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Study of Copy Number Variants as A Prognostic Marker In Chronic Myeloid Leukaemia

Dr. Sachin Bharti[1], Dr. Nikesh Kumar[2], Dr. Amit Kumar[3]
1.Assistant prof. Department of General medicine, KMC Katihar
2.Senior Resident, Department of General medicine, AIIMS, Patna
3. Senior Resident, Department of General medicine, AIIMS, Patna
Corresponding Author- Dr. Sachin Bharti

Abstract
Background*: Chronic Myeloid Leukaemia is the most common adult leukaemia in the Indianpopulation and is one of the most extensively studied human malignancy. The discovery of tyrosine kinase inhibitors - the first rationally designed drug in human history – at the beginning of 21st century dramatically changed CML landscape. Thanks to this wonder drug, our patients now can have nearly normal life expectancy. However response to TKIs is highly variable among population.*
Methodology*: The study was done on bone marrow samples of patients who are newly diagnosed or on follow up at Katihar medical college katihar. Study duration period of Two years. The samples are collected when they come for diagnosis or when clinically indicated. DNA is isolated from these samples and fold change of genes of samples compared to healthy control is determined using Real Time PCR technique calculated by comparative Ct method.*
Conclusion*: In the era of Tyrosine Kinase Inhibitors the role of pre treatment clinical staging like EUTO score has lost it's clinical significance. Treatment response based indicators are the reliable prognostic tools at present to predict response which needs a minimum of 3 months of assessment, during which patient can clinically worsen. In our novel study we identify two genes APO1.11 and PRDM12 , the fold change of which can predict the response to therapy.*
Keywords*; CML, PRDM12, Tyrosine kinase Inhibitors.*

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I. Introduction

In 1845, Edinburgh pathologist John Hughes Bennett reported a "Case of Hypertrophy of the Spleen and Liver in which Death Took Place from Suppuration of the Blood" in the *Edinburgh Medical Journal.* Only a few weeks later, Rudolf Virchow in Berlin published a very similar case. Although one cannot know for sure, these two patients probably represent the first descriptions of the disease that later became known as chronic myeloid leukemia (CML). While Bennet thought that the patient had an infection, Virchow suspected a neoplastic disorder that he soon called white blood disease or leukemia. In 1872, Ernst Neumann observed that leukemiacells originated in the bone marrow. The next decades saw the differentiation into myeloid versus lymphoid and acute versus chronic leukemias. A real quantum leap, however, was the discovery by Philadelphia cytogeneticists Peter Nowel and David Hungerford of an abnormally small G-group chromosome thatwe now call the Philadelphia chromosome (Ph). This was a seminal step, as it unequivocally proved that cancer was a problem of DNA. Thirteen years later Dr Janet Rowley recognized that Ph was the product of a reciprocal translocation between chromosomes 9 and 22. In the 1980s, the translocation partners were identified as BCR and ABL, followed by the discovery that unregulated tyrosine kinase activity is critical to BCR-ABL's ability to transform cells. A faithful murine disease model was established in 1990. the Indian Ramayana more than 2000 years earlier. In the 1920s, splenic irradiation was introduced for symptomatic relief. Effective control of blood counts became feasible with busulfan (1959), followed 10 years later when the better-tolerated hydroxyurea became available, probably the first intervention with a (modest) prolongation of survival. A breakthrough was achieved in the mid-1970s when the Seattle group reported the disappearance of the Ph chromosome in CML patients who underwent allotransplant, the first cures of CML. Soon later, interferon-α was found to induce durable complete cytogenetic responses and long-term survival, although in only a small fraction of patients. In 1992, Alexander Levitzki proposed the use of ABL inhibitors to treat leukemias driven by ABL oncogenes. At about the same time, scientists at Ciba-Geigy had synthesized a potent inhibitor of ABL that was termed GCP57148B and is now known as Imatinib. Clinical trials initiated by Brian Druker, much against the skepticism of the manufacturer, rapidly established the compound's activity in patients with CML and

revolutionized CML therapy. In 2008, the majority of patients diagnosed with chronic phase CML can expect to have durable responses with good quality of life. For that 20% to 30% who fail Imatinib, second-line inhibitors are an effective salvage strategy. However, once the disease has progressed beyond the chronic phase, allotransplant is still therecommended treatment for all eligible patients. CML has served as a paradigm for cancer therapy, and it is likely that it will continue to be the case as we start to transform profound responses into definitive 'cures'

