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Serum Calcium And Phosphateleveelsin Patients With Chronic Myeloid Leukemia Taking Different Dose of Tyrosine Kinase Inhibitors.

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Abstract:

Objective: chronic myeloid leukemia (CML) is a myeloproliferative confusion characterized by the occurrence of an acquired mutation which affect the hematopoietic stem cell, treatment of CML with tyrosine kinase inhibitors (TKI) has resulted in high response rates compared to the interferon alpha and hydroxy urea. However, the long-term consequences of TKI had an effect on calcium (Ca++) and phosphate(PO4-2) levels which may have adverse events on the cardiac and skeletomuscular contraction.

Methods: in a cross-sectional study, 95 patients with CML receiving different TKI treatment for at least one year duration were randomly divided in to three groups: group (1) who received imatinib myselate (Gleevec) 400mgper oral per day,the second group were patients received gleevec 600mg-800mg/day, while the third group patients were received nilotinib 800mg/day as a second line therapy after failure to imatinib mylselate response .

Results: in the present study,serum Ca++ level was a significant lower statistical different with $p < 0.05$ when compared with serum level of PO4-2inpatients treated with imatinib and nilotinib with lower serumCa++ and PO4-2levelsin imatinibpatientsgroup than patientsused nilotinib group with p-value 0.049 and 0.005 subsequently.

Conclusion: imatinib therapy with the long term use may cause hypocalcemia and hypophospatemia without significant correlation more than treatment with nilotinib where significant correlation was between serum Ca++ and PO4-2and these changes may give an idea that disturbance of serum Ca++, PO4-2was multifactorial.

Key words: chronic myeloid leukemia,serum calcium and phosphatelevels, tyrosin kinase inhibitors.

الخلاصة:

الهدف: سرطان الدم النخاعي المزمن هو ارتباك تكاثري نقوي يتميز بحدوث طفرة مكتسبة تؤثر على الخلايا الجذعية المكونة للدم، وقد أدى علاجه مع مثبطات التيروزين كيناس إلى معدلات استجابة عالية بالمقارنة مع ألفا إنتيرفيرون و هيدروكسي اليوريا. ومع ذلك، فإن عواقب مثبطات التيروزين كيناسعلى المدى الطويل كان لها تأثير على الكالسيوم والفوسفورو قد يكون لها أحداث سلبية على انكماش القلب والهيكل العظمي العضلي.الطرق: في دراسة مستعرضة ، تم تقسيم 95 مريضا مصابين بسرطان الدم النخاعي المزمن ويتلقون علاج مثبطات التيروزين كيناس بجرع مختلفة لمدة سنة واحدة على الاقل بشكل عشوائي الى ثلاث مجموعات: المجموعة (1) الذين تلقو اماتينيب ميسيلات 400 ملغ في اليوم الواحد عن طريق الفم، المجموعة (2) تم تلقي المرضى اماتينيب بجرعة 600-800 ملغ في اليوم ،في حين تلقى مرضى المجموعة (3) نيلوتينيب 800 ملغ في اليوم كعلاج الخط الثاني بعد الفشل في استجابة اماتينيب ميلسيلات.النتائج: في الدراسة الحالية كان مستوى مصل الدم للكالسيوم احصائيا معنويا اقل من 0.05 عند مقارنتة مع مص الدم للفوسفات في المرضى الذين يتعالجون مع إماتينيب و نيلوتينيب ولوحظ قلة مستوى مصل الدم للكالسيوم والفوسفات للمرضى الذين يتعالجون بالإماتينيب اكثر من النيلوتينيب.الخلاصة: قد يسبب العلاج إماتينيب مع استخدام على المدى الطويل نقص كلس الدم ونقص بوتاسيوم الدم دون علاقة كبيرة أكثر من العلاج مع نيلوتينيب حيث كان هناك ارتباط كبير بين المصل كالسيوم والوفسفات وان هذه التغيرات تعطي فكرة ان اضطراب مصل الدم للكالسيوم والفوسفات لها عدة سياقات.

