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ORIGINAL RESEARCH

Clinico-Pathological Factors and AR-LBD Mutations in Early and Late Castration-Resistant Prostate Cancer

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Background: Prostate cancer (PCa) is not well understood because of its enormous biological heterogeneity and unreliable progression. We conducted this retrospective analysis to examine the variables predicting early and late progression to castration-resistant PCa (CRPC) for better management of this disease.

Methods: This single institutional retrospective study was conducted from January 2018 to January 2022. A total of 98 consecutive men meeting with the diagnosis of CRPC as per the inclusion criteria were included in the study and were stratified in four quartiles on the basis of time to CRPC (time to castration resistance [TTCR]) development. Early CRPC (1st quartile, TTCR = 6–12 months) and late CRPC (4th quartile, TTCR = 38–120 months) were then compared on the basis of different clinical, pathological and AR-LBD sequence to find the correlation with response duration.

Results: Median time to develop castration resistance was 25 ± 26.44 months. The mean age of the patients was 66.8 ± 9.20 years and median baseline PSA was calculated 100±685.06 ng/mL respectively. Higher Gleason score (≥7–10) was found to be significantly associated with early development of CRPC ($p<0.001$) and lower nadir PSA was significantly indicating late CRPC progression ($p<0.005$). No mutations were found in androgen receptor exon-5, 6, 7 except a homozygous mutation in the 7th intronic region, which is involved in splice variants formation playing noteworthy role in CRPC development.

Conclusion: Time for metastatic PCa to CRPC ranges from 6–120 months revealing its heterogeneous nature. Early age presentation in the clinic and high initial PSA and high grade (GS>7) at diagnosis were positively associated with early CRPC while lower nadir PSA was correlated with late CRPC progression. No remarkable genomic mutations were discovered. Therefore, more data are needed and further research is required with large no. of patients to discover the predictive prognostic biomarkers for better patients' management.

Keywords: prostate cancer, castration resistance, early and late progression, PSA, androgen receptor, PCa

Introduction

Prostate cancer (PCa) is one of the most common cancers among men.[1] A total of 14,14,259 new cases of PCa were reported globally in 2020. Prostate carcinoma is the second-leading cause of death in males after lung cancer, causing 3,75,304 deaths in 2020 worldwide.[2] Among this global burden, 2.9% was contributed by India with 41,532 PCa cases.[3,4] For primary, locally advanced and high risk metastatic cases, androgen deprivation therapy (ADT) such as bilateral orchiectomy and gonadotropin-releasing hormone analogs treatment is the main and effective one. Despite standard ADT, almost every hormone sensitive PCa (HSPC) patient leads to the development of castration resistance. However this has been observed in the clinic that the time involved in the development to castration resistant PCa (CRPC) after ADT, is highly varied. A number of studies have focused on the factors correlated with the development of HSPC to CRPC.[5]

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A number of theories and mechanisms for the development to castration resistance have been proposed in various studies.[6,7] Despite the fact that CRPC was previously assumed to be androgen-independent, new research shows that androgen signaling is frequently maintained through a variety of non-traditional pathways and alterations involving both the androgenic ligand and the androgen receptor (AR). Somatic mutations of the ligand binding domain (LBD) which result in drug resistance to anti-androgens has been reported in literature.[8,9] However, reports on co-relation and impact of gene-mutations on development of CRPC are lacking.

PCa exhibit enormous biological heterogeneity. Understanding and predicting the progression to CRPC is critical for better PCa management and patients' risk-stratification. In this study, we attempted to determine the significance of clinico-pathological risk factors for PCa as well as focusing on AR-LBD mutations that may confer survival benefit in androgen depleted status and others that may render it susceptible to androgen depletion, leading to early or late development to CRPC.

Materials and Methods

Study Approval and Patients' Population

In this study, a total of 98 CRPC cases were enrolled at a single tertiary care center from January 2018 to January 2022. Clinic-pathological variables including the age at presentation, baseline prostate specific antigen (PSA), Gleason score, metastatic status (PET-CT/bone scan/MRI findings), nadir PSA and treatment modalities were extracted retrospectively from the patients' records. Approval was obtained from the Institute Ethics Committee All India Institute of Medical Sciences, New Delhi, India (Ref. No.: IEC-628/03.11.2017). regarding human subjects' involvement. Also, the written informed consent was obtained from each patient at the time of registration and sample collection. Exclusion criteria were unknown follow-up status or unknown status regarding the time point of progression to CRPC (n = 3). CRPC status was defined in accordance with the EAU guidelines ie PSA progression of three consecutive rises of PSA values or a 50% increase of absolute PSA values over PSA nadir under HSPC treatment combined with a testosterone level<50ng/dl.