II. Objectives

The aim of the project is to assess prognostic significance of Copy number variations of 2 specific genes APO1.11 and PRDM12 in CML patients.

III. Review of Literature

Chronic myeloid leukemia (CML) is probably the most extensively studied human malignancy. The discovery of the Philadelphia (Ph) chromosome in 1960 as the first consistent chromosomal abnormality associated with a specific type of leukemia wasa breakthrough in cancer biology. It took 13 years before it was appreciated that the Ph chromosome is the result of a t(9;22) reciprocal chromosomal translocation(6) and another 10 years before the translocation was shown to involve the *ABL*proto- oncogene normally on chromosome 9 and a previously unknown gene on chromosome 22, later termed *BCR* for breakpoint cluster region. The deregulated Abl tyrosine kinase activity was then defined as the pathogenetic principle, and the first animal models were developed. The end of the millennium sees all this knowledge transferred from the bench to the bedside with the arrival of Abl-specific tyrosine kinase inhibitors that selectively inhibit the growth of *BCR-ABL*–positive cells in vitroand in vivo. Essential features of the Bcr-Abl protein, Mutational analysis identified several features in the chimeric protein that are essential for cellular transformation**.** Deregulation of the Abl tyrosine kinase Abl tyrosine kinase activity is tightly regulated under physiologic conditions. The SH3 domain appears to play a critical role in this inhibitory process because its deletion or positional alteration activates the kinase. On exposure of cells to oxidative stress such as ionizing radiation, this small protein is oxidized and dissociates from Abl, whose kinase is in turn activated. Activated signaling pathways and biologic properties of BCR-ABL–positive cells Three major mechanisms have been implicated in the malignant transformationby *Bcr-Abl*,namely altered adhesionto stroma cells and extracellular matrix, constitutively active mitogenic signaling and reduced apoptosis. A fourthpossible mechanism is the recently described proteasome-mediated degradation of Abl inhibitory proteins. Jak-Stat pathway: In contrast to the activation of the Jak-Stat pathway by physiologic stimuli, Bcr-Abl may directly activate Stat1 and Stat5 without prior phosphorylation of Jak proteins. PI3 kinase pathway: PI3 kinase activity is required for the proliferation of BCR- ABL–positive cells. Myc pathway: Overexpression of Myc has been demonstrated in many human malignancies. Inhibition of apoptosis. Degradation of inhibitory proteins: The recent discovery that Bcr-Abl induces the proteasome-mediated degradation of Abi-1 and Abi-2,proteins with inhibitory function, may be the first indication of yet another way by which Bcr-Abl induces cellular transformation, About 30 to 50% of patients with CML diagnosed in the United States are asymptomatic. The disease is found on routine physical examination or blood tests. CML can be classified into three disease phases: chronic phase (CP), acceleratedphase (AP), and blast phase (BP). Common signs and symptoms of CML in CP, whenpresent, result from anemia and splenomegaly. These include fatigue, weight loss, malaise, easy satiety, and left upper quadrant fullness or pain. Rare manifestations include bleeding (associated with a low platelet count and/or platelet dysfunction), thrombosis (associated with thrombocytosis and/or marked leukocytosis), gouty arthritis (from elevated uric acid levels), priapism (usually with marked leukocytosis or thrombocytosis), retinal hemorrhages, and upper gastrointestinal ulceration and bleeding (from elevated histamine levels due to basophilia). Leukostatic symptoms (dyspnea, drowsiness, loss of coordination, and confusion). Splenomegaly is the most consistent physical sign in CML, and is detected in 50–60% of cases. Hepatomegalyis less common (10–20%). Lymphadenopathy and infiltration of skin or other tissues are uncommon. When present, they favor Ph-negative CML or AP or BP of CML. Patients with accelerated or blastic phase CML may receive initial therapy with TKIs to reduce the CML burden, and be considered for early alloSCT. Response rates with combinations of TKIs and chemotherapy are 40% in nonlymphoid BP CML and 70– 80% in lymphoid BP CML. At present, *alloSCT* is the only curative therapy for accelerated and BP CML. Patients with cytogenetic clonal evolution as the only AP criterion have a long-term event-free survival rate. A limitation of fluorescent in situ hybridisation (FISH) methodology is that it cannot detect smaller micro-deletions. Quantitative detection of duplications and deletions can be done by using Comparative Genomic Hybridisation techniques and Single Nucleotide Polymorphism genotype arrays have very low resolutions in order to detect microdeletions and microduplications.

