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Cytogenetic and molecular genetic studies of number of chronic mylogenous leukemia in Erbil province

Mustafa S. Al-Attar and Govand M. Qader
University of Salahaddin-Erbil, College of science, Biology Department

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***Corresponding Author:**
Mustafa S. Al-Attar
Email:
mustafa.mustafa@su.edu.krd

ABSTRACT

Chronic myeloid leukemia (CML) is amyeloprolirative disorder characterized by the Philadelphia chromosome originates from the translocation of chromosome 9 and 22 t (9;22) (q34;q11), creating a BCR-ABL fusion gene which results in the constitutive activation BCR-ABL tyrosine kinase. Patients of BCR-ABL fusion gene positive in (CML) are treated by an effective therapy called Imatinib (Gleevec). Point mutations alter the conformation of the ATP binding site that disturbs the binding of therapy to its target which leading to imatinib resistance. Fifty CML patients were collected from Nanakali Hospital in Erbil city which were diagnosed by physicians. The age of the patients from 9 to 80 years, 62% were male and 39% were female, median age of the patients were 38 years. The present study deals with two different aspects; conventional cytogenetic (G-banding) analysis to diagnose the Philadelphia chromosome as a (CML) marker and using Allele Specific Oligonucleotide Polymerase Chain Reaction (ASO-PCR) assay for screening the mutant allele at the three codons 351, 311 and 315 of the BCR-ABL ATP binding domain in Imatinib resistant CML patients. The phenotype of fifteen CML patients were successfully analyzed, thirteen patients were in complete cytogenetic response state (Philadelphia 0%) only two of those patients showed the Philadelphia chromosome. Three point mutations T1052C, T932C and C944T were identified by allele specific oligonucleotide polymerase chain reaction (ASO-PCR). One patient showed T1052C mutation, T932C was detected in three patients and two patients showed the C944T ATP binding domain mutation. In conclusion, conventional cytogenetic and molecular genetic tests are complementary techniques to show the exact types of abnormalities, which allow better evaluation of the genomic aberration involved in CML patients, specifically for kinase domain mutation that plays an important role in different diagnosis, prognosis, therapy treatment and drug resistant management of chronic myeloid leukemia.

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal disorder of myeloid stem cells (Witte, 2001; Mark *et al.*, 2006), accounting 15% of adult leukemias (Kaur *et al.*, 2012). It was first recognized in 1845 (Rachel & Mary, 2007; McCann, 2012). The median age of CML patients is 45 to 55 years (Faderl *et al.*, 1999). It is the first malignant disorder which is found to be associated with a chromosome aberration called Philadelphia (Ph) chromosome (Chin *et al.*, 1996 ;Hamad *et al.*, 2013).

The high exposure to ionizing radiation, pesticide, and herbicide are factors that lead to CML (Moloney, 1987; Kaur *et al.*, 2012). The typical symptoms of CML are leukocytosis, fatigue, splenomegaly, thrombocytosis and anemia (Faderl *et al.*, 1999; Nestal *et al.*, 2012).

The induction of the genomic aberration which lead to the formation of *BCR-ABL fusion genes* are due to environmental factors, life style, natural individual genotype difference, exposure to radioactivity and carcinogen substances play an important role (Iqbal, 2011; Bhat *et al.*, 2012). Cytogenetic analysis is the standard tool for initial evaluation, diagnosis, management of hematological malignancy of a patient that suspected to cancer (Goh *et al.*, 2006) and used as a prognostic indicator for monitoring therapy (Parikh and Tefferi, 2012). Also it provides evidence of the progression of disease at an earlier phase than hematological marker (Alachkar *et al.*, 2013) by detecting various chromosomal aberrations and has a significant role at the time of CML diagnosis (Razelle *et al.*, 1990; Boronova & Sotak, 2007).

The first an older therapy for CML is Arsenic trioxide that has apro-apoptic effect which increase apoptosis and a significant decline BCR-ABL protein level (Cheryl, 2012; Rachel & Mary, 2007; Melda & Guray, 2013).

Hydroxyurea is an inhibitor of the ribonucleotide reductase as well as decrease the tumor mass, however hydroxyurea prolonged the duration of chronic phase as compared with busulfan while busulfan is an alkalating agent that used as a second line treatment after hydroxyurea resistant or intolerance that act as anti-tumor activity at stem cell level (Hehlmann *et al.*, 1993; Paci, 2013). In contrast, interferon alpha used in response to antigenic stimuli such as those that occur with viral infection and malignant disease as well as it has pleiotropic effect involving antiviral, antiproliferative and antiangiogenic activities (Faderl *et al.*, 1999), however interferon alpha prolonged survival as compared to hydroxyurea with low risk due to expose of significant toxicity (Eiring *et al.*, 2011).

When a fragment of *ABL gene* is fused with *BCR gene* thus the activation loop in *BCR-ABL fusion gene* would be in the open conformation and phosphorylation, in contrast when Imatinib binds with an active site of the tyrosine kinase at ATP binding site, Imatinib mesylate switches off downstream signaling, cells stop proliferating and apoptosis by preventing tyrosine autophosphorylation (Guido, 2003; Al-achkar *et al.*, 2009; Dhara *et al.*, 2010).

Despite response to Imatinib in the majority of patients with chronic myeloid leukemia, CML patients show primary or acquire resistance (Parker *et al.*, 2011). Generally there are two kinds of BCR-ABL mechanism resistant, first BCR-ABL independent mechanism of resistance belong to decreasing in the intracellular level of Imatinib due to complication with drug efflus, drug influx or drug concentration which lead to signal activation of Ras/MEK and STAT (Hamad *et al.*, 2013).

