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Research Article

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Pharmacogenetics and Molecular study on BCR/ABL Inhibitors connected with Chronic Myeloid Leukemia; Genotype variability polymorphism among Babylon population

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ABSTRACT

Our study aimed to investigate the association between multidrug resistance BCR/ABL1 gene polymorphisms associated with chronic myeloid leukemia (CML). The BCR/ABL1 oncogene contributes to the progress of CML clones. BCR/ABL played significant roles in the pathogenesis in CML. Expression of BCR-ABL in patients were 88.9%, while in control was 6.7%

Keywords: BCR/ABL; CML; Genotype; Pharmacogenetics

INTRODUCTION

Leukemia is an undermining clonal issue of the hematopoietic system with astounding heterogeneity and ghastly expectation. Starting late, the recurrence of leukemia extended gradually [1]; regardless, the cautious characteristic segments of leukemia have not been totally enlightened [2]. Epidemiological and case control considers have found that basic biological exposures add to the pathogenesis of leukemia [3]. In addition, past confident quality association techniques and genome wide connection focuses on have perceived the presence of obtained genetic vulnerability to this ailment [4 7]. Subsequently, it is generally viewed as that the causation is multifactorial and that the relationship between exogenous or endogenous exposures and innate frailty accept a basic part in the etiology of leukemia [2].

Notwithstanding being an outstandingly specific and suitable sub-nuclear treatment for curbing TK, its therapeutic reasonability can be limited in a couple of patients who develop a resolved phenotype at disease onset or in the midst of development to both the animated stage and/or sway crisis [8, 9]. An uplifting in the dose of imatinib from 400 mg/d to 600-800 mg/d can be permitted in patients who had a basic hazardous response or treatment frustration, as portrayed by the European Leukemia Net [10]. However these helpful date-books are not recommended for patients with imatinib extremism, or who make bone marrow aplasia as a discretionary impairment to imatinib and/or interferon treatment [11, 12]. A choice is to use diverse TKIs, for instance, dasatinib or nilotinib [13, 14]. These are more customarily used as a piece of patients with changes in the BCR-ABL quality which could deliver dissatisfaction of accommodating response to imatinib. Another proposed TKI drug-resistance framework is this can be made by an inadequate solution obsession at the intracellular level, as the outcome of element transport being mediated by the over-verbalization of the ABC-transporters P-glycoprotein (P-gp) and/or BCRP (chest illness safe protein), encoded by the MDR1/ABCB1 and ABCG2 qualities independently [15, 16]. Assortments in single-nucleotide polymorphisms (SNPs) of MDR1/ABCB1 have been portrayed as potential components related to the clinical-helpful improvement of a couple of diseases [14].

The up-control of drug transporters (ABCB1-ABCG2) is one of specific explanations behind Imatinib resistance, since it can be effluxes through MDR1 (ABCB1) transporters (17). Upregulation of prescription transporters results in redesigned opportunity of pharmaceutical from the phone realizing decreased drug availability and medicine resistance. Along these lines, the examination of G2677T polymorphism in exon 21 of MDR1 quality will be helpful in dose change of imatinib, which should be fruitful if there ought to emerge an event of Imatinib safe individuals (18, 19). (2)

As a result of the hugeness of choosing the piece of SNPs in the response to the CML treatment, this study was expected to investigate the allele and genotype frequencies of SNPs ABCB1 C3435T and ABCG2 C421A in CML patients encountering IM treatment and to make sense of if particular genotype case of these SNPs have any effect in mediating extraordinary response and impenetrability to IM. The second objective was to source out the allele and genotype frequencies between average controls and CML patients in our masses and evaluate their contributory part in interceding CML helplessness peril (20).

EXPERIMENTAL SECTION

Study Subjects:
The practical side of the study was done in the period between "September 2015 until July 2016".One hundred and ninety samples were collected. Two enrolled groups of subjects were involved in this study.

Patients:
This study includes 90 patients with Chronic Myeloid Leukemia (CML) admitted to Marjan Hospital. Patients included (51 males and 63 females), with an age range (8-76) years, they were diagnosed by specialist physicians and selected in the current study. Blood and serum samples taken from every patient and control having thoroughly examined.

Laboratory criteria for diagnosis; Complete Blood Count (C.B.C.) and Fluorescent In Situ Hybridization (FISH)

Healthy control group:
Sixty of actual healthy persons from various Iraqi populations were arbitrarily involved in the study.