IV. Material And Methods

A prospective study, Total 42 patients were included in our study, All patients with CML who are newly diagnosed or on treatment, followed upfor a minimum of at least 3 months at KMC Katihar. The study was done on bone marrow samples of patients who are newly diagnosed or on follow up at Katihar medical college katihar, Bihar. Study duration period of Two years. The samples are collected when they come for diagnosis or when clinically indicated. DNA is isolated from these samples and fold change of genes of samples compared to healthy control is determined using Real Time PCR technique calculated by comparative Ct method.

Inclusion criteria

CML patients newly diagnosed or in chronic/ accelerated / blast crisis phase .Given informed consent for study.

Exclusion criteria

Other active malignancies.

On irregular treatment.

COLLECTION OF SAMPLES

42 new and previously diagnosed CML patients were included in the study, Informed consent of patients taken, in accordance with the declaration of Helsinki.The study was approved by the institutional ethical committee, The bone marrow samples of the study population was taken when they come for diagnosis and when clinically indicated. DNA is an extremely stable molecule with a half life of more than 500yrs, provided it is protected from nucleases, as in cell nucleus. In DNA isolation we use many buffers to protect from nucleases, precipitated out in 70% ethanol. The isolated DNA was suspended in 20µl of nuclease free water and stored at -30 °C.300Ml whole blood was taken in a 1.5ml eppendroff and 900µl of 1 x RBC lysisbuffer was added and mixed well by inverting the tubes.

The tubes were incubated at 4°C (on ice) for 10-15 min, and inverted occasionally. Tubes were centrifuged at 1400 rpm for 20 sec at room temperature. The supernatantobtained was discarded.

Reagent to be added	Volume of the reagent
Forward primer	0.5µl
Reverse primer	0.5µl
Nuclease free water	3µl
DNA	1µl
2X SYBR Green master mix	5µl
Total volume	10µl

Each of these reactions were set up in triplicate along with the Negative control (devoid of template) for each gene. GAPDH used was used as the endogenous control. The patients were followed up for variable periods, with a minimum of 3 months anda maximum of 2 yrs depending on the time of enrollment into study.

V. Results

COMORBIDITY	NO. OF PATIENTS	PATIENT	OTHER MEDICATIONS
Hypertension	3	P6,P12,P33	Losartan,AtenololAmlodipine.
Diabetes mellitus	2	P9,P12	insulin
Hypothyroidism	4	P13,P33, P40,P42	Thyroxin
Bronchial asthma	2	P2,P14	Beta2 agonist
Colloid goitre	1	P37	-
Anaemia	1	P8	-
Old Ca cervix	1	P14	-
Deep vein thrombosis	1	P30	Off anticoagulants
Seizure disorder	1	P30	phenytoin
Myelofibrosis	1	p2	-
C4-6 discectomy	1	p5	-
TKI induced NSIP	1	P10	-
Pancytopenia	1	P22	-

