Association of PTEN gene 32bp deletion variant with development of CML: A Case-Control study

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Abstract : PTEN (Phosphatase and Tensin Homolog), a tumor suppressor and an important second messenger involved in the AKT signaling pathway, is known to be mutated in several cancers including chronic myeloid leukemia (CML). CML is a myeloproliferative disorder characterized by the t(9;22) translocation coding for the chimeric protein, BCR-ABL. The tumor suppressor PTEN plays a critical role in the pathogenesis of CML, through non genomic loss of function mechanisms, such as protein down-regulation and impaired nuclear/cytoplasmic shuttling. The present study is an attempt to evaluate the association of PTEN 32bp deletion polymorphism with CML in regional state of Southern India. Blood samples (CML cases, age and gender matched controls from local population) were collected and analyzed for the PTEN 32bp deletion polymorphism. DNA was isolated by salting out method and genotyping was carried out by PCR and analyzed on 4% gel. Odds ratio, allele and genotype frequencies were calculated using SNPSTATs. D/D genotype of PTEN 32bp gene polymorphism was found to confer reduced risk for CML development indicating the protective nature of D allele for CML susceptibility. The results indicated that the polymorphism is strongly associated with CML susceptibility but not with progression. However, this is the first report on PTEN gene polymorphism in CML. Hence, further larger sample based and functional studies are required to validate its role in CML.

IndexTerms **- CML, BCR-ABL, PTEN, SNP.**

I. INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disorder of hematopoietic stem cells, characterized by a specific chromosomal aberration, the Philadelphia[Ph] chromosome harboring reciprocal translocation, t(9;22)(q34;q11). CML progresses through 3 clinical phases: the chronic phase (CP) is followed by an accelerated phase and then by blast crises. The molecular consequence of the translocation is a novel fusion gene, BCR-ABL, which encodes an oncoprotein with aberrant tyrosine kinase activity (Daley GQ. et. al 1990; Kelliher M et. al 1990). Leukemic transformation process and progression is driven by BCR-ABL activity through several pathways (Stefan F et. al 1999; Ren R 2002) which are blocked efficiently by selective Tyrosine Kinase Inhibitors (TKIs) mainly Imatinib Mesylate, the frontline treatment. BCR-ABL dependent pathways were relatively under expressed during imatinib treatment but the other signaling pathways, including PI3K/AKT pathway are involved in activation of cell adhesion, proliferation, differentiation and inactivation of tumor suppressors indicating the implication of many other genes in the development of CML. Activation of these alternate pathways may allow progression even with therapeutic blockade of BCR-ABL activated pathways.

PTEN (Phosphatase and Tensin Homolog), located on chromosome 10q23.31, is known to be a tumor suppressor which is mutated in a large number of cancers at high frequency. PTEN mainly involved in the homeostatic maintenance of the phosphatidylinositol 3 kinase (PI3K) AKT cascade, influences a number of cellular processes relevant to tumor genesis including regulation of proliferation and survival, cell migration and invasion, genomic instability, induction of cell cycle check points in response to DNA damage and stem cell self-renewal in several cancers including CML. It is a key component of PIK3 pathway associated with inhibition of AKT signaling pathway (Song MS et al., 2012). More than 150 unique mutations in PTEN are listed in the Human Gene Mutation Database. The majority of PTEN germline mutations (76%) result in truncated protein, dysfunctional protein or haplo insufficiency which results in the inability of inhibition of AKT phosphorylation. PTEN loss causes increased proliferation, de-differentiation and progression in prostate stem cells and the number of tumor initiating cells is enhanced due to PTEN loss in mouse model of leukemia (Michele M. et al., 2015). Despite unique and precise BCR-ABL tyrosine kinase inhibitors, complete eradication of CML is still facing limitless challenges. Point mutations in BCR-ABL gene accelerates the risk of CML by down regulating PTEN activity and increasing drug resistance in leukemia stem cells (Peng et al., 2010; Panuzzo C et al., 2014).

In the present study, we focused on the analysis of PTEN 32-bp Ins/Del, rs34421660, in CML. Previous studies on this SNP reports revealed that loss of PTEN function by deletion was strongly associated with the intraductal papillary mucinous neoplasms (IPMN) of pancreas (Garcia-Carracedo et al., 2013). Whereas in another study, deletion variant was associated with decreased hepatocellular carcinoma (HCC) risk in Chinese Han population (Ding, Gao, Liu, Xu, & Liu, 2010). However, in context of Indian population and or Asian population in connection with CML is yet to be studied.