الكلمات الرئيسية: سرطان الدم النخاعي المزمن، ومستويات الكالسيوم والفوسفات في الدم، ومثبطات التيروزين كيناس

Abbreviations:
Chronic myeloid leukemia (CML)
Tyrosine kinase inhibitors (TKI)
Calcium &phosphate (Ca++, PO4-2)
Philadelphia chromosome (Ph)
Mesenchymal stem cells (MSCs).

Introduction:

Chronic myeloid leukemia (CML) is defined as a myeloproliferative disease that is characterized by inflexible presence of the philadelphia chromosome (Ph) or the BCR/ABLfusion gene [1]. It was originated in a pluri potent stem cell common to all three hematopoietic lineages, resulting in overproduction of myeloid cells in all stages of maturation [2]. TKI are highly effective drugs in the upfront treatment of CML [3] with betteroutcome and over all survival [4]. AmongTKI used in the treatment of CML, nilotinib and imatinib mesylate. These medications inhibits numerous signaling kinases pathways, like BCR-ABL, C- kit receptor (is a form of receptor tyrosine kinase and a form of tumor marker), and the platelet derivative growth factor receptors α and β, all of which are related with the disease [5].

However, TKI are well tolerated compared with traditional chemotherapies, but they were associated with some side effects which may range from mild to sever degree, like edema, muscle cramps, musculoskeletal pain, nausea,cardiac toxicity, diarrhea, and skin rash. But there is worry about lasting consequences of inhibition of these TKI, one of these consequencesis long term effect of TKI on some minerals like Ca++ and PO4-2[6].

The homeostatic manage of serum level of PO4-2& Ca++ levels was achieved by three ways; first is reabsorption of PO4-2& Ca++ by the kidneys,secondly is an absorption of dietary PO4-2and Ca++ by the gut, and dissolution of PO4-2and Ca++from bone. Therefore, it was conceived that changes in serum Ca++ &PO4-2levels in CML patients using TKI treatment,could be due to one of these mechanisms or combination of these[6]. Also theincidence of subclinical tubular or glomerular defects, and due to the complex cellular and hormonal mechanisms involved in the regulation of phosphate/ calcium metabolism at the bone, intestinal, and renal level. It is, therefore, essential that all patients with more hypothyroidism be evaluated for the blood levels of calcium, phosphate and magnesium, which will affect a variety of metabolisms and clinical manifestations in these patients, in observation of providing individualized disease management strategies for those patients [7].

The inhibition of C-kit within cajal cells (intestinal pacemaker cells) could play a role in the incidence of diarrhea in patients treated with imatinab. An apparent consequence of diarrhea was malabsorption which could reason a reduction in serum Ca++ levels, resulting in a minor hyperparathyroidism which causes improved secretion of the phosphate and subsequent reduction in phosphate [8].

While in other studies, the inhibitory outcome of imatinib on osteoclast action and production of mesenchymal stem cells (MSCs), causing a variety of non hematologic adverse events [6].

In adults, Berman et al.(2006) noted that, patients experienced hypophosphatemia and a compensatory increase of parathyroid hormone during the treatment with imatinib .

Another study reported that imatinab could be exerting a direct result on renal tubular TKI receptors, resulting in comparative hypocalcaemia. Secondly, imatinib as a portion of a family of protein TKI can stimulate marked changes in cell excitability and ion homeostasis and indeed, imatinib has activity in the blockage of low voltage activated T-type calcium channels in the human embryonic kidney cells. Thus imatinib could have unfocused effect on calcium homeostasis that is not TKI receptor-mediated [9].

On the other hand, severe hypophosphataemia has infrequently been reported in coincidence with blast cell

proliferation in haematological patients. Hypophosphataemia in those cases was regularly attributed to the excessive uptake of the phosphate by proliferating cells [10]. Nonetheless, TKI treatment was related with decreased re-absorption of phosphate by the kidneys, suggesting renal phosphate excretion which may cause low serum PO4-2[6].

In a biphasic manner, the bone formation is primarily increased while with time its return and exaggerated by impairment of both: decreased resorption by osteoclasts and decreased development by osteoblasts. Which is because of the inhibition of osteoclasts by blocking Ca++ and Platelet derived growth factor response signaling, and establishment of osteoblasts'activity through the reduction of Platelet derived growth factor response by imatinib. The resultant positive property on bone mineral trabecular bone volume and density may propose increased bone potency in CML patients [11].