COMPARISON OF SOKAL ,EUTOS AND EURO SCORING WITHCLINICAL RESPONSE

CLINICAL STAGING	RESPONDER		NON RESPONDER		CLINICAL RESPONDERVs NON RESPONDER (P) *
	MEDIAN	STD. DEVIATION	MEDIAN	STD. DEVIATI N	
SOKALSCORE	0.210	0.315	0.1900	0.2263	0.990
EURO SCORE	60	223.8	60	323.85	0.791
EUTO SCORE	0.79	0.184	0.76	0.3194	0.120

DURATION OF FOLLOW UP IN PREVIOUSLY UNTREATEDPATIENTS

	MONTHS
MEDIAN	10.4375
STD DEVIATION	5.32877
TOTAL	16

MONOTHERAPY

THERAPY	NUMBER
IMATINIB	33
NILOTINIB	1
TOTAL	34

CHR and molecular response in previously untreated patients

PREVIOUSLY UNTREATED PATIENTS 26*	CHR 26	RESPONDERS 15
		NON RESPONDERS 11
	MOLECULAR RESPONSE 17	RESPONDERS 9
		NON RESPONDERS 8

One patient is BCR ABL negative CML,Molecular response could be assessed in 18 newly diagnosed and 13 previously diagnosed patients. CHR – complete haematological response APO 1.11 was studied in 41 patients. PRDM12 was studied in 37 patients.

FOLD CHANGE IN GENES AND MR-PRDM12

	Attained MR	Not attained MR
MEDIAN	0.8902	0.4204
STD. DEVIATION	1.079	1.779
TOTAL	1	

COMPARISON BETWEEN MOLECULAR NONRESPONDER ANDRESPONDER

Analysis of previously treated patients for fold change in genes between molecular responders and non responders is not significant for APO1.11 gene(P 0.348) Analysis of previously treated patients for fold change in genes between molecular responders and non responders is not significant for PRDM gene (P0.398)

VI. Discussion

A total of 42 patients was grouped into previously diagnosed and newly diagnosed patients. APO1.11 analysis was done on 41 patients and PRDM12 analysis done on 37patients. Test for significance was done to find association between fold change of these genesand treatment response. Test for significance by Mann Whitney U test is significant for APO1.11(P <0.001) and not significant for PRDM12(P 0.104) between responders and non responders for fold change in genes on follow up. This indicates that less the fold change of APO1.11 gene at the time of diagnosis, the patients tends to respond poorly to therapyon follow up in

terms of complete haematologic remission.

Molecular response based on BCR ABL transcript levels

Test for significance by Mann Whitney U test is not significant for APO1.11(P 0.139) and PRDM12(P 0.724) between responders and non responders for fold change in genes on follow up. This indicates that the fold change of APO1.11 and PRDM12 gene at the time of diagnosis has no significant association with response to therapy on follow up in terms of molecular remission. Test for significance by Mann Whitney U test is not significant for APO1.11(P 0.348) and PRDM12(P 0.398) between molecular responders and non responders at the time of assessment. **Comparison of fold change of genes in previously treated and untreated patients** Comparison of mean fold change in genes between previously treated and untreated patients shows a relatively less fold change of genes in non responders compared toresponders(P 0.997 for APO1.11 and P0.028 for PRDM12 by student T test) and themean fold change of genes is low in previously untreated patients who on follow upwere poor responders, comparable to previously treated patients who had poorresponse at assessment.

Assuming that the fold change of APO.11 and PRDM 12 does not change with treatment, we can infer that the fold change at beginning of treatment predicts subsequent treatment response .The mean haemoglobin levels, total count and platelet count had no significant association with treatment response(P value 0.407,0.642,0.642 respectively). Anaemia is a major presenting sign in most patients at time of diagnosis (p 0.00). There is no significant difference between males and females in clinical response (P0.724). The clinical stage scoring systems at time of diagnosis had no significant association with subsequent treatment response (SOKAL score -P 0.990,EURO score-P 0.791, EUTOS score -P 0.120).

