ORIGINAL ARTICLE ◄

Expression of A Novel Tumor Associated Antigen (Rhamm/cd 168) in Cml Patients. Clinical Implications and Potentiels for Specific Immunotherapy.

*Heba M Gouda, MD, Mohsen M Abdel Mohsen, MD**

**Department of Clinical and Chemical Pathology, Department of Medical Oncology, Faculty of Medicine, Cairo University.*

✉ *Corresponding Author: Heba M Gouda, Department of Clinical and Chemical Pathology, Department of Medical Oncology, Faculty of Medicine, Cairo University, E-mail: goudamed@hotmail.com*

Key words: CML, RHAMM, CD168, Leukemia Associated Antigen.

Submitted: 05 January 2009; Accepted: 30 January 2009

ISSN: 2070-254X

Abstract

RHAMM/CD168 is a cell surface receptor for hyaluronan, a glycoaminoglycan that plays a fundamental role in cell growth, differentiation and motility. It is one of the leukemia-associated antigens (LAA) identified in patients with myeloid leukemias. In the present study we aimed at studying the expression of RHAMM in chronic CML patients as an early diagnostic or prognostic procedure and a potential target structure for cellular immunotherapies.

Patients and Methods: RHAMM expression was tested in peripheral blood mononuclear cells of 60 CML patients divided into 2 groups, group A: 44 chronic phase CML patients, group B: 16 accelerated/blastic phase patients as well as 15 healthy volunteers by RT-PCR.

Results: demonstrated that 31.8% of our chronic CML patients showed positive RHAMM expression in contrast to 93.7% in the accelerated/blastic phase patients. Moreover within the chronic phase patients the RHAMM positive patients had a significant higher level of bcr-abl/abl ratio. This highlighted the contribution of RHAMM expression with CML disease progression.

Conclusion: the high prevalence of RHAMM expression in CML patients especially the accelerated/blastic phase favors the use of RHAMM R3 peptide for patient vaccination together with conventional therapy in order to achieve complete molecular remission or at least prevent disease progression.

Introduction

Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia (Ph) chromosome, which results from a reciprocal translocation between the long arms of the chromosomes 9 and 22 t(9;22)(q34;q11). This translocation creates two new genes, BCR-ABL on the 22q- (Ph chromosome) and the reciprocal ABL-BCR on 9q-. The BCR-ABL gene encodes for a 210-kD protein with deregulated tyrosine kinase (TK) activity, which is crucial for malignant transformation in CML. (1) The chronic (CP) of CML is characterized by excess numbers of myeloid cells that proliferate extensively. Between 90% and 95% of patients will be diagnosed in this phase of the disease.

If untreated, within an average of 4 to 6 years, the disease transformed through an "accelerated phase" (AP) to an invariably fatal acute leukemia, also known as blast crisis. Disease progression is likely due to the accumulation of molecular abnormalities that lead to a progressive loss of the capacity for terminal differentiation of the leukemic clone. The current treatment goals for the patient with CML are to maintain remission and prevent progression of the disease to accelerated phase (AP) or blast phase (BP) while minimizing any therapy-related toxicity .(2)

During the 1900s, radiation, busulfan, hydroxyurea (cytoreductive therapy), interferon-_ (IFN-_), and stem cell transplantation were developed for other indications, tried broadly, and found to have activity in CML. Allogeneic stem cell transplantation is the only proven curative therapy, however, toxicity is formidable, with treatment- related mortality reported in the 30% range. Thus, effective therapy that maintains the patient with CML in CP with minimal toxicity is the goal for treatment of modern therapies (3).

