PRAME and WT1 Genes expression in Chronic Myeloid Leukemia Patients: Clinical importance and future prospects

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Abstract

Objectives: This study was designed to declare the frequency of expression of PRAME and WT-1 genes in Egyptian patients with chronic myeloid leukemia during chronic phase and accelerated/blastic transformation phase of the disease.

Patients and methods: RT-PCR technique was used to detect the expression of PRAME and WT-1 genes as well as BCR-ABL (p210) and ABL transcripts in peripheral blood samples of 30 CML patients. The BCR-ABL/ABL ratio was estimated by densitometric analysis. Twenty healthy volunteers were subjected to the same analysis as a control group.

Results: The control subjects were negative for PRAME, WT-1 and BCR-ABL. mRNA expression of PRAME gene was detected in 22/30 (73.3%), while WT-1 gene was expressed in 15/30 (50%) of CML patients. PRAME expression was significantly higher in patients in blastic transformation, while there was no significant difference in the expression of WT1 between CML patients in the chronic or the blastic transformation phase. BCR-ABL/ABL ratio was significantly higher in CML patients in the blastic transformation phase which reflects the disease progression.

Conclusion: PRAME and WT-1 are tumor associated antigens that could be relevant for follow up of CML patients as well as being promising targets for future cancer immunotherapy.

Introduction

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease with distinct biological and clinical features. CML usually presents in chronic phase. Without curative intervention, chronic-phase CML will invariably transform through a phase of "acceleration," often heralded by the appearance of increased immature myeloid cells in the bone marrow and peripheral blood, as well as new cytogenetic changes in addition to the Philadelphia chromosome (Ph). Progression then proceeds to blast crisis, with immature blast cells overwhelming the production of normal hematopoietic elements. Blast crisis is highly resistant to treatment. The median time from diagnosis of chronic phase CML to progression to accelerated phase is 3–4 years, but the range of timing is quite broad, encompassing from 0.5 to 15 years (1).

There are several treatment options for CML. All treatments are more successful when administered during the chronic phase disease than in accelerated or blast phase. The only known curative therapy for CML is stem cell transplantation (SCT), a complex and potentially toxic modality that carries a high potential for morbidity and mortality (2). Non-transplant therapy includes IFN-α, which produces a major reduction in the proportion of Ph-positive cells and extends the natural history of the disease in 10–20% of patients, with some alive and in remission for >10 years (3). IFN-α has largely been replaced by the tyrosine kinase inhibitor, Imatinib Mesylate, which suppresses the Ph to the point where it is undetectable by cytogenetic evaluation (a complete cytogenetic remission or CCR) in >70% of newly diagnosed patients with chronic-phase CML disease (4). The long-term duration of such responses is unknown, as is potential for cure with imatinib. Complete molecular response, defined by the absence of the Bcr-Abl fusion mRNA by RT-PCR, is unusual in patients with CCR (5), and patients who achieve a molecular response, but have imatinib discontinued, show a relatively rapid reemergence of disease (6). Resistance to imatinib occurs (especially in advanced phase disease) often accompanied by point mutations in the tyrosine kinase domain of the ABL gene (7).

Alternative treatment options to achieve remissions are therefore still needed to combat CML. For this purpose, immunotherapy trials using mostly dendritic cell-based vaccination strategies should be conducted (8). Tumor associated antigens (TAA) provide attractive targets for cancer specific immunotherapy as PRAME (9) and WT-1 (10).

The possibility that PRAME is a leukemia antigen recognized by cytotoxic T cell (CTL) was evaluated by Steinbach et al. (11) and it was found that PRAME- positive leukemia cell lines and fresh leukemia cells were susceptible to lysis by the specific CTL. The sensitivity of CML to donor lymphocyte infusion after allogenic stem cell transplantation suggests that this tumor can be highly susceptible to cellular immunotherapy targeted to tumor associated antigens, so PRAME –CTLs or vaccines may thus be of value for patients with CML (12).