Blood Sampling:
About five milliliters of venous blood were collected from each patient in the study. The blood was divided into two parts: one part (about two milliliters) was collected into EDTA containing tubes. The second part of the blood was placed in gel tube for thirty minutes, then transferred to plain tube and serum was obtained by centrifugation at 3000 rpm for 15 min ; after that the serum collected and kept in the freezer (-20 ºC) until it was used for immunoassay.

Isolation of genomic DNA:
Genomic DNA was used for molecular study by sequestered from the fresh blood which collected which collected in tubes of anticoagulant EDTA and for frozen blood samples we recommended using protease K were applied using for DNA purification; Promega Wizard genomic kits. The isolation of DNA depended on the 5 stage procedure utilizing salting out techniques (Sambrook et al., 1989):

- Lysis of the RBCs in the Cell Lysis Solution.
- Lysis of the WBCs and their nuclei in the Nuclei Lysis Solution.
- The cellular proteins were then removed by a salt out precipitation step using the Protein Precipitation Solution.
- The genomic DNA was concentrated and desalted by Isopropanol precipitation.
- The genomic DNA was rehydrated using the DNA Rehydration Solution.

Isolation kit Components:

Components	Amount	Components	Amount
Cell Lysis Solution	500 ml	Protein Precipitation Solution	125 ml
Nuclei Lysis Solution	250 ml	DNA Rehydration Solution	100 ml

The Estimation of DNA Concentration and Purity:
The DNA concentration of samples was estimated by using the Nano drop by putting 2.5µl of the extracted DNA in the machine to detect concentration in ng/µL and the purity detected by noticing the ratio of optical density (OD)

260/280 nm to detect the contamination of samples with protein. The accepted 260/280 ration for purifying DNA was between 1.7-1.9.

Tris Borate EDTA Buffer preparation (1X TBE)
This solution was prepared by adding 900 ml Distill water to 100 ml 10X TBE (Promega/ Germany) ,forming 1 liter of(1x) TBE buffer (Sambrook and Russel, 2001).

Preparation of agarose gel:
1- The amount of 1 X TBE (100 ml) was taken in a beaker
2- Agarose powder (1.5 gm) was added to the buffer
3- The solution was heated to boiling using a microwave oven for 2 min.
4-Ethidium Bromide (1 µl) of (10mg/ml) was added to the agarose solution.
5- The agarose was stirred in order to be mix and avoid making bubbles.
6- The solution was left to cool down at 50 – 60 oC.

Casting of the horizontal agarose gel:
Subsequent to settling the brush in 1 cm a long way from one edge, the agarose game plan was filled the gel plate. The agarose was allowed to bond at room temperature for 30 minutes. The modified brush was absolutely removed and the gel plate was set in the gel tank. The tank was stacked with 1 X TBE support until it accomplished 1-2 mm over the surface of the gel.

DNA Loading & Electrophoresis:
DNA (3 µl) was mixed with (2 µl) loading dye. The samples loaded carefully into the individual wells of the gel, and then electrical power was turned on at 70 volt for 1 hour, afterwards the DNA moved from cathode (-) to anode (+) poles. The Ethidium Bromide stained bands in the gel were visualized using UV. Transilluminator at 350 nm and photographed.

PCR Technique:
In this study four types of PCR were used include ARMS, RFLP, multiplex and conventional PCR, to detect mutation genes by using ten primers as shown in the following table. The primers were supplied by Bioneer (Korea) Organization as a lyophilized result of various picomols fixations. Lyophilized preliminary was disintegrated in a free DNase/RNase water to give a final concentration of 100 pmol/µl and kept as a stock in -20ºC, to prepare 10µM concentration as work primer re suspended 10 pmol/µl in 90 µl of free DNase/RNase to reach a final concentration 10µM (21, 22, 23, 24).

Detection of BCR-ABL kinase
By using conventional PCR, BCR-ABL was analyzed with modification of the method that described by (Evans H. and et al., 2013). Sets of primer manufactured by Bioneer, Korea. The primer sequences:

5' - TTCAGAAGCTTCTCCCTGACAT - 3'
5' - TGTTGACTGGCGTGATGTAGTTGCTTGG - 3'

PCR optimization was done as a first step by using a gradient temperature. This is highly important to determine the optimum annealing temperature. The PCR reaction mixture for gradient consisted of 5µl template DNA, 5µl master mix, 5µl of each forward and reverse primer in 20 µl of total reaction volume. PCR condition of gradient is shown in the following table.