The recognition of the BCR-ABL gene and corresponding protein as the preeminent mutation driving CML led to the synthesis of small-molecule drugs, designed to interfere with BCR-ABL tyrosine kinase activation by competitive binding at the ATP-binding site. The first tyrosine kinase inhibitor (TKI), introduced into clinical practice in 1998, was imatinib mesylate. Imatinib became the first choice drug in chronic phase CML, because of its high efficacy, low toxicity and ability to maintain durable hematological and cytogenetic responses. However, approximately 20-25% of patients initially treated with imatinib needed alternative therapy, due to drug intolerance or drug resistance.(1)

The most common cause of imatinib resistance is the selection of leukemic clones with point mutations in the abl kinase domain. These mutations lead to amino acid substitutions and prevent the appropriate binding of imatinib. Genomic amplification of BCR-ABL, modulation of drug efflux or influx transporters, and Bcr-Abl-independent mechanisms also play important roles in the development of resistance. Persistent disease is another therapeutic challenge and may in part, be due to the inability of imatinib to eradicate primitive stem cell progenitors. Thus finding inhibitors acting on targets found in the signaling pathways downstream of Bcr-Abl, such as the Ras-Raf-mitogen-activated protein kinase and phosphatidylinositol-3may have an important contribution to final therapy outcome whether as alternative therapy or as combination therapy. (4)

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 specific graft versus leukemia effect after allogeneic stem cell transplantation without aggravating the graft versus host disease. For an effective specific immunotherapy in CML, the

use of leukemia-associated antigens (LAAs) with an optimal expression pattern is required. (5) To design a specific immunotherapy for leukemia patients, the identification of leukemia-associated antigens (LAAs) is a pivotal step. Antileukemic effects after hematopoetic stem cell transplantation for myeloid leukemias are observed and might be related to the recognition of LAAs (6)
Among these leukemia-associated antigens (LAAs) that induce a humoral immune response in CML patients is the receptor for hyaluronic acid-mediated motility (RHAMM) (5,6)
RHAMM/CD168 is a receptor for hyaluronan, a glycoaminoglycan that plays a fundamental role in cell growth, differentiation and motility. RHAMM is a cell-surface receptor and belongs to the group of extracellular matrix molecules. The gene for RHAMM is localized on the human chromosome band 5q33.2. RHAMM is highly expressed in different tumor cell lines and in multiple myeloma. The receptor for hyaluronan-mediated motility RHAMM exerts different functions in the cell as well as on the cell membrane. RHAMM can be exported to the cell surface where it binds hyaluronic acid (HA) and interacts with the HA receptor CD44. Processes like cell motility, wound healing and invasion are modulated by RHAMM. Intracellularly,RHAMM is associated with the cytoskeleton, microtubules, centrosomes and the mitotic spindle. It participates in the control of mitotic spindle stability and integrity.
Furthermore, RHAMM is overexpressed in several cancer tissues. It was found that RHAMM expression is differentially regulated during the cell cycle and that cell cycle-dependent synthesis of RHAMM is controlled by its promoter on the transcriptional level. RHAMM protein levels follow mRNA expression in the early phases of the cell cycle. However, they already peak in S phase and decrease before the maximum of RHAMM mRNA expression is reached in G(2)/M. Furthermore, RHAMM expression is downregulated by the tumor suppressor p53. Additionally, p53-dependent downregulation is consistent with an oncogenic function of RHAMM and the recently reported tumor-suppressive function of CD44 transcriptional repression by p53. Anti RHAMM antibodies block the migration of cells to the process of tissue injury and angiogenesis including EC, macrophages, smooth muscle cells and fibroblast. (7)

No expression of RHAMM was described on non-activated normal B cells in blood, spleen, or lymph nodes (8). Over-expression of RHAMM is essential for ras-mediated transformation and it is associated with the development of metastases. Metastases showed a significantly higher expression level of RHAMM than the primary tumor, and the expression is strongly correlated with overexpression of both ras and extracellular signal-regulated protein kinase (ERK) Expression of RHAMM mRNA and protein expression levels was detected in all leukemia cell lines (8). Giles et al. (9) demonstrated by immunocytologic staining that RHAMM/CD168 is expressed both in the cytoplasm and the surface of leukemic blasts, opening a new strategies for potentially using RHAMM targeting antibody therapies in analogy to the CD33 directed antibody therapy.

The aim of this study is to detect RHAMM as an immunogenic antigen in chronic myeloid leukemia that may be useful as an early diagnostic or prognostic procedure and a potential target structure for cellular immunotherapies and antibody-based therapies.

