Assessment of Nuclear Factor Kappa B Among Chronic Myeloid Leukemia Patients

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Abstract
Chronic myeloid leukemia (CML) model of disease characterized by Philadelphia chromosome as a result of the t(9;22) reciprocal translocation between chromosomes 9 and 22 and the translocation occur in hematopoietic stem cell The activation of Nuclear Factor Kappa B signaling has been observed to increase significantly in leukemic stem cells exposed to chemotherapy. The aim of study to investigate the level of Nuclear factor kappa b in patient that treated with chemotherapy and compare the concentrations of NF kb in two type of therapy. The research included 50 individuals from the Iraqi National Hematology Center/Al-Mustansiriyah University and Baghdad Teaching Hospital diagnosed with Chronic Myeloid Leukemia, alongside 50 control participants matched for physical characteristics. The age range of the patients was between 20 and 70 years. The quantification of Nuclear Factor Kappa B concentration in plasma was determined through a quantitative sandwich enzyme immunoassay method (ELISA).The number of total males with CML was low (23 out of 50 ; 46%) while the total females with CML was (27 out of 50; 54%). This study showed increased level of nuclear factor kappa b concentration in cml patients plasma that treated with glavic (*2.105 ± 0.503*) pg/ml compared with concentration of healthy group plasma (***1.468 ±0.741***) pg/ml. While the mean of Erythrocyte sedimentation rate in Cml patients was (23.0 ± 18.4) mm/hour while the mean of Erythrocyte sedimentation rate in healthy control was (6.04 ± 2.42) mm/hour. Our findings indicate that the levels of NK-KB exhibit a significant elevation in CML patients who treated with Glavic and Tasigna. Erythrocyte sedimentation rate in Cml patients was elevated.
Keyword: Nuclear Factor Kappa b , Chronic Myeloid leukemia , Erythrocyte Sedimentation Rate.

Introduction:

Chronic myeloid leukemia (CML) model of disease characterized by Philadelphia chromosome as a result of the t(9;22) reciprocal translocation between chromosomes 9 and 22 and the translocation occur in hematopoietic stem cell the patients who had positive Philadelphia ch.9 was translocation of ABL proto-oncogene found[1] The emergence of the p210-BCR-ABL isoform is accountable for the manifestation of the CML phenotype, whereas the shorter p190-BCR-ABL isoform is associated with the onset of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). Nonetheless, it's noteworthy that the occurrence of the p210-BCR-ABL isoform extends beyond CML, being detectable in approximately 10% to 20% of adult patients and a minor fraction of pediatric cases diagnosed with ALL[2,3,4].

Additionally, a tiny area of chromosome 22 measuring up to 5.8 kb was found to contain every translocation breakpoint and was given the designation "breakpoint cluster region" (BCR). [5] . there were three type of Chronic myeloid leukemia disease accelerate phase (AP) ,chronic phase (CP), Blast Phase (BP) each type of three phases depend of ratio of Blast [6]. Nuclear factor kappa B (NF-κB) was originally identified as a transcription factor present in the B cell nucleus that binds to the immunoglobulin κ light chain enhancer.[7].

The Nuclear Factor-kappaB (NF-κB) family of transcription factors initially garnered attention within the field of immunology because of its capacity to control the expression of cytokines and functional enzymes, particularly following the stimulation of various immune receptors. These receptors are integral components of the immune response, encompassing those associated with T and B-cell activation. [8,9,10] The expression of the Bcr/Abl fusion oncoprotein is characterized by its inherent kinase activity .[11]

The activation of Nuclear Factor Kappa B signaling has been observed to increase significantly in leukemic stem cells exposed to chemotherapy, as reported in a study by Zhou et al[12]. in 2015. In mammals, there are five NF-κB proteins, namely RelA (p65), RelB, c-Rel, NF-κB1, and NF-κB2. NF-κB1 and NF-κB2 are initially synthesized as larger precursors with molecular weights of 105 kDa (p105) and 100 kDa (p100), respectively. These precursors undergo cleavage to generate biologically active p50 and p52 subunits, respectively. The canonical NF-κB pathway relies on the activation of the IKK (inhibitor κB[IκB] kinase) complex, which comprises IKKα,