II. METHODOLOGY

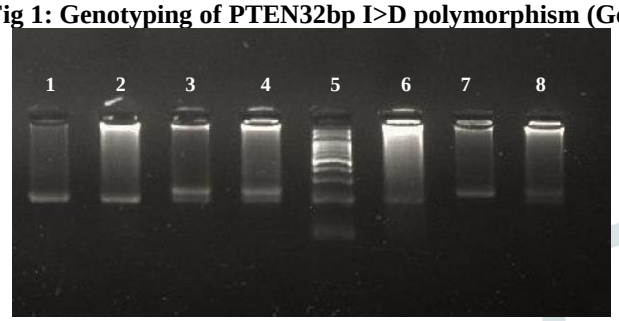
544 primary CML cases reported at Nizam Institute of Medical Sciences, Hyderabad were recruited in the present study after obtaining informed consent. 514 age and gender-matched controls were obtained from the local population without a family history of any cancers. Only primary Ph+ve CML cases with the confirmed diagnosis were included in the study.

Epidemiological information such as gender, age at the time of diagnosis, occupation, area of living, habits, and diet were collected through personal interviews and the clinical information was noted down from the tumor registry with the help of medical oncologist which included: Phase of CML at the time of diagnosis, baseline clinical characteristics such as WBC count, Platelet count, Blast %, Basophils, Eosinophils and Spleen size. All the patients were followed up to assess the drug (imatinib) response at Hematological, Cytogenetic and Molecular levels as well as the progression into advanced phase. The response was categorized into major and poor responders (intermediate and minor) based on specific criteria. The study was approved by the Institutional

Ethics Committee for Biomedical Research, Osmania University and an Ethical committee of Nizams Institute of Medical Sciences, Hyderabad.

Genomic DNA was isolated by non-enzymatic salting out method from 5 ml of blood samples collected in EDTA vacutainers from both patients and controls. Genotyping was carried out by PCR- Direct Genotyping method wherein the reaction was performed using 50 ng of DNA as template in 10 µl reaction mix comprising of 10 mM each of dNTP mix, 30 pmol each of forward and reverse primers (Fwd: 5′-CATTAGAGCTGCGATTGGACCG-3′, Rev: 5′-GCTCCCTCGGGAGGTTTGGT-3′, 0.5U Taq Polymerase, and Milli-Q water. The PCR conditions included 35 cycles of initial denaturation (95°C for 5 minutes), denaturation (95°C for 30 seconds), annealing (60°C for 30 seconds), extension (72°C for 23 minute) and final extension (720C for 10 minutes). The PCR products were analyzed on 3.5% agarose gel. The PCR products for insertion and deletion alleles comprised 241 and 209 bp respectively. The data obtained was subjected to various statistical analyses like SNPSTATs (http://bioinfo.iconcologia.net/snpstats/start.htm) – for calculating the odds ratio, Hardy-Weinberg equilibrium frequencies. Allele frequencies and chi-square were tested using online statistical tools (http://www.had2know.com/academics/hardy-Weinberg-equilibrium-calculator-2-alleles.html, http://www.quantpsy.org/chisq/chisq.htm).

Fig 1: Genotyping of PTEN32bp I>D polymorphism (Gel Picture)



Lane 3, 4, 8- ID genotype (241bp, 209bp)
Lane 7– II genotype (241bp)
Lane 1, 2, 6- DD genotype (209 bp)
Lane 5 – ladder (100 bp)

III. RESULTS AND DISCUSSION

Table 1: Distribution of genotypes and allele frequencies of PTEN 32bp Insertion/Deletion polymorphism among controls and CML cases according to different genetic models

MODEL	GENOTYPE	CONTROL	CASE	OR (95% CI)	P-VALUE
		N=514(48.6%)	**N=544(51.4%)**		
Codominant	I/I	187 (36.4%)	227 (41.7%)	1.00	
	I/D	160 (31.1%)	174 (32.0%)	0.90 (0.67-1.20)	
	D/D	167 (32.5%)	143 (26.3%)	**0.71 (0.52-0.95)**	**0.07#**
Dominant	I/I	187 (36.4%)	227 (41.7%)	1.00	**0.07#**
	I/D-D/D	327 (63.6%)	317 (58.3%)	0.80 (0.62-1.02)	
Recessive	I/I-I/D	347 (67.5%)	401 (73.7%)	1.00	**0.03***
	D/D	167 (32.5%)	143 (26.3%)	**0.74 (0.57-0.97)**	
Overdominant	I/I-D/D	354 (68.9%)	370 (68.0%)	1.00	0.76
	I/D	160 (31.1%)	174 (32.0%)	1.04 (0.80-1.35)	
ALLELE FREQUENCIES					
Wild Allele	I	534(51.9)	628(57.7)	1.00	**0.007***
Variant Allele	D	494(48.1)	460(42.3)	**0.79(0.68-0.94)**	
Hardy Weinberg Equilibrium : Controls p<**0.0001**; Cases p<**0.0001**					
OR- Odds ratio adjusted by age and sex; p value- χ2 p value; *p<0.05; #p<0.10					