WT-1 mRNA down regulation during differentiation in leukemic cell lines, and the induction of differentiation by blocking WT-1 transcription via antisense suggest that WT-1 is indeed involved in the differentiation of hematopoietic precursor cells and possibly in leukemogenesis (13). Also, high WT-1 expression after induction therapy predicts high risk of relapse in chronic myeloid leukemia (14). It was reported that WT-1 might be a suitable target for new therapeutic strategies using siRNA in leukemia cells (15).

Yong et al. (10) reported that WT-1 was consistently over expressed in advanced

phase of CML in all CD34+ subpopulation and mature progenitors of chronic phase CML which suggests that the most primitive haematopoeitic stem cells in advanced phase of CML could be targets for WT-1 peptide based vaccines, which in combination with PRAME could additionally improve targeting differentiated progeny, and benefit patients responding sub-optimally to tyrosine kinase inhibitors or enhance graft versus leukemia effect in SCT patients.

The present work aimed at studying the frequency of expression of PRAME and WT-1 genes in 30 Egyptian CML patients in chronic and blastic transformation phases to identify the gene signature associated with the phase of the disease.

Patients and Methods

This study was carried out on 30 chronic myeloid leukemia patients, as well as 20 age and sex matched healthy volunteers as a control group. The patients were randomly chosen from outpatient clinic or inpatients of department of Clinical Oncology Kasr El Aini Teaching Hospital, Cairo University. An informed consent was taken from both patients and control individuals. Diagnosis of CML cases was based on clinical and laboratory assessment according to *Kantarjian et al*. (3). The definition of chronic, accelerated and blast crisis was based on the criteria of the WHO (16), thus, chronic phase was defined as <10% blasts, the accelerated phase was defined as 10–19% blasts and blastic crisis was defined as >20% blasts. The patient group was classified into two groups as follows:

- **Group A:** Twenty two patients of chronic myeloid leukemia cases in the chronic phase (22/30 - 73.3%).
- **Group B:** Eight patients of chronic myeloid leukemia in accelerated/blastic transformation phase (8/30 - 26.6%).

All patients were subjected to the following:

- Full history taking, thorough clinical examination with careful notation and assessment of clinical signs relevant to CML as splenomegaly, hepatomegaly and anemic symptoms.
- Routine laboratory investigations as differential blood count (CBC), liver and kidney functions, serum uric acid, LDH and coagulation profile.
- Cytochemical studies including leukocyte alkaline phosphatase (LAP score) and myeloperoxidase for patients in accelerated/blastic transformation phase.
- Bone marrow aspiration, conventional cytogenetic analysis for Philadelphia chromosome.
- Detection of BCR-ABL (p210) and ABL transcripts by reverse transcriptase - polymerase chain reaction (RT-PCR) to estimate the BCR-ABL/ABL ratio which reflects disease control (i.e. the response to therapy) as described by *Schmitt et al*. (17).
- PRAME and WT-1 genes expression in peripheral blood mononuclear cells of CML patients as well as the control group by reverse transcriptase – polymerase chain reaction (RT-PCR) according to *Greiner el al*. (18).

Five ml of blood were withdrawn from every patient as well as the healthy volunteers in a sterile EDTA vacutainer. The mononuclear cells are separated by Ficoll-Hypaque density gradient separation. Total RNA extraction from peripheral blood MNCs was done using High pure RNA isolation kit (Fermentas TM Germany, catalogue no; 1828665). Isolated mRNA was reverse transcribed into cDNA using cDNA synthesis kit (FermentasTM Germany, catalogue no; 1975234) and stored at -20°C until used for PCR amplification. PCR amplification was performed in a final volume of 50 μl (1 ul DNA, 25 μl 2XPCR Master Mix [#K0171 Lot no; 00022525, Fermentas™ - Germany], 1 μl primer; each sense and antisense and 22 μl distilled water). The PCR reaction was carried out in the DNA thermal cycler (Perkin Elmer 9600). β-actin was amplified as an internal control to study the purity of the extracted RNA as described by *Dias et al*. (19). The following primer sequences were used:

- PRAME: Forward primer: 5'-GTC CTG AGG CCA GCC TAA GT -3', Reverse primer: 5'- GGA GAG GAG GAG TCT ACG CA -3' (Fermentas™ – Germany).
- WT-1: Forward primer: 5'- ATG AGG ATC CCA TGG GCC AGC A -3', Reverse primer: 5'- CCT GGG ACA CTG AAC GGT CCC CGA -3' (Fermentas™ – Germany).
- β-actin : Forward primer: 5'- TCA TGT TTG AGA CCT TCA A-3', Reverse primer: 5'- GTC TTT GCG GAT GTC CAC G -3' (Fermentas™ – Germany).

For PRAME gene reverse transcription: Initial denaturation at 95 oC for 20 seconds, annealing at 64 oC for 10 seconds, and extension at 72 oC for 40 seconds. This was repeated for 40 cycles. For WT-1, initial denaturation at 95 oc for 15 seconds, annealing at 62 oC for 10 seconds, and extension at 72 oC for 22 seconds. This was repeated for 45 cycles. For β-actin, incubation for 30 minutes at 50°C, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 68°C for 2 minutes, final extension 68°C for 7 minutes.

For BCR-ABL and ABL transcripts, the following primer sequences were used:

- BCR-ABL: Forward primer: 5'-TTC AGA AGC TTC TCC CTG ACA T- 3', Reverse primer: 5'- CGG CTC TCG GAG ACG TAG A- 3' (Fermentas™ – Germany).
- ABL: Forward primer: 5'- GAT ACG AGG GAG GGT GTA CCA - 3', Reverse primer: 5'-CTC GGC CAG GGT GTT GAA - 3' (Fermentas™ – Germany).

The thermocycler program for BCR-ABL and ABL was done according t0 *Schmitt et al*., (17) initial denaturation step at 95 oc for 60 seconds followed by 45 cycles of 1 second at 95 oC, 10 seconds at 64 oC and 26 seconds at 72 oC. The amplified products were analysed by gel electrophoresis using 4% agarose gel (Promega cat no.V3121), stained with ethidium bromide then the electrophoretic pattern was visualized under UV light trans-illumination. The sample was considered positive when a clear sharp band was observed at the specific molecular weight; 661 bp for β-actin, 764 bp for PRAME and 480 bp for WT-1.

Densiometric analysis of the results

The density of the amplified product of different samples was assessed by Biometra BioDocAnalyze Digital Gel Documentation System. Expression of BCR-ABL to ABL (R) was calculated according to the formula: R = densiometrical units of the BCR-ABL /densiometrical units of ABL. Relative expression (R1) of WT-1, PRAME, BCR-ABL and ABL were calculated according to the formula: R1 = densiometrical units of the amplified gene /densiometrical units of β-actin gene.

Statistical analysis

Data were summarized and presented in the form of mean, range and standard deviation as descriptive statistics. Descriptive statistics and statistical comparison were performed using the statistical software program SPSS (version 15). Group comparison was done using analysis of variance (ANOVA test). Correlation analysis was evaluated using the Pearson coefficient or the 2 sided Fisher>s exact test. Non parametric data were analyzed using the Chi-square test. For all of the above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value). Probability value (p-value) of more than 0.05 was considered non-significant, while p-value equal or less than 0.05 indicated a significant result.

Results

The present study was carried out on 30 chronic myeloid leukemia patients. Fifteen patients were females and fifteen were males. Their age ranged from 27 - 68 years. Twenty age and sex matched normal volunteers were included in this study as a control group. The patients were classified according to the stage of the disease into two groups as follows:

- Group (A): Chronic myeloid leukemia patients in the chronic phase (CML-CP).
- Group (B): Chronic myeloid leukemia in accelerated/blastic transformation phase (CML-AP/BC).

All the normal volunteers enrolled in the study did not express BCR-ABL, PRAME or WT-1 genes.