While the BCR-ABL dependent mechanism of resistance involves duplication or over amplification of BCR-ABL oncogene that leads to elevated ABL kinase, among them the most frequent dependent mechanism is

mutation in the ABL kinase domain. Mutation can affect directly the proper binding of Imatinib to the target molecule as well as binding of ATP (Alimena, 2009; Hamad *et al.*, 2013).

The BCR-ABL domain mutation include of four major regions P-loop ATP-binding site, catalytic domain and activation loop, the two major categories of mutation have been defined as those at positions where direct contact with imatinib occurs and those that affect the conformation of BCR-ABL thus preventing imatinib binding. The first category mutation namely T315I located in the ATP-binding site that prevents the formation of hydrogen bonds with imatinib and F359V mutation located in the catalytic domain. The second category comprises mutations in the P-loop (Y253H and E255K) and H396R/P mutation in A-loop (Tali & Ninette, 2012; Menon, 2013).

The most frequent mutations are namely C944T (cytosine to thymine substitution at *ABL gene* in position 944 I exon3) which lead to amino acid change of threonine to isoleusine at position 315(Thr315Ile). T932C (thymine to cytosine substitution at *ABL gene* position 932) causing change the amino acid of phenylalanine to leucine at position 311(Phe311Leu) and T1052C (thymine to cytosine substitution at *ABL gene* position 1052) which converts the amino acid methionine to threonine at position 351(Met351Thr) that forms apart of *BCR-ABL fusion gene* and hence BCR-ABL onco-protein (Catherine *et al*., 2002; Mahon, 2006).

The aims of the current study were diagnose the Philadelphia chromosome by cytogenetic analysis and then confirmation of it's by screening various mutations (T1052C, T932C and C944T) in the BCR-ABL ATP binding domain by allele specific oligonucleotide polymerase chain reaction (ASO-PCR) assay.

MATERIALS AND METHODS

Sample Collection

A total of three to five ml venous blood samples of fifty CML patients were collected from Nanakali Hospital in Erbil city which were diagnosed by physicians, fifteen of the samples were subjected to the cytogenetic technique. Sample collection and transport was completed at the same day on ice. The age of the patients from 9 to 80 years, 62% were male and 39% were female, median age of the patients were 38 years.

A-Cytogenetic method:

Cytogenetic analysis:

Peripheral blood samples were collected in lethium heparin tubes (Gersen & Keagle, 2005). It was performed on (24-48) hours on peripheral blood cell culture (Joaquín *et al*., 2000; Farkhondeh *et al*., 2001) using standard protocol for preparation of GTG-banding metaphase (Talwar, 2003; Boronova & Sotak, 2007) and chromosome karyotype was described according to International System Cytogenetic Nomenclature (ISCN) guidelines for human Cytogenetic Nomenclature, using cytovision system for image analysis (Qureshi, 2008). The number of metaphase were analyzed on each sample were at least 20 metaphases (Al-achkar *et al*., 2007). The prepared metaphase slides (metaphase analyzing) had been read in laboratory of medical genetics in Iran/Tehran.

The absence of any Ph+ metaphase cells is indicating a complete cytogenetic response (CyR) (Alice *et al*., 2007).

Preparation of Metaphase Banding Human Chromosome:

The protocol used according to (Moorhead *et al*., 1960; Fan, 2003; Gersen & eagle, 2005).

Specimens Collection and Handling:

Three ml of venous blood was drew by a sterile syringe and collected in a vacuum tubes containing anticoagulant lithium heparin. Specimens were transported on ice box and were kept in refrigerator at 4°C till culturing. The specimens were cultured within 24 hours of collection for best result.

Karyotype Procedure (Culture of leukocytes):

0.5ml of heparinized whole blood was mixed with 4.5ml of PAA culture medium that contain all requirements like (RPMI 1640,Phytohaemagglutinin, Fetal bovine serum, L-glutamine and antibiotics) for cell proliferation. The cultured cells were incubated at 37°C for (65-70) h.

B- Molecular method:

DNA Extraction:

Genomic DNA was extracted from whole blood by using Genomic DNA kit (Genaid, UK), depending on manufacturer's instructions. The qualification and quantitification of extracted DNA were done by gel electrophoresis and Nano-Drop respectively.

Allele specific Oligonucleotide Polymerase Chain reaction (ASO-PCR)

To determine the most useful protocol in clinical practice (Catherine *et al*., 2002) was developed ASO-PCR assay using primer sets detecting the three common point mutations in *BCR-ABL fusion gene* ATP binding domain known as T1052C (Met351Thr), T932C (Phe311Leu) and C944T (Thr315Ile). ASO-PCR assay can amplified the mutated or wild type sequences in CML patients specifically in the PCR reaction (Zafar, 2004; Kim, 2006; Aamir *et al*., 2011).

Allele specific oligonucleotide polymerase chain reaction technique uses three primers, first primer wild type allele specific second primer mutant allele specific and third common primer. The primer wild type and mutant allele specific usually differ by only one nucleotide that gives them a high specifity to the corresponding wild type or mutant allele, the two allele specific primer are combined with the common primer in separate reaction tubes (Delia *et al*., 2008), the primers are listed in (Table 1). The tubes were placed in the PCR machine for amplification depending on the PCR program file in (Table 2 and 3). Then the PCR product was run on agarose gel electrophoresis (2%).

PCR Mix preparation:

A 20 µl PCR reaction was performed containing 2µl of DNA, 2X lyophilized Accupower master mix (Bioneer, Korea) and 1.5µl was added for each of the forward and reverse primers then the mixture was completed by adding 15 µl of nuclease free water.

Table 1: Identities and sequences of primers used in ASO-PCR assay for mutant allele detection in CML Gleevec resistant patients (Catherine *et al*., 2002).