VII. Conclusion

For previously untreated(newly diagnosed) patients, test for significance by Mann Whitney U test for fold change in genes and subsequent treatment response by CHR is significant for APO1.11(P 0.000). Comparison of mean fold change in genes between previously treated and untreated patients shows that the fold change of PRDM 12 at beginning of treatment predicts subsequent treatment response by molecular response (p0.028).

References

[1]. Goldman JM. Chronic myeloid leukemia: a historical perspective. SeminHematol. 2010 Oct;47(4):302–11.
[2]. WHO | Cancer [Internet]. WHO. 2015 [cited 2015 Jul 5]. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/
[3]. Höglund M, Sandin F, Simonsson B. Epidemiology of chronic myeloid leukaemia: an update. Ann Hematol. 2015 Apr;94 Suppl 2:S241–7.
[4]. Agarwal MB. Importance of early and deeper responses to long-term survival in CML patients: Implications of BCR-ABL testing in management of CML in Indian setting. Indian J Med Paediatr Oncol Off J Indian Soc Med Paediatr Oncol. 2014;35(1):10–6.
[5]. Bansal S, Prabhash K, Parikh P. Chronic myeloid leukemia data from India.
[6]. Indian J Med Paediatr Oncol Off J Indian Soc Med Paediatr Oncol.2013;34(3):154–8.
[7]. Rowley JD. A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and GiemsaStaining. Nature. 1973 Jun 1;243(5405):290–3.
[8]. Pratibha S Amare, Cytogenetics Laboratory, Tata Memorial Hospital, Parel,Mumbai - 400012, Indi. Chronic Myeloid Leukemia-cytogenetics and moleculargenetics.
[9]. Cilloni D, Saglio G. Molecular Pathways: BCR-ABL. Clin Cancer Res. 2012 Feb 15;18(4):930–7.
[10]. Deininger MWN, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood. 2000 Nov 15;96(10):3343–56.
[11]. Rohon P. Biological therapy and the immune system in patients with chronic myeloid leukemia. Int J Hematol. 2012 Jul;96(1):1–9.
[12]. Talpaz M, Kantarjian HM, McCredie KB, Keating MJ, Trujillo J, Gutterman J. Clinical investigation of human alpha interferon in chronic myelogenous leukemia. Blood. 1987 May;69(5):1280–8.
[13]. Chronic Myelogenous Leukemia: Update on Biology and Treatment: Page 3 of 3 Cancer Network Dr.K.Pavithran, MD,DM, Mathew Thomas, MD,Department of Haematology, Medical College Hospital,, Trivandrum-695011. TREATMENT OF CHRONIC MYELOID LEUKEMIA - AN UPDATE.
[14]. Chronic Myelogenous Leukemia (CML) Guidelines: Management of Chronic Myelogenous Leukemia (CML). 2015 Aug 26 [cited 2015 Sep 15]; Available from:
[15]. TP, Hochhaus A, Branford S, Müller MC, Kaeda JS, Foroni L, et al.Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the International Randomized Study of Interferon and STI571 (IRIS). Blood. 2010 Nov 11;116(19):3758–65.
[16]. Total DNA isolation protocol,Primer Digital,2015.
[17]. Absolute vs. Relative Quantification for qPCR [Internet]. [cited 2015 Oct 7]. www.thermofisher.com/in/en/home/life-science/pcr/real-time-pcr/qpcr-education/absolute-vs-relative-quantification-for-qpcr.html
[18]. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CTmethod. Nat Protoc. 2008 Jun;3(6):1101–8.
[19]. Four tips for RT-qPCR data normalization using reference genes Biogazelle.[cited2015Oct7]