Group (A): The main clinical and laboratory data of CML patients in the chronic phase of the disease are presented in table (1). mRNA expression analysis of the studied genes revealed that BCR-ABL and ABL genes were expressed in 22/22 (100%) of cases. PRAME was expressed in 15 / 22 (68.2%) of cases while WT-1 was expressed in 11/22 (50%) of cases. Seven cases showed dual expression of PRAME and WT-1 (7/22 – 31.8%). Comparison between PRAME and WT-1 positive and negative patients revealed no statistically significant difference as regard their clinical and laboratory data ($p>0.05$).

Group (B): The main clinical and laboratory data of CML patients in the accelerated/blastic transformation phase of the disease are presented in table (2). mRNA expression analysis of the studied genes revealed that BCR-ABL was expressed in 22/22 (100%) of cases, while ABL was expressed in 7/8 (87.5%) of cases. PRAME was expressed in 7/ 8 (87.5%) of cases while WT-1 was expressed in 4/8 (50%) of cases. Four cases showed dual expression of PRAME and WT-1 (4/8– 50%). Comparison between PRAME and WT-1 positive and negative patients revealed no statistically significant difference as regard their clinical and laboratory data ($p>0.05$). Among CML patients in the accelerated/blastic transformation phase, there was a positive correlation between BCR-ABL/ABL ratio and the percentage of myeloblasts in their peripheral blood ($p=0.026$, $r=0.77$).

Discussion

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease characterized by a reciprocal t(9;22)(q34;q11) chromosomal translocation, which creates the Philadelphia chromosome and leads to the expression of the BCR-ABL fusion protein, whose deregulated constitutive tyrosine kinase activity is responsible for leukemogenesis. Most patients present in the chronic phase, in which malignant progenitor cells proliferate rapidly but retain much of their ability to differentiate, with the disease later evolving into the accelerated phase (AP) and blastic crisis. The AP is an intermediate stage in which patients show signs of disease progression and is characterized by increased refractoriness to standard therapy (20).

Recently, the introduction of imatinib mesylate (Gleevec/ Novartis), an inhibitor of the BCR-ABL tyrosine kinase activity, has strongly improved the therapy of CML, leading to a significant prolongation of hematologic and cytogenetic remissions and the mean survival time without inducing major adverse effects(21). Nevertheless, resistance to imatinib treatment is a problem. In most cases, point mutations or an amplification of the BCR-ABL gene are responsible for the loss of efficacy, but also BCR-ABL–independent mechanisms like a differential gene expression in resistant cells have been described (22).

Alternative treatment options to achieve remissions are therefore still needed to combat CML, especially in patients resistant to the standard forms of therapy, including IFN- or imatinib. Immunotherapy for leukemia patients, aiming at the generation of anti-Leukemic T cell responses, could provide a new therapeutic approach to eliminate minimal residual disease (MRD) cells in acute myeloid leukemia (AML) (23).

Various solid tumours express PRAME as sarcomas, small-cell lung cancers, renal cancers and head and neck cancers. Its expression has been found in many haematopoietic neoplasia including acute and chronic leukaemias, multiple myeloma and lymphoma (24). In addition, PRAME was demonstrated to be a useful marker for detection of minimal residual disease (MRD) in patients with leukemia, particularly those leukaemias in which tumor specific markers are currently unavailable. It encodes an antigen recognized by autologous cytotoxic T lymphocytes (CTL), it was found that PRAME- positive leukemia cell lines and fresh leukemia cells were susceptible to lysis by the specific CTL (11). So PRAME is a good candidate for tumour immunotherapy and is a useful marker gene for detection of minimal residual disease (24).

