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Role of Human Organic Cation Transporter1 (*SLC22A1)* Gene Polymorphisms in Modifying Response to Imatinib in CML Patients

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ABSTRACT

Background: Human organic cation transporter1 (*hOCT1*,*SLC22A1*), an influx transporter, is responsible for the uptake of Imatinib into chronic myeloid leukemia (CML)cells. Variation in clinical response to Imatinib has been observed with two nonsynonymous SNPs in *hOCT1* gene, namely M420del and M408V in some populations.
Aims: The study aimed to study hoct1 gene polymorphism M420del & M408V in CML patients treated with imatinib & their effect on hematological and molecular response to imatinib .Also, the synergistic effect of M408V & M420del on hematological and molecular response to imatinib were studied. Additionally, correlation of these polymorphisms with hoct1 gene expression was studied.
Methodology: Newly diagnosed CML patients, in the age group 18-80 years, with diagnosis confirmed by qualitative PCR for BCR-ABL1 fusion gene, who were to be initiated on Imatinib therapy, were included in the study. Detection of BCR-ABL1 fusion gene transcripts for confirmation of diagnosis of CML by Qualitative multiplex RT-PCR, evaluation of hOCT1 gene expression by Real Time quantitative RT-PCR (qPCR) and detection of gene polymorphism in hOCT1 gene: SNPs M420del (rs35191146) and M408V (rs628031) was done by Allele Specific PCR (AS-PCR).
Results: It was observed that patients having normal homozygous genotype for M408V and mutant homozygous or heterozygous genotype for M420del had an increased tendency towards imatinib resistance as compared to patients who had normal homozygous genotype for both the polymorphisms. On the other hand, this effect was not seen in patients with mutant homozygous or heterozygous genotypes for both polymorphisms.
Conclusions: Mutant M420del allele may be linked to poor outcome of imatinib treatment in CML, however simultaneous presence of mutant M408V allele appears to circumvent this effect.

Key words: Human organic cation transporter1 (*hOCT1*,*SLC22A1*), gene polymorphism, CML patients, imatinib

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of pluripotent stem cells which is characterized by the Philadelphia (Ph) chromosome which is formed due to a balanced reciprocal translocation between chromosome 9 and 22 t(9;22)(q34.1;q11.2).[1] The incidence of CML varies from approximately 0.7 per 100,000 persons in Sweden and China, to approximately 1.5 per 100,000 persons in Switzerland and the United States[2]. In India, CML is the commonest adult leukaemia, with the annual incidence ranging from 0.8–2.2/100,000 population in males and 0.6–1.6/100,000 population in females[3]. Imatinib, a tyrosine kinase inhibitor, is a highly effective drug used for patients with CML. Response to imatinib treatment is measured in terms of hematologic, cytogenetic, and molecular parameters, as proposed by the European leukemia Net (ELN)[4,5]. Human organic cation transporter1 (*hOCT1*,*SLC22A1*), an influx

transporter, is responsible for the uptake of Imatinib into chronic myelod leukemia (CML)cells.[6] Variation in clinical response to Imatinib has been observed with two nonsynonymous SNPs in *hOCT1* gene, namely M420del and M408V in some populations.[7,8]

AIMS : The study aimed to study hoct1 gene polymorphism M420del & M408V in CML patients treated with imatinib & their effect on hematological and molecular response to imatinib .Also, the synergistic effect of M408V & M420del on hematological and molecular response to imatinib were studied. Additionally, correlation of these polymorphisms with hoct1 gene expression was studied.

