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Association of BCR-ABL transcript variants with different blood parameters and demographic Characteristics of chronic myeloid leukemia patients in a tertiary care center of North-East India.

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ABSTRACT

Chronic Myeloid Leukaemia (CML) is distinguished by the existence of *BCR-ABL* transcript gene possess its common variant subtype B3A2, B2A2 among population.The objective of present investigation was to find the different variants of *BCR-ABL* subtypes and their influence on demographic and haematological characteristics in the CML patients attending in the OPD of Assam Medical College & Hospital, Dibrugarh, Assam, India.*BCR-ABL* positive patients numbering 218 were enrolled for this investigation. RNA was extracted and complementary DNA was prepared with the aid of reverse transcriptase polymerase chain reaction method. To identify the *BCR-ABL* transcript type of the patients, a qualitative polymerase chain reaction was carried out with one specific primer. Out of 218 CML positive cases, 143 (65.60%) patients were found to express B3A2 transcript, whereas 74 (33.94%) cases were found to be positive for B2A2 variant and the remaining 1 (0.46%) case possess both subtypes of RNA B3A2/B2A2. In this study the B3A2 and B2A2 ratio was recorded as 1.93: 1 (approx. 2:1) which greatly differs from global ratio (3:1) irrespective of gender. The B3A2 variant was more common among the age group of 41-50 while B2A2 was common in 31-40 age (p=0.043). Upon analysing different patients parameters, pain in abdomen, joint pain/ body ache, fever was found to be significantly associated with disease condition (p=0.041<0.05). The B3A2 subtype showed higher level of total haemoglobin and WBC count than B2A2 subgroup. The diverse type of *BCR-ABL* transcript is reflected by WBC and platelet counts at diagnosis, which is a distinct phenotype and disease biology. In this study, occupational data showed majority of the CML positive patients from agriculture and tea tribe community and harbouring both type of transcripts. Further analysis of different life style factors showed close association with the drinking and smoking habits (p=0.001). B3A2 and B2A2 ratio (1.93: 1) was not identical with global ratio (3:1). The B3A2 variants is common in 41-50 age group and B2A2 in 31-40 age (p=0.043). Agriculture and tea tribe occupation has maximum numbers of positivity consuming alcohol and tobacco (p=0.001). Among the nine demographic parameters constituting a total of 27 sub-indices, 10 indices have significant correlation (p>0.05). Prevalence of B3A2 transcript was more abundant among male patients revealing gender skewed distribution.

KEYWORDS: CML, BCR-ABL, qPCR qualitaive, RNA, haematological parameters.

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INTRODUCTION

CML is a type of neoplasm of white blood cells, characterized by unregulated growth, predominantly myeloid cells in bone marrow, and the accumulation of these cells in the blood[1]. CML represents 14% of all cases of leukemia (Leukemia and Lymphoma Society, 2004). CML occurs rarely in children which makes upto 10%, in adults represents about 20% of all leukemia[1]. It accounts for 50-70% of leukemia in India with an annual incidence of 1-2 per lac population. The median age at presentation in India is 50 years[2]. A high prevalence of CML is observed in North East Assam because annualy, 20-30 CML cases are diagnosed in AMCH, Dibrugarh.

CML is characterized by the presence of the Ph chromosome, which results from a reciprocal translocation between chromosomes 9q34 and 22q11 regions[3]. This translocation fuses the breakpoint cluster region of BCR and ABL genes forming *BCR-ABL* oncogene in exon B2 or B3 at BCR gene and exon A2 of ABL gene to create B2A2 or B3A2[4]. Since the *BCR-ABL* kinase is active in >90% of CML cases[5], frequency of fusion oncogenes associated with leukemia can vary in different ethnic groups, which may have a significant influence on the management and prognosis of this type of malignant blood diseases.

Most of the patient suffering from CML are found to have an elevated level of neutrophil count. Even though a presumptive diagnosis can be made, based on clinical features and routine blood work, a definitive diagnosis of CML requires either the demonstration of the Philadelphia chromosome translocation, or BCR-ABL fusion gene (qRT-PCR) expression. Both the frequency of presence and impact of different transcripts of phenotypic characteristics among CML patients were varied and it is a conflicting issue[6].