IKKβ, and NF-κB essential modulator (NEMO or IKKγ). Activation of this complex leads to the phosphorylation and subsequent degradation of IκB, resulting in the release and activation of NF-κB. On the other hand, the noncanonical NF-κB pathway involves the activation of NIK (NF-κB-inducing kinase) and IKK, which triggers the processing of p100 into the transcriptionally active p52 form. Additionally, phosphorylation of p105 by the classical IKK complex initiates its polyubiquitination, degradation, and subsequent release of the transcriptionally active p50 subunit.[13]

Signaling initiated by the Bcr/Abl kinase activates various survival pathways, one of which involves the activation of NF-κB . Activation of NF-κB by the Bcr/Abl fusion oncoprotein leads to an increased translocation of the transcriptionally active subunit, p65, into the nucleus. This translocation may be attributed to p65 hypophosphorylation, which occurs independently of IKK [14]. These pathways ultimately culminate in the upregulation of antiapoptotic proteins such as Bcl-XL, conferring a growth advantage to clonal Bcr/Abl+ cells. The constitutive activation of NF-κB has been consistently demonstrated in late-stage chronic myeloid leukemia (CML) across various research studies.[15]

Both imatinib and nilotinib fall under the category of tyrosine kinase inhibitors (TKIs) and are employed in the treatment of chronic myeloid leukemia (CML) and other related conditions.[16] Despite their shared classification and similar modes of action, they exhibit distinct characteristics: Imatinib, among the early TKIs developed, finds utility in the treatment of various medical conditions, encompassing chronic-phase CML, accelerated-phase CML, and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL).[17] It is also a recognized therapeutic option for gastrointestinal stromal tumors (GISTs). [18] Nilotinib, conversely, is primarily prescribed for the treatment of chronic-phase and accelerated-phase CML in both adult and pediatric patients. Additionally, it is indicated for adult patients with Ph+ ALL who display resistance or intolerance to alternative therapeutic approaches.[19]

Both medications operate by inhibiting the activity of the BCR-ABL protein, which results from the Philadelphia chromosome translocation and drives the uncontrolled proliferation of

cancerous cells in CML and Ph+ ALL. Nevertheless, nilotinib demonstrates superior potency as a BCR-ABL inhibitor when compared to imatinib.[20]

Imatinib exhibits a lower level of selectivity and potency in inhibiting BCR-ABL, and it also affects other tyrosine kinases, including c-KIT and PDGF-R While Nilotinib, in contrast, boasts higher selectivity and binding affinity for the BCR-ABL protein. It is strategically designed to address some of the resistance challenges encountered with imatinib.[21]

Over time, certain CML patients may develop resistance to imatinib. In such instances, clinicians may contemplate a transition to a second-generation TKI, such as nilotinib, as it is specifically engineered to combat specific imatinib-resistant mutations [22].

Patients ,Materials and Methods

The research included 50 individuals diagnosed with chronic Myeloid Leukemia in Iraq, along with 50 control participants who were matched in terms of physical characteristics. The age of the patients ranged from 20 to 70 years. This investigation took place at the National Center of Hematology - Mustansyriah University, spanning from January 2021 to June 2023. A skilled clinical hematologist conducted a thorough physical examination for each patient, collecting data on pertinent medical conditions.

To quantify the concentration of Nuclear factor kappa b in serum, a quantitative sandwich enzyme immunoassay (ELISA) approach was employed. The ELISA kit utilized for this purpose was sourced from R&D Systems, USA.

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken.

Statistical analysis

The researches examine the mean, standard deviation (SD), and person correlation of discrete variables. Minitab version 21 is used to evaluate the category variables. P less than 0.01 was the threshold for statistical significance.