In the hematopoietic system, WT-1 mRNA was detected at high levels in spleen and at low levels in bone marrow (BM) where it is confined to CD34+ progenitor cells during early hematopoietic differentiation. This transcription factor is normally expressed in immature CD34+ progenitor cells, and differentiation is associated with WT-1 down regulation (13). Expression of WT-1 gene has been found in most cases of acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and myelodysplastic syndrome (25). The minimal residual disease of leukemia can be detected in normal peripheral blood mononuclear cells by means of the quantitation of WT1 mRNA (WT1 assay) using reverse transcriptase-polymerase chain reaction. Thus, the WT1 assay makes it possible to rapidly assess the effectiveness of treatment and to evaluate the degree of eradication of leukemic cells in individual leukemia patients. Furthermore, the WT1 assay can continuously assess the disease progression of CML and predict the evolution of chronic phase to blastic phase. In CML and Ph positive ALL patients, WT1 expression levels changed in parallel with the BCR-ABL expression pattern as the disease progressed or responded to effective treatment (26). WT1 protein is highly immunogenic, thus, patients with WT1-expressing tumors produce antibodies and cytotoxic T-lymphocytes against WT1 protein, indicating that WT1 protein is a promising tumor antigen (WT1 peptide-based cancer immunotherapy) (27).

The current study aimed at detecting the frequency of expression of PRAME and WT-1 genes in Egyptian CML patients as immunogenic antigens in chronic myeloid leukemia that may be useful as prognostic markers, to monitor the response to therapy, early detection of blastic transformation as well as potential target structures for cellular immunotherapies and/or antibody-based therapies especially for patients resistant to the standard forms of therapy.

In the present study, the mRNA expression of different immunogenic antigens was evaluated in peripheral blood mononuclear cell samples from 30 chronic myeloid leukemia patients as well as 20 healthy volunteers as a control group. All the control subjects were negative for PRAME and WT-1 which agrees with the results presented by several researchers (13, 28). The antigen PRAME showed positive mRNA expression in 22/30 (73.3%) of CML patients; in group A (CML-CP), it was expressed in 15/22 (68.2%) of the patients while it was expressed in 7/8 (87.5%) of the patients in group B (CML-AP/BC). This agrees with Schmitt et al., (17) where PRAME was expressed in 58% of cases in the chronic phase and in 70% of cases in blastic transformation phase. On the contrary, the studies of Matsushita et al. (28) and Paydas et al. (29) revealed that PRAME expression in both chronic and accelerated phases of CML was lower than that reported in

the present study as it was 23% and 22% in the chronic phase and 36.8% and 20% in the blastic transformation phase respectively.

In another study by Roman-Gomez et al. (9), PRAME expression in chronic phase CML patients was lower than that detected in the present study as it was expressed in 36% of CML patients in the chronic phase, while the percentage of PRAME positive patients in the blastic transformation phase (70%) which is in accordance to our results (9).

On comparing PRAME positive and negative patients either in the chronic or the blastic transformation phase, no statistically significant difference could be detected between the two groups as regards the clinical and laboratory data. Similarly, Roman-Gomez et al. (9) and Paydas et al. (30) stated that in their studies for PRAME expression by quantitative RT-PCR, there was no statistically significant difference between PRAME positive and negative patients as regard their age, gender, clinical and hematological findings.

WT-1 gene was expressed in 15/30 (50%) of CML patients. In group A, it was expressed in 11/22 (50%) of patients while it was expressed in 4/8 (50%) of the patients in group B. No statistically significant difference was found between patients in the chronic or blastic transformation phase of CML as regard the expression of WT-1 gene either as percentage expression or the level of expression detected by densitometry (p=1 and 0.21 respectively). This is in concordance with Schmitt et al. (17) where WT-1 was expressed in 53% of cases in the chronic phase and in 50% of cases in blastic transformation phase. On the contrary, Cao et al. (30) and Kang et al. (31) reported that WT-1 expression levels in CML patients in the accelerated phase or blastic crisis were strikingly higher than those in CML patients in the chronic phase of the disease, in which WT-1 expression was undetectable or showed low expression, however, that can be attributed to the large sample size included in their studies. Also, the study of Rosenfeld et al. (32) revealed over expression of WT-1 in the blastic crises but not in the chronic phase.