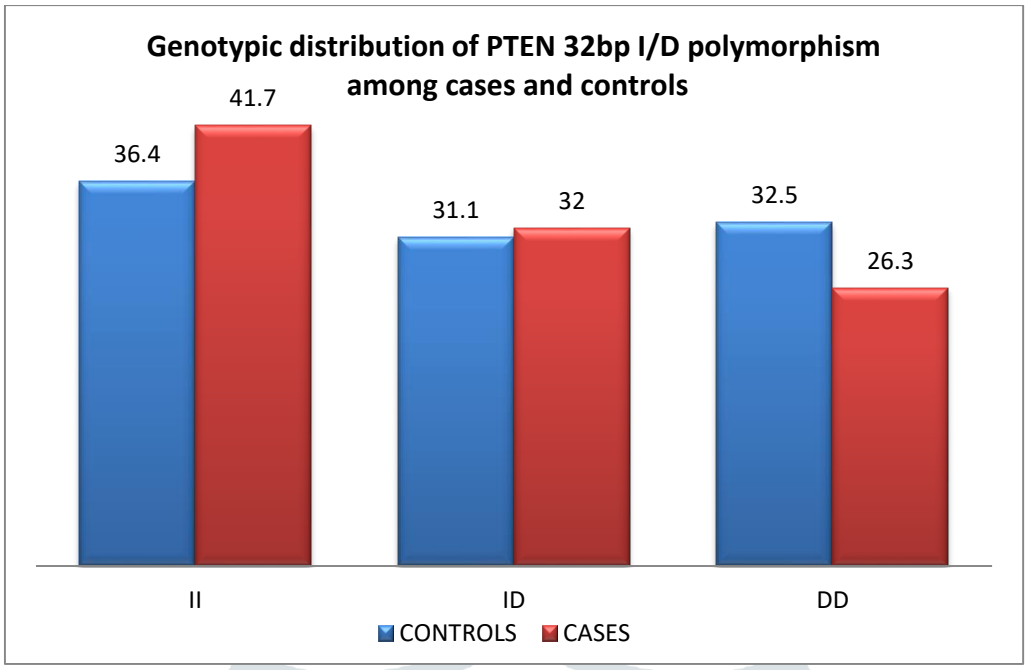


Figure 2: Genotypic distribution of PTEN 32bp I/D polymorphism among cases and controls

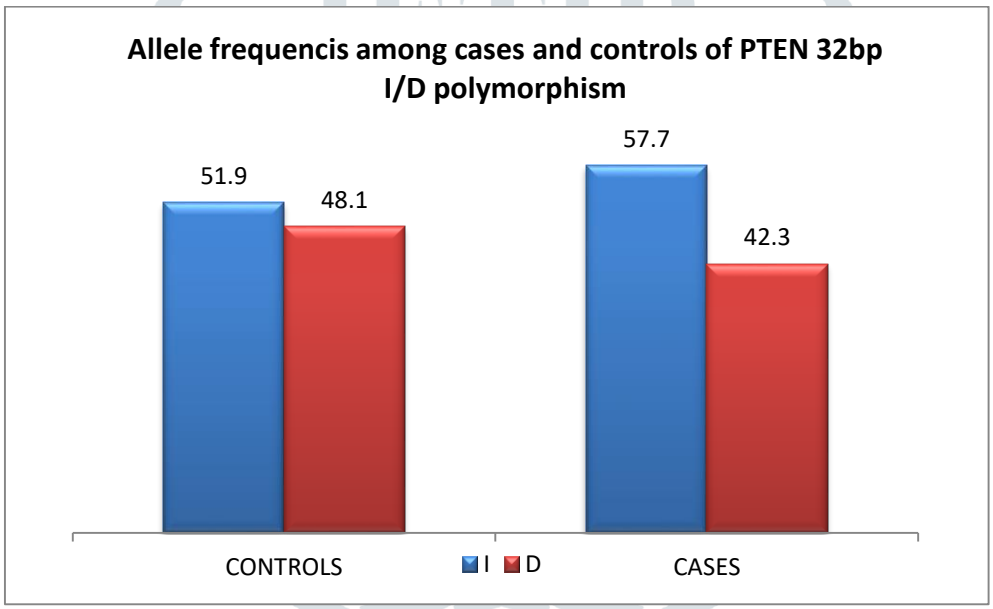


Figure 3 : Allele frequencis among cases and controls of PTEN 32bp I/D polymorphism