Result

In this study, the number of total males with CML was low (23 out of 50 ; 46%) while the total females with CML was (27 out of 50; 54%) Table (1). CML patients were investigated., The mean age of CML patients was (46.25 ± 2.77) years and control mean age was (47.80±1.34) years. Table (1).

Table (1) Age and Gender distribution among Patients & Control groups-

Item	Mean age	Male	Female	Total
Patients	**48.54± 2.77**	**23(46%)**	**27(54%)**	**50(100%)**
Control	**47.80± 1.34**	**25(50%)**	**25 (50%)**	**50(100%)**

This study showed increased level of nuclear factor kappa b concentration in cml patients plasma that treated with glavic (*2.105 ± 0.503*) pg/ml compared with concentration of healthy group plasma (***1.468 ±0.741***) pg/ml .there was a significant difference between patient and healthy donors (p=0.01) Table (2).

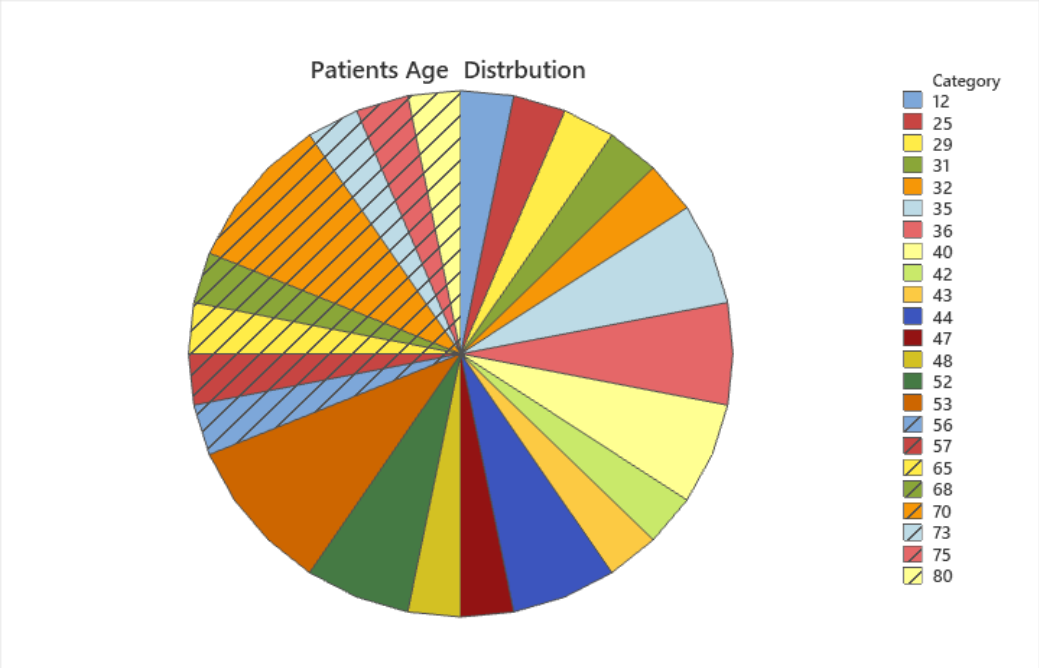

Figure (1) : Distribution of CML patients according Age

This study include 50 patients of Cml and fifty healthy control the mean of white blood cells in treated patients was (6.75 ± 2.35) 10^3/µL and the mean of white blood cells in healthy control was (6.57 ± 1.88) 10^3/µL . There was no significant difference between two group Table 2 .