Mutation	Primer Type	Polarity	Recognition sequence (5'—3')	Amplicon length (bp)
T1052C	NP	Forward	**CCA CTC AGA TCT CGT CAG CCA<u>T</u>***	**112 (bp)**
	ASO	Forward	**CCA CTC AGA TCT CGT CAG CCA <u>C</u>***	
	Common	Reverse	**GCC CTG AGA CCT CCT AGG CT**	
T932C	NP	Forward	**CAC CCG GGA GCC CCC G<u>T</u>***	**172 (bp)**
	ASO	Forward	**CAC CCG GGA GCC CCC G<u>C</u>***	
	Common	Reverse	**CCC CTA CCT GTG GAT GAA GT**	
C944T	NP	Forward	**GCC CCC GTTCTA TAT CATCA<u>C</u>***	**158 (bp)**
	ASO	Forward	**GCC CCC GTTCTA TAT CATCA<u>T</u>***	
	Common	Reverse	**GGA TGA AGT TTT TCT TCT CCA**	

Table (2): Thermal cycling conditions of ASO-PCR technique for mutation C944T and T932C (Catherine *et al.*, 2002).

Steps	Tempreture (°C)	Time	Number of cycles
Initial denaturation	**94**	**12 minutes**	**1**
Denaturation	**95**	**1 minute**	**35**
Annealing	**64**	**1 minute**	
Extension	**72**	**1 minute**	
Final extension	**72**	**5 minutes**	**1**

Table (3): Reaction cycling program of ASO-PCR technique for mutation T1052C (Catherine *et al.*, 2002).

Steps	Tempreture (ºC)	Time	Number of cycles
Initial denaturation	**94**	**12 minutes**	**1**
Denaturation	**95**	**1 minute**	
Annealing	**68**	**1 minute**	**35**
Extension	**72**	**1 minute**	
Final extension	**72**	**5 minutes**	**1**

Results and Discussion:

Cytogenetic studies:

Out of the fifteen diagnosed CML patients, two CML patients showed philadelphia positive Figure (2) while thirteen patients' specimen have a normal karyotype that is philadelphia negative Figure (1). In the current study the number of philadelphia positive was lower than that reported by Al -Attar, (2004), who found fourteen positive cases among twenty six patient samples. Philadelphia negative belong to many reasons one of them reported by Razelle *etal*., (1990) that ph- can be exists at the time of CML diagnosis patients but this disorder are not clinically distinguishable from those with philadelphia positive.

On other hand Current results were supported by the previous finding such as Goh *et al.*, (2006) illustrated that the analyzed metaphase were philadelphia negative this is due to complete cytogenetic response or masked philadelphia chromosome (Hochhaus, 2006; Boronova & Sotak, 2007) Table (4) shows the cytogenetic analysis of CML patient.

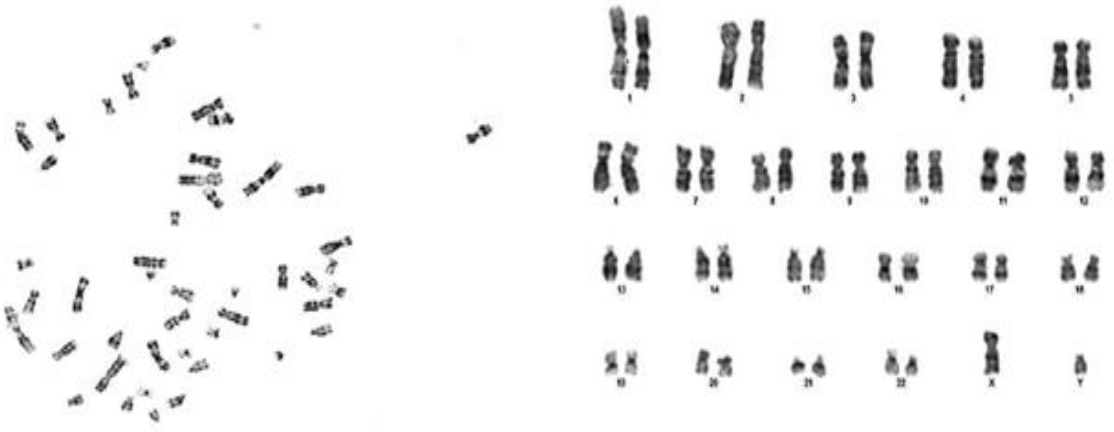

Figure (1): GTG-banding of human chromosome. 46,XY Normal male karyotype (negative Philadelphia chromosome) in CML patient (1000X).

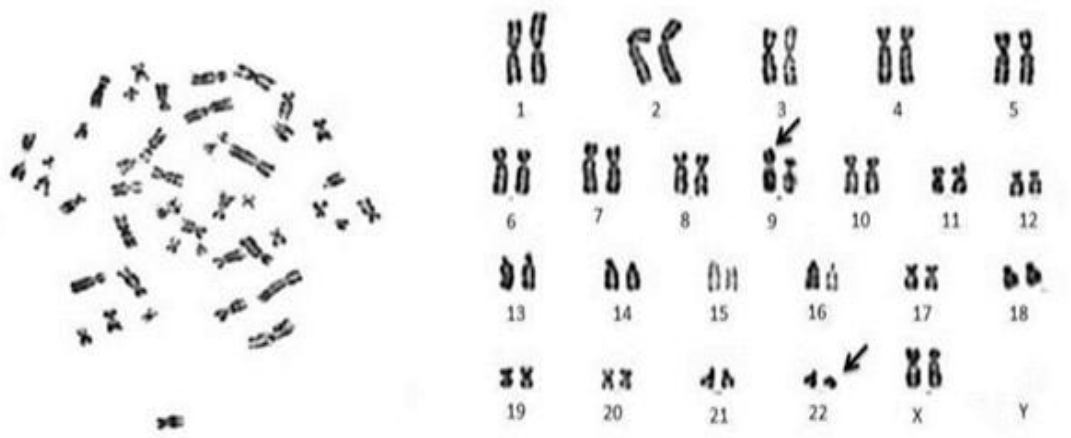

Figure (2): GTG-banding of human chromosome. Female 46,XX,t(9;22) that revealed Philadelphia chromosome in CML patient (1000X).