On comparing WT-1 positive and negative patients either in the chronic or the blastic transformation phase, no statistically significant difference could be detected between the two groups as regards the clinical and laboratory data. No statistically significant correlation could be detected between WT-1 expression and BCR-ABL/ABL ratio in neither group A nor B. This is in accordance with Kang et al. (31) and Schmitt et al. (17) who did not find a statistical correlation between WT-1 expression level and BCR-ABL/ABL ratio. Another study by Rosenfeld et al. (32) reported a significant positive correlation between BCR-ABL/ABL ratio which reflects the disease control and WT-1 level being significantly higher among CML patients in blastic transformation phase.

As regard BCR-ABL/ABL ratio, there is a positive correlation between this ratio and the percentage of myeloblasts in the peripheral blood in CML patients either in chronic phase (p=0.021, r=0.49) or blastic transformation phase (p=0.026, r=0.77). This reflects disease progression from chronic to accelerated/blastic transformation phase. This ratio was significantly higher in CML patients in blastic transformation phase. This is in accordance with the studies of Schmitt et al. (17) and Kantarjian et al. (33) who stated that there is a good concordance between the ratio of BCR-ABL/ABL transcripts and the cytogenetic response to imitinib, that's why quantitative PCR studies for this ratio provide a useful tool to monitor patients with CML on imatinib mesylate therapy.

Specific immunotherapies for CML patients targeting T-cell antigens might eliminate residual CML cells after chemotherapy, in combination with imatinib or other tyrosine kinase inhibitors and might enhance a graft versus leukemia effect after allogenic BMT without aggravating graft versus host disease (18). Both human studies in vitro and moue models in vivo have demonstrated that the leukemia associated antigens (LAAs) such as the fusion protein BCR-ABL, Wilm's tumor protein, PRAME and Proteinase 3 may serve as efficient targets for cellular immunotherapies. Pilot clinical trials have been initiated in acute and chronic leukemia and other haematological malignancies, which include vaccination of patients with synthetic peptide derived from these leukemia associated antigens (LAAs) (Dao & Scheinberg (34).

In conclusion, PRAME and WT-1 being tumor associated antigens could be considered as prognostic markers that can be used for monitoring therapy in PRAME and/or WT-1 positive CML patients. Moreover, PRAME and WT-1 seem to be favourable candidate for future cancer immunotherapy.

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Tables

Table 1: The Main Characteristics of CML Patients Included in Group (A).

	CML patients in Chronic phase (CML-CP) (Number 22 / 30)		
Clinical Data	**Age (years)**	27 – 68 years	
	Sex (M/F)	**Male**	10/22 (45.5%)
		Female	12/22 (54.5%)
	Splenomegaly (Number- %)	22/22 (100%)	
	Hepatomegaly (Number-%)	6/22 (27.3%)	
	Anaemic symptoms (Number- %)	2/22 (9.1%)	
Laboratory Data	**Hemoglobin level (gm/dl) (range)**	7.6 – 16 (12.1 ± 1.6)	
	Total leucocytic count (Range - x10³/cmm)	24.1 – 280 (148.5 ± 80)	
	Platelets (Range - x10³/cmm)	209 – 448 (236.7 ± 89.5)	
	Basophil count (%)	1 – 13 (5.3 ± 3.4)	
	Myeloblasts (%)	0 – 8 (3.1 – 2.1)	
	NAP score	1 – 10 (6.1 ± 2.7)	
	Philadelphia chromosome	21 / 22 (95.5%)	
	BCR-ABL fusion gene	22 / 22 (100%)	

NAP score: Neutrophil alkaline phosphatase score.

Table 2: The Main Characteristics of CML Patients Included in Group (B).