Patients and Methods

This study was carried out on60 chronic myeloid leukemia patients, as well as 15 age and sex matched healthy volunteers as a control group. The patients were randomly chosen from outpatient clinic or in patients of the new Kasr el Aini teaching hospital, Cairo University. An informed consent was signed by both patients and control individuals.

Patients were stratified into 2 distinct groups: group A: 44 patients in chronic phase and group B: 16 patients in accelerated/blastic crisis phase.

In group A, patient's age ranged between 27 and 58 years. They were 20 females and 24 males.

In group B, patient's age ranged between28 and 58 years. They were 10 female and 6 male.

ALL Patients and controls were analyzed for several clinical and laboratory findings, including: .full history taking, thorough clinical examination, complete panel of routine laboratory work up including lap score. Detection of Philadelphia chromosome by conventional cytogenetic study, detection of bcr-abl translocation, bcr-abl/abl ratio and RHAMM(CD 168) expression by RT-PCR.

Detection of RHAMM gene expression by RT-PCR
Five ml of blood were withdrawn from every patient as well as the healthy volunteers in a sterile EDTA vaccutainer. The mononuclear cells are separated and preserved at - 20 oc. Total cellular RNA was extracted from the monnuclear cells using the QIA amp RNA blood Mini kit (QIAGEN, Catalogue number. 52304), followed by c-DNA preparation using Revert AidTM First strand cDNA synthesis kit (Fermentas, K1621). A volume of 5 μl cDNA was added to a final PCR reaction mixture of 25 μl containing 12.5 μl Master Mix (Fermentas K0171 which contains TaqDNA polymerase in reaction buffer, MgCl2 and dNTPs), 1 μl of 10 μM of each of the forward and reverse RHAMM specific primers and 1 μl of 10 μM of each of the forward and reverse primers of β-actin. For standardization, expression of RHAMM was correlated with the expression of the house keeping gene β-actin. The primers for RHAMM: forward primer: 5>-CAG GTC ACC CAA AGG AGT CTC G-3>, reverse primer: 5>-CAA GCT CAT CCA GTG TTT GC-3>. For β-actin: forward primer: 5>-GCA TCG TGA TGG ACT CCG-3>, reverse primer: 5>-GCT GGA AGG TGG ACA GCG A-3> (Fermentas™ – Germany). PRC protocol for RHAMM was performed as described by (8)

The following thermocycler program was performed : initial denaturation at 95 oC for 1 minute, annealing at 60 0 C for 1 minute, and extension at 72 0 C for 1 minute. This was repeated for 36 cycles. The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide. The electrophoretic pattern was visualized under UV light then photographed using a Polaroid camera with a red orange filter. The sample was considered positive when a clear sharp band was observed at the specific molecular weight; 661 bp for β-Actin and 565 bp for RHAMM.
The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide. The electrophoretic pattern was visualized under UV light then photographed using a Polaroid camera with a red orange filter. The sample was considered positive when a clear sharp band was observed at the specific molecular weight; 661 bp for β-Actin ,565 bp for RHAMM

Bcr-abl/abl ratio
Bcr-abl/abl ratio was done by RT- PCR followed by densitometric analysis of the products and ratio calculation. The density of the amplified product of different samples was assessed by densitometric documentation program (version 0.3).

For bcr-abl the following primers were used: forward primer: 5> TTC AGA AGC TTC TCC CTG ACA T,and reversed primer 5> CGG CTC TCG GAG GAG ACG TAG A(10). As for abl ,the following primers were used: forward primer-5> GTC TGA GTG AAG CCG CTC GT, and reverse primer 5>GGC CAC AAA ATC ATA CAG TGC A; reactions had place in the same tube, where abl served also as internal contol for the reaction. The following thermocycler program was followed: initial denaturation at 95 oC for 1 minute, annealing at 64 0C for 10 second, and extension at 72 0 C for 26 second. This was repeated for 45 cycles (11)

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 14). Group comparison was done using analysis of variance (ANOVA 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 less than 0.05 indicated a significant result.

Results

The current study included 60 CML patients divided into 2 distinct groups according to criteria described by(12).