CML is one of the most common diseases of adult hematopoietic tissues in North-East. Our study has been aimed with demographic and haematological characterization of *BCR-ABL* positive CML patients, to establishing a cost effective diagnostic method.

MATERIALS AND METHODS

The present investigation was conducted after due ethical clearance obtained from the AMCH, Dibrugarh. All essential clinical and laboratory epidemiological information was recorded.

Inclusion and exclusion criteria:

Only those CML patients who have voluntarily given the consent for participation in the study were taken for this study.

Procedure:-

Blood samples were collected in EDTA vial. RNA was isolated using QIAamp RNA Blood Mini Kit (QIAGEN). A Nano drop (MRC, Model: NANO-200) was used to estimate the purity and concentration of RNA. cDNA was prepared using *Verso cDNA Synthesis Kit,* (Thermoscientific, USA).

BCR-ABL fusion was determined qualitatively from cDNA using specific primers. BCR forward Primer (5´-GGAGCTGCAGATGCTGAC-3´) and ABL Reverse Primer (5´-TCAGACCCTGAGGCTCAA-3´). Along with one Housekeeping Primer (β-globulin), was also used to avoid the false negative results. PCR master mix was prepared using 2X master mix (Promega PCR Master Mix) mixing with 1 µg of cDNA, 10pM of primer and the 25 µl of final volume was adjusted with Nuclase free water.

PCR reactions were carried out for (Arktik Thermo-scientific) initial denaturation for 3 min at 95°C followed by 35 cycles of denaturation at 95°C for 45s, annealing at 57°C for 30s and extension at 72°C for 45s. The final elongation was at 72°C for 7 min. 2% Agarose gel were used for PCR visualize with gel documentation system (Science instruments, CLINX).

Statistical analysis

All the results were expressed from three parallel measurements and carried out SPSS version 19.0 (SPSS Inc., Chicago, USA)[7].

RESULTS

Table 1. Baseline demographic characteristics of the study group

Variable	Category	No. Of patients	Percentage of total
Gender	Male	144	66.06
	Female	74	33.94
Age	13-20	15	6.88
	21-30	42	19.27
	31-40	51	23.38
	41-50	64	29.35
	51-60	27	12.39
	61-70	17	7.81
	71-80	2	0.93
Educational level	Low	71	32.57
	Intermediate	85	38.99
	High	62	28.44
Residence	Rural	154	70.64
	Urban	64	29.36
Family History	No	218	100
	Yes	0	0
Occupation	Agriculture & Tea tribe	97	44.50
	Labours	53	24.31
	Others	68	31.19
Diet	Vegetarian	18	8.26
	Non-Veg	200	91.74
Habbit	Smoking	6	2.75
	Alcoholic	44	20.18
	Tobacco	49	22.48
	No Habits	119	54.59
Chief Problem	Pain Abdomen	68	31.19
	Joint pain/ BodyAche	70	32.11
	Fever	76	34.86
	Easy Fatigability	96	44.04
	Giddiness	55	25.23
	Decrease Appetite	25	11.47
	Early Satiety	31	14.22
	Cough	46	21.10
BCR-ABL Subtype	B3A2	143	66.20
	B2A2	74	33.34
	Both	1	0.46

The values are mean ± SD of three independent determinations at 5% level of significance. Data was analyzed using Analysis Of Variance (ANOVA).

Demographic characteristics interpretation of the population

The various epidemiological characteristics of total 218 positive cases have been presented with an average age of 67 years (13–80 years) 78.88% cases were younger than 50 years.

BCR-ABL positive CML occurred commonly in patients of age between 11-80 years. More than two/third of patients had either low or intermediate educational level, and resided in rural areas. Most of the CML positive patients were belonging to the occupation with agriculture and tea tribe (44.50%). Tobacco habituate was recorded (22.18%) but maximum patients (54.59%) had no such habits. Chief problems encountered among the positive patients were pain of abdomen (31.19%) and joint pain or body ache (32.11%). Along with this the easy fatigability (44.04%) and fever (34.86%) symptoms were commonly present among the patients.