Table (4): Patient's characteristics and results of cytogenetic analysis.

Sample	Age	Sex	Therapy	Karyotype
Case 1	70	M	Nilotinib	Ph-
Case 2	59	F	Gleevec	Ph-
Case 3	40	M	Nilotinib	Ph-
Case 4	58	M	Nilotinib	Ph-
Case 5	38	M	Gleevec	Ph-
Case 6	49	M	Nilotinib	Ph-
Case 7	13	F	Gleevec	Ph+
Case 8	52	M	Gleevec	Ph-
Case 9	40	F	Gleevec	Ph-
Case 10	45	M	Gleevec	Ph-
Case 11	55	F	Gleevec	Ph-
Case 12	30	M	Nilotinib	Ph-
Case 13	39	F	Gleevec	Ph-
Case 14	55	F	Gleevec	Ph+
Case 15	51	M	Gleevec	Ph-

Molecular studies:

Molecular detection of BCR-ABL kinase domain mutation that associated with Imatinib resistance:

Mutation analysis was performed by using a sensitive technique ASO-PCR for CML cases. Out of the 50 chronic phase (CP) CML patients examined according to the presence of the BCR-ABL tyrosine kinase mutation, six patients (12%) were found to harbor the mutant allele at the three codon 351, 311 and 315 of the *BCR-ABL fusion gene* Table (5). Thirty four patients (68%) had the wild type allele of 351, 311 and 315, the remaining patients were 10 (20%) that there were no any indicator (normal band) on the gel electrophoresis to have wild type of the 311 and 315 alleles.

Delia *et al*., (2008) which supports that the mutant specific primer was designed for high specifity, it binds when the exact complementary sequence for it must be present in DNA of the CML patients otherwise amplification is not occurred, then we confirmed our results on the same patients by using normal primer and also there was no any normal bands after running PCR products on the gel electrophoresis, in this case we were interpreted that these CML patients (20%) have another mutation.

Each of these mutations were detected alone which are in concordance with results of (Catherine *et al*., 2002; Aamir *et al*., 2011) and there was no any double mutation in a single patient which is disconcordance with the results that reported by (Zafar, 2004).

Our data showed that the BCR-ABL Met351Thr (T1052C) already described gene mutation in one of the fifty CML cases with using ASO-PCR technique that supported by the previous study such as (Catherine *et al*., 2002; Zafar, 2004), Figure (3) shows specific band (112bp) after amplification the target sequence of CML patient with normal primer and one of them not amplified with normal primer. Figures (4) show wild (normal) and mutated type allele of 351.This mutation can be partially impairs the binding of the Imatinib to its target and thus lead to partial drug resistance that overcome actively by administering full therapeutic dose escalation of Imatinib as a first line therapy from 600mg/day to 1600mg/day (Zafar, 2004; Hochhaus, 2006). The incidence of T1052C (1 of 50 patients, 2%) was lower than that reported by (Catherine *et al*., 2002), (1 of 24, 4.1%) and (Zafar, 2004), (6 of 68, 4.4%).

Table (5): Patient's characteristics and positive results of different types of ATP Binding Domain Mutation by ASO-PCR.

ID Sample	Age	Sex	Mutation type
Case 2	**59**	**F**	**T1052C**
Case 5	**38**	**M**	**T932C**
Case 6	**41**	**M**	**T932C**
Case 7	**40**	**F**	**T932C**
Case 29	**35**	**F**	**C944T**
Case 39	**40**	**M**	**C944T**

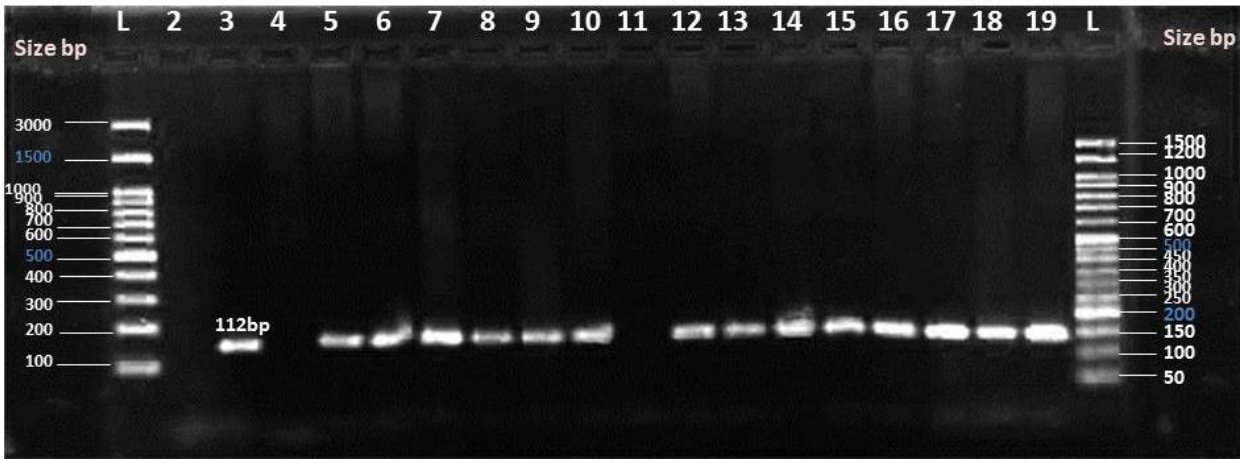

Figure 3: Analysis of T1052C mutation by ASO-PCR. Lane 1 and 20: DNA ladder of 1kb and 50bp respectively, Lane 2: Negative control, Lane 3: Positive control (Control DNA amplified with normal primer), Lane 4: Control DNA not amplified with mutant specific primer, Lane 11: Patient sample not amplified with normal primer and the rest are Patient samples amplified with normal primer.