	CML patients in accelerated/blastic transformation phase (CML-AP/BC) (Number 8 / 30)		
Clinical data	**Age (years)**	33 - 58 years	
	Sex (M/F)	**Male**	5/8 (62.5%)
		Female	3/8 (37.5%)
	Splenomegaly (Number- %)	8/8 (100%)	
	Hepatomegaly (Number-%)	2 /8 (25%)	
	Anaemic symptoms (Number- %)	5/8 (62.5%)	
Laboratory data	**Hemoglobin level (Range – gm/dl)**	7.2 – 13 (10.1 ± .3)	
	Total leucocytic count (Range - x10³/cmm)	72.6 – 181 (116.5 ± 36)	
	Platelets (Range - x10³/cmm)	55 – 117 (97.6 ± 18.5)	
	Basophil count (%)	4 – 16 (9.6 ± 4)	
	Myeloblasts (%)	17 – 34 (24.1 ± 6)	
	NAP score	2 - 8 (4.2 ± 1.9)	
	Philadelphia chromosome	8 / 8 (100%)	
	BCR-ABL fusion gene	8 / 8 (100%)	

NAP score: Neutrophil alkaline phosphatase score.

Table 3: Comparison Between the Two Studied Groups As regard BCR-ABL/ABL Ratio, PRAME and WT-1 Expression.

	Group (A) (CML-CP)	***Group (B) (CML-BC)***	***P-value***	***Significance***
BCR-ABL/ABL ratio *(Range, mean ± SD)*	0.1-3.78 (1.38 **±** 1.26)	1.86 – 4.3 2.86 **±** 0.92	0.001	**S**
PRAME expression *(RT-PCR)*	15/22 (68.2%)	7/8 (87.5%)	0.039	**S**
WT-1 expression *(RT-PCR)*	11/22 (50%)	4/8 (50%)	1	NS
PRAME expression/ *Densitometric analysis (mean ± SD)*	(0.57 **±** 0.38)	(0.58 **±** 0.41)	0.92	NS
WT-1 expression/ *Densitometric analysis (mean ± SD)*	(0.46 **±** 0.43)	(0.46 **±** 0.49)	0. 96	NS

S= Statistically significant, NS= Statistically not significant.

As regard BCR-ABL/ABL ratio, there is a positive correlation between this ratio and the percentage of myeloblasts in the peripheral blood in CML patients either in chronic phase (p=0.021, r=0.49) or blastic transformation phase (p=0.026, r=0.77). This reflects disease progression from chronic to accelerated/blastic transformation phase as the ratio was significantly higher in CML patients in blastic transformation phase (p= 0.026).

Figures

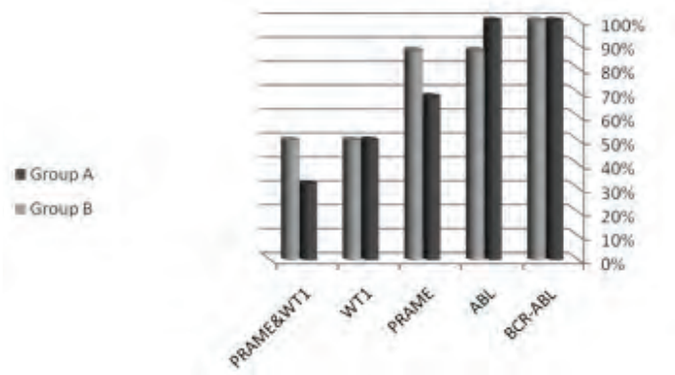

Fig 1. mRNA Expression of the Studied Genes in Both Groups.
On comparing mRNA expression of the studied genes in the chronic phase versus the accelerated/blastic transformation phase, the BCR-ABL/ABL ratio was significantly higher among CML patients of group (B) (p=0.001), while no statistical significance difference was noticed as regard PRAME and WT-1 levels by densitometric analysis (p=0.11 and 0.21 respectively). Regarding the percentage of PRAME expression, it was significantly higher among CML patients in group (B) (p= 0.039) (Table-3).