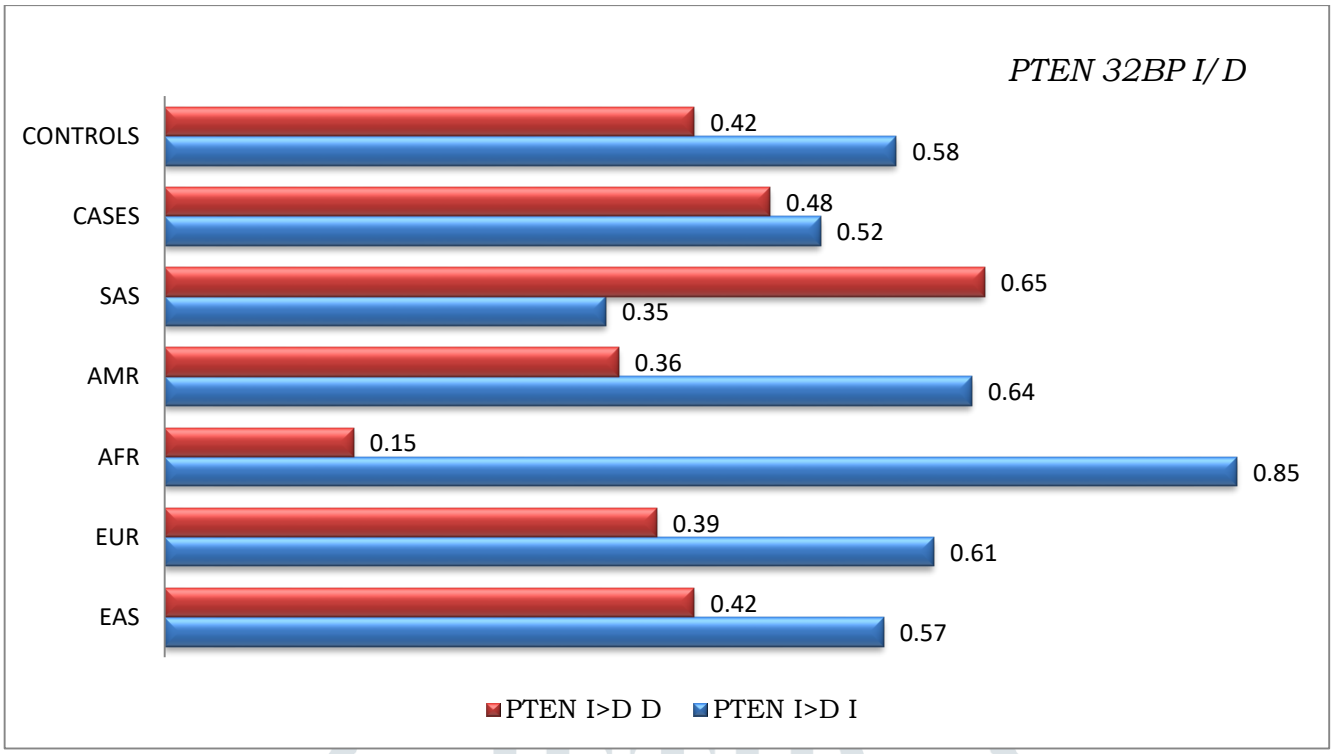


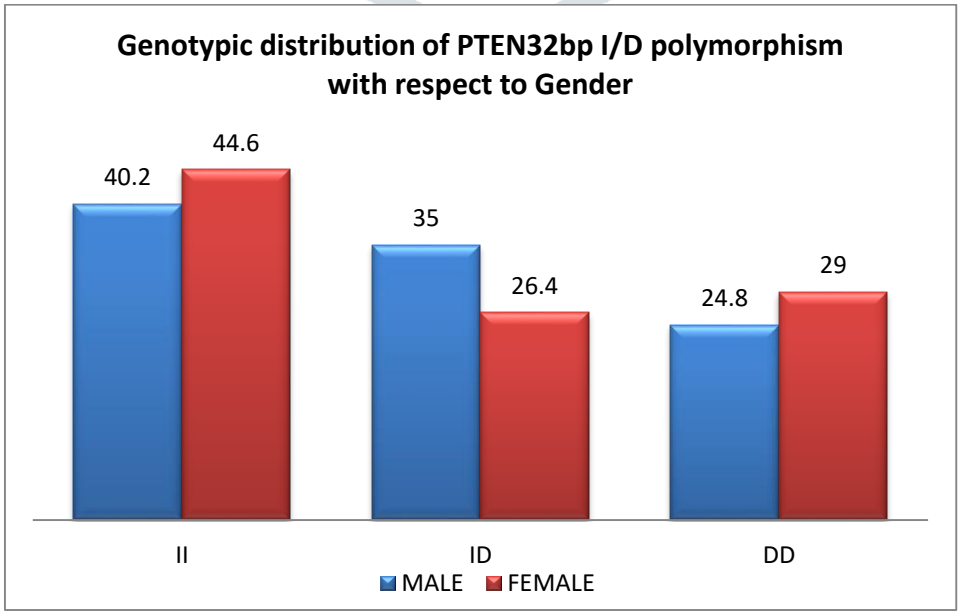
Figure 4: HAPMAP OF PTEN 32bp I/D POLYMORPHISM