The present study was aimed to report the changes in serum levels of Ca++ and PO4-2in CML patients receiving different doses of imatinib therapy and patients receiving nilotinib therapy during regular daily follow up of CML patients.

Materials and methods

Ninety five patients with CML conducted in a cross-sectional study at National Center of Hematology /Baghdad-Iraq from November/2015 up to October/20016. The study was permitted by the review ethical committee of hematology. The patients were categorizedinto: group (1) taking imatinib myselate 400mg/day,. group (2) taking imatinib myselate 600-800mg per day, while the third group taking nilotinib 800mg /day used after imatinib failure treatment response (second line therapy). All groupsconceived their treatmentfor one year at least and more.

Calcium and phosphate levels assessments were measured by means of an automated clinical chemistry analyzer (Humastar 200; Human Diagnostics, Germany) by venous blood sampling of 5ml from allgroup of patients and compared with normalcontrol group. The serum Ca++ and PO4-2levels normal range is (2.02–2.60mmol/l), (0.81–1.62mmol/l) consequently.

Exclusion criteria:

Patients who were hypertensive, smokers and diabetic, or with other chronic disease or getting treatment other than TKI

Ethical approval: all procedures performed in this study concerning human participants were in agreement with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later comparable or amendments ethical standards.

Statistical Analysis:

All data were presented in straightforward measures of frequency, mean, percentage, , range (minimum-maximum values) and standard deviation . The significance difference of unusual means (quantitative data) was tested using students- t -test for difference between two independent means. Statistical significance was measured when the P- value was ≤ 0.05.

Results:

The present study, table (1) showed spatients characteristic with mean age group 40.24±13.01 (25-75) years old and male: female proportion (1.02:1) .

According to treatment type, 66.3% of patients received imatinib; 33.6% nilotinib. The duration of treatment for patients enrolled in this study receiving imatinib ranged 1-134 months, while duration of nilotinib treatment ranged (2-23) months.

Table-1: The CML patient's characteristic.

Patient's characteristics	Results
Age (Mean±SD)	40.24±13.01 (25-75years old)
Sexual characteristics	Male 48 (50.51%) Female 47 (49.4%)
Male to female proportion	1.02:1
Management type	Imatinib 63 (66.3%) Nilotinib 32 (33.6%)
Imatinib treatment doses	400mg 55 (87.3%) 600mg 8 (12.6%)
Management duration in months with imatinib Mean±SD (Range)	52.3±38.6(1-133)
Management duration in months with nilotinib Mean±SD (Range)	12.7±6.8(2-22)

A significant statistical variation in serum Ca++ and $PO4^{-2}$appeared between imatinib and nilotinib treatments with lesser serum levels of Ca++ and $PO4^{-2}$in imatinib treated CML patients (group1 and 2) than those with nilotinib treatment (group3) as shown in Table (2).

Table-2: Serum calcium and phosphatein CML patients according to different TKI treatment (Mean±SD)

Parameters (mmol/l)	imatinib (n=63)	nilotinib (n=32)	P value
SerumCa++ level	1.85±0.16 (1.4-2.1)	1.90±0.18 (1.7-2.3)	0.05*
Serum $PO4^{-2}$ level	1.08±0.28 (0.6-1.70)	1.32±0.49 (0.40-2.6)	0.0051*
-Normal range of Calcium is (2.02-2.60mmol/l) and for Phosphate is (0.81-1.62 mmol/l) -Data were existing as mean±SD()			
*Significant difference between two independent means at *P<0.05*.			

About 11.1% of patients using imatinib with 600mg/day may record changes in both serum calcium and phosphate in the different patients groupswith no significant difference.(table3).