Table 2. Haematological data of CML patients

Variable	Mean ± SD
Hb (gm/dl)	14.66±81.71
Platelet Count (lakh /mm3)	2.88±1.94
ESR mm AEFH	38.02±27.57
WBC Cells/µL	2.44±9.62
Blasts	10.16±17.37
RBS (mg/dl)	101.17±23.58
Creatinine (mg/dL)	1.23±2.56
Uric acid (mg/dL)	7.03±1.88
Urea (mg/dL)	20.23±9.08
Neutrophil (%)	44.13±18.57
Lymphocyte (%)	12.11±15.94
Monocyte (%)	2.37±4.17
Eosinophil (%)	4.06±5.68
Basophil (%)	4.19±3.87
Promylocyte	9.52±9.17
Mylocytes	16.82±7.84
Metamylocytes	13.89±7.88

The mean blood count showed that Hb level were higher 14.66 gm/dl. Total WBC 2.44 lacs/ µL and uric acid (7.03) were higher than the standard values. The differential leukocyte count showed reduced neutrophil 44.13%.

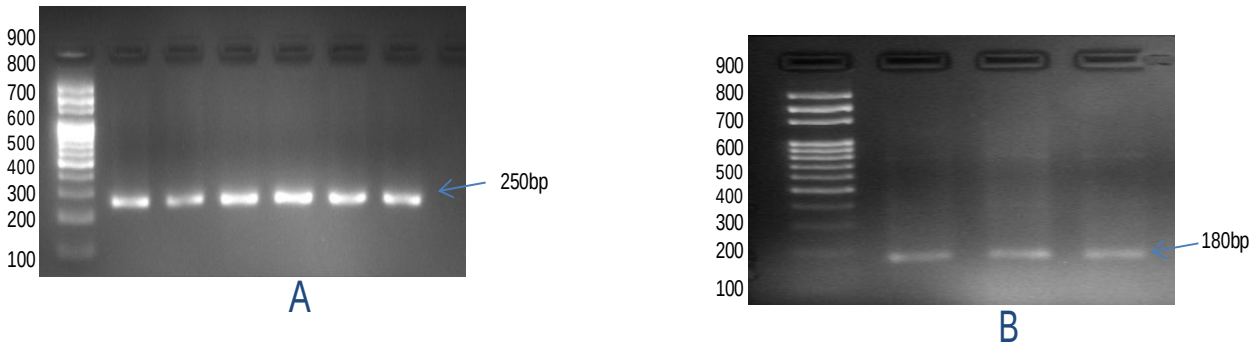

Fig. 1. Gel electrophoresis of multiplex RT-PCR products visualized under UV light after staining with ethidium bromide. M: 100–1000 bp DNA ladder; A-represent B3A2 variant (250 bp length product); B-represents B2A2 variant (180 bp length product);

Haematological characteristics of the study population

A total of 143 (66.20%) of *BCR-ABL* positive patients were found to express B3A2 transcript, whereas the remaining 74 (33.34%) cases were positive for B2A2 variant. The remaining 1 (0.46%) patient coexpressed two types of mRNA B3A2/ B2A2.

Table 3. Patients Haematological data of the Chronic myeloid leukemia patients

Variable	Category	No of Patients	%
Hb (gm/dl)	>10	51	23.40
	7-10	77	35.32
	<7	90	41.28
Platelet Count (lakh/mm3)	<1.0	21	9.63
	1.0-<1.5	21	9.63
	1.5-<4.5	95	43.58
	4.5-6.0	68	31.19
	>6	13	5.97
ESR mm AEFH	<40	71	32.57
	>40	147	67.43
WBC Cells/µL	0.1-0.5	35	16.06
	0.5-1	12	5.50
	1-2	108	49.54
	>2	63	28.90
Blasts	<10	79	36.24
	10-19	8	3.67
	>20	131	60.09
RBS	<80	6	2.75
	80-150	210	96.33
	>=150	2	0.92
Creatinine (mg/dL)	<0.6	5	2.29
	0.6-1.4	46	21.10
	>=1.4	167	76.61
Uric acid (mg/dL)	<2.4	1	0.46
	2.4-7	44	20.18