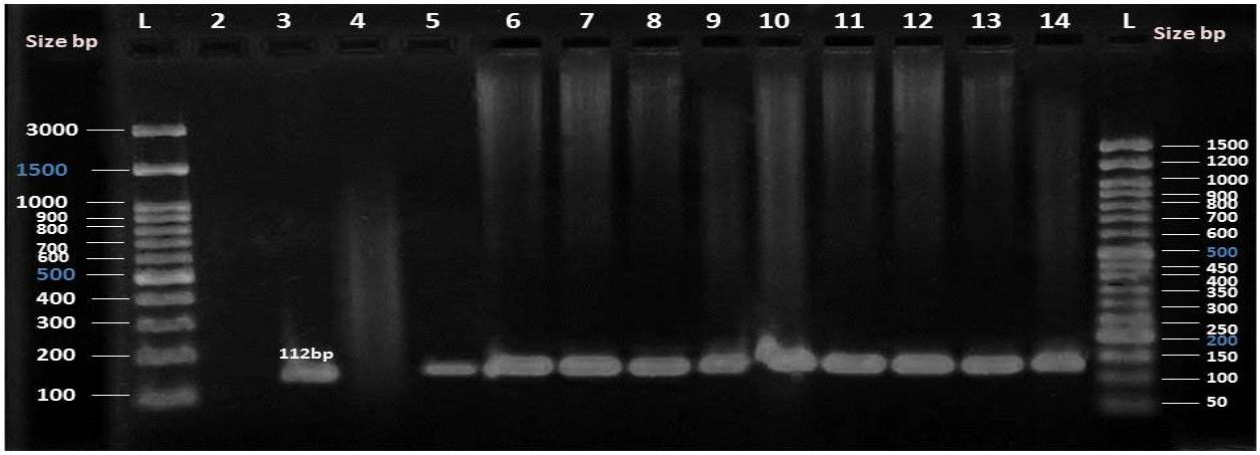

Figure 4: Analysis of T1052C mutation by ASO-PCR. Lane 1 and 15: DNA ladder of 1kb and 50bp respectively, Lane 2: Negative control, Lane 3: Positive control (Control DNA amplified with normal primer), Lane 4: Control DNA not amplified with mutant specific primer, Lane 5: Patient sample amplified with mutated specific primer T1052C and Lane 6-14: Patient samples amplified with normal primer.

BCR-ABL ATP binding mutation (T932C) of the ABL gene was checked by ASO-PCR (Zafar, 2004). Figure (5) shows specific band (172bp) of CML patients with normal primer and three mutations Phe311Leu (T932C) out of the fifty CML patients after using ASO-Primer for their amplifications. Figure (6) shows three CML patients were not amplified with normal primer while the same patients were positive for mutated primer like in previous figure and the rest are patient samples amplified with normal primer. The incidence of T932C mutation (3 of 50 CML patients, 6%) was higher than that reported by (Catherine *et al*., 2002) (1 of 24 CML patients, 4.1%) but lower than that found by (Aamir *et al*., 2011) (7 of 40 CML patients, 17.75%).

The first identified mutation in CML patients was the C944T (T315I) in a male 53 years old that described by Gorre *et al* in 2001 (Francis *et al*., 2007; Massimo & Giuliana, 2009). Cytosine to Thymine mutation at position 944 of *ABL gene* (Al-achkar *et al*., 2013) was detected by ASO-PCR assay (Waggas & Abdel-Muhsin, 2011; Maysaa, 2013). Figure (7) shows three CML patients that not amplified with normal primer then we used ASO-Primer for the same samples, we observed the band of specific PCR product (158bp) of mutated *ABL gene* Thr315Ile (T315I) on the gel electrophoresis by amplification of DNA patient with mutant specific primer (ASO-Primer). This mutation was formed by a single amino acid substitution at location 315 (Gorre *et al*., 2001), normally site chain of threonine 315 provide oxygen atom that form a crucial hydrogen bond with Imatinib (Gorre *et al*., 2001) but in the absence of an oxygen atom in the substitution isoleucine prevents bond formation that lead to inhibit binding (Massimo & Giuliana, 2009). C944T mutation completely impaired the therapy binding leading to complete drug resistance. Complete Imatinib resistance can be controlled by combination of therapies (Zafar, 2004). Imatinib plus arsenic (Hamad *et al*., 2013), Imatinib plus Cytarabine (Kantarjian *et al*., 2003), interferon alpha plus Cytarabine (Vigil *et al*., 2011) And Imatinib plus pegylated interferon alpha are used for those CML patients whom show complete resistance to Imatinib (Zafar, 2004; Jayakar, 2012; Radich, 2012). Figure (8) shows those patients were amplified with normal primer. The incidence of C944T was lower than that showed in the previous study by (Gorre *et al*., 2001) (6 of 9, 66.6%), (Catherine *et al., 2002*) (3 of 24, 12.5%), (Zafar, 2004) (6 of 68, 8.8%) and (Aamir *et al*., 2011) (3 of 40, 7.5%).