Table-3: Serum calcium and phosphatein CML patients according different doses of imatinib treatment (Mean±SD)

Parameters (mmol/l)	Imatinib management dosage		P value
	400mg (patients no.=56)	600mg (patients no=7)	
SerumCa++ level	1.85±0.16(1.4-2.1)	1.78±0.19 (1.4-2.08)	0.411
Serum $PO4^{-2}$ level	1.08±0.28 (0.60-1.70)	0.95±0.28 (0.61-1.29)	0.249
- Normal range of Calcium is (2.02-2.60mmol/l) and for Phosphate is (0.81-1.62 mmol/l) -Data were existing as mean±SD()			
*Significant difference between two independent means at *P<0.05*			

The statistical results comparing serum Ca++and $PO4^{-2}$with the normal control values within different management of CML patients are shown in (Table 4).

Table -4: Comparison the number of CML patients on different TKI therapy according to serum level of calcium and phosphatewith normal level values.

Parameters (mmol/l)		Management type				P value
		Imatinib		Nilotinib		
		no.	%	no.	%	
Serum Ca++ level	Low range	54	85.7	26	86.7	0.901
	Normal range	9	14.3	6	1873	
Serum $PO4^{-2}$level	Low range	10	15.9	3	10.0	0.062
	Normal range	51	81.0	24	75	
	High range	2	3.2	5	16.7	
Normal range of Calcium is (2.02-2.60mmol/l) and for Phosphate is (0.81-1.62 mmol/l)						
*Significant difference between two independent means at *P<0.05*. *no: number of patients						

While table 5 shows, the statistical difference in serum Ca++ and $PO4^{-2}$levels of CML patients with different imatinib doses with the normal control values.

Table -5: Comparison the number of CML patients on different doses of imatinib therapy according serum level of calcium and phosphate with normal level values.

Parameters (mmol/l)		Imatinib management dose				
		400mg		600mg		P-value
		Number	%	Number	%	
Serum Ca++ level	Low range	48	85.7	6	85.7	0.1
	Normal range	8	14.3	1	14.3	
Serum $PO4^{-2}$level	Low range	7	12.5	3	42.9	0.110
	Normal range	47	83.9	4	57.1	
	High range	2	3.6	-	-	

Normal range of Calcium is (2.02-2.60mmol/l) and for Phosphate is (0.81-1.62 mmol/l)
*Significant difference between two independent means at $P<0.05$.

Discussion:

The improvement made in the understanding of chronic myeloid leukemia because the identification of a common chromosomal deviation to the introduction of gradually effective TKI was unprecedented in cancer [12] .Chronic myeloid leukemia occur more frequently in adults than in children [13], as the risk of getting CML increased with age. This disease was slightly more common in males than in females [14]. In this cross-sectional study, patients mean age was 40.24±13.01 (25-75) years old with male: female proportion 1.02:1, Dhahii etal(2011) showed that Iraqi CML studies concerning the age and gender proportion were34.71±1.02 (9-70) years and male: female ratio was 1:1.62 [15], while Jamal *et* al(2014); recorded a mean age of 40.45 ± 2.56 ranging from (25-75) years with male: female proportion 1.12:1[16].

The prolong duration uses of imatinib more than nilotinib therapy in the present study showed the median period of using imatinib and nilotinib were 52.3±38.6(1-133) and 12.7±6.8(2-22) consequently, beside the presence 11.1% of patients using imatinib with 600mg/day may play a role in the changed for both serum Ca++ and $PO4^{-2}$in different patients groups. This result also agrees with ENEST trial study (2012) [17].

About 85% of studied patients including both imatinib groups and nilotinib therapy group had hypocalcemia whencompared with the normal range of Ca++ and $PO4^{-2}$but without significant difference between them. The study showed significant lower mean levels of serum Ca++ in imatinib patients groups than nilotinib patients group (group3) while there was no significant difference change in mean level of Ca++ between the two groups of imatinib (group 1and 2).

On the other hand, hypophosphotemia percentage was about 12.5%, 43% and 10% of patients received imatinib 400m/day, 600mg/day and nilotinib consequently while only about 3% and16% of both imatinib groups and of nilotinib group had hyperphosphatemia accordingly, without significant differences between all patients groups also.