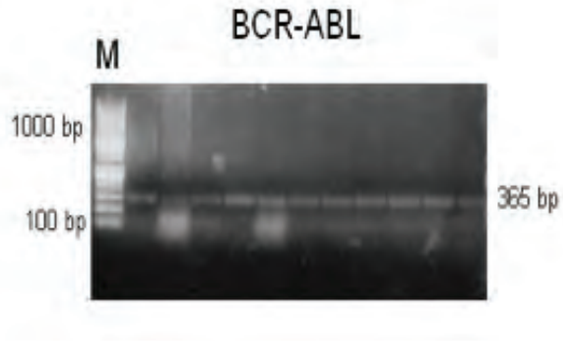

Fig 2. Agarose Gel Electrophoresis Analysis of BCR- ABL gene.
M: PCR marker (1001000-900-800-700-600-500-400-300-200- bp).
BCR-ABL gene was expressed in 100% of CML cases.

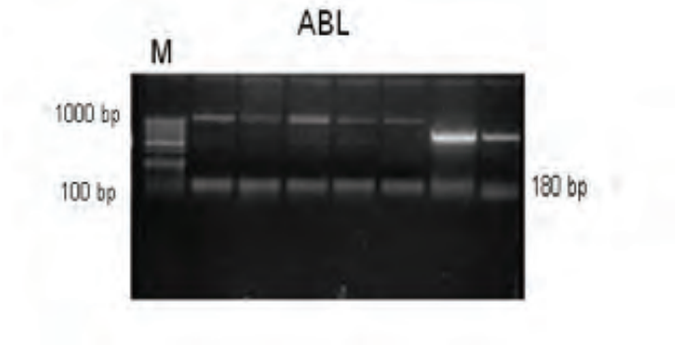

Fig 3. Agarose Gel Electrophoresis Analysis of ABL gene.
M: PCR marker (1001000-900-800-700-600-500-400-300-200- bp).
ABL gene was expressed in 100% of cases in group A and in 87.5% of cases in group B.

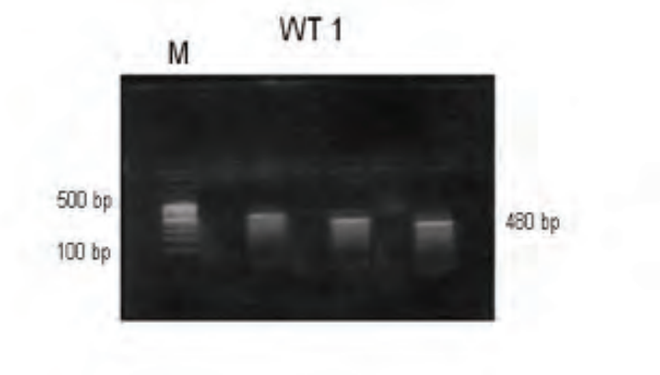

Fig 4. Agarose Gel Electrophoresis Analysis of WT 1 gene.
M: PCR marker (100500-400-300-200- bp).
WT 1 gene was expressed in 50% of cases in group A and in 50% of cases in group B.

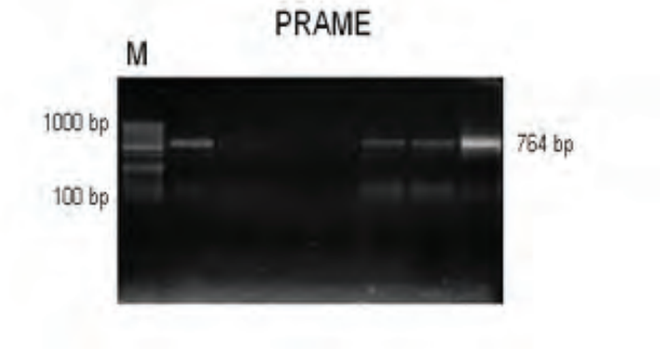

Fig 5. Agarose Gel Electrophoresis Analysis of PRAME gene.
M: PCR marker (1001000-900-800-700-600-500-400-300-200- bp).
ABL gene was expressed in 68.2% of cases in group A and in 87.5% of cases in group B.