METHODOLOGY

This was a hospital based prospective study. Thirty Chronic myeloid leukemia patients in chronic phase CP-CML from Lok Nayak Jai Prakash Hospital (LNJP), New Delhi, India, who met the inclusion criteria, were recruited in the study. Thirty age and sex matched healthy volunteers were recruited in the study as controls. Newly diagnosed CML patients, in the age group 18-80 years, with diagnosis confirmed by qualitative PCR for BCR-ABL1 fusion gene, who were to be initiated on Imatinib therapy were included in the study. Chronic MyeloMonocytic Leukemia (CMML), BCR-ABL1 positive adult ALL patients and other myeloproliferative disorders were excluded from the study. Patients who had previously undergone any treatment for chronic myeloid leukemia were also excluded. Detailed clinical examination (relevant findings like spleen size noted)

STUDY PLAN

A peripheral blood sample, 5mL from cases and 3mL from controls collected for molecular studies, in an EDTA vial by venipuncture, after taking informed consent. Hematological laboratory tests: complete blood count (Hb, TLC, DLC, total platelet count) were done before initiation of imatinib. Detection of BCR-ABL1 fusion gene transcripts for confirmation of diagnosis of CML was done by Qualitative multiplex RT-PCR., evaluation of hOCT1 gene expression was done by Real Time quantitative RT-PCR (qPCR) and detection of gene polymorphism in hOCT1 gene: SNPs M420del (rs35191146) and M408V (rs628031) was done by Allele Specific PCR (AS-PCR). Quantitative REAL-TIME PCR for BCR-ABL1 fusion gene to assess molecular response (BCR-ABL1/ABL1 %) was done once either at 6 months or at 12 months after initiation of imatinib therapy. Hematological response (Hb, TLC, DLC and platelet count) was assessed at regular intervals during the duration of the study.

❖ **MOLECULAR ANALYSIS**

1. **Isolation of peripheral blood leucocytes from whole blood**
   Blood was collected in EDTA vial and kept in standing position. After 15-20 minutes buffy coat appeared at the top of the vial and all the red cells were in the lower layer. This buffy coat was carefully pipetted out in two 1.5mL Eppendorf tubes and the remaining blood sample was stored at -80 ^{o} C. Out of the two Eppendorf tubes one tube which contained 200µL of buffy coat was immediately used for RNA extraction and other tube was stored at -80 ^{o} C for subsequent use.
2. **RNA Extraction from CML patient samples:**
   Modified acid guanidiniumthiocyanate-phenol-chloroform (TRIZOL) RNA extraction method was used for RNA extraction from PBLs of CML cases. (PaulD. Siebert and Alex Chenchik 1993).
3. **RNA Extraction From Control Samples:**
   RNA from control samples was extracted by using Total RNA Mini Kit (Blood/Cultured Cell) from Gene Aid Biotech Ltd., Taiwan using protocol as per manufacturer's instructions. The concentration of extracted RNA was checked by using Nanodrop and quality

was checked by running 2 µL of each sample on 2 % agarose gel.

4. **c-DNA synthesis protocol:**
   Extracted RNA was reverse transcribed to c-DNA by RT-PCR.cDNA was synthesized using the Verso cDNA synthesis kit (Thermo Scientific, EU) using protocol as per the manufacturer's instructions. A parallel PCR was performed on each sample using primers specific for the constitutively expressed β- actin gene which was used as endogenous control.(Table 1)
5. **DNA isolation from whole blood:**
   Genomic DNA was isolated from whole blood by using Genomic DNA Mini Kit (Blood/ Cultured Cell) from Gene Aid Biotech Ltd., Taiwan as per the manufacturer's protocol.
6. **Multiplex RT –PCR for detection of BCR-ABL1 fusion gene transcripts:**
   Diagnosis of CML was confirmed by Multiplex RT-PCR which allows simultaneous detection of all the BCR-ABL1 fusion gene transcripts in addition to normal BCR gene as an internal control. cDNA synthesized from the total RNA(as described previously) was used in multiplex PCR .The sequence of oligonucleotide primer sequences used for this multiplex PCR are shown in Table 2. The expected band size for different BCR-ABL1 fusion transcripts were: 808bp - normal BCR,481 bp - e1a2,385 bp - b3a2 ,310 bp - b2a2,103bp – b2a3 & 209bp – b3a3.