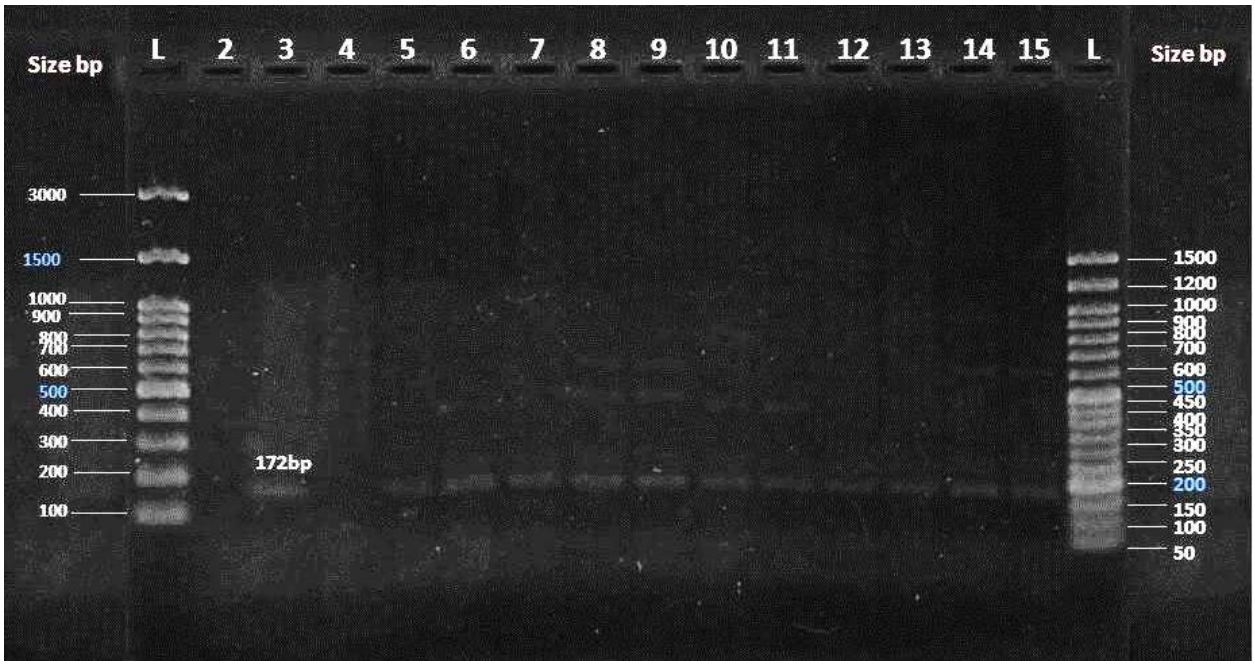

Figure 5: Agarose gel Electrophoresis showing 172bp ASO-PCR fragments corresponding to BCR-ABL Gene ATP binding domain T932C mutation. Lane 1 and 15: DNA ladder of 1kb and 50bp respectively, Lane 2: Negative control, Lane 3: Positive control (non CML DNA amplified with normal primer), Lane 4: non CML not amplified with mutant specific primer, Lane 5,6 and 7: Patient sample amplified with mutated specific primer T932C and Lane 8-15: Patient samples amplified with normal primer.

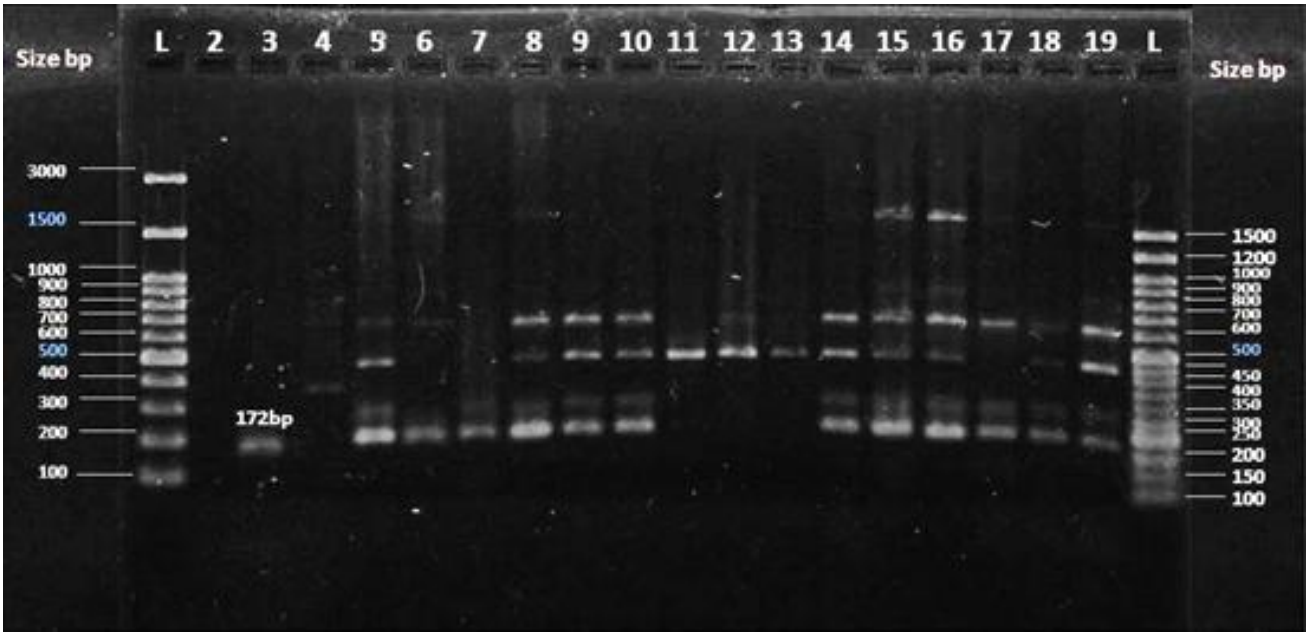

Figure 6: Agarose gel Electrophoresis showing 172bp ASO-PCR fragments corresponding to BCR-ABL Gene ATP binding domain T932C mutation. Lane 1 and 19: DNA ladder of 1kb and 50bp respectively, Lane 2: Negative control, Lane 3: Positive control (non CML DNA amplified with normal primer), Lane 4: non CML DNA not amplified with mutant specific primer, Lane 11,12 and 13: Patient samples not amplified with normal primer and the rest are Patient samples amplified with normal primer.