Table 2 : Comparison between white blood cell (WBC) patients and healthy control

Parameter	No.	Mean ±SD	P-value	T. test
Patients WBC× 10^3/µL Mean ± SD	50	6.75 ± 2.35	0.734	.34
Control WBC× **10^3/µL Mean ± SD**	50	6.57 ± 1.88		

(P<0.01)*

While the mean of Erythrocyte sedimentation rate in Cml patients was (23.0 ± 18.4) mm/hour while the mean of Erythrocyte sedimentation rate in healthy control was (6.04 ± 2.42) mm/hour .there was a significant differences between two group **(P<0.01)* table(3).**

Table 3 :Comparison between erythrocyte sedimentation rate(ESR) patients and healthy control (P<0.01)*

Parameter	No.	Mean ±SD	P-value	T. test
Patients ESR mm/hour	50	23.0 ± 18.4	0.002*	3.78
Control ESR mm/hour	50	6.04 ± 2.42		

The result showed increased level of nuclear factor kappa b concentration in cml patients plasma that treated with ***Imatinit & nilotinib*** (*2.10 ± 0.503* , 2.34 ± 0.584) pg/ml compared with concentration of healthy group plasma (1.468 ±0.741) pg/ml .there was a significant difference between patient and healthy donors (p=0.01) Table (4,5).

Table 4: Difference concertation between patients treated by glavic and healthy control(P<0.01)*

NF-Kb Conc. with Glavic (imatinit)	***No.***	***Mean ±SD***	***P-value***	***T – test Value***
Patients pg/ml	***25***	***2.10 ± 0.503***	***0.001***	***3.56****
Control pg/ml	***25***	***1.468 ±0.741***		

Table 5: Difference concertation between patients treated by tasigna and healthy control(P<0.01)*

NF- KB Conc. with Tasigna (nilotinib)	*No.*	*Mean ±SD*	*P-value*	*T – test Value*
Patients pg/ml	*25*	*2.34 ± 0.584*	*0.000*	*4.62**
Control pg/ml	*25*	*1.468±0.741*		

In this study, we analyze patients chronic myeloid leukemia . Fifty chronic myeloid leukemia patients are studied and divided into two groups according to type of therapy (Glavic ; Tasigna). The mean of NF-kB serum concentration for patients treated with Glavic was (2.105 ± 0.503) pg/ml and NF-kB concentration for patients who took Tasigna was (2.34 ±0.584) pg/ml , there was no significant difference among two group according the type of therapy these results shown in table(6).

Table (6):NF-kb conc. according to type of therapy

NF-Kb Conc. among glavic (imatinit) and tasigna (nilotinib)	*No.*	*Mean ±SD*	*P-value*	*T – test Value*
Patients (Glavic) pg/ml	*25*	*2.105 ± 0.503*	*0.134*	*1.52*
Patients (Tasigna) pg/ml	*25*	*2.34 ±0.584*		

Discussion:

Chronic myeloid leukemia one of most malignancies widespread in Iraq[23]. Chronic myeloid leukemia (CML) does not have a significant gender preference. It can affect both men and women. The incidence of CML increases with age and can occur in both genders. As with many cancers, the risk of CML is not determined solely by gender but can be influenced by a variety of factors, such as genetics, exposure to certain substances, and other environmental factors.in this study revealed their was no predominance in gender while there was increase the disease with age this result agreed with pervious studies [24,25]. The concentration of NF-KB in patients

with chronic myeloid leukemia and in both patients who treated with a *imatinit and nilotinib* was elevated the results was agree with pervious studies [26] . The association of NF-κB signaling pathways with conditions such as cancer, diabetes, neurological disorders, and even memory has been widely observed. Consequently, NF-κB serves as a pivotal factor in comprehending not only the fundamental biological aspects but also the pathogenesis of these conditions. As a result, it has been extensively investigated in these various contexts. The number of white blood cell is in patients of cml was equivalent to healthy control that belong all patient treated with chemotherapy so the lymphocyte return to normal range while the erythrocyte sedimentation rate was increased in patients compared with healthy control this result was agree with previous studies [27] The prognosis in different malignancies is influenced by factors such as the nature of the underlying disorder, the stage and duration of the illness, and the type and intensity of the antitumor treatment prescribed. Furthermore, an increased ESR level has been recognized as a prognostic indicator associated with a negative impact on the survival of individuals with cancer [28,29].