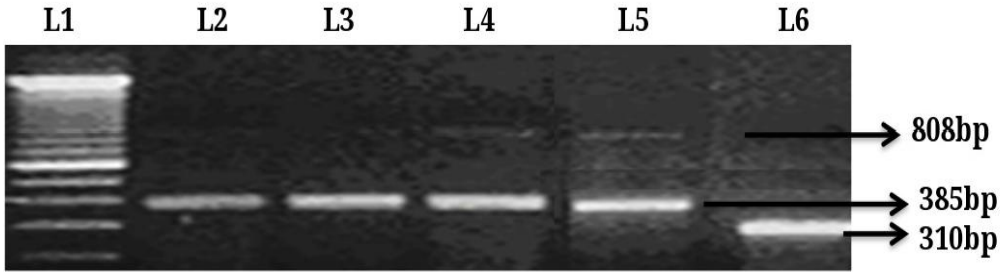

FIGURE 1: Ethidium Bromide stained gel electrophoresis image of BCR-ABL transcripts by multiplex RT-PCR. L1.100bp ladder, L2, L3, L4, L5. b3a2 transcript (385 bp). L6. b2a2 transcript (310bp).

7. **Expression of hOCT1 gene in CML patients and controls**

After cDNA quality was checked it was used to study the expression of hOCT1 gene in patient and control samples by quantitative Real Time PCR using Rotor Gene Q (Qiagen) analyzer. Thermo Scientific Maxima SYBR Green qPCR master mix was used for quantative real-time PCR which contains Hot start Taq polymerase, SYBR Green qPCR buffer, SYBR Green I dye, reference dye and dNTP. βactin gene was used as internal control. In this PCR, amplification of hOCT1 gene was compared with the amplification of β actin gene and relative expression was calculated. Melting curve analysis was done in the temperature range 35°C to 95°C for assessment of homogeneity of the qPCR products. Gene expression levels were calculated based on the $\Delta\Delta C_t$ method.

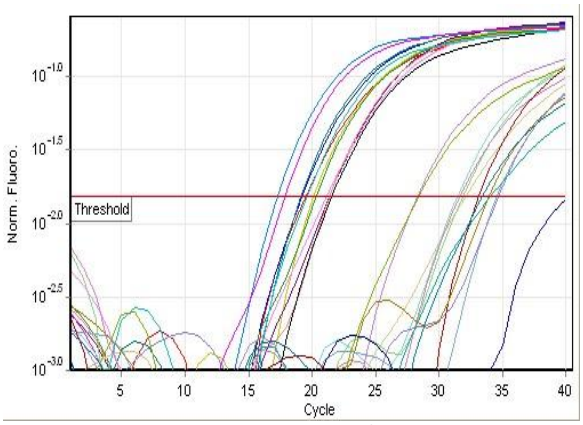

Figure 2 : Gene expression curve for hOCT1 gene.

8. <u>hOCT1 gene polymorphism study in CML cases and controls :</u>

SNPs M408V (rs628031)& M420del (rs35191146) were studied in 30 CML cases and 30 controls by AS-PCR. Genomic DNA extracted from whole blood of CML cases and controls was used in AS-PCR. The primers for both the SNPs were designed using *Primer3* primer designing tool.

