential biomarker for the diagnosis and/or prognosis of CML deserves further consideration.

The fact that treated CML erythrocytes failed to express similar elevations in both basal and H_2O –induced MDA suggests a possible antioxidant properties of imatinib therapy used. In the present study expressed as a decreased susceptibility to oxidative stress. However, several studies have shown that many anticancer drugs (imatinib) exert their therapeutic activity through induction of oxidative stress in affected cells (reviewed by Udensi and Tchounwou, 2014). Such controversial role of antioxidant stress in CML disease cannot the clarified at present. Further studies are required to address the possible role of oxidative stress in the etiology and progression of CML state as well as its role as a mechanism of the antileukemic therapy in CML.

Table (3-3) Plasma GSH and red cell MDA levels in apparently healthy control and CML patients.

		CML Subjects	
Parameters	Apparently healthy controls (n = 13)	Newly diagnosed (n = 13)	Treated (n = 15)
Plasma (GSH) (μg/ml)	38.05 ± 9.45 [a]	8.60 ± 6.90 [b]	36.71 ± 9.04 [a]
Red cell MDA(nmol/gHb) Basal	6.47 ± 4.29 [a]	12.69 ± 7.24 [b]	8.01 ± 8.14 [ab]
H_2O_2 (2.5 mM)-induced	9.17 ± 6.92 [a]	21.34 ± 11.34 [b]*	12.30 ± 6.09 [a]

Values are expressed as mean ± standard deviation (SD)

Values with different letters are significantly different (P<0.05)

n = number of subjects

b = referred to significantly different

*Significantly different (P<0.05) from basal MDA levels in newly diagnosed CML patients.

Conclusions

1- The present study revealed the presence of alterations in the immune system of the CML state, manifested by an elevation in the TNF-α levels in the newly diagnosed CML patients.

2- The decline of plasma GSH levels, together with the increase in both basal and H_2O_2-induced MDA levels in erythrocytes of newly diagnosed CML patients suggest the presence of increased susceptibility to oxidative stress in CML.

3- The ability of imatinib therapy to normalize most of the observed changes are suggestive of a possible antioxidant activity of the drug.

Recommendations

Further studies are required to investigate the following in large number of samples:

1. The total antioxidant status in erythrocytes of newly diagnosed CML patients.
2. The possible antioxidant properties of the anti-leukemic therapy, imatinib in treated CML patients.

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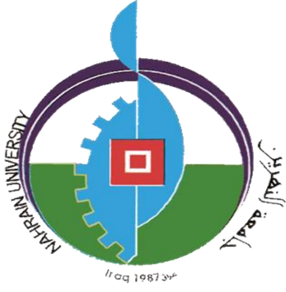
الخلاصة

ابيضاض الدم النقوي المزمن هو اضطراب التكاثر النقيي المزمن الناجم عن الجين الورمي BCR/ABL وتتميز بالتوسع النسيلي من الخلايا الاصلية المكونة للدم وخلايا الدم النخاعي الناتجة عن الانتقال الجيني (09:22). الهدف من هذه الدراسة هو للتحري عن المقاييس المناعية والكيمياحيوية المتضمنة في ابيضاض الدم النقوي المزمن في عينات الدم من المرضى المشخصين حديثا. ايضا تم التحري عن التاثير الدوائي لل (imatinib و linotinib) على هذه المقاييس في الاشخاص المصابين بابيضاض الدم النقوي المزمن و المعالجين. تم الحصول على عينات الدم من 71 ذكر وانثى (مشخص حديثا او معالج) تم ارسالهم الى المركز الوطني لامراض الدم, الجامعة المستنصرية و دائرة مدينة الطب/المختبرات التعليمية, بغداد. عدد المرضى المصابون بابيضاض الدم النقوي المزمن تتضمن 15 مريض مصاب حديثا (7 ذكور و 8 اناث) مع متوسط عمر 49.7±10.6 ومعدل عمر 32-69 سنة. والاشخاص الاخرين المعالجين و عددهم 56 شخص (29 ذكر و 27 انثى) مع متوسط عمر 54.4±13.3 سنة ومعدل عمر من 31-75 سنة متضمنة في هذه الدراسة. وكذلك تضمنت الدراسة ايضا 37 شخص طبيعي (21 ذكر و 16 انثى) مع متوسط عمر 47±11.84 سنة و معدل عمر 30-75 سنة.