Group A : 44 chronic CML patients and group B: accelerated /blastic crisis patients as well as 15 healthy individual as control group.

In group A : 14 /44 patients(31.8%) showed positive RHAMM expression by conventional RT PCR, while no RHAMM expression was detected in the peripheral blood mononuclear cells of the control group. Comparison between RHAMM positive and negative patients regarding their clinical and laboratory findings is presented in Table (1). There were no statistically significant differences between RHAMM positive and negative patients as regard their clinical data. As regard the laboratory data only the percent of peripheral blood basophils and the bcr-abl/abl ratio were significantly higher in RHAMM positive chronic CML patients with a p value 0.024 and 0.001 respectively.

Table 1: Comparison between RHAMM positive and negative chronic CML patients regarding their clinical and laboratory findings.

While in group B (accelerated/blastic crisis phase) 15/16 (93,7% of cases) showed positive RHAMM expression by conventional RT-PCR.

Table 2: shows the summary of clinical and laboratoy data of the 16 cases studied in group B.

Figure (1): RHAMM gene expression by RT-PC.

Discussion

The advent of tyrosine kinase inhibitors (TKIs) started a new era in the management of chronic myeloid leukemia (CML). Imatinib, the first TKI to be approved for the treatment of CML and the current standard first-line therapy, has significantly improved the prognosis of patients with CML. Nevertheless, a minority of patients in chronic-phase CML and even more patients with advanced-phase disease demonstrate resistance to imatinib or develop resistance during treatment leading to inevitable disease progression. In 40% to 50% of cases, this is attributed to the development of mutations that impair the ability of imatinib to bind to and inhibit the constitutively active Bcr-Abl kinase. Consequently, researchers have developed novel, strategies that can overcome not only Bcr-Abl-dependent mechanisms of resistance, but also those that are Bcr-Abl-independent, suh as targeted immunotherapy (13)

Targeted immunotherapies require the identification and characterization of appropriate antigen structures. Initially, T-cell based cancer vaccines were designed for patients with solid tumors after the definition of suitable tumor-associated antigens. Several immunological and even clinical responses prompted researchers and clinicians to extend the spectrum of cancer vaccines towards hematologic malignancies such as acute and chronic myeloid leukemia. The graft-versus-leukemia (GVL) effect observed after allogeneic stem cell transplantation and donor lymphocyte infusions strongly suggests that T lymphocytes play a major role in the rejection of leukemic cells. Therefore, immunotherapy directed against leukemia associated antigens might elicit specific immune responses that could eliminate minimal residual disease after chemotherapy, or enhance the GVL effect after hematopoietic stem cell transplantation. (14)

Peptide-based vaccines are able to induce cytotoxic T-lymphocyte responses that kill leukaemia cells. Based on this , pilot clinical trials have been initiated in chronic and acute myeloid leukaemia and other haematological malignancies, which include vaccination of patients with synthetic peptides derived from these LAAs. Results from these trials show that peptide vaccines are able to induce immune responses that are associated with clinical benefit. These early clinical results are promising and provide valuable information for future improvement of the vaccines.(15).

An ideal LAA that qualifies as a potential target for immunotherapies should be expressed preferentially in leukemic blasts, but neither on haematopoietic stem cells nor on normal tissues (16). Several leukemia-associated antigens (LAA) have been identified in patients with chronic myeloid leukemia as BAGE, BCL-2, , PRAME, proteinase 3, RHAMM, and WT-1 (5). RHAMM is an antigen eliciting both humoral and cellular immune responses in patients with AML, CML , myelodysplastic syndrome and multiple myeloma (17).

In the present study we focused on studying the RHAMM expression by conventional RT-PCR in peripheral blood mononuclear cells of 44 chronic myeloid leukemia patients and 16 blastic/acceleratic phase patients as well as 15 healthy normal volunteers as a control group.

All the control subjects were negative for RHAMM . This is in agreement with Greiner et al. (6,8,14,16,18).