	>=7	173	79.36
Urea (mg/dL)	<10	1	0.46
	10-40	214	98.16
	>=40	3	1.38
Neutrophil (%)	<40	163	74.77
	40-80	53	24.31
	>80	2	0.92
Lymphocyte (%)	<20	94	43.12
	20-40	115	52.75
	>40	9	4.13
Monocyte (%)	<2	67	30.73
	2-10	149	68.35

Age	Total	Male	Female	HB	WBC	Platelate	Neu	Lym	Mon	Eos	Baso	ESR
11-20	15	6	6	7.68	1.47	3.44	60.17	16	2.67	2.83	1.67	22.83
21-30	42	27	14	8.98	1.78	2.81	47.82	13.24	2.12	3.23	4.61	45.37
31-40	51	33	21	8.50	1.68	2.83	40.06	13.32	2.81	5.17	4.95	29.55
41-50	64	50	15	8.84	1.92	3.03	44.67	13.13	2.45	4.22	3.70	34.21
51-60	27	14	13	8.64	1.74	3.31	43.13	6.88	1.87	3.33	4.73	49.25
61-70	17	12	5	8.51	1.11	2.19	42.73	12.17	1.25	4.47	3.08	49
71-80	2	2	0	11.75	2.6	4.25	36	2	1	4	9	12

	>10	2	0.92
Eosinophill (%)	<1	4	1.83
	1-6	21	9.63
	>6	193	88.53
Basophill (%)	0-1	90	41.28
	>1	128	58.72

All the haematological data were recorded and has been presented in the above table in the form of number of patients. There was low level of haemoglobin (41.28%) and neutrophil (74.77%) were recorded; and high level of ESR (67.43%), eosinophil (88.53%), basophil (58.72%), WBC (78.4%), blasts (60.09%), creatinine (76.6%), uric acid (79.36%), urea (98.16%), lymphocyte (68.35%) and monocyte (52.75%).

Table 4. Haematological characteristics of BCR-ABL positive chronic myeloid leukemia patients.

BCR-ABL positive CML occurred commonly in between the age of 11-80 years, men (66.06%) and Women (33.94%). Highest numbers of positive patients were found in the age of 41-50 (64 numbers). The range of haematological characteristic of BCR-ABL positive chronic myeloid leukemia patients plays a significant role in determining the disease. This experiment showed it is more prominent in the middle age patient.

Table 5. Haematological characteristics of BCR-ABL positive chronic myeloid leukemia patients.

Age	Uric Acid	Urea	Creatinine	Blast	Pro mylocyte	Mylocyte	Meta mylocyte	RBS	B2A2	B3A2	Both
13-20	7.76	16.78	0.94	5	8.67	20.33	21.67	112.33	7	5	-
21-30	6.41	17.61	0.76	13.17	14.84	16.58	11.67	99.68	12	27	-
31-40	7.05	20.12	2.89	11.6	9.13	15.27	12.96	92.28	21	30	1
41-50	7.00	21.40	0.91	6.92	7.31	15.32	14.54	106.89	17	47	-
51-60	7.02	27.25	0.96	6.57	7.46	19.46	14	87.92	11	16	-
61-70	7.73	21.25	0.94	20	10.43	17.71	12	120.13	3	14	-
71-80	8.9	17.3	-	5	8	5	32	83	-	2	-

The other haematological characteristics of BCR-ABL positive chronic myeloid leukemia patients were uric acid, urea, creatinine, blast promylocytes, mylocytes meta mylocytes and RBS.

The highest cases of incidence showing B2A2 mutation were present 21 were found in the age group of 31-40. The other type of CML transcripts viz., B3A2, were found highest 47 no of cases were recorded in the age group of 41-50. Along with this a rare case of presence of both variants were observed in the age group of 31-40.