There was significant lower mean level of serum $PO4^{-2}$in imatinib patients group (group1 &2) when it compared with mean serum level of $PO4^{-2}$for nilotinib group(group 3), while there was a lower mean level of serum $PO4^{-2}$in imatinib patients group received 600mg/day than those received 400mg/day but without significance difference. Hasan etal(2015)study showed significant hypophosphatemia and no significance hypocalcemia between patients received imatinib and nilotinib [18].

Some researchers suggested that imatinib cause an increase in bone formation by increasing the osteoblast differentiation and functions and also decrease bone resorption by decrease of osteoclast numbers and functions where it might be lead to an increase in bone formation and then hypocalcemia and hypophosphatemia. The net results of this inhibition were tough to predict with respect to the dynamic condition of modeling and remodeling in the growing skeleton [6].

According to the type of TKI treatment we analyzed the correlation between Ca++ and $PO4^{-2}$, in studied groups, all groups containing nilotinib treatment, data showed strong significant correlation between Ca++ and $PO4^{-2}$and this may give idea that the effect of nilotinib treatment was mostly related to disturbance of the normal physiological distribution of Ca++ and $PO4^{-2}$in the body. Where in both imatinib groups there was no significant correlation between serum level of Ca++ and $PO4^{-2}$and this may be due to long duration of using imatinib treatment leading to compensation of the Ca++ and $PO4^{-2}$disturbance, unlike those patients on nilotinib treatment, compensating mechanism due to longer duration of treatment with imatinib treatment [18].

The changes in serum levels of each of Ca++ and $PO4^{-2}$in CML patients using TKI, may be not only due to effect of TKI on the bone, but effect of TKI on gastro intestinal tract absorption and renal excretions are another mechanism for these changes [19].

Berman et al(2006), reported enhance urinary $PO4^{-2}$output in patients managed with imatinib compared with normal controls. Also observed that patients managed with imatinib had considerably minor levels of the bone resorption marker CTX-1 compared with healthy controls and reported that patients managed with imatinib for an undetermined amount of time did not have significantly changed levels of bone-specific alkaline phosphatase, relation to normal controls. And the study recorded for the first time 16 patients who experienced hypophosphatemia and a compensatory increase of parathyroid hormone during treatment with imatinib [5]. Sullivan *et* al(2009), mentioned that the biochemical varity persist but do not progress during 2years of treatment with imatinib [20].

Conclusion:

Long term treatment effect withimatinib therapyunlike those patients on nilotinib treatment, compensating mechanism due to longer duration of treatment with imatinib treatment may cause significant hypocalcemia and hypophospatemia more than nilotinib treatment which may give idea that the effect of nilotinib treatment was mostly related to disturbance of the normal physiological distribution of Ca++ and $PO4^{-2}$in the body with significant correlation in patients who treated with nilotinib.Whilethedifferent imatinib doseshad no significant correlation between serum Ca++ and $PO4^{-2}$levels.

These changes may give an idea that disturbance of serum Ca++, $PO4^{-2}$is multifactorial and TKI treatment may have a rule in one or more than one mechanism relating to Ca++ and $PO4^{-2}$homeostasis.

References:

1- Vardman JW, Harris NL & Brunning RD . The world health organization (WHO) classification of the myeloid neoplasms. Blood 2002; 100:2292-2302.

2- Pavón V, Gómez R, Jaime JC, Hernández P, Arencibia A, Espinosa-Martínez E et al. Introduction of Imatinib as First-line Therapy for Chronic Myeloid Leukemia in Cuba. MEDICC Review2011; 13:35-40.

3- Jaeger BA, Tauer JT, Ulmer A, Kuhlisch E, Roth HJ, Suttorp M. Changes in bone metabolic parameters in children with chronic myeloid leukemia on imatinib treatment. Med Sci Monit2012; 18:CR721–CR728.

4- Yeung DT&Branford S. Monitoring disease response in chronic-phase chronic myeloid leukemia: the age of molecular assays? Hematology Am Soc Hematol Educ Program.2012:111-114. doi: 10.1182/asheducation-2012.1.111.

5- Berman E, Nicolaides M, Maki RG, Fleisher M, Chanel S, Scheu K et al. Altered Bone and Mineral Metabolism in Patients Receiving Imatinib Mesylate . N Engl J Med.2006;354:2006-2013.