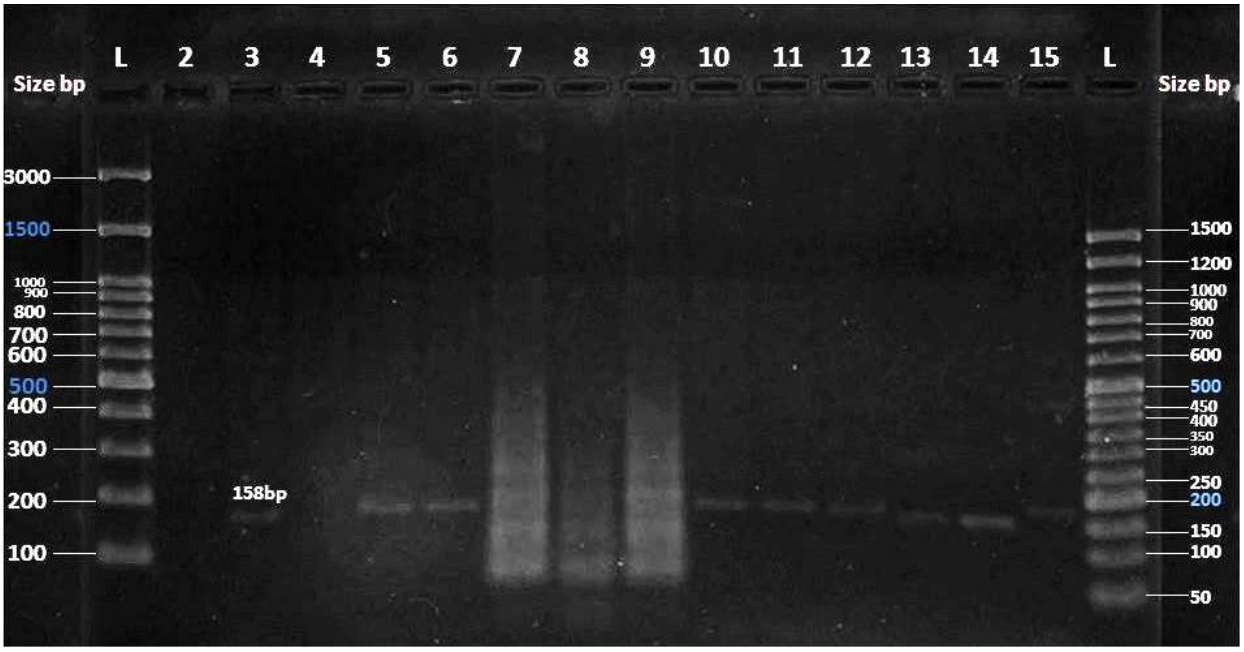

Figure 7: Detection of mutation C944T by ASO-PCR. Lane 1 and 15: DNA ladder of 1kb and 50bp respectively, Lane 2: Negative control, Lane 3: Positive control (Normal DNA amplified with normal primer), Lane 4: Normal DNA not amplified with mutant specific primer, Lane 5, 6 and 10: Patient samples amplified with ASO-Primer,Lane 7-9: CML patient samples not amplified with normal primer and the rest Patient samples amplified with normal primer.