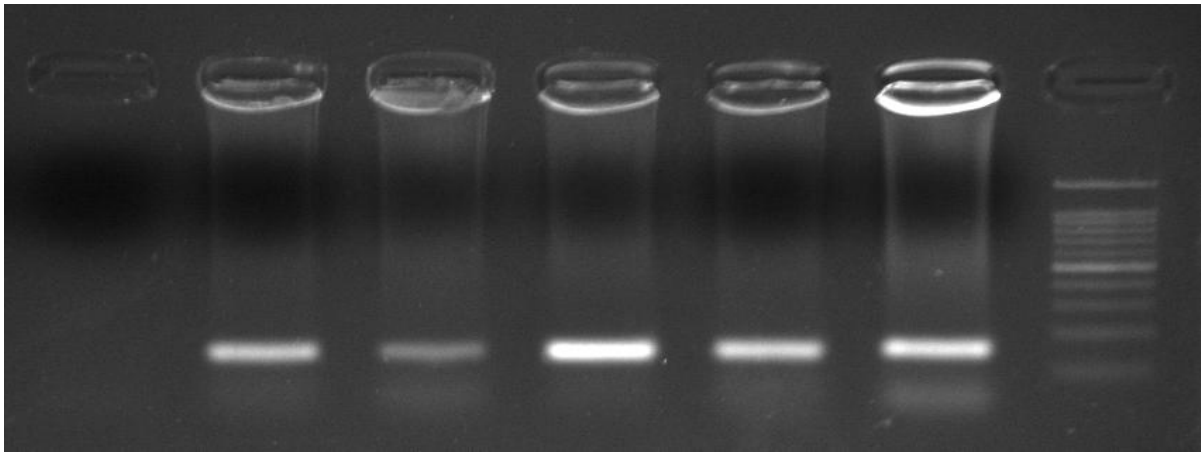

FIGURE 3: Ethidium Bromide stained gel electrophoresis image showing band for M408V polymorphism (amplicon size 158 bp) and 100bp ladder.

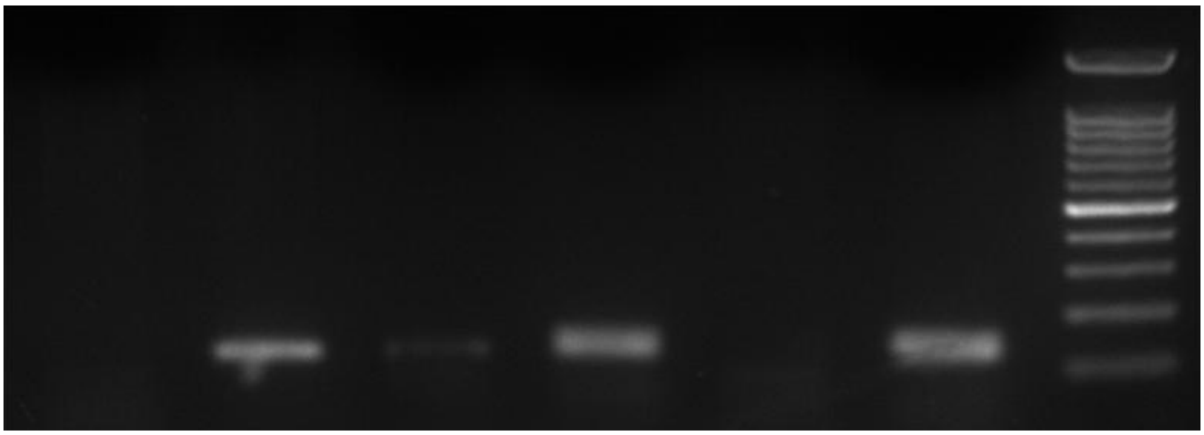

FIGURE 4: Ethidium Bromide stained gel electrophoresis image showing band for M420del polymorphism (amplicon size 135 bp) and 100bp ladder.

TABLE 1 : Table showing sequences of different primers used in the PCR reactions done in the study.

Primer sequences for βactin PCR	
Primer	**Sequence**
βactin Forward primer	5′CGACAACGGCTCCGGCATGTGC3′
βactin Reverse primer	5′CGTCACCGGAGTCCATCACGATC3′
Primer Sequences used for Multiplex RT-PCR for BCR-ABL1 fusion gene	
Primer code	Primer Sequence
C5e	5'-ATAGGATCCTTTGCAACCGGGTCTGAA-3'
B2B	5'-ACAGAATTCCGCTGACCATCAATAAG-3'
BCR-C	5'-ACCGCATGTTCCGGGACAAAAG-3'
CA3	5'-TGTTGACTGGCGTGATGTAGTTGCTTGG-3'
Primers used for hOCT1 gene expression	
Primer	Sequence
Forward primer	5′-GGGCAGCCTGCCTCGTCAT-3′
Reverse primer	5′-ACCTCCCTCAGCCTGAAGAC-3′
Primer sequences used in AS-PCR	
M420del (Amplicon size : 135bp)	
Forward primer –Normal (wild) :	5′--GCAGCCTGCCTCGTCATG-3'
Forward primer-Mutant	5′--GCAGCCTGCCTCGTCATA-3'
Common Reverse primer:	5′-CATCTTTGTTCTCATTCCAGAGGC-3'
B. M408V (Amplicon size : 158bp)	
Forward primer –Normal (wild) :	CGCATCTACCCCATGGCCA
Forward primer-Mutant	CGCATCTACCCCATGGCCG
Common Reverse primer:	GAGGCTTATCAAAGAGTCACAACAC