Study Design

On the basis of time to castration resistance (TTCR), all the enrolled subjects were stratified into four quartiles. First quartile of the patients was defined as early CRPC and forth quartile as late CRPC. Further, these early and late responders were compared on the basis of different clinical parameters such as Age, PSA at diagnosis, Gleason Score, Nadir PSA, metastatic site, treatment modalities till CRPC development and AR-LBD genomic mutations to find co-relation among them.

Genomic PCR and Sanger Sequencing

Genomic DNA was extracted from the blood samples of each patient by QIAamp DNA blood mini kit using manufacturer's protocol. Primers for the exon 5, 6, 7 and 8 of AR-LBD were designed using Primer Blast tool of NCBI. The sequences of the primers were as follows: AR-5 (FP: CAACCCGTCAGTACCCAGAC; RP: AGCTTCACTGTCACCCCATC), AR-6 (FP: AGAGACATTCCCTCTGGGCT; RP: GGGCATTCCCTGCACTTCTA), AR-7 (FP: CTAATGCTCCTTCGTGGGCA; RP: CAACAGGTGGTGCCAGACTC), AR-8 (FP: GTTGGGGAAGAGGCTAGCAG; RP: GGCACTGCAGAGGAGTAGTG). Amplified gene fragments were sequenced using the Sanger's sequencing protocol (Sanger F, Nicklen S, Coulson AR. 1977). Big-Dye Terminator v3.1 cycle Sequencing Kit was used for sequencing as per the manufacturer's instructions on ABI 3500 Genetic Analyzer.

Statistics and Data Analysis

Significance of the clinic-pathological parameters among the data was checked using Stata, version 14.0 software (StataCorp, College Station, Texas). Level of significance was set at $p<0.05$. Chromas Software V2.4.1 (available at http://www.technelysium.com.au/chromas.html) and SnapGene software (from Insightful Science; available at snapgene.com) was used for viewing, editing and analyzing the chromatogram images of all the sequences. Variants were analyzed

using the BioEdit Sequence Alignment Editor V7.2.5 (Hall, T.A., 1999) and Mutation Surveyor V4.0 software package (Software Genetics, State College, PA, USA).

Results

A total of 98 eligible CRPC cases were enrolled, however, selection criteria resulted in 95 eligible CRPC patients and the clinic-pathological characteristics are summarized in Table 1. Median time to develop castration resistance was 25 ± 26.44 months (mean 30.7, range 6–120) and the mean age of the patients was 66.8 ± 9.20 (median 67, range 46–91) respectively. Median baseline PSA was calculated 100 ng/mL ranging from 8.7 to 1175 ng/mL. At diagnosis, 36.8% cases were found to have low grade PCa (GS≤7) whereas 63.2% patients were grouped as high grade PCa (GS>7).

On the basis of time involved in development of CRPC, all the enrolled subjects were stratified into four groups as shown in Figure 1. First quartile or early CRPC (25.2% percentile; n = 24) and forth quartile or late CRPC (25.2% percentile; n = 24) acquired resistance in a median time period of 9 months (range 6–12 months) and 62 months (range 38–120 months) respectively. Four factors were found to be associated with CRPC progression (Table 2). High grade Gleason score (>7) at diagnosis was significantly associated with early CRPC development ($p<0.001$; Figure 2b) while lower nadir PSA was indicating the late CRPC progression (Figure 2a). Advance presentation to clinic at earlier ages with higher baseline PSA was associated with early development to CRPC but was not significant ($p>0.05$; Figure 2c and d).

A total of 95 samples were sequenced from both the sides. All completely sequenced and manually curated sequences were uploaded into Mutation Surveyor for variant calling. One sample was found to have homozygous mutation while a heterozygous missense mutation was observed in two samples at the same position as given in Table 3.

Table 1 Baseline Characteristics of Overall Participants and Participants Grouped into 1st Quartile Defined as "Early CRPC" and 4th Quartile as "Late CRPC"