In the chronic phase patients group(group A) ,RHAMM expression was detected in 14/44 patients (31.8%)this is agreement with Greiner et al (8,16),and GREINER AND SCHMITT 2008 (5)who found 38- 50%RHAMM expression in CML patients by RT-PCR and a similar pattern was detected when serologic response to RHAMM epitope R3 was found in 31% f CML patients . However schmitt et al (10) found in a series of 34 CML 28/34 (83%) RHAMM expression in chronic CML patients..

Comparing RHAMM Positive to RHAMM negative chronic CML patient ,RHAMM positive patients showed a significant higher peripheral blood basophilia and a highly significant higher bcr-abl /abl ratio .this is in agreement with Schmitt et al (10) who found prevelant RHAMM expression with high bcr-abl/abl ratio and no RHAMM expression in patient with complete molecular remission.

As regard the accelerated/blastic phase,15/16(93.7%) showed positive RHAMM

expression ,this was in accordance with Schmit et al (10)and Greiner and Schmitt (5) who demonstrated 100% RHAMM expression in accelerated/blastic phase . It is noteworthy that patient expressing LAA on their leukemic blasts had better therapy outcome and expression of theses LAA on lekeumic progenitors yield better post-tranplantation outcome in CML patients (19).

For sufficient presentation of peptides inducing Specific T Cells, MHC molecules and costimulatory molecules are necessary CD34+cml expressed HLA-ABC and HA- DR but they lack all the costimulatory molecules, this might be essential for the process of tumor escape of CML cells especially the progenitor CML cells.

After donor lymphocyte infusion(DLI), the donor derived T cell clones recogninse mature monocytes and myeloid cells but not immature progenitors of the bone marrow. Therefore DLI seem to be dependent on specific lysis of progenitor cells by specific cytotoxic T cells, however a specific CD8+T cell response against RHAMM derived R3 peptide in CML patients and a specific lysis of RHAMM expressing leukemic cells was detected by(5).

Many of the identified LAAs are now used for vaccinations, in many clinical trial worldwide whether as peptide derived antigens or vaccination using dendretic cells to induce specific immune response against LAAs in CML and other myeloid leukemias (20) . Immunologic and clinical response had proved to be very promising, in reaching complete remission, maintaining major genetic remission, or at least prevention of disease progression. (5).

Vaccination strategies inducing effector T cells against bcr-abl , rhamm and other LAAs expressed in CML are promising approach to enhance specific immune responses against CML.

Our work suggest the concomitant use of RHAMM R3 peptide vaccination with conventional CML therapy due to its high prevalence especially in accelerated phase ,in order to achieve complete molecular remission of our patient.

References

1. Fausel C. Targeted chronic myeloid leukemia therapy: seeking a cure. J Manag Care Pharm. Oct;13(8 Suppl A):8-12.2007.

2. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML.. Blood. Dec 15;112(13):4808-17.2008.

3. Gora-Tybor J, Robak T. Targeted drugs in chronic myeloid leukemia. Curr Med Chem.;15(29):3036-51.2008.

4. Melo JV, Chuah C. Novel Agents in CML Therapy: Tyrosine Kinase Inhibitors and BeyondHematology Am Soc Hematol Educ Program.:427-35. 2008.

5. Greiner J and Schmitt M. Leukemia-Associated Antigens (Laas) As Target Structures For A Specific Immunotherapy In Chronic Myeloid Leukemia (CML).: Eur J Haematol. Feb 12 .2008

6. Jochen Greiner, Li Li, Mark Ringhoffer, Thomas F. E. Barth, Krzysztof Giannopoulos, Phillipe Guillaume, Gerd Ritter, Markus Wiesneth, Hartmut Döhner, and Michael Schmitt l. Identification and characterization of epitopes of the receptor for hyaluronic acid-mediated motility (RHAMM/ CD168) recognized by CD8_ T cells of HLA-A2-positive patients with acute myeloid leukemia. Blood;106:938-945.2005.

7. Sohr S and Engeland K. RHAMM is differentially expressed in the cell cycle and downregulated by the tumor suppressor p53Cell Cycle. 2008 Nov 1;7(21):3448-60. 2008.