Table 6. Baseline demographic characteristics of the study group

Variable	Category	BCR-ABL fusionvariants		*P*value
		B3A2	B2A2	
		N=143	N=74	
Gender	Male	91(63.64)	52(70.27)	0.060
	Female	52(36.36)	22(29.73)	0.184
Age	13-20	5(3.50)	10(13.51)	0.043
	21-30	29(20.28)	13(17.57)	
	31-40	30(20.98)	21(28.38)	
	41-50	47(32.87)	16(21.62	
	51-60	17(11.89)	10(13.51)	
	61-70	13(9.08)	4(5.41)	
	71-80	2(1.40)	0(0)	
	Low	43(19.72)	28(37.84)	0.080

Educational level	Intermediate	68(47.55)	17(22.97)	0.172
	High	32(22.38)	29(39.19)	0.008
Residence	Rural	100(69.93)	54(72.97)	0.054
	Urban	43(30.07)	20(27.03)	0.072
Family History	No	143(100)	74(100)	0.042
	Yes	0	0	
Occupation	Agriculture or tea tribe	65(45.45)	32(43.24)	0.080
	Labours	35(24.48)	17(22.97)	0.172
	Others	43(30.07)	25(33.78)	0.008
Diet	Veg	12(8.39)	6(8.11)	0.054
	Non-Veg	132(91.61)	68(91.89)	0.072
Habbit	Smoking	14(9.79)	8(10.81)	0.013
	Alcoholic	30(20.98)	16(21.62)	0.126
	Tobacco	33(23.77)	14(18.92)	0.001
	NoHabbits	66(45.46)	36(51.35)	0.378
Chief Problem	Pain Abdomen	51(35.66)	17(22.97)	0.142
	Joint pain/ Body Ache	50(34.97)	19(25.68)	0.041
	Fever	45(31.47)	31(41.89)	0.027
	Easy Fatigability	66(46.15)	30(40.54)	0.078
	Giddiness	38(26.57)	17(22.97)	0.423
	DecreaseApetite	18(12.59)	7(9.46)	0.766
	Early Satiety	21(14.69)	10(13.51)	0.370
	Caugh	26(18.18)	20(27.03)	0.518

Demographic data based on BCR-ABL fusion transcripts

The association of B3A2 and B2A2 transcripts with the patient's demographic information is displayed and each data of age, educational level (high), residence (rural), family history, occupation (others), diet (veg), habit (smoking, tobacco) and problems (joint pain/body ache, fever) had significant association (p>0.05). Majority of patients were male whether it may be B3A2 (n=91; 63.64%) or B2A2 (n=52; 70.27%) trancripts. There were 52 (36.36%) in B3A2 and 22 (29.73%) in B2A2 variants incase of females. Male to female ratio of study population was 1.93:1. In this study the B3A2 and B2A2 ratio was recorded as 1.93:1. (Table>6). The majority of patients lived in rural areas B3A2(69.93%) and B2A2 (72.97%) respectively. Most of the CML patients were intermediately literate (66.77%) in B3A2 and highly literate in B2A2 (60.81%).

Table 7. Patient's haematological data based on *BCR-ABL* fusion transcript types

Variable	Mean ± SD		
	BCR-ABL fusion variants		
	B3A2	B2A2	*P*-value
Hb (gm/dl)	17.87±98.86	7.96±2.66	0.641
Platelet Count (lakh/mm3)	2.83±1.73	2.95±2.33	0.009
ESR mm AEFH	25.75±24.22	43.48±33.05	0.285

WBC Cells/µl	2.74±11.59	1.79±1.50	0.845
Blasts	9.55±17.12	11.15±18.31	0.557
RBS	103.11±25.30	97.21±20.27	0.064
Creatinine (mg/dl)	0.90±0.33	1.77±4.13	0.269
Uric acid (mg/dl)	6.91±1.89	7.25±1.87	0.066
Urea (mg/dl)	20.20±9.08	20.44±9.31	0.244
Neutrophil	43.64±16.29	45.62±22.61	0.590
Lymphocyte	12.66±16.62	11.12±14.75	0.226
Monocyte	2.49±4.89	2.13±2.23	0.498
Eosinophill	4.05±4.52	4.11±7.58	0.681
Basophill	4.53±4.21	3.6±3.05	0.412
Promylocyte	10.45±10.78	8.06±4.98	0.967
Mylocytes	16.60±7.31	17.12±8.92	0.424
Metamylocytes	12.82±7.74	16±7.91	0.018