تم دراسة مستويات ال TNF-α و IFN-α في البلازما و كذلك المستضدات اللمفاوية المرتبطة, الفروع CD4و CD8. ومن اجل التحري عن دور الاكسدة في الفيزيولوجيا المرضية لابيضاض الدم النقوي المزمن, تم تقدير مستويات الكلوتاثيون (GSH) في البلازما. وايضا تم فحص خلايا الدم الحمراء للبحث عن قابلية تحسسها للاكسدة المحدثة بواسطة بيروكسيد الهيدروجين. المعيار الذي تم قياسه في هذا المجال هو malondialdehyde (MDA) (مؤشر اكسدة الدهون).

النتائج التي تم الحصول عليها اوضحت ان هناك زيادة هامة لل TNF-α في البلازما في الاشخاص المصابين بابيضاض الدم النقوي المزمن المشخصين حديثا (لكن ليس الاشخاص المعالجين). وأظهرت الفروع اللمفاوية (CD4 وCD8) زيادة هامة في الاشخاص المصابين حديثا بالمرض. ولكن, الاشخاص المصابين بابيضاض الدم النقوي المزمن المعالجين باستخدام (imatinib او nilotinib) أظهروا انخفاض هام للقيم مقارنة بالاشخاص المصابين حديثا بالمرض و كذلك الاشخاص الطبيعيين. أوضحت النتائج ايضا وجود انخفاض هام في مستويات الكلوتاثيون في البلازما في الاشخاص المصابين حديثا بالمرض. علاوة على ذاك, يوجد ايضا زيادة هامة بمستويات كل من المستوى الاساسي لل MDA وكذلك مستوى ال MDA المحدث مختبريا بواسطة بيروكسيد الهيدروجين في خلايا الدم الحمراء للاشخاص المصابين حديثا بالمرض. وأظهرت الدراسة ايضا وجود زيادة هامة في مستويات الكلوكوز في البلازما للاشخاص المصابين حديثا بالمرض. وأظهر الاشخاص المعالجين باستخدام (imatinib او linotinib) القابية لخفض هذه القيم, ولكن لا يزال وجود زيادة هامة مقارنة بالاشخاص الطبيعيين. وعلى نحو مشابه, عكست مستويات ال HbA1c وجود ارتفاع فقط في الاشخاص المصابين حديثا بالمرض.

واستنتجت الدراسة الحالية وجود تغييرات في الجهاز المناعي في حالة ابيضاض الدم النقوي المزمن. وأن الانحدار بمستويات الكلوتاثيون, بالتزامن مع الارتفاع بمستويات ال MDA الاساسية والمحدثة بواسطة بيروكسيد الهيدروجين. في خلايا الدم الحمراء للاشخاص المصابين حديثا بالمرض, يوحي وجود زيادة في التحسس للاكسدة في حالة ابيضاض الدم النقوي المزمن. وأن الفعالية المضادة لابيضاض الدم لل imatinib و linotinib لتعديل اغلب هذه التغييرات, يوحي باحتمال وجود فعالية مضادة للاكسدة لهذين العقارين.



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تقييم عامل نخر الورم الفا و الانترفيرون الفا مع بعض المعايير الكيمياحيوية في مجاميع مختلفة لمرضى ابيضاض الدم النقوي المزمن في بغداد

رسالة

مقدمه الى مجلس كلية العلوم / جامعة النهرين

كجزء من متطلبات نيل درجة الماجستير في علوم التقانة الاحيائية

من قبل

محمد مازن ناجي

بكالوريوس 2012

اشراف

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