ORIGINAL ARTICLE

Serum PSA variation and reference change value in Indian patients with prostate cancer and benign prostate hyperplasia: implications for serial monitoring

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Abstract

Background: Regular evaluation of Prostate Cancer (PCa) is not widely practiced in India, leading to delayed diagnoses based on elevated Prostate-Specific Antigen (PSA) or abnormal Digital Rectal Examination (DRE) findings. Conventional reference intervals might not indicate clinically meaningful alterations due to intra-individual biological variation. *Aim and Objectives:* To calculate the Reference Change Value (RCV) for PSA using analytical (CVA) and biological variation (CVI), and to evaluate its application in clinical settings in distinguishing PCa from Benign Prostate Hyperplasia (BPH) in Indian cases. *Material and Methods:* Total of 60 male patients (30 PCa, 30 BPH) with two PSA values were included. The levels of serum PSA were measured by ECLIA on Roche Cobas e401. RCV was determined by means of formula: RCV $2^{1/2}$*Z*$(CVA^2+CVI^2)^{½}$, with CVI from the EFLM database. Statistical analysis was performed using SPSS v20.0. *Results:* RCV thresholds were ±21.5% (increase) and −17.7% (decrease). PSA levels were markedly reduced in 76.7% of PCa vs. 46.7% of BPH patients ($p = 0.033$). RCV exceedance correlated with lower Gleason scores ($p = 0.013$) and was the only significant predictor of PCa diagnosis (OR 6.81, $p = 0.038$). *Conclusion:* RCV-based interpretation improves PSA diagnostic utility by accounting for biological variation. This approach may support more individualized and accurate clinical decision-making in PCa evaluation.

Keywords: Prostate Cancer, Prostate-specific Antigen, Benign Prostate hyperplasia, Biological Variation, Reference Values

Introduction

In India, Prostate Cancer (PCa) screening is still uncommon, and most cases are evaluated only after symptoms appear. Consequently, patients often come with symptoms of Lower Urinary Tract (LUTS) that are frequently related to Benign Prostatic Hyperplasia (BPH). PCa is often detected incidentally during the investigation of the lower urinary tract manifestations in combination with serum Prostate Specific Antigen (PSA) and Digital Rectal Examination (DRE) serving as initial assessments. When abnormalities are noted, Transrectal Ultrasonography (TRUS)-guided needle biopsies are typically performed to confirm the diagnosis [1]. A PSA level above 4 ng/mL, particularly in conjunction with an abnormal DRE, commonly prompts biopsy. However, this threshold, largely adopted from Western populations, may not be universally applicable, as PSA levels are influenced by ethnicity, age, environmental exposures, lifestyle, and metabolic factors [2]. PCa remains among the most frequently identified cancers in men worldwide. According to current

estimates, it is expected to account for approximately 313,780 new cases and 35,770 deaths globally in 2025 [3]. In India, the burden of PCa is also rising. As per the Global Cancer Observatory (GCO) 2022, the country is projected to report approximately 41,736 new cases by 2025 [4]. Clinical decisions are frequently guided by diagnostic data gathered from standard population reference ranges, many of which are subjected to significant intra-individual variation [5]. When the index of individuality (II) for an analyte falls below 0.6, conventional reference ranges become less reliable in spotting atypical results [6]. II is calculated using intra-individual variation (biological variation) and inter-individual variation [7]. A key challenge for clinicians lies in determining whether a given PSA value warrants further diagnostic evaluation. Hence, it is crucial to understand the extent of random variation in PSA levels that may occur between consecutive measurements [8]. Prior studies in the Indian population have highlighted the limitations of traditional PSA levels and advocated for the use of more accurate biomarkers and multivariable assessment strategies in PCa evaluation [9]. While these approaches focus largely on diagnostic performance, the current study addresses a distinct but complementary gap by calculating a PSA-specific Reference Change Value (RCV). This is achieved by integrating laboratory-derived analytical variation (CVA) with established within-subject biological variation (CVI) from European Federation of Laboratory Medicine (EFLM) data. Individuals' PSA values were assessed against the calculated RCV to determine significant changes, which were then correlated with histopathological findings to derive clinically meaningful insight.

Material and Methods

This was an observational study conducted over time with a retrospective analysis from January 2023 to December 2024 in the outpatient clinic of Urology at a tertiary care teaching hospital in Belagavi. The study was approved by the Institutional Ethics Committee (Ref. No. KAH ER/EC/22-23/229/8).