Haematological data based on BCR-ABL fusion transcript types

Haematological characteristics of CML patient differentiated by *BCR-ABL* transcripts are presented and differences in Hb, ESR, WBC, blasts, RBS, creatinine, basophil, promylocytes and metamylocytes level were not significant and the values are below the minimum limit of normal in both (B2A2 and B3A2) transcripts in most of the cases. Except platelet and metamylocytes count all the haematological parameters' level had no significant correlationships (p>0.05). Transcript stratified platelet count was significantly higher than the normal level, with B3A2 subtype showing 2.83 and B2A2 subtype 2.95. Leukocyte count revealed minor increase of basophil and eosinophil for both transcripts. However, there were no significant differences between the two transcripts in eosinophil but in case of basophil it is slight higher in B3A2 (4.53) than B2A2 (3.6).

Additionally, the patients with B3A2 subtype showed a significantly higher HB (17.87), WBC (2.74), RBS (103.11), Basophil (4.53) and promylocyte (10.45) compared to those with B2A2 transcript. The haematological data of different subtypes has been presented in the Table>8. Low level of haemoglobin (32.87%, 58.11%) was recorded; platelet count showed high range and high levels in ESR (65.73%,70.27%), WBC (49.65%, 48.65%), blasts (59.44%,60.81%), creatinine (78.32%,72.97%) and uric acid (76.92%,83.78%) in B3A2 and B2A2 variants respectively was observed in the prevalent cases.

Haematological indices based on BCR-ABL fusion transcript types

There was no significant correlation between various parameters of different age groups.The lowest level of maximum characteristics were recorded in the age group of 61-40. Highest number

of B2A2 transcripts patients was 16 belonging to the age group of 31-40 of which male patients were 14 and female were 7.

Notable RBC and platelet indices of CML patient population stratified by *BCR-ABL* transcripts are presented. Additionally, patients with B3A2 subtype showed significantly higher number with 47 in the age group of 41-50 of which 37 were male and 10 were female. The HB (47.52), WBC (6.34), lymphocyte (16.00), uric acid (8.16) and creatinine (1.04) level in the age group of 31-40. In comparison the lowest value were recorded in the age group of 13-20. The lowest average value of HB (0.46), platlet (2.38) and RBS (82) were recorded.

Monocyte, uric acid and urea indices were not significantly different between the two subtypes. However, for most of these indices viz. HB, WBC, platelet, eosinophil, basophil, promylocytes and metamylocytes, patients with B3A2 transcript showed slightly higher values than those with B2A2 variant. On the other hand neutrophil, lymphocytes, ESR, blast, mylocytes and RBS were significantly higher in those with B2A2 transcript as compared to those with B3A2 transcript observed.

Table 8. patients haemotological data in number of incidence

Variable	Category	No of Patients	
		BCR-ABL fusionvariants	
		B3A2(%)	B2A2(%)
Hb (gm/dl)	>10	38(26.57)	12(16.22)
	7-10	58(40.56)	19(25.68)
	<7	47(32.87)	43(58.11)
Platelet Count (lakh/mm3)	<1.0	9 (6.29)	12(16.22)
	1.0-<1.5	10(6.99)	11(14.86)
	1.5-<4.5	63(44.06)	31(41.89)
	4.5-6.0	54(37.76)	14(18.92)
	>6	7(4.89)	6(8.11)
ESR mm AEFH	<40	49(34.17)	22(29.73)
	>40	94(65.73)	52(70.27)
WBC Cells/µL	0.1-0.5	24(16.78)	11(14.86)
	0.5-1	5(3.50)	7(9.46)
	1-2	71(49.65)	36(48.65)
	>2	43(30.07)	20(27.03)
Blasts	<10	55(38.46)	24(32.43)
	10-19	3(2.10)	5(6.76)
	>20	85(59.44)	45(60.81)