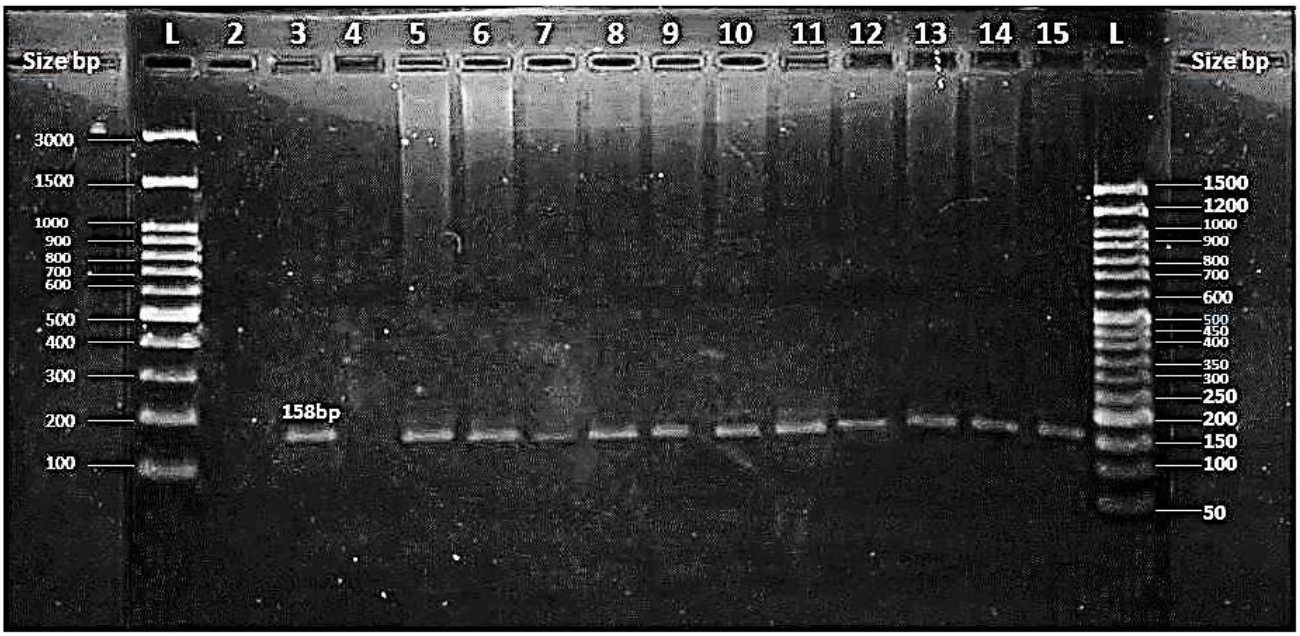

Figure 8: Detection of mutation C944T by ASO-PCR. Lane 1 and 15: DNA ladder of 1kb and 50bp respectively, Lane 2: Negative control, Lane 3: Positive control (Normal DNA amplified with normal primer), Lane 4: Normal DNA not amplified with mutant specific primer and Lane 5-15: Patient samples amplified with normal Primer.

Imatinib has fascinated the physicians, clinicians, patients and researchers by its high effectiveness against BCR-ABL positive CML patients (Zafar, 2004), that stops the oncogenic activity of BCR-ABL onco-protien by binding to its ATP binding site on the ABL kinase leading to inhibition of substrate phosphorylation of protein BCR-ABL and preventing the activation of downstream signaling pathways (Hofmann *et al.*, 2003; Mathisen *et al.*, 2011).

Imatinib interacts with kinase through hydrogen bond; any mutation affecting amino acid residues in the active site of ABL could induce a structural change effect, making the drug unable to inhibit ABL kinase activity (Catherine *et al*., 2002).

The molecular genetic changes (DNA damage) in CML patients affect the action of targeted therapy causing drug resistance like Imatinib, specifically that forms abnormal *BCR-ABL fusion gene* that lead to genomic instability of leukemic clone (Cortes, 2009) which induce point mutation in BCR-ABL kinase domain leading to shortened survival of CML patients due to failure to response to Imatinib. On other hand exposure to Imatinib may induce the genetic instability eventually cause the high frequency of mutation in wild type ABL kinase domain (Neil *et al*., 2002).

The most frequent mechanism that change Imatinib sensitivity in BCR-ABL transformed cells (Massimo & Giuliana, 2009; Mathisen *et al.*, 2011) and finally cause Imatinib resistant is mutation in the BCR-ABL kinase domain that impair Imatinib binding either by direct affect the proper binding of Imatinib to the target molecule binding of ATP or lead to conformation changes of the protein which reduce the affinity to Imatinib (Hochhaus, 2006; Massimo & Giuliana, 2009) The occurrence of the mutations in patients whom develop Imatinib resistant is from 30% to 90% (Delia *et al*., 2008). Allele specific oligonucleotide ASO-PCR is a very specific and sensitive technique for detection of known mutations (Catherine *et al*., 2002), short turnaround time of analysis, lower cost and simple procedure (Kim, 2006). Direct sequencing of the ABL kinase domain is the most common mutation analysis technique but has a limited sensitivity for detection (Waggas & Abdel-Muhsin, 2011; Parker *et al.*, 2011) so ASO-PCR method is more sensitive than mutation detection by sequencing of ABL ATP binding domain because DNA sequencing is useful to detect point mutation when proportion of mutated cells is more than 30% (Catherine *et al*., 2002; Zafar, 2004). Also sequence analysis for mutation detection is quite costly, time consuming and laborious (Zafar, 2004; Massimo & Giuliana, 2009).

On the other hand ASO-PCR is suitable for rapid and routine screening of mutations and with a satisfying cost effectiveness value (Delia *et al*., 2008). The ASO-PCR was founded that able to detect one mutated cell Imatinib resistant cell out of 10,000 normal cells (Zafar, 2004). Strong specifity of the method was demonstrated by the constant lack of amplification of mutant allele specific fragment in healthy individuals who served as a control for the study (Catherine *etal*., 2002 ; Delia *et al*., 2008). *BCR-ABL fusion gene* ATP binding domain mutation have been founded in another tyrosine kinase inhibitor like Nilotinib and Dasatinib which cause resistance formation in Nilotinib and Dasatinib (Simona *et al*., 2007; Michele *et al*., 2013). The mutation number that formed by Nilotinib treatment is lower than that formed by Gleevec treatment (Massimo & Giuliana, 2009) and the mechanism of Nilotinib resistance belong to over expression of *BCR-ABL fusion gene* (Nestal *et al.*, 2012).

The best way to avoid Imatinib resistance in CML can be achieved by dose escalation. Administering full therapeutic doses as early as possible such as 800mg/day as a first line therapy in newly diagnosed chronic phase

CML patients causes the maximal depletion of leukemic cells (Jeffrey & Helen, 2003; Hochhaus, 2006). Also higher doses (standard dose, 800mg/day) of Imatinib reinduce hematologic response or impair cytogenetic response (Kantarjian *et al*., 2003).

BCR-ABL positive CML patients require immediate attention not only by local physicians and oncologists but international community should also go many stages to work in collaboration with local personnel for this issue, to find out the reasons, molecular maker of the disease and drug targets.

Conclusion

Conventional cytogenetic and molecular genetic tests are complementary techniques to show the exact types of abnormalities, that allow better evaluation of the genomic aberration involved in CML patients, specifically for kinase domain mutation that plays an important role in different diagnosis, prognosis, therapy treatment and drug resistant management of CML patients like selection the specific tyrosine kinase inhibitor when Imatinib mesylate treatment has been failed.

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