Conclusion: our findings indicate that the levels of NK-KB exhibit a significant elevation in CML patients who treated with Glavic and Tasigna. Erythrocyte sedimentation rate in Cml patients was elevated . This association may play a pivotal role in disease progression and contribute to resistance against therapeutic interventions.

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Conflicts of interest

There are no conflicts of interest.

Ref….

1. Bennett, J.H.(**1845)** Case of Hypertrophy of the Spleen and Liver, which Death Took Place from Suppuration of the Blood. Edinb. Med. Surg. J., 413–423.

2. Melo JV.(1997) BCR-ABL gene variants. Baillières Clin Haematol.;10:203–22.
3. Westbrook CA, Hooberman AL, Spino C, Dodge RK, Larson RA, Davey F, Wurster-Hill DH, Sobol RE, Schiffer C, Bloomfield CD.(1992) Clinical significance of the BCR-ABL fusion gene in adult acute lymphoblastic leukemia: a Cancer and Leukemia Group B Study (8762) Blood.;80:2983–90.
4. Suryanarayan K, Hunger SP, Kohler S, Carroll AJ, Crist W, Link MP, Cleary ML. (1991)Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. Blood.;77:324–30.
5. Minciacchi, V. R., Kumar, R., & Krause, D. S. (2021). *Chronic Myeloid Leukemia: A Model Disease of the Past, Present and Future. Cells, 10(1), 117.* doi:10.3390/cells10010117 .
6. Jamieson, C.H.; Ailles, L.E.; Dylla, S.J.; Muijtjens, M.; Jones, C.; Zehnder, J.L.; Gotlib, J.; Li, K.; Manz, M.G.; Keating, A.; (**2004)** Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis, C.M.L. N. Engl. J. Med., 351, 657–667.
7. Ghosh, S., & Karin, M. (2002). *Missing Pieces in the NF-κB Puzzle. Cell, 109(2), S81–S96.*
8. Ghosh S, May MJ, Kopp EB. (1998)NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol.;16:225–60.
9. Bonizzi G, Karin M.(2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol.;25:280–8.
10. Carrà G, Torti D, Crivellaro S, Panuzzo C, Taulli R, Cilloni D, Guerrasio A, Saglio G, Morotti A. (2016)The BCR-ABL/NF-κB signal transduction network: a long lasting relationship in Philadelphia positive Leukemias. Oncotarget. Oct 4;7(40):66287-66298.

11. Delaval B, Lelievre H and Birnbaum D (2005) Myeloproliferative disorders: the centrosome connection. *Leukemia* **19**: 1739–1744.
12. Zhou J, Ching YQ and Chng WJ (2015) Aberrant nuclearfactor-kappa B activity in acute myeloid leukemia:from molecular pathogenesis to therapeutic target.Oncotarget6, 5490–5500.
13. Chorzalska, A., Ahsan, N., Rao, R.S.P., Roder, K., Yu, X., Morgan, J., Tepper, A., Hines, S., Zhang, P., Treaba, D.O., Zhao, T.C., Olszewski, A.J., Reagan, J.L., Liang, O., Gruppuso, P.A. and Dubielecka, P.M. (2018), Overexpression of Tpl2 is linked to imatinib resistance and activation of MEK-ERK and NF-κB pathways in a model of chronic myeloid leukemia. Mol Oncol, 12: 630-647.
14. Reuther JY, Reuther GW, Cortez D, Pendergast AM and Baldwin Jr AS (1998) A requirement for NF-kappaB activation in Bcr-Abl-mediated transformation. *Genes Dev.* **12**: 968–981.
15. Braun, T., Carvalho, G., Fabre, C. Grosjean J. , Fenaux P. , Kroemer G. *(2006)* Targeting NF-κB in hematologic malignancies. *Cell Death Differ* **13**, 748–758.
16. Kantarjian HM, Hochhaus A, Saglio G, De Souza C, Flinn IW, Stenke L, Goh YT, Rosti G, Nakamae H, Gallagher NJ, Hoenekopp A, Blakesley RE, Larson RA, Hughes TP.(2011) Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. Lancet Oncol.Sep;12(9):841-51.
17. Gordon, J.K., Martyanov, V., Magro, C. *et al.(* (2015). Nilotinib (Tasigna™) in the treatment of early diffuse systemic sclerosis: an open-label, pilot clinical trial. *Arthritis Res Ther* **17**, 213 .