The Study adhered to the ethical standards of the Declaration of Helsinki and its subsequent amendments. One hundred and thirty male patients, enrolled consecutively to TRUS-guided prostate biopsy were approached for participation. Referrals were made from both outpatient and inpatient departments based on increased serum PSA concentrations, irregular findings on DRE, or LUTS suggestive of prostatic disease. Comprehensive information regarding the study was explained to each participant in their regional language, and written consent was obtained once they were fully informed about their involvement and the review of their medical records. Male patients who consented to participate, and had complete clinical and laboratory histories were included. Patients with missing PSA level data (n = 34), ongoing or prior hormone therapy (n = 30), and additional therapies that might affect PSA levels (n = 6) were excluded from the study. After applying these criteria, 60 participants constituted the final group analysis.

All recruitment and data handling procedures followed the original cross-sectional study design. The original study cohort comprised 65 patients with PCa and 65 with BPH. For the present analysis, a subset of 30 PCa and 30 BPH patients was selected according to the availability of complete PSA data collected at two separate time points, alongside both measurements performed under standardized

laboratory conditions. An equal number of patients were included to ensure balanced comparative analysis of PSA variation and minimize potential statistical bias.

PSA concentrations in serum were recorded retrospectively from the patients in whom PSA testing was performed at two defined time points: once prior to biopsy and again about fourteen days later in clinical follow-up. These measurements were performed as part of the research protocol and documented in hospital records. Only participants with two clearly documented PSA values were included for analysis.

Concentrations of serum PSA were measured using an Electrochemiluminescence Immunoassay (ECL IA) on a Cobas e401 analyser (Roche Diagnostics, Germany) in an accredited laboratory by the official accreditation board for testing and calibration laboratories under the Quality Council of India (NABL). Analysis of all samples was conducted on the same day using identical lot numbers of reagents, calibrators, and controls to minimize analytical variation. Two levels of internal quality control material, one within the normal range and one pathological, were performed within every batch to ensure analytical validity. Measurements were performed by applying the same calibration curve. An analysis of descriptive data was done to summarize clinical and laboratory data. Continuous variables were reported as median along with the Interquartile Range (IQR), and categorical variables as frequencies and percentages. Comparisons between PCa and BPH groups were categorized by the Mann–Whitney U test for continuous variables alongside chi-square tests for categorical variables, with p value <0.05 being considered significant. The Statistical Package for the Social Sciences (IBM SPSS), version 20.0 was used for data analysis. The percentage change in PSA was computed using the following formula between the two time points:

Percent change = (PSA2 - PSA1 / PSA2) × 100

The CVA for PSA in our laboratory was determined to be 1.80%. The CVI was obtained from the EFLM Biological Variation Database [10].

The RCV was calculated to determine whether observed changes in PSA exceeded expected analytical and biological variation. RCV was computed using the following formula:

$$RCV = \sqrt{2}^{1/2} * Z * \sqrt{(CVA^2 + CVI^2)}^{1/2}$$

A two-sided RCV was computed using a 95 percent confidence interval (Z = 1.96), and the assumption that 95 percent of the variation in stable patients reflected combined analytical and biological variation.

II values were obtained using the following calculation:

$$II = CV_I / CV_G$$

The Analytical Performance Specifications (APS) for the uncertainty (%I), bias (%B), and total error (%TE) are stated in the below formula:

$$I\% = 0.5 \times CVI; \quad B\% = 0.25 \times \sqrt{(CVI^2 + CVG^2)}^{1/2}$$
$$TE\% = 1.65 \times I\% + B\%$$

Results

A cohort of 60 patients was selected for the analysis, comprising 30 with PCa and 30 with BPH. As summarized in Table 1, no significant difference was found in median age among the groups (PCa: 73.0 years; BPH: 69.0 years; p = 0.807). However, serum PSA measurements at both time points demonstrated a notable rise in the PCa group. The

median first PSA value was 24.10 ng/mL in PCa and 11.36 ng/mL in BPH (p = 0.009). Significant variation in the second PSA value was also observed across groups (23.36 ng/mL vs. 8.47 ng/mL; p = 0.039). Although the percent change in PSA was greater in PCa (15.34%) than BPH (12.56%), the change was not significant on statistical testing (p = 0.089).

The II for PSA was calculated to be 0.16. Based on CVA and CVI values, the asymmetric RCVs were computed as +21.5% for a notable increase and −17.7% for a notable decrease in PSA levels, as detailed according to the data in Table 2.

Using these thresholds, PSA changes were classified. As presented in Table 3 (Figure 1), 23 PCa patients (76.7%) demonstrated a significant decrease in PSA (below −17.7%) compared to 14 BPH patients (46.7%) (Chi-square test, p = 0.017; continuity-corrected p = 0.033).