Variable		Overall	Early CRPC	Late CRPC
		n = 98	n = 24	n = 24
Time to develop CRPC	Median	25	9	54.5
	(IQR)	(6–120)	(6–12)	(38–120)
Age at diagnosis	Median	67	63.6	68.6
	(IQR)	(46–91)	(47–80)	(65–78)
PSA at diagnosis	Median	100	100.0	50.1
	(IQR)	(8.7–4442.0)	(8.7–1175.0)	(8.8–1000)
Gleason Score at diagnosis	≤7	35	1	15
	>7	60	23	6
	Not Known	3	–	3
Nadir PSA	Median	0.48	2.41	0.06
	(IQR)	(0–1128)	(0.03–500.8)	(0.00–12.7)
Primary metastatic	Yes	86	24	19
	No	12	–	5
Metastatic site	Bones only	58	21	13
	Lymph Nodes only	11	1	4
	Multiple sites	13	1	–
	Reports N/A	4	1	2
Local/Primary therapy	Only medical castration	22	4	5
	Surgical+ Medical castration	76	20	19
Treatment till CRPC	ADT alone	82	21	18
	ADT+RARP/Prostatectomy	6	–	4
	ADT+ RT/CT	8	1	2
	ADT+ Abiraterone/CT	2	2	–

Abbreviations: IQR, inter quartile range; ADT, androgen deprivation therapy; RT, radiotherapy; CT, chemotherapy; RARP, robotic assisted radial prostatectomy.

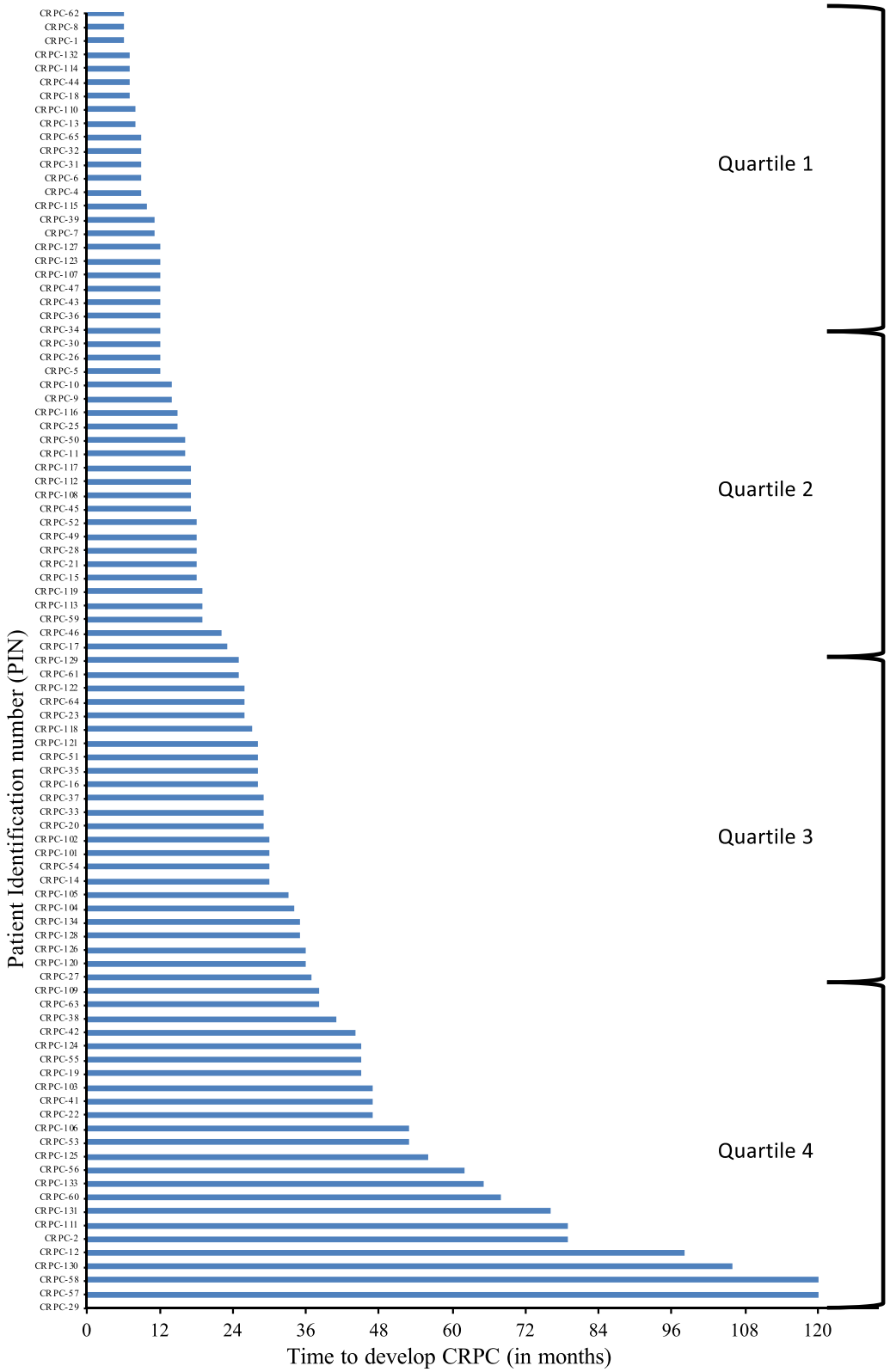

Figure 1 Graph showing the time taken by the individual case (6–120 months).