Genotypic distribution of PTEN gene polymorphism with respect to confounding variables

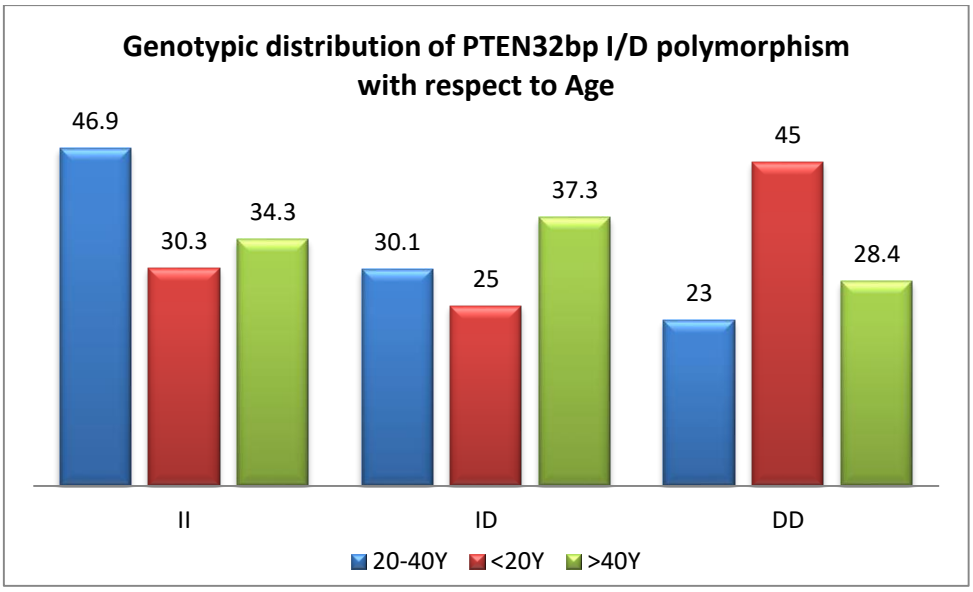
Table 2: Genotype distribution of PTEN32bp I/D polymorphism with respect to Epidemiological variables

VARIABLES	II N (%)	ID N (%)	OR (95% CI)	DD N (%)	OR (95% CI)	p-value	I N (%)	D N (%)	OR (95% CI)	p-value
GENDER										
Male	141(40.2)	123(35.0)	1.00	87(24.8)	1.00	0.12	405(57.7)	297(42.3)	1.00	0.97
Female	86(44.6)	51(26.4)	0.68(0.45-1.04)	56(29.0)	1.06(0.69-1.62)		223(57.8)	163(42.2)	1.00(0.78-1.28)	
AGE AT ONSET										
20-40 yrs	157(46.9)	101(30.1)	1.00	77(23.0)	1.00	**0.004***	415(61.9)	255(38.1)	1.00	**0.004***
< 20 years	12(30.0)	10(25.0)	1.30(0.54-3.11)	18(45.0)	**3.06(1.40-6.67)**		34(42.5)	46(57.5)	0.65 (0.33-1.31)	
>40 years	58(34.3)	63(37.3)	**1.69(1.09-2.61)**	48(28.4)	**1.69(1.05-2.70)**		179(53.0)	159(47.0)	1.26 (0.90-1.76)	

OR- Odds ratio adjusted by age and sex; p value- $\chi 2$ p value; *p<0.05; #p<0.10



Figures 5: Genotypic distribution of PTEN32bp I/D polymorphism with respect to Gender