18. Quintas-Cardama A, Cortes J.(2007) Nilotinib therapy in chronic myelogenous leukemia. Drugs Today (Barc). Oct;43(10):691-702.
19. Wang Z, Jiang L, Yan H, Xu Z, Luo P. (2021) Adverse events associated with nilotinib in chronic myeloid leukemia: Mechanisms and management strategies. *Expert Rev Clin Pharmacol* :14(4):445–56.
20. Iurlo A, Cattaneo D, Bucelli C, Breccia M. (2021) Dose optimization of tyrosine kinase inhibitors in chronic myeloid leukemia: A new therapeutic challenge. *J Clin Med* 10(3):515.
21. Thielen N, Visser O, Ossenkoppele G, Janssen J.(2016) Chronic myeloid leukemia in the Netherlands: a population-based study on incidence, treatment, and survival in 3585 patients from 1989 to 2012. Eur J Haematol.;97:145–54.
22. Hughes TP, Munhoz E, Aurelio Salvino M, Ong TC, Elhaddad A, Shortt J, et al. (2017)Nilotinib dose-optimization in newly diagnosed chronic myeloid leukaemia in chronic phase: final results from ENESTxtnd. Br J Haematol.;179:219–28.
23. Iraqi cancer registry ,2012.
24. Berger, U., Maywald, O., Pfirrmann, M. (2005)Gender aspects in chronic myeloid leukemia: long-term results from randomized studies. Leukemia 19, 984–989 .
25. Lee JP, Birnstein E, Masiello D, Yang D, Yang AS. Gender and ethnic differences in chronic myelogenous leukemia prognosis and treatment response: a single-institution retrospective study. J Hematol Oncol. 2009 Jul 24;2:30.
26. Mostafizar M, Cortes-Pérez C, Snow W, Djordjevic J, Adlimoghaddam A, Albensi BC. Challenges with Methods for Detecting and Studying the Transcription Factor Nuclear Factor Kappa B (NF-κB) in the Central Nervous System. Cells. 2021 May 28;10(6):1335. doi: 10.3390/cells10061335. PMID: 34071243; PMCID: PMC8228352.

27. Tas F, Erturk K. (2017)Elevated erythrocyte sedimentation rate is associated with metastatic disease and worse survival in patients with cutaneous malignant melanoma. Mol Clin Oncol. Dec;7(6):1142-1146. doi: 10.3892/mco.2017.1440. Epub 2017 Oct 4. PMID: 29285390; PMCID: PMC5740825.
28. Seong MK. Prognostic Inflammation Score in Surgical Patients with Colorectal Cancer. J Korean Med Sci. 2015 Dec;30(12):1793-9. doi: 10.3346/jkms.2015.30.12.1793. Epub 2015 Nov 30. PMID: 26713054; PMCID: PMC4689823.
29. Strojnik T, Smigoc T, Lah TT. Prognostic value of erythrocyte sedimentation rate and C-reactive protein in the blood of patients with glioma. Anticancer Res. 2014 Jan;34(1):339-47. PMID: 24403485.
30. Al-Hashemi, Hassnien Samir; Rahman, Sabah A. Hameid A.1; Shabeeb, Zeyad Ahmed. Expression of immune checkpoint molecules in Iraqi acute myeloid leukemia patients. Iraqi Journal of Hematology 10(1):p 1-16, Jan–Jun 2021. | DOI: 10.4103/ijh.ijh_46_20