Table (1) gradient condition for BCR-ABL

Step	Temperature C°	Time/min.	Cycles
Initial denaturation	94	1	1
Denaturation	94	1	40
Annealing Zones	56-58-60-62-64-66	1	40
Extension	72	1	40
Final extension	72	10	1
Storage	4		

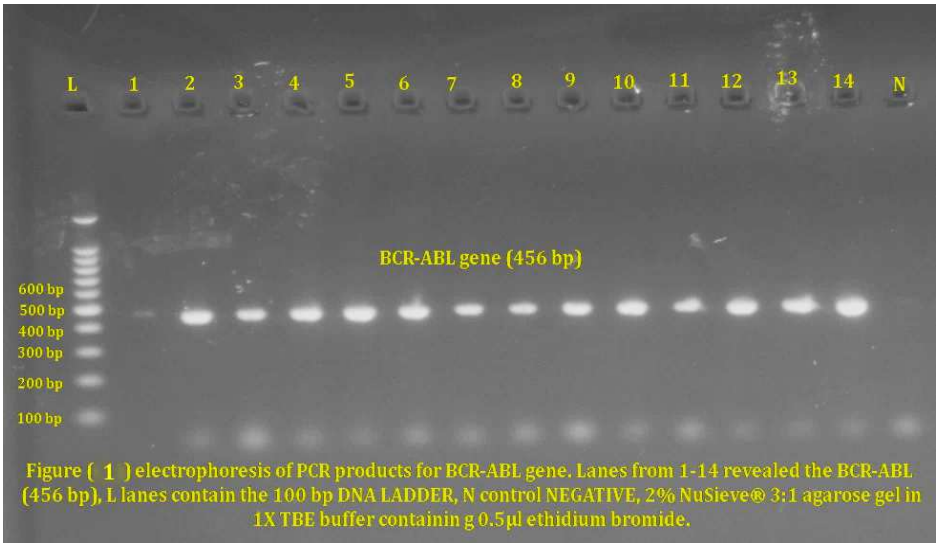

Figure (1) electrophoresis of PCR products for BCR-ABL gene. Lanes from 1-14 revealed the BCR-ABL (456 bp), L lanes contain the 100 bp DNA LADDER, N control NEGATIVE, 2% NuSieve® 3:1 agarose gel in 1X TBE buffer containin g 0.5µl ethidium bromide.

After the determination of optimum annealing temperature for BCR-ABL genes by selecting the clearest band which is 62 C°, PCR mixture was 5µl DNA, 5µl master mix, 1.5 forward and reverses primer. PCR conditions were performed as in the following tables .Amplicon were analyzed on 2% agarose gel tainted with 0.5 µg/ml ethidium bromide. The BCR-ABL gene produce 456bp. (Evans H. and et al., 2013)

Table (2) PCR condition for BCLR-ABL

Step	Temperature C°	Time/min.	Cycles
Initial denaturation	94	1	1
Denaturation	94	1	40
Annealing	62	1	40
Extension	72	1	40
Final extension	72	10	1
Storage	4		

Table (3) Sequence of primers

GENE	Primer		bp.
Bcr-ABL Kinase	Forward	5' - TTCAGAAGCTTCTCCCTGACAT - 3'	456
	Reverse	5'TGTTGACTGGCGTGATGTAGTTGCTTGG3'	

RESULTS AND DISSCISION

Detection of BCR-ABL gene

Philadelphia chromosome manifestation of the, the t (9; 22) chromosomal translocation and the creation of the BCR/ABL fusion protein is the hallmark of chronic myeloid leukemia (Zhu, Y. and Qian, S. X., 2014; Cai, J., 2014). The BCR/ABL1 oncogene contributes to the progress of CML clones (25).BCR/ABL played significant roles in the pathogenesis in CML (26). Expression of BCR-ABL in patients were 88.9% , while in control was 6.7% as show in table(4) ,this explain the close correlation between the incidence of disease and BCR-ABL percentage., thereby it will use in the assessment of prognosis, treatment and management of CML patients (27).

Table (4) the relation of BCR-ABL kinase and some clinical parameters

BCR-ABL Kinase gene			
Criteria	Control	CML	P value
	Mean SD	Mean SD	
Age	49.2+9.11	46.8+13.2	NA
TNF (pg/ml)	52.9+12.8	219.8+19.7	<0.005
IL-18 (pg/ml)	50.1+9.5	339.2+29.7	
IL-6 (pg/ml)	4.2+1.1	26.1+2.7	
IL-1 (pg/ml)	16.3+3.9	57.4+11.5	
IL-2 (pg/ml)	56.7+14.2	132.4+15.3	
Ph+	96.3	0	

PCR amplification for BCR-ABL gene was made for 90 patient samples, PCR product was 456 bp as one band on 2% agarose as revealed in figure 1.