Table 2 List of Variables Found to Be Associated with Early and Late Development of CRPC

Variable	Early CRPC	Late CRPC	p-value
Gleason Score >7	23	6	**<0.005**
Nadir PSA	2.41	0.06	**<0.005**
Mean Age at diagnosis (95% CI)	63.6 (59.27–67.97)	68.6 (65.28–72.05)	0.06
Mean Baseline PSA (95% CI)	280.0 (122.85–437.25)	156.95 (48.95–264.94)	0.203

Note: P value < 0.05 signifies statistical significance and shown in bold.

Discussion

Various studies[10,11] had tried to develop the understanding on the role of different clinical and pathological factors to understand PCa aggressiveness and metastatic potentials. However, factors contributing to CRPC development have largely remained unexplored despite knowing the fact that after standard ADT, PCas rapidly relapse after castration therapy and progress to CRPC stage. This has also been observed that the time involved in the development of CRPC after ADT may vary from few months to several years.[12,13]

Mechanism of development of castration resistance variably depends on tumor heterogeneity and selection pressure exerted via drug treatment.[14,15] Any drug treatment invariably leads to decrease in the population of sensitive cells and subsequent increase in the drug resistant cells. This drug resistance could be either acquired through somatic mutations during tumor development or there could be certain germ-line mutation providing susceptibility or resistance mechanism to prostate cells in certain clinical conditions.[16]

In our study, we tried to find out the correlation of the clinic-pathological characteristics of patients with CRPC progression by parting the cases into four quartiles according to TTCR. This TTCR has been studied as a predictive biomarker for disease assessment and to determine sequential treatment options.[12]

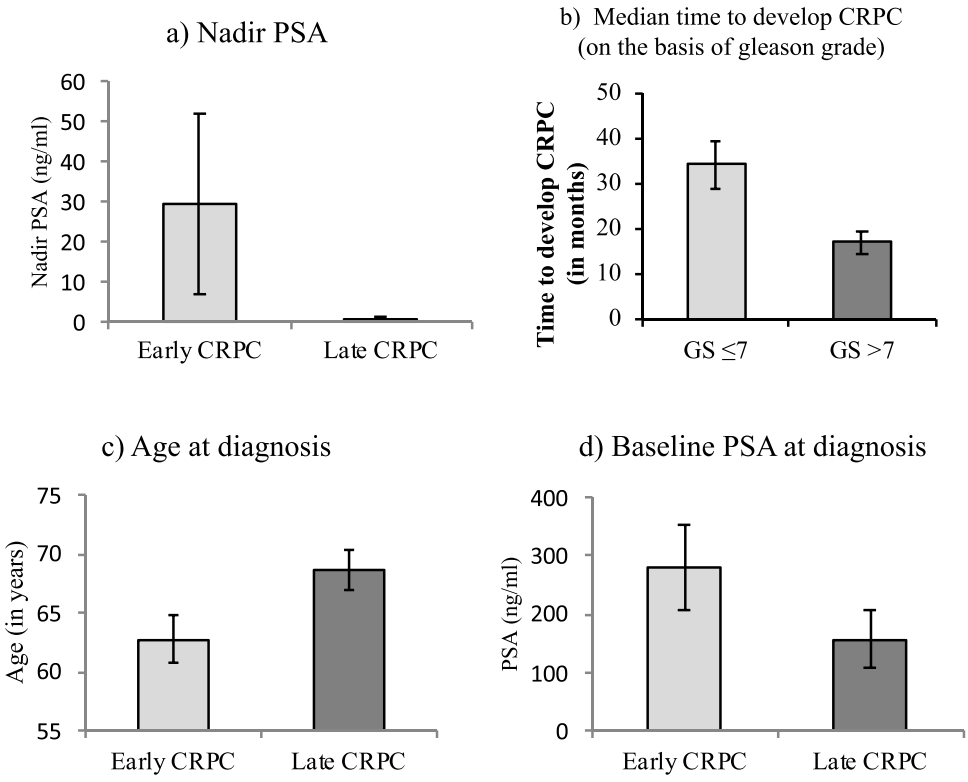

Figure 2 Graphs showing the level of significance among Early CRPC & Late CRPC. (**a**) Nadir PSA. (**b**) Median time to develop CRPC (on the basis of Gleason grade. (**c**) Age at diagnosis. (**d**) baseline PSA at diagnosis.