9. Quantitative REAL-TIME PCR for BCR-ABL1 fusion gene

A peripheral blood sample was collected at follow-up either at 6 months or at 12 months after initiation of imatinib therapy to assess the molecular response. Molecular response was assessed by calculating BCR-ABL1/ABL1 ratio. This was done by quantification of BCR-ABL1 p210b2a2 or b3a2 transcripts. *Ipsogen* BCR-ABL1 Mbcr kit(from QIAGEN, Netherlands,) was used for this purpose and protocol followed was as per the manufacturer's instructions. For each gene (ABL1 and BCR-ABL1), raw Ct values obtained from plasmid standard dilutions were plotted according to the log copy number (3,4 and 5 for C1,C2 C3 ; and 1,2,3,4,5 for F1, F2, F3, F4, F5).The ABL1 standard curve equation was used to transform raw Ct values for the unknown samples into ABL1 copy numbers ($ABL1_{CN}$). The BCR-ABL1 standard curve equation was used to transform raw Ct values for the unknown samples into BCR-ABL1 copy numbers ($BCR\text{-}ABL1Mbcr_{CN}$). Normalized copy number (NCN) was calculated using the formula : NCN = [$BCR\text{-}ABL1Mbcr_{CN}$ / $ABL1_{CN}$] * 100

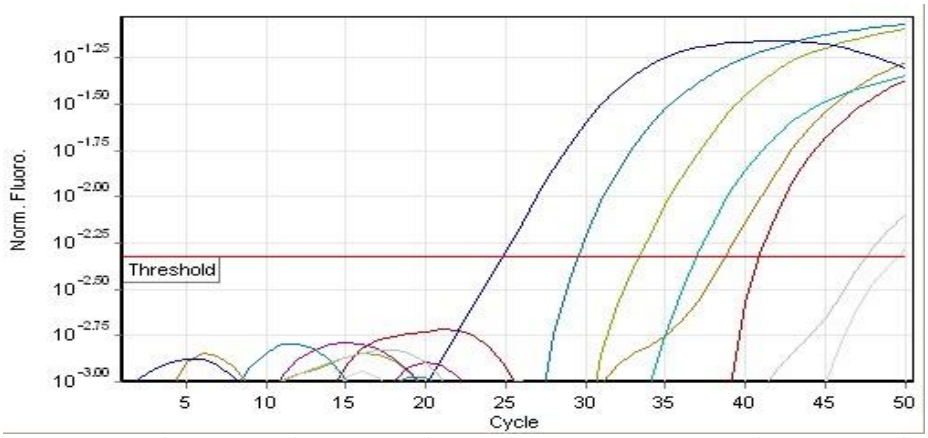

Figure 5: Standard amplification curve for BCR/ABL for Quantitative REAL-TIME PCR for BCR-ABL1 fusion gene

STATISTICAL ANALYSIS: All statistical analysis was performed using SPSS software22.0. Variables were presented as range, Mean ± SD and median value. Fisher exact, Mann Whitney-U and Kruskal Wallis tests were used to estimate the statistical significance of differences observed between the groups. $p<0.05$ was taken as statistically significant.

RESULTS

Thirty Chronic myeloid leukemia patients in chronic phase and thirty age and sex matched healthy volunteers were recruited in the study as controls. Demographic profile of cases and controls is depicted in Table 2.

TABLE 2 : Age and sex distribution of cases and controls

	CML cases (n=30)	Controls (n=30)	P VALUE
Age distribution			
Mean± S.D. (yrs)	39.70 ± 18.04	39.27 ± 16.00	0.906
Range(yrs)	18-80	20-75	
Sex distribution			
Males(%)	22 (73.34)	19(63.34)	0.580
Females(%)	8 (26.67)	11(36.67)	

The clinical and hematological parameters were analyzed at baseline and at follow up as depicted in Table 3.