RBS	<80	1(0.70)	5(6.76)
	80-150	140(97.90)	69(93.24)
	>=150	2(1.40)	0(0)
Creatinine (mg/dL)	<0.6	4(2.80)	1(1.35)
	0.6-1.4	27(18.88)	19(25.68)
	>=1.4	112(78.32)	54(72.97)
Uric acid (mg/dL)	<2.4	0(0)	1(1.35)
	2.4-7	33(23.08)	11(14.86)
	>=7	110(76.92)	62(83.78)
Urea (mg/dL)	<10	0(0)	1(1.35)
	10-40	142(99.30)	71(95.95)
	>=40	1(0.70)	2(2.70)
Neutrophil (%)	<40	110(76.92)	52(70.27)
	40-80	33(23.08)	20(27.03)
	>80	0(0.00)	2(2.70)
Lymphocyte (%)	<20	62(43.36)	32(43.24)
	20-40	74(51.75)	40(54.05)
	>40	7(4.90)	2(2.70)
Monocyte (%)	<2	45(31.47)	22(29.73)
	2-10	96(67.13)	52(70.27)
	>10	2(1.40)	0(0.00)
Eosinophill (%)	<1	0(0.00)	4(5.41)
	1-6	11(7.69)	10(13.51)
	>6	132(92.31)	60(81.08)
Basophill (%)	0-1	60(41.96)	30(40.54)
	>1	83(58.04)	44(59.46)

DISCUSSION AND CONCLUSION

Our study demonstrated that BCR-ABL positive CML frequently involved older patients (median age 30-50 years), including both male (66.06%) and female (33.94%) patients. Earlier report had revealed that the patients with sixth or seventh decade were predominant in the case of CML screening[8,9,10,11]. Furthermore, some studies[8,9,10] have described prevalence of male BCR-ABL negative CML while in some studies such data have not been seen. Female representation of CML positive cases were described more, accounting to 57% from an Italian study[11].

Table 9 Incidence of B2A2 and B3A2 transcripts in Eastern African, European, and eastern countries

(Source: Khazaal et al.)[4]

SlNo	Country	No. ofpatients	BCR -ABL fusiongene		
			B3A2%	B2A2%	Both%
1	**Presentstudy**	218	65.60	33.94	0.46
2	Iraq[5]	100	59	39	-
3	India[23]	170	63.53	36.36	-
4	Iran[24]	85	62.35	29.41	-

5	Syria[18]	45	51.1	46.7	-
6	Pakistan[25]	23	26	65	-
7	Saudi Arabia[26]	30	63.33	36.66	-
8	Korea[27]	548	67.66	32.34	-
9	Japan[28]		67.9	30.2	-
10	Germany[17]	1.105	44.89	40.81	-
11	Bulgaria[7]	98	54	45	-
12	UK[29]	71	39	31	-
13	Brazil[30]	203	64	34	-
14	USA[31]	481	41	42	-
15	Argentine[32]	24	37.5	41.7	-
16	Ecuador[33]	40	5.4	94.6	-
17	Mexico[16]	250	.35	48	-
18	Tunisia[34]	45	64	36	-
19	Sudan[22]	46	41.9	53.5	-

In a large number of foregoing table containing data of East to West, the B3A2 is dominant over B2A2 variant in CML patients. The current investigation too shows a relatively higher number of CML positive patients with dominance of B3A2 subtype.

The difference in the above results may be attributed to four reasons. Among the first one is the ethnic diversity of the patient cohort included in the study, since diverse community carried diverse descendancy in genetic background reasonably having different sub-types. Second reason may probably be the diverse geographical dissemination. Thirdly, the size of sample might influence the results as maximum number sample size includes more representative and replications. Lastly, the time of sampling; blood sample collection from freshly analysed patients accuracy is more than that of during imatinib treatment (though they contain imatinib resistance), since the tyrosine kinase inhibitor (TKI) may to influence the less abundant variants like e1a2 [12]. Among the diverse variants at the beginning both B3A2 and B2A2 coexpressed but with the progression of disease only one transcripts prevail[13].

There was a noteworthy interdependence observed among gender and BCR-ABL variants that is presented in Table>6. The global findings on account of relationship between gender and different variants were conflicting. This study revealed the predominance of both subtypes in males compared to females. In Mexico CML studies[14] revealed that higher patients of male was for B3A2 whereas female for B2A2. This result was concordant with a previous Indian study but different from the current findings.