The relationship between RCV exceedance and Gleason score was explored in PCa patients (Table 4). RCV exceedance was observed in 7 of 9 patients (77.8%) with a Gleason score ≤ 6, compared to 6 of 21 patients (28.6%) with a Gleason score ≥ 7. A statistically meaningful association was observed ($\chi^2 = 6.21$, p = 0.013). Binary logistic regression analysis (Table 5) was conducted to identify predictors of PCa. Among the variables analyzed, only RCV exceedance was significant statistically (B = 1.918, p = 0.038),

Table 1: Comparison of age and PSA parameters between BPH and PCa patients

Variable	PCa	BPH	*p*
Age	73.0 (42.0 – 85.0)	69.0 (42.0 – 86.0)	0.807
1st PSA	24.10 (1.15 – 228.92)	11.36 (0.69 – 71.89)	0.009*
2nd PSA	23.36 (0.15 – 215.90)	8.47 (0.40 – 69.55)	0.039*
Percent change (%)	15.34 (2.04 – 86.96)	12.56 (0.56 – 51.42)	0.089

*II=0.16 for PSA, *p-value is significant. Data presented as median (range). PSA; prostate-specific antigen, PCa; prostate cancer, BPH; benign prostatic hyperplasia.*

Table 2: Asymmetric reference change values for prostate-specific antigen at 95% confidence interval

Variable	Median	Upper CI	Lower CI
CVI	6.80	6.20	7.50
CVA	1.80	-	-
RCV Threshold (Increase)	+21.5	19.6	23.8
RCV Threshold (Decrease)	-17.7	-16.4	-19.2

CVI= within-subject biological variation; CVA= analytical coefficient of variation; RCV= reference change value; CI= confidence interval

Table 3: Classification of PSA change based on RCV in PCa and BPH groups

Classification	PCa (n = 30)	BPH (n = 30)	*p*
Significant Decrease (-17.7%)	23	14	
No Significant Change	7	16	0.017* (χ^2)

**Statistically significant at p < 0.05 (Chi-square test), RCV=Reference change value; PCa= prostate cancer; BPH= benign prostatic hyperplasia*

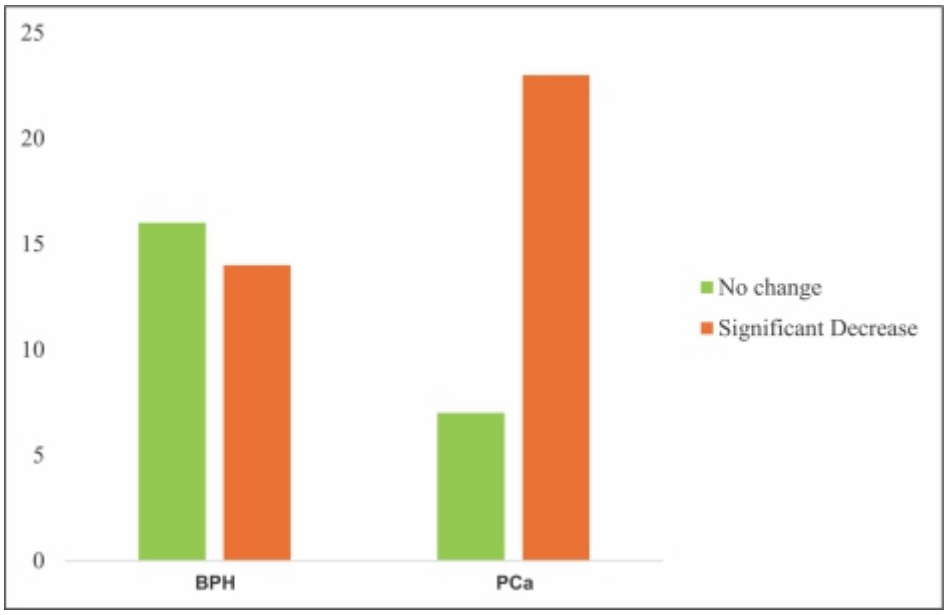

Figure 1: Distribution of PSA changes in PCa and BPH patients based on RCV

Table 4: Cross-tabulation of RCV exceedance status and Gleason score categories among PCa patients

	GS ≤ 6	GS ≥ 7	Total	*p*
Not exceed RCV	2	15	17	**0.013***
Exceeds RCV	7	6	13	
Total	9	21	30	

*Chi-square test: $\chi^2 = 6.21$, df = 1, p = 0.013*Statistically significant at p < 0.05. RCV = Reference change value; GS = Gleason score*

Table 5: Binary logistic regression predicting PCa diagnosis (PCa vs BPH)

Predictor	B	SE	Wald χ^2	p-value	OR (Exp(B))	95% CI for OR
RCV change	1.918	0.923	4.321	**.038***	6.807	[1.112, 41.674]
1st PSA	-0.056	0.073	0.603	.437	0.945	[0.819, 1.091]
2nd PSA	0.008	0.084	0.009	.923	1.008	[0.852, 1.192]

**Statistically significant at p < 0.05. OR = odds ratio; CI = confidence interval.*

resulting in an odds ratio of 6.81 (95% CI: 1.11–41.67). Neither first nor second PSA values showed predictive significance (p = 0.437 and p = 0.923, respectively).