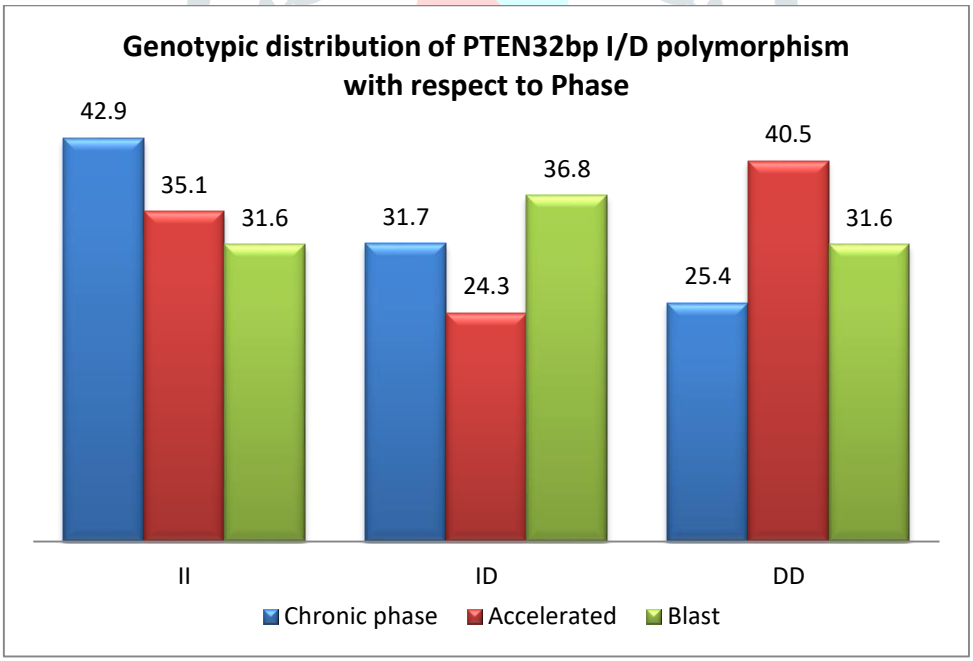


Figures 6: Genotypic distribution of PTEN32bp I/D polymorphism with respect to Age

Table 3: genotype distribution of PTEN 32bp I/D polymorphism with respect to Phase

PHASE OF CML	II N (%)	ID N (%)	OR (95% CI)	DD N (%)	OR (95% CI)	p-value	I N (%)	D N (%)	OR (95% CI)	p-value
Chronic phase	203(42.9)	150(31.7)	1.00	120(25.4)	1.00	0.29	556(58.8)	390(41.2)	1.00	0.10
Accelerated	13(35.1)	9(24.3)	0.94(0.39-2.25)	15(40.5)	1.95(0.90-4.24)		35(47.3)	39(52.7)	1.59(0.9 9-0.55)	
Blast	6(31.6)	7(36.8)	1.58(0.52-4.79)	6(31.6)	1.69(0.53-5.36)		19(50.0)	19(50.0)	1.43(0.75-2.73)	

OR- Odds ratio adjusted by age and sex; p value- $\chi 2$ p value; *p<0.05; #p<0.10

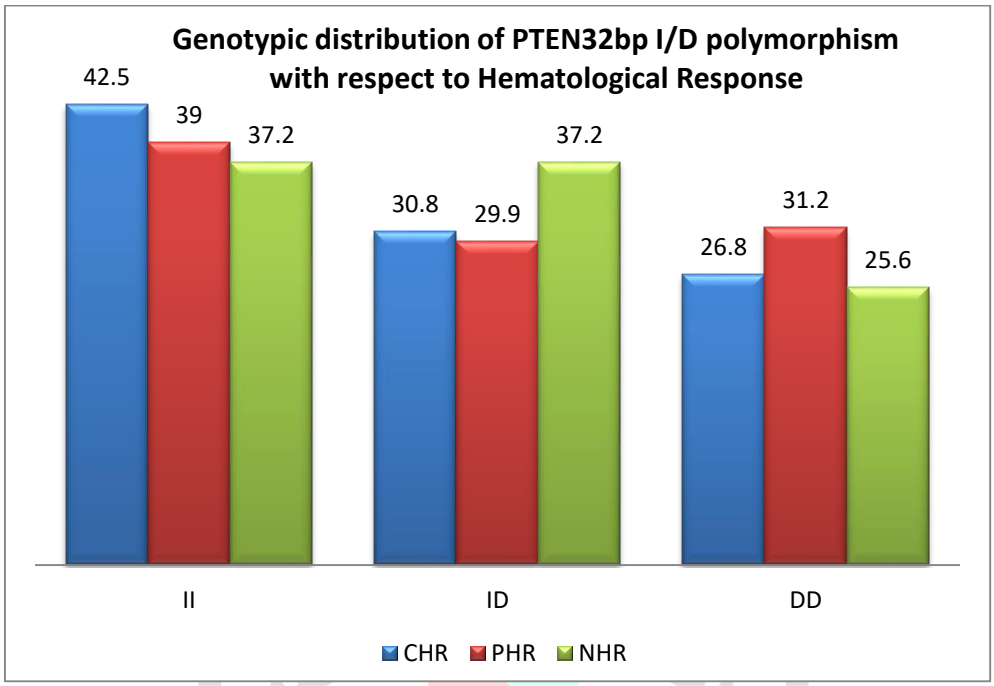


Figures 7: Genotypic distribution of PTEN32bp I/D polymorphism with respect to Phase