Furthermore, in few studies[15,16,17] did not revealed any relation between male-female and *BCR-ABL* fusion. However, some other investigations recorded contradiction in results, where in the females

showed B3A2 fusion more frequently whereas male group showed more of B2A2 fusion frequency. Saudi Arabia, German and Sudanese CML[18,19,20] reported higher number of males with B2A2 and females with B3A2. Present investigation comprised 143, 74 and 1 number of patients with B3A2, B2A2 and both respectively. From the above studies it could be concluded that method of detection, size of sample and the ethnicity of the studied community is responsible for such dissimilarity.

Table10. Incidence of B2A2 and B3A2 transcripts in India

SlNo	Country	No. Of patients	BCR -ABL fusiongene		
			B3A2%	B2A2%	Both
1	**Present study**	218	65.60	33.94	0.46
2	India[35]	400	72	26	2
3	India[36]	122	56.56	27	0.49
4	India[37]	87	26.44	34.48	32.15
5	India[38]	208	66.82	28.84	3.36
6	India[23]	170	63.53	36.36	-

The incidences of presence of different variants as recorded in a few studies from India have been presented and it was revealed that the prevalence of B3A2 is more abundant than that of B2A2 transcripts except the data of that of Upadhyay et al.[21].

Table 11. Published studies about the association between *BCR-ABL* transcript types and Laboratory data in patients with CML(Source: Khazaal et al.)[5]

SlNo	Study	BCR- ABL Fusion Transcripts			
		B3A2		B2A2	
		WBC	Platelet	WBC	Platelet
1	**Present Study**	↑	nd		nd
2	Mondal et al.[23]	↑			↑
3	Khazaal et al.[4]	↑			↑
4	Kagita et al.[24]	↑	↑		
5	Balatzenko et al.[6]		↑		
6	Ayatollahi et al.[25]	nd		nd	
7	Al-Achkar et al.[16]		↑	↑	
8	Hanfstein et al.[15]	↑			↑
9	Vasconcelos et al.[26]		↑	↑	
10	Bennour et al.[27]		↑		
11	Deb et al.[28]	↑			↑
12	Polampalli et al.[29]	nd		nd	
13	Rosas-Cabral et al.[30]	nd	↑	nd	
14	Perego et al.[31]		↑		
14	Inokuchi et al.[32]		↑		

A few investigations revealed remarkable results that WBC count has increase in B3A2 CML patients and B2A2 showing high platelet count. In contrast to this some reports were opposite, having high platelet count and leukocyte in B3A2 and B2A2 variants, respectively. Still, some other studies found no differences in this regard. In the present study increased level of WBC for both and platelet in B3A2 have been found.

The lack of consistency in the correlation among particular variants, platelet and WBC was described by different researchers. One group ascribed this might be due to the conformational changes among the two variants as B2A2 subtype is shorter than B3A2 fusion protein which has another 25 amino acids present in *BCR* region[22]. With regard to WBC indices, they were comparable between B3A2 and B2A2 expressing patients with significant differences. On the other hand, platelet indices show no significant variations between the two groups.

The results presented in table11 comprise of data from two[23,24] CML investigation reports from India. The haematological data recorded from India showed significant differences in association with different transcripts. In the present study WBC content is higher in B3A2 subtypes in comparison to B2A2 and platelet content shows no difference. Whereas higher content of platelet revealed by Mondal et al.[23] in B2A2 and Kagita et al.[24] reported in case of B3A2. The WBC report is concordant with both the Indian investigations. The platelet content of this investigation also revealed significant association (p=0.009).

In conclusion, all studied positive CML patients expressed the *BCR-ABL* subtype of the fusion gene; a preponderance of B3A2 over B2A2 variant; a sex skewed distribution in *BCR-ABL* transcript types with both B3A2 and B2A2 transcript which is more prevalent in males and the type of *BCR-ABL* transcript is reflected by different haemoglobin, WBC and platelet counts, which might represent a distinct phenotype and disease biology. At last, the frequency of BCR-ABL B3A2 and B2A2 mutation is most concordant and higher level of WBC with most other CML studies. We recommend identifying the *BCR-ABL* transcript type in every CML patient at diagnosis along with the cytogenetic study, linking the detected transcript with the patient's clinical findings.

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