8. Greiner J, Ringhoffer M, Taniguchi M, et. al. RHAMM is a new immunogenic tumor-associated antigen overexpressed in acute and chronic myeloid leukemia. Exp Hematol. 30: 1029-1035.2002.

9. Giles F.J, Keating A, Goldstone A.H, Avivi I, Willman C.L, Kantarjian H.M: Acute myeloid leukemia. Hematology (Am Soc Hematol Educ Program), 73-110. 2003.

10. Schmitt M, Li L, Giannopoulos K, Chen J, Brunner C, Barth T, Schmitt A, Wiesneth M, Döhner K, Döhner H, Greiner J. Chronic myeloid leukemia cells express tumor-associated antigens eliciting specific CD8+ T-cell responses and are lacking costimulatory molecules. Exp Hematol. Dec;34(12):1709-1.2006.

11. Hagop M. Kantarjian,1 Moshe Talpaz,Jorge Cortes, Susan O'Brien, Stefan Faderl,Deborah Thomas, Francis Giles, Mary Beth Rios, Jianqin Shan, and Ralph Arlinghaus. Quantitative Polymerase Chain Reaction Monitoring of BCR-ABLduring Therapy with Imatinib Mesylate (STI571; Gleevec) in Chronic-Phase Chronic Myelogenous Leukemia Clinical Cancer Research Vol. 9, 160–166, January 2003.

12. Richard S,Larson And Steven N.Wolff. chronic myeloid leukemia, wintrobe's clinical haematology 10 th edition p2342-2373,Williams and wilkins 1999.

13. Jabbour E, Cortes J, O>Brien S, Giles F, Kantarjian H. New targeted therapies for chronic myelogenous leukemia: opportunities to overcome imatinib resistance. Semin Hematol. Jan;44(1 Suppl 1):S25-31.2007.

14. Greiner J, Döhner H, Schmitt M. : Cancer vaccines for patients with acute myeloid leukemia--definition of leukemia-associated antigens and current clinical protocols targeting these antigens. Haematologica. Dec;91(12):1653-61.2006.

15. Dao T and Scheinberg D.A. : Peptide vaccines for myeloid leukaemias. Best Pract Res Clin Haematol. Sep;21(3):391-404.2008.

16. Jochen Greiner, Mark Ringhoffer, Masanori Taniguchi, Thomas Hauser, Anita Schmitt, Hartmut Dohner and Michael Schmitt : Characterization of several leukemia associated antigens inducing humoral immune response in acute and chronic leukemia. Int J.Cancer: 106, 224-231.2003.

17. Schmitt M, Schmitt A, Rojewski M.T, Chen J, Giannopoulos K, Fei F, Yu Y, Götz M, Heyduk M, Ritter G, Speiser D.E, Gnjatic S, Guillaume P, Ringhoffer M, Schlenk R.F, Liebisch P, Bunjes D, Shiku H, Dohner H, Greiner J. RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. Blood:. Feb 1;111(3):1357-65.2008.

18. Jochen Greiner, Mark Ringhoffer, Masanori Taniguchi, Li Li, Anita Schmitt, Hiroshi Shiku, Hartmut D¨Ohner and Michael Schmitt : mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the developmentof specific immunotherapies. Int. J. Cancer: 108, 704–711.2004.

19. Yong AS, Rezvani K, Savani BN, Eniafe R, Mielke S, Goldman JM, Barrett AJ. High PR3 or ELA2 expression by CD34+ cells in advanced-phase chronic myeloid leukemia is associated with improved outcome following allogeneic stem cell transplantation and may improve PR1 peptide-driven graft-versus-leukemia effectsBlood. Jul 15;110(2):770-5.2007.

20. van de Loosdrecht A.A, van den Ancker W, Houtenbos I, Ossenkoppele G.J, Westers T.M : Dendritic cell-based immunotherapy in myeloid leukaemia: translating fundamental mechanisms into clinical applications. Handb Exp Pharmacol. ;(188):319-48.2009

Tables

Table 1: Comparison between RHAMM positive and negative chronic CML patients regarding their clinical and laboratory findings.