TABLE 3 : Clinical and hematological parameters at baseline and at follow up

Parameter	Mean±S.D. at baseline (n=30)	Mean±S.D.after 6 months of imatinib therapy (n=30)	Mean±S.D.after 12 months of imatinib therapy (n= 10)
1.Hb (gm%)	11.01±2.08	10.61±1.73	10.29±1.46
2.TLC(*10^9/L)	203±176.73	6.82±1.63	6.47± 2.21
3.PLATELET COUNT (*10^9/L)	318.30±166.75	166.40±42.16	148.20±43.27
4. SPLEEN SIZE (cms below costal margin)	11.93±2.98	Not palpable	Not palpable
5.BLASTS(%)	3.73±1.20	00±0.000	0.30±0.95
6.BASOPHILS(%)	2.23±0.89	1.63±0.85	1.90±0.87
7.EOSINOPHILS(%)	4.03±1.84	2.73±0.82	1.70±0.67

hOCT1 gene expression was analyzed by quantitative real time PCR in 30 CML patients with respect to 30 healthy controls The fold change in expression of hOCT1 gene ranged from 0.02 to 96.33 (Table 4).

TABLE 4: Fold change in hOCT1 gene expression

Range of fold change in hOCT1 gene expression	Median value	Number of cases with high expression	Number of cases with low expression
0.02-96.33	5.6	15(50%)	15(50%)

Study of hOCT1 gene polymorphisms

hOCT1 gene polymorphism study was done by AS-PCR for 2 SNPs, M408V & M420del, in all the 30 CML and 30 control samples.

There were three genotypes for the two SNPs: normal (wild) homozygous (N) , mutant homozygous (M) and heterozygous (NM). The frequencies of these genotypes and the allelic frequencies for the wild allele and the mutant allele in both cases and controls for both the SNPs are depicted in Table 5.

It was studied whether there was any association of the three genotypes with achievement of Complete Hematological Response at 3 months or not. As depicted in Table 6 & 7, no significant association was found. It was studied whether there was any association of the three genotypes with the type of molecular response (optimal, warning or failure) achieved and again no significant association was found. (Table 8)

TABLE 5 : Genotype & allelic frequencies for SNPs in cases & controls for M408V and M420del polymorphisms

Genotype frequencies for SNPs in cases & controls			
M408V genotype	**CML cases, n (%)**	**Controls, n (%)**	**p-value**
N	9 (30)	17(56.66)	0.0779
M	3 (10)	3(10)	
NM	18 (60)	10(33.33)	
M420del genotype	**CML cases, n (%)**	**Controls, n (%)**	**p-value**
N	22(73.33)	26(86.66)	0.515
M	3 (10)	2(6.66)	
NM	5 (16.67)	2(6.66)	
Allele frequencies for SNPs in cases and controls			
M408V			
Allele	Cases	controls	p-value
Wild	0.6	0.7333	0.121
Mutant	0.4	0.2666	
M420del			
Allele	Cases	controls	p-value
Wild	0.8166	0.9	0.19
Mutant	0.1833	0.1	

TABLE 6 : Association of the three genotypes of M408V with achievement of Complete Hematological Response at 3 months and time to complete hematological response (THR).

Association of M408V with CHR at 3 months			
M408V genotype	**CHR at 3 months present, n**	**CHR at 3 months absent , n**	**p-value**
N	7	2	0.732
M	3	0	
NM	16	2	
Total	26	4	
Association of M408V with THR			
M408V Genotype	**THR Range(months)**	**THR mean ± S.D. (months)**	**p-value**
N	2.5-4	2.61±0.82	0.341
M	1.5-3	2.16±0.76	
NM	1-4.5	2.16±1.01	

TABLE 7 : Association of the three genotypes of M420del with achievement of Complete Hematological Response at 3 months and time to complete hematological response (THR).