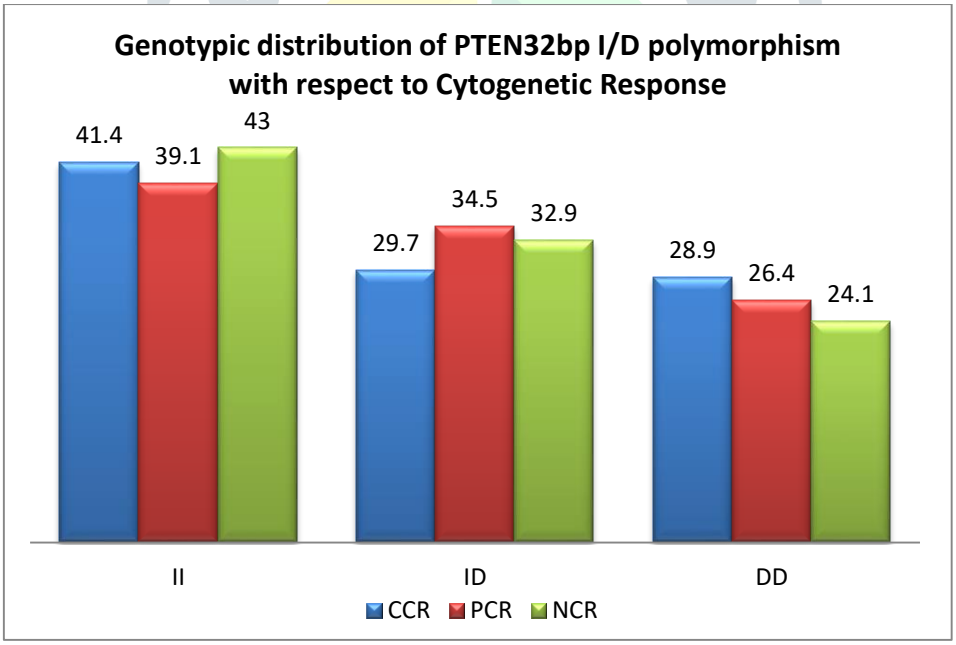
Table 4: genotype distribution of PTEN 32bp I/D polymorphism with respect to Imatinib response

Variable	II N (%)	ID N (%)	OR (95% CI)	DD N (%)	OR (95% CI)	p-value	I N (%)	D N (%)	OR (95% CI)	p-value
HEMATOLOGICAL RESPONSE										
CHR	127(42.5)	92(30.8)	1.00	80(26.8)	1.00	0.84	346(57.9)	252(42.1)	1.00	0.66
PHR	30(39)	23(29.9)	1.06(0.58-1.94)	24(31.2)	1.27(0.69-2.33)		83(53.9)	71(46.1)	1.17(0.82-1.68)	
NHR	16(37.2)	16(37.2)	1.38(0.66-2.90)	11(25.6)	1.09(0.48-2.47)		48(55.8)	38(44.2)	1.09(0.69-1.71)	
CYTOGENETIC RESPONSE										
CCR	99(41.4)	71(29.7)	1.00	69(28.9)	1.00	0.87	269(56.3)	209(43.7)	1.00	0.77
PCR	34(39.1)	30(34.5)	1.23(0.69-2.19)	23(26.4)	0.97(0.53-1.79)		98(56.3)	76(43.7)	1.00(0.70-1.41)	

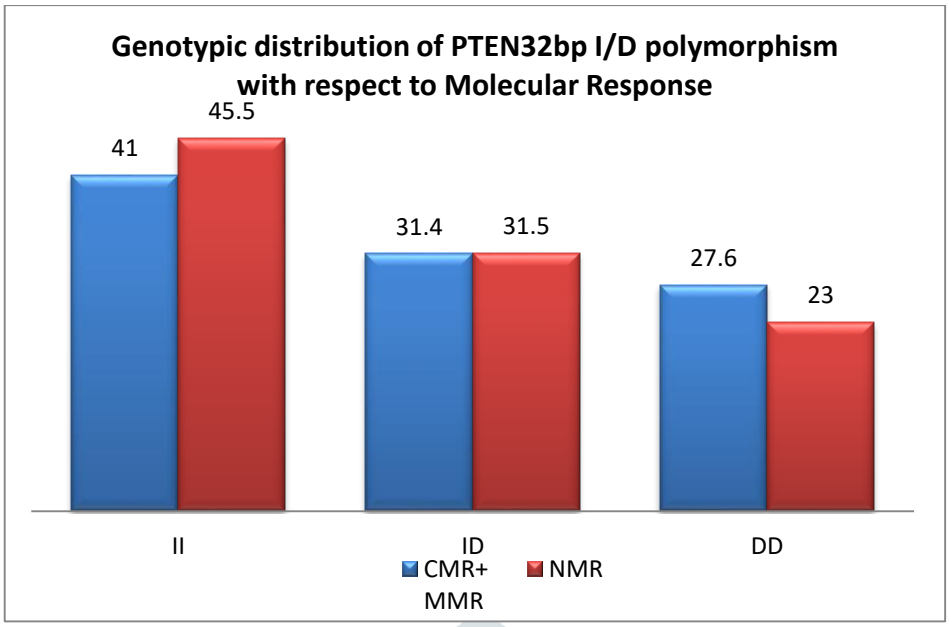
NCR	34(43)	26(32.9)	1.07(0.59-1.93)	19(24.1)	0.80(0.42-1.52)		94(59.5)	64(40.5)	0.88(0.61-1.26)	
MOLECULAR RESPONSE										
CMR MMR	86(41.0)	66(31.4)	1.00	58(27.6)	1.00	0.55	238(56.7)	182(43.3)	1.00	0.21
NMR	75(45.5)	52(31.5)	0.90(0.56-1.46)	38(23)	0.75(0.45-1.25)		202(61.2)	128(38.8)	0.88(0.61-1.26)	
OR- Odds ratio adjusted by age and sex; p value- $\chi 2$ p value; *p<0.05; #p<0.10										