Discussion

The biological process of life is constantly evolving. Individual genetic traits, environmental influences, exposure variables, and numerous additional unidentified causes will all influence the laboratory parameters. Therefore, population-based reference ranges commonly used in medical laboratories may not always be sufficient for accurate clinical evaluation at the individual level [5]. In India, PSA is mainly employed as a diagnostic rather than a screening test, largely resulting from the relatively lower observed frequency of PCa [11]. Underreporting along with limited extensive population-based cancer registries have documented insufficient information about the actual incidence of PCa in India [12]. Although the Indian Council of Medical Research reported incidence rate is less than previously reported in the Western countries, it's more than what is observed in other Asian and African nations. Even in the non-urban setting, PCa is steadily rising, and it places a significant burden on India's health care system [13].

Importantly, RCV-based interpretation differs fundamentally from absolute PSA thresholds. While traditional PSA cutoffs are broadly employed as well as have also drawn critique for their specificity, leading to unnecessary biopsies and overtreatment. Studies by Eickelschulte S et al. and others have argued that PSA alone is a poor discriminator between aggressive and indolent PCa, emphasizing that better risk stratification is required [14]. In contrast, RCV considers the individual's inherent biological fluctuation, offering a more refined view of significant change. Despite this, there is disagreement among authors on the superiority of RCV or similar kinetic approaches. Campbell *et al.* reported that PSA kinetics, including velocity and doubling time, add minimal predictive value unless PSA changes are large and consistent [15]. Moreover, using RCV in PCa may be questioned, since the biological variation assumption underlying RCV calculation may not always hold. Despite this, the results of this study have established that even in a disease state, RCV is useful in differentiating between benign and malignant cases and identifying patients diagnosed with disease of potentially greater aggressiveness.

Interestingly, the regression model indicated that RCV exceedance was the only significant predictor of PCa diagnosis, while absolute 1st and 2nd PSA values were not. This result is consistent with new

literature that suggests serial changes in PSA may reflect disease dynamics better than single-point measurements. Yet, Poppel *et al.*, as per the Prostate Cancer Prevention Trial, concluded that PSA velocity doesn't give predictive accuracy over total PSA alone [16], casting doubt on the incremental benefit of serial measurements in all clinical settings.

We also observed a statistically significant correlation among the RCV exceedance and higher Gleason scores, indicating that PSA decline beyond biological variation was connected to tumor aggressiveness. This result is in line with previous research by Kobayashi *et al*. [17], who identified PSA kinetics as predictors of high-grade disease. The study by Okwor *et al*. showed a weak or inconsistent association of PSA velocity, especially in low-risk PCa [18]. In India, although some multicenter studies have determined reference intervals for PSA and other biomarkers [19-22], the implementation of RCV to clinical populations remains largely unexplored. As far as we are aware, this constitutes among the first Indian studies to apply RCV methodology to differentiate PSA changes in PCa and BPH patients, demonstrating its feasibility and potential clinical utility.

The present research introduces the concept that incorporating RCV to PSA interpretation may improve diagnostic precision, particularly in borderline cases. In addition, computation of the II for PSA, rarely described in earlier studies, highlighted its exceptionally low value (0.16), reinforcing the inadequacy of population-based reference intervals. This finding further supports the use of RCV as a more appropriate interpretative metric for interpreting serial PSA results on an individual basis.

Limitations

This study is not without limitations. The CVI and CVG values used for RCV calculation were sourced from the EFLM Biological Variation database instead of from our own cohort. Although this represents a widely accepted practice, it may introduce imprecision if our population's biological variation differs due to ethnic, regional, or clinical factors. Finally, applying RCV in cases of potentially unstable disease biology (rapidly progressing PCa) must be done cautiously, as it assumes relative biological stability.

Conclusion

This study demonstrates the clinical utility of RCV-based PSA interpretation, offering a more personalized alternative to static cutoffs. By applying the EFLM-derived CVI value, we show that RCV can meaningfully differentiate between PCa and BPH, even without population-specific BV data. These findings highlight the feasibility of integrating RCV into routine practice and underscore the requirement for locally tailored BV studies in Indian populations.

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