Association of M420del with CHR at 3 months			
M420del genotype	**CHR at 3 months present, n**	**CHR at 3 months absent , n**	**p-value**
N	20	2	0.284
M	2	1	
NM	4	1	
Total	26	4	
Association of M420del with THR			
M420del Genotype	**THR Range(months)**	**THR mean ± S.D. (months)**	**p-value**
N	1-4.5	2.18±0.94	2.0
M	2-3.5	2.83±0.76	3.0
NM	1.5-4	2.5±1.0	2.0

TABLE 8 : Association of the three genotypes of M408V & M420del with achievement of Molecular Response.

M408V Genotype	**Optimal response, n=**	**Warning,n=**	**Failure ,n=**	**p-value**
N	5	3	1	0.629
M	3	0	0	
NM	9	4	5	
Total	17	7	6	
M420del Genotype	**Optimal response, n=**	**Warning,n=**	**Failure ,n=**	**p-value**
N	13	4	5	0.125
M	0	2	1	
NM	4	1	0	
Total	17	7	6	

Study of the synergistic effect of M408V & M420del on hematological and molecular response to imatinib

Patients were divided into 4 groups as follows to study the synergistic effect of the 2 polymorphisms, SNP M408V & M420del.(table 9)

It was analysed whether the number of patients with attainment of CHR at 3 months was different among the groups B,C and D as compared to group A or not. As shown in Table 10, no significant difference was found. It was also analysed whether the type of molecular response achieved (optimal, warning or failure) was different among the groups B,C and D as compared to group A or not. As shown in Table 11 no significant difference was found between groups A&B and A&D. But, the difference was significant for groups A&C as well as C&D.

TABLE 9: Group division to study synergistic effect of M408V&M420 del

GROUP		**No.of patients**	**Percentage(%)**
A	• Patients having Normal homozygous genotype for both M420del &M408V	5	16.66
B	• Patients having M420 Normal homozygous genotype and M408V mutant homozygous or M408V heterozygous genotype	17	56.66
C	• Patients having M408V Normal homozygous genotype and M420del mutant homozygous or M420del heterozygous genotype	4	13.33
D	• Patients having mutant homozygous or heterozygous genotype for both M420del &M408V.	4	13.33

TABLE 10 : Study of the synergistic effect of M408V & M420del on hematological response to imatinib

Comparison of groups A & B			
GROUP	**CHR at 3 months present ,n=**	**CHR at 3 months absent ,n=**	**p-value**
A	5	0	1.0
B	15	2	
Comparison of groups A& C			
GROUP	**CHR at 3 months present ,n=**	**CHR at 3 months absent,n=**	**p-value**
A	5	0	0.1666
C	2	2	
Comparison of groups A& C			
GROUP	**CHR at 3 months present ,n=**	**CHR at 3 months absent,n=**	**p-value**
A	5	0	1.0
D	4	0	

TABLE 11 : Study of the synergistic effect of M408V & M420del on molecular response to imatinib

Comparison of groups A & B				
	No. of cases with Optimal response,n=	**No. of cases with Warning response,n=**	**No. of cases with Failure response,n=**	**p-value**
Group A	5	0	0	0.1573
Group B	8	4	5	
Comparison of groups A& C				
	No. of cases with Optimal response,n=	No. of cases with Warning response,n=	No.of cases with Failure response,n=	p-value
Group A	5	0	0	0.0079*
Group C	0	3	1	
Comparison of groups A& D				
	No. of cases with Optimal response	No. of cases with Warningresponse,n=	No. of cases with Failureresponse,n=	p-value
Group A	5	0	0	1.0
Group D	4	0	0	
Comparison of groups C & D				
	No.of cases with Optimal response,n=	No. of cases with Warning response,n=	No. of cases with Failure response,n=	p-value
Group C	0	3	1	0.02*
Group D	4	0	0	

Study of association of hOCT1 gene expression with SNPs M408V and M420del

It was analysed whether there was any association between the three genotypes (N,M,NM) in the two SNPs with gene expression or not. As shown in table 12, no significant association was seen.