Figures 8: Genotypic distribution of PTEN32bp I/D polymorphism with respect to Hematological Response



Figures 9: Genotypic distribution of PTEN32bp I/D polymorphism with respect to Cytogenetic Response



Figures 10: Genotypic distribution of PTEN32bp I/D polymorphism with respect to Molecular Response

D/D genotype of PTEN 32bp gene polymorphism was found to confer reduced risk for CML development (D/D- 26 % in cases and 32 % in controls).The same trend is observed with D allele frequency (42.3%) conferring reduced risk. The D allele frequency (42%) in control group of our study is similar to that of EAS (42%) population. The genotype distribution of PTEN 32bp I/D polymorphism among cases showed deviation from Hardy-Weinberg Equilibrium indicating the SNP association. The D/D genotype having conferring to reduced risk was clearly observed in 20-40 age group (23.0%) as compared to I/D (30.1%) and I/I (46.9%) genotypes. With respect to phase at diagnosis DD genotype frequency was elevated in Accelerated Phase than Blast Phase. Further strongly indicating reduced risk conferred by DD (40.5%) genotype. When compared to imatinib response the D/D genotype frequency were slightly reduced among non-responders as compared to partial and complete responders. However the polymorphic distribution did not show variation with other confounding variables.

CONCLUSION

The results of the study suggest that PTEN as an independent prognostic marker for treatment and monitoring in CML cases. D/D genotype of PTEN 32bp gene polymorphism was found to confer reduced risk for CML development. However, future larger studies are needed to evaluate the clinical values of PTEN in CML susceptibility.

IV. ACKNOWLEDGMENT

We would like to thank all the patients (CML) and volunteers who gave consent to participate in the study. The financial assistance from UGC-MJRP project (F43-350/2014(SR)) and Department of Science and Technology (OU-DST)-PURSE-II program, Osmania University is greatly appreciated.

REFERENCES

[1] Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukaemia in mice by the P210 bcr/abl gene of the Philadelphia chromosome. Science 1990; 274:824-830.

[2] Ding, J., Gao, Y., Liu, R., Xu, F., & Liu, H. (2010). Association of PTEN Polymorphisms with Susceptibility to Hepatocellular Carcinoma in a Han Chinese Population. DNA and Cell Biology, 30(4), 229–234.

[3] Garcia-Carracedo, D., Turk, A. T., Fine, S. A., Akhavan, N., Tweel, B. C., Parsons, R.,Su, G. H. (2013). Loss of PTEN expression is associated with poor prognosis in patients with intraductal papillary mucinous neoplasms of the pancreas. Clinical Cancer Research, 19(24), 6830–6841.

[4] Kelliher M, Mc Laughlin J, Witte O, Rosenberg N. Induction of a chronic myelogenous leukaemia-like syndrome in mice with v-abl and bcr/abl. The Proceedings of the National Academy of Sciences USA 1990; 87:6659-6653.

[5] Michele M. , Italia F., Fabiana C., Ursula C. I., Anais D. C., Nicola I., Carmen M. A., Nuzzo,1 Vanja V., Sabrina V., Francesco C., and Ludovica, C. (2015) PTEN: Multiple Functions in Human Malignant Tumors. Frontiers in Oncology 2015; 5: 24.

[6] Panuzzo C, Crivellaro S, Carrà G, Guerrasio A, Saglio G, Morotti A. BCR-ABL promotes PTEN downregulation in chronic myeloid leukemia. PloS One. 2014;9: e110682.

[7] Peng, C., Chen, Y., Yang, Z., Zhang, H., Osterby, L., Rosmarin, A. G., & Li, S. (2010). PTEN is a tumor suppressor in CML stem cells and BCR-ABL-induced leukemias in mice. Blood, 115(3), 626–635.

[8] Ren R. The molecular mechanism of chronic myelogenous leukemia and its therapeutic implications: studies in a murine model. Oncogene 2002; 21(56):8629-8642.

[9] Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol. 2012;13: 283–296.

[10] Stefan F, Moshe T, Zeev E, Susan OB, Razelle K, and Kantarjian HM. The Biology of Chronic Myeloid Leukemia. New England Journal of Medicine 1999; 341:164-172.