		RHAMM POSITIVE (n=14)	RHAMM NEGATIVE (n =30)	P value
Clinical Data	Age (years)	42.57+12.35	42.27±10.10	0.952
	Sex:			
	male	8(57.1%)	12(40%)	0.476
	Female	6(42.9%)	18(60%)	
	Splenomegaly:			0.949
	absent	2 (14.3%)	4(13.3%)	
	present	12 (75.7%)	26(86.7%)	
	Hepatomegaly:			0.272
	Present	6(42.9%)	6(20%)	
	Absent	8(57.1%)	24(80%)	
	Anaemic manifestations:			1
	Present	2(14.3%)	4(13.3%)	
	absent	12(85.7%)	26(86.7%)	
	Hb (g/dl)	11.2 ± 1.35	11.70±1.63	0.523
	WBC (x103/cm3)	168.98±85.06	139.08± 79.66	0.431
	Platelet (x103/cm3)	210.71±91.73	248.93±89.00	0.364
	PB basophils (%)	7.71±5.02	4.26±1.66	0.024
	PB blasts (%)	1.35±4.14	2.66±2.28	0.132
	Ph chromosome:			0.122
	Present	12(85.7%)	30(100%)	
	absent	2(14.3%)	0(0%)	
	Lap score			0.823
		10(71.4%)	20(66.7%)	
	Bcr-abl/abl ratio	4(28.6%)	10(33,3%)	0.001

Table 2: shows the summary of clinical and laboratoy data of the 16 cases studied in group B.

		CML patient (Number = 16)	
Clinical data	Age (years) (range, mean ± SD) 30-58,mean39.88±8.408		
	Sex (M/F) (number-%)	Males= 10 / 16 (62.5%) Females= 6/ 16 (37.5%)	
	Anaemic manifestations(number -%)	Absent	6/16 (37.5%)
		Present	10/16 (62.5%)
	Hepatomegaly (number -%)	Absent	12/16 (75%)
		Present	4/16(25%)
	Splenomegaly (number -%)	Absent	0/16(0%)
		mild	4/16(25%)
		huge	12/16 (75%)
laboratory data	Haemoglobin level (gm/dl) (range, mean ± SD)	7.2-11(9.26±1.5)	
	Total leucocytic count (X10³/cmm) (range, mean ± SD)	72.6-525 (191.77± 143.49)	
	Platelet Count (X10³/cmm) (range, mean ± SD)	55-117(97.62 ± 18.59)	
	Peripheral blood blasts (%) (Range, mean ± SD)	4-22% (15.87 ± 6.10)	
	Peripheral blood basophils (%) (Range, mean ± SD)	4-16% (9.62 ± 4.03)	
	Philadelphia chromosome (Number - %)	Absent	3-16(18.7%)
		Present	13/16(81.2%)
	Lap score	2-8(4.25±1.98)	
	Bcr-abl/abl ratio (Range, mean ± SD)	0.0-3.8(2.33±1.18)	
	RHAMM expression (Number - %)	Negative	1/16(6.25%)
		Positive =	15/16(93.75%)

Figures

Lane; 1 2 3 4 5 6 7 8

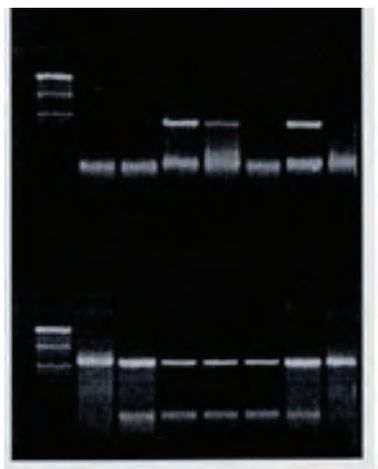

Fig 1. RHAMM gene expression by RT-PC.

- Upper panel: Amplification with RHAMM specific primers showing 565bp PCR product. Lane 4,5,7 show +ve expression, Lane 2,3,6,8 show negative expression.
- Lower panel: the same RNA as in lanes given above amplified with β-actin specific primers (661 bp) serves as an internal control for the quality of RNA.
- Lane 1; 100- 1500 bp ladder size marker.