6- Vandyke K, Fitter S, Dewar AL, Hughes TP, Zannettino AC. Dysregulation of bone remodeling by imatinib mesylate. Blood 2010;115:766-774.

7- Susanna TY, Sagayaraj A, Shashidhar KN, Gomathi M,and Mahesh V. A correlative study of thyroid profile and mineral status in patients with hypothyroidism - a hospital based case control study.Asian J Pharm Clin Res. 2016: 9: 292 -294

8- Franceschino A, Tornaghi L, Benemacher V, Assouline S, Gambacorti-Passerini C. Alterations in creatine kinase, phosphate and lipid values in patients with chronic myeloid leukemia during treatment with imatinib Haematologica.

haematol 2008;93: 317-318; doi:10.3324/.11680

9- Zekri JM, Robinson MH, Woll PJ. Relative Hypocalcaemia and Muscle Cramps in Patients Receiving Imatinib for Gastrointestinal Stromal Tumour Sarcoma. 2006;:48948

10- Ra'anani P, Lahav M, Prokocimer' M, Poles L, Theodor E. Life threatening hypophosphataemia in a patient with Philadelphia chromosome positive chronic myelogenous leukaemia in acute blastic crisis postgrad Med J1992; 68: 283-286.

11- Matti BF, Naji AS, Alwan AF.Evaluation of the Safety of Imatinib Mesylate in 200 Iraqi Patients with Chronic Myeloid Leukemia in the Chronic Phase: Single-Center Study. Turk J Hematol,2013; 30:387-393.

12- Cortes J. and Kantarjian H. How I treat newly diagnosed chronic phase CML. Blood, 2012;120:1390-1397.

13- Liu T. Identification of cooperating genetic events in acute leukemia. 2009; Ph.D [Dissertation. Aus Hebei China: Basel University]

14- American Cancer Society.Leukemia--Chronic Myeloid(Myelogenous).2012.

15- Dhahii MA, Murad NS, Matti BF. Evaluation of Molecular Response to Imatinib in Iraqi Chronic Myeloid Leukemia Patients Using Real Time – Reveres Transcriptase-Polymerase Chain Reaction (RT-RT-PCR) – Taqman. Journal of Molecular Biology Research,2011; 1: 47-54

16- Jamal QW, Dhahi MA and Mattii BF. Molecular screening for E255k and F359V Mutations in Non-Responders Chronic Myeloid Leukemia Iraqi Patients to Imatinib Mesylate Therapy. International Journal of Biology2014; 6:139-141.

17- Kantarjian HM, Kim DW, Issaragrisil S, Clark RE, Reiffers J, Nicolini FE, Pasquini R, Kalaycio M, Hughes TP, MBBS, Hochhaus A, Kemp C, Fan X, Waltzman RJ, Saglio G & Larson RA. Enestnd 4-Year (y) Update: Continued Superiority of Nilotinib Vs Imatinib in Patients (pts) with Newly Diagnosed Philadelphia Chromosome–Positive (Ph+) Chronic Myeloid Leukemia in Chronic Phase (CML-CP).Blood(ASH Annual Meeting Abstracts).2012; 120: Abstract 1676.

18- Hasan BF, Matti BF, Hameed RY. Evaluation of Electrolytes Disturbances in Iraqi Chronic Myeloid Leukemia Patients treated with Nilotinib with Monitoring of Response by FISH Study. Baghdad Science Journal2015; 12:110-118.

19- Jönsson S, Olsson B, Ohlsson C, Lorentzon M, Mellström D, Wadenvik H. Increased cortical bone mineral-ization in imatinib treated patients with chronic myelogenous leuke- mia. Haematologica.2008; 93: 1101–1103.

20- O'Sullivan S, Horne A, Wattie D, Porteous F, Callon K, Gamble G, et al.Decreased bone turnover despite persistent secondary hyperpara-thyroidism during prolonged treatment with imatinib. J Clin Endocrinol Metab 2009; 94:1131-6. doi: 10.1210/jc.2008-2324.