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Authors' Disclosures of Potential Conflicts of Interest
No potential conflicts of interest relevant to this article were reported.

REFERENCES

[1] Siegel R, Naishadham D and Jemal A: **2012**. *CA Cancer J Clin* **2012**. 62: 10-29.
[2] Inaba H, Greaves M and Mullighan CG: *Lancet* **2013**.381: 1943-1955.
[3] Greaves M: *Nat Rev Cancer* **2006**. 6: 193-203.
[4] Sherborne AL, Hosking FJ, Prasad RB, et al: *Nat Genet* **2010**. 42: 492-494.
[5] Vijayakrishnan J and Houlston RS: *Haematologica* **2010**. 95: 1405-1414,
[6] Wang Y, Chen J, Li J, et al: *Gene* **2013**. 524: 203-207.
[7] Speedy HE, Di Bernardo MC, Sava GP, et al: *Nat Genet* **2014**.46: 56-60.
[8] Druker BJ, Guilhot F, O'Brien SG, et al. *N EnglJ Med* **2006**. 355:2408-17.
[9] Hochhaus A, Kreil S, Corbin AS, et al. *Leukemia* **2002**.16:2190-6.
[10] Deininger MW, Druker BJ. *Cancer Cell* **2004**.6:108-10.
[11] Baccarani M, Saglio G, Goldman J, et al. *Blood* **2006**. 108:1809-20.
[12] Khan KA, Junaid A, Siddiqui NS, Mukhtar K, Siddiqui S. *J Coll Physicians Surg Pak* **2008**. 18:176-8.
[13] Alabdulaaly A, Rifkind J, Solow H, Messner HA, Lipton JH. *Leuk Lymphoma* **2004**.45:175-7.
[14] Henkes M, van der Kuip H, Aulitzky WE. *TherClin Risk Manag* **2008**. 4:163-87.
[15] Mahon FX, Hayette S, Lagarde V, et al. *Cancer Res* **2008**. 68:9809-16.
[16] Shukla S, Sauna ZE, Ambudkar SV. *Leukemia* **2008**.22:445-7.
[17] Tanabe M, Ieiri I, Nagata N, et al. *J PharmacolExpTher* **2001**.297:1137-43.
[18] Gréen H, Söderkvist P, Rosenberg P, Horvath G, Peterson C. *Clin Cancer Res* **2006**.12:854-9.
[19] Gurney H, Wong M, Balleine RL, Rivory LP, McLachlan AJ, Hoskins JM, Wilcken N, Clarke CL, Mann GJ, Collins M, Delforce SE, Lynch K, Schran H,. *Clinical Pharmacology & Therapeutics*, **2007**. 82(1): 33-40.
[20] Tagawa, H. Saitoh, N. Fujishima, T. Yoshioka, M. Hirokawa and K. Sawada, *J Human Genet*. **2010**. vol. 55, pp. 731-737.
[21] Tasahel Hamid Al-Dulaimi, HawraaWahab Aziz, Ali Hussein Al-Marzoqi, SaadAbd Al-Aziz, SoadAbdAlmahdiMohsin. *Medical Journal of Babylon*. **2015**. Vol. 12- No. 2.
[22] Raad Abbas Kadhim, Ali Hussein Al-Marzoqi, Zahraa Mohammed Al-Taee. *Al-Kufa University Journal for Biology* **2016**. VOL.8. NO.2.
[23] Lena Fadhil, Ali Hussein Al-Marzoqi, Zahraa Mohammad Al Taee, and Ammar A Shalan. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* **2016**. 7(1): 590-598.
[24] Ali Hussein Al-Marzoqi, NisreenKaddimRadi, AmmalRaqibShamran. *World Journal of Pharmaceutical Research.* Vol 4, Issue 4: 102-113.
[25] Rondanin, R *Bioorg Med Chem Lett.* **2014**.24, 4568–4574.
[26] Li, Y. *PLoS One*. **2013**.. 8, 276–284.
[27] Ali H. Al-Marzoqi, Hawraa J. Kadhim, Ammal R. Shimran, Nebras M. Al-Khafaji, Sabreen A. Kamal, RusulAdilNaji, ZubaidaAmer Faisal, Sarah Abbas Fakhri and RedaahAbd-Alrazzak *Journal of Chemical and Pharmaceutical Research*, **2016**, 8(4):919-927.