Table 3 Mutations Identified in AR-LBD in CRPC Patients

	CRPC-35	CRPC-31	CRPC-54
Mutation Type	Homozygous	Heterozygous	Heterozygous
Chromosome Position	X:66942837	X:66943580	X:66943581
Mutation	179464G>A	180207T>TC:887M>M/T	180208G>GA:887M>M/T
Exon	Intronic region	Exon-8	Exon-8
Effect of mutation	11bp away from the 3' end of exon-7	Mis-sense mutation	Mis-sense mutation
Time to develop CRPC	28	9	30
Age at diagnosis	60	78	75
Baseline PSA	100	16.8	1373.0
Gleason Score	7	9	7
B/L orchiectomy	Done	Done	Done
Nadir PSA	1.85	0.6	1.93
Metastatic/Localized	Metastatic	Metastatic	Metastatic

Note: The clinical parameters of CRPC-35, CRPC-31, CRPC-54 has been given in the table.

As reported by Aly, Markus et al.[17] Gleason score ≥8 was found to be associated with higher all-cause mortality than GS≤6. Our study also came to the conclusion that early CRPC development is closely connected with high Gleason scores. In our early CRPC group, 95.8% of cases had GS>7, indicating a very strong link of Gleason score with the early advancement to CRPC but only 25% of patients were diagnosed with high grade (GS>7) in our late CRPC group, indicating a stronger response to ADT and a delayed progression to CRPC.

Additionally, we observed in our population that instances with lower nadir PSA levels following androgen depletion, indicating a better response to ADT, had a higher likelihood of late CRPC progression. These findings are consistent with the previous studies where higher baseline PSA as well as high nadir PSA was found to be linked with shorter overall survival and progression free survival in alliance with TTCR.[18,19]

As reported in prior studies, our study also suggested the positive correlation of higher baseline PSA level with early CRPC progression.[20,21] However the p-value was not up to significant level as the p-value depends on the total number of cases studied; if a larger number of patients are included, a large-scale study could establish a cut-off and more clearly demonstrate the significance of the baseline PSA.

Age is one of the foremost criteria while deciding course of treatment for the patient. Kimura et al (2018)[4] suggested that age was positively associated with PCa incidence. Similar findings were made in our study as well where we discovered that the preliminary presentation to clinic at early age was associated to early CRPC development whereas a later PCa diagnosis led to a better response to initial hormone therapy. This correlation, however, was not determined to be significant in terms of p-value, maybe as a result of the complex PCa tumourigenesis process that involves a variety of confounding factors.

Earlier, number of studies[21,22] has correlated progression to CRPC with age, baseline PSA, Gleason grade and nadir PSA level as we observed in our data as well. A number of genome based studies had discovered significant variations including AR gene amplification and mutations in AR-LBD associated with CRPC progression and drug resistance to anti-androgens.[23,24] Specifically in AR-LBD, a homozygous mutation was observed in the 7th intronic region. As reported in literature, this intronic region is involved in splice variants formation during post transcriptional modifications and play role in CRPC development[19,25] however, more detailed mechanisms are not known. Additionally, a mis-sense heterozygous mutation in exon-8 was discovered in two samples, however we were unable to find any link of these mutations to the development of CRPC.

Limitations of this study include small sample size, heterogeneity among data and somewhat retrospective nature of the study. Some clinical data were retrospectively collected which resulted in incomplete retrieval of some information. Also, our study did not include any other prognostic variables such as prostate volume, PHI, castrate testosterone levels, no. and type of bone(s) involved.

Conclusion

In conclusion, our work is significant because it confirms earlier findings and shows the association between the several clinical, pathological, and biological genetic characteristics involved in the early and late development of CRPC. The variability in TTCR is a result of the heterogeneity of prostatic cancer. An accurate prediction of this TTCR may indicate need to more aggressive treatment modalities for early CRPC predictors, which could increase overall survival. To determine the function of alternate or non-androgen controlled pathways in the development of mCRPC, it is urgently necessary to examine and interpret genomic data in large sample sizes. Understanding the factors involved in the resistance mechanisms and development of an algorithm or clinical score on those factors may dramatically improve treatment protocols. Therefore, there is a need for predictive prognostic biomarkers as well as an increasing number of different therapy alternatives that can aid in improving patient risk classification and extending survival in mPCa patients.

Ethical Statement and Consent to Participate

All methods were carried out in accordance with the AIIMS, New Delhi, guidelines and the regulations. All experimental protocols were approved by our Institutional and/or licensing committee. Informed consent was obtained from all subjects. This study complies with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

There is no conflict of interest among authors.

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