TABLE 12:Association ofhOCT1expression with M408V & M420del genotypes in cases

M408V Genotype	High expression , n=	Low expression , n=	p-value
N	4	5	0.34
M	3	0	
NM	8	10	
Total	15	15	

M420del Genotype	High expression , n=	Low expression , n=	p-value
N	11	11	0.108
M	0	3	
NM	4	1	
Total	15	15	

DISCUSSION

Giannoudis A et al[9.10] have reported that hOCT1 SNPs M420del (rs35191146) and M408V (rs628031) alter imatinib uptake. They have also reported that M420del modifies clinical outcome in imatinib-treated CML cases and is associated with imatinib resistance.

In the present study, the two hOCT1 polymorphisms M408V & M420del were studied in CML cases and controls by AS-PCR.

In CML cases, the genotype frequencies for M408V normal homozygous, mutant homozygous and heterozygous genotypes were 30%, 10% and 60 % respectively whereas in controls they were 56.66%, 10% and 33.33 % respectively. This difference was however found to be not significant statistically.

The allelic frequency for M408V normal allele was found to be 60% and for the mutant allele was found to be 40% in the cases. On the other hand, in the controls, they were 73.33% and 26.66% for the normal and mutant alleles respectively. Giannoudis A et al have reported the allelic frequency for M408V to be 59.8% in the European-Americans.

The association of M408V genotypes with hematological response was studied. It was seen that 77.77% of cases with normal homozygous genotype had achieved CHR by the end of 3 months,

90.47% of those with the mutant allele had it. This difference was not found to be statistically significant.

Regarding the association between the polymorphism and molecular response, it was seen that 55.55% of cases with normal homozygous genotype had an optimal response whereas 57.14 % of cases with the mutant allele had it. This difference was however not found to be significant.

Moreover it was seen that there was no significant association between the polymorphism and both the prognostic scores.

In case of M420del, in CML cases the frequencies for genotypes were 10%, 73.33% and 16.67% for mutant homozygous, normal homozygous and heterozygous genotypes respectively whereas in the control group they were, 6.66%, 86.7% and 6.66% respectively. This difference was found to be statistically non-significant.

The allelic frequencies for the normal and mutant alleles in the cases were 81.66% and 18.33%respectively. While in the control group they were observed to be 90% for the normal allele and 10% for the mutant allele. Giannoudis A et al have reported the allelic frequency for M420del to be 18.5% in European-Americans (according to the National Center for Biotechnology Information).

The association of M420del with hematological response was studied and it was seen that 90.90% of cases with normal homozygous genotype had achieved CHR by the end of 3 months. On the other hand, only 75% of the cases with mutant allele had it. However, this observation was found to be not significant.

In the case of molecular response, it was seen that while 59.09% of the cases with normal homozygous genotype had an optimal response, only 50% of cases with mutant allele had it. This difference was not found to be significant statistically.

The synergistic effect of hOCT1 M408V & M420del on hematological and molecular response was studied and it was observed that although hematological response was not significantly associated, molecular response was altered by the combined effect of these SNPs. It was observed that patients having normal homozygous genotype for M408V and mutant homozygous or heterozygous genotype for M420del had an increased tendency towards imatinib resistance as compared to patients who had normal homozygous genotype for both the polymorphisms. On the other hand, this effect was not seen in patients with mutant homozygous or heterozygous genotypes for both polymorphisms.

These findings are similar to that of Giannoudis A et al who confirmed in a functional assay that M420del decreased imatinib uptake, whereas M408V counteracted this effect.

STRENGTHS & LIMITATIONS

As per our knowledge, not much work has been done on hOCT1 polymorphisms and their role in response to Imatinib in CML patients, in the Indian population.[11] As per our findings, these SNPs in *hOCT1* gene occur at reasonable frequencies in Indian population, to be of clinical interest as predictors of response to imatinib in CML.

The findings of this study however, need to be confirmed in a bigger sample size including patients from multiple health-care centres and broader demographics.

CONCLUSIONS

Mutant M420del allele may be linked to poor outcome of imatinib treatment in CML, however simultaneous presence of mutant M408V allele appears to circumvent this effect.

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