EPCA2.22: A Silver Lining for Early Diagnosis of Prostate Cancer

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Purpose: To investigate whether EPCA-2 (a prostate matrix nuclear protein) can be a more helpful marker in prostate cancer diagnosis.

Materials and Methods: 176 patients enrolled in this study had abnormal prostate specific antigen (PSA) or digital rectal examination and were candidates for prostate needle biopsy. Blood samples were obtained from each patient prior to biopsy and the samples were frozen for EPCA-2 measurement. Patients diagnosed with cancer were assigned to the case group and those with benign prostate hyperplasia (BPH) were included in the control group. Univariate and multivariable analyses were done to assess the relationship between different independent variables with cancer diagnosis. The diagnostic power of EPCA-2 for cancer was estimated at different levels of PSA according to the ROC curve.

Results: The mean(± SD) age of cancer cases was 70.33(± 9.02) years while it was 63.34(± 9.47) years for BPH cases (P < .01). EPCA-2 and PSA were also significantly different between cancer and BPH cases ($P < .001$). The multivariable logistic regression showed that EPCA-2 has a significant relationship with cancer diagnosis (OR=1.009, $P = .021$). After controlling other variables following stratification for PSA, it was shown that EPCA-2 and cancer were correlated just when PSA was >10 ($P < .001$). AUC was 0.694 for cancer prediction by EPCA-2 when PSA was >10 ng/mL.

Conclusion: EPCA-2 has the power of differentiating BPH from cancer in prostate cancer suspects. This suggests that EPCA-2 can be helpful in diagnosing prostate cancer and can be a preventive test to avoid unnecessary biopsies considering PSA and age of the patient.

Keywords: EPCA; prostate cancer diagnosis; PSA

INTRODUCTION

The discovery and increasing use of PSA, as a screening test since 1980, has lifted prostate cancer (PCa) to the most frequent neoplasia in men of developed countries. It is estimated that 900,000 new cases of PCa leading to 258,000 PCa-related deaths worldwide in 2008 are diagnosed; it proposed a rise to 1.7 million diagnoses and an annual mortality rate of 0.5 million men in 2030[1]. Prostate cancer incidence in Europe is estimated at 416,700 new cases in 2012 resulting in 92,200 cancer deaths per year[2]. In USA, it was estimated that 233,000 new cases would be diagnosed and 29,480 cancer deaths would occur during 2014[3]. In Iran, the incidence rate of PCa (11.25: 100,000) is lower than the western countries[4] and this might be attributed to the nutrition pattern of the country consuming less red meat (36.3Kg/year) than the world's average per capita rate (41.90 Kg/year) according to Current Worldwide Annual Meat Consumption per capita. Notwithstanding its revolutionary role in prostate cancer diagnosis, PSA is a tissue marker with restrictions due to its lack of specificity for PCa cells, the serum level of which may change following inflammation, infection or manipulation of the prostate. Racial and geographical variations of serum PSA level should be added to the limitations of interpreting its results, as well. It is inevitable to investigate a tumor marker with high specificity to avoid unnecessary biopsies in cases with elevated prostate specific antigen (PSA) and normal digital rectal examination (DRE). EPCA (Early Prostate Cancer Antigen), primarily introduced by Dhir et al.[4], is a nuclear matrix protein that has shown to be associated with prostate cancer and may be used as a specific tumor marker rather than a tissue marker for prostate cancer diagnosis individually or in combination with PSA. They were able to measure anti-EPCA antibodies in prostate biopsies with negative results to predict prostate cancer development after 5 years. Further immunohistochemical analyses documented a sensitivity and specificity of >80% for detecting prostate cancer[4,5]. There are two unrelated types of nuclear matrix proteins found in serum, assisting urologists with diagnosing prostate cancer; the proteins are called EPCA and EPCA-2 due to their date of discovery, respectively[6]. Three epitopes including EPCA-2.22, EPCA-2.19, and EPCA-2.24 are defined for EPCA-2[7]. It was observed that serum levels of EPCA-2.22 higher than 30 ng/mL were associated with a sensitivity of 94% for PCa diagnosis while maintaining 92% specificity[8,9]. Besides differentiating BPH from PCa, EPCA-2.19 and EPCA-2.22 assays were able to diagnose and localize prostate cancer from the metastasis[10,11].

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Table1: Clinical characteristics of patients according to their final diagnosis.

Risk factors	Outcomes		OR(CI)	*P*-value
	BPH; N (%)	Cancer; N (%)		
Cardiovascular diseases	23(21.5)	14(20.6)	0.94(0.44 – 1.99)	0.88
Diabetes	13(12.1)	11(15.9)	1.37(0.57 – 3.26)	0.47
Hypertension	28(26.2)	19(27.5)	1.07(0.54 – 2.12)	0.84
Renal failure	3(2.8)	5(7.2)	2.70(0.62 -11.71)	0.16
Foot fracture	4(3.7)	4(5.8)	1.58(0.38 - 6.55)	0.52
Colon surgery	0(0)	2(2.9)	-	0.07
Prostate surgery	5(4.7)	6(8.7)	1.94(0.56 -6.63)	0.28
History of prostatitis	4(3.7)	0(0)	-	0.10
Urinary Tract Infection	2(1.9)	0(0)	-	0.25
Medication	49(48.0)	29(46.8)	0.95(0.50 -1.78)	0.87
Family history of prostate disorder	50(46.7)	26(37.7)	0.68(0. 37 – 1.27)	0.23
Urinary Tract Obstruction	33(30.8)	14(20.3)	0.57(0.27 – 1.16)	0.12
Hematuria	27(25.2)	20(29.0)	1.20(0.61 – 2.38)	0.58
Fever	9(8.4)	7(10.1)	1.22(0.43 – 3.47)	0.69

Abbreviations: CI, confidence interval; N, number

This study was conducted to investigate the efficacy of EPCA-2.22 as an epitope of EPCA-2 in differentiating PCa from benign prostate hyperplasia (BPH) in candidates of prostate biopsy due to elevated PSA and/or abnormal DRE.

MATERIALS AND METHODS

176 prostate biopsy candidates with elevated PSA and/or abnormal DRE were enrolled in the study. Blood samples were obtained from patients (5cc) to measure serum EPCA-2 and PSA levels. The serum level of EPCA-2.22 epitope of EPCA-2 was measured using ELISA method (CUSABIO Kit). After measuring EPCA-2, all patients underwent Transrectal Ultrasound guided biopsy of the prostate using the 10 core biopsy method (standard method in our center). According to pathology reports, patients were divided into BPH (N = 107 patients) and PCa (N = 69 patients) groups. Paraclinical and physical examination results as well as demographic information of the patients were gathered.

Statistical analysis

The relationship between different independent variables and the outcome (PCa versus BPH) was estimated using univariate tests (chi 2, fisher exact, t-test). Different independent variables were applied in a multivariate logistic regression and remained in the final model based on the backward method using Wald test (entry: 0.05, removal: 0.1).The ROC curve and AUC as well as sensitivity, specificity, positive and negative predictive values were used for estimating the diagnostic power of EPCA-2.

Table2: Multivariable logistic regression results; dependent variable: cancer Vs BPH

	B	S.E.	OR(exp b)	*p*-value
Age	.077	.024	1.080	.00
Medication in use	-.061	.39	.941	.87
Prostate in family	-.145	.396	.865	.713
EPCA	.009	.004	1.009	.021
PSA	-.007	.015	0.993	.62

RESULTS

Pathology reports revealed 107 BPH and 69 PCa diagnosed cases. The mean(± SD) age of the patients was 63.34 (± 9.47) years and 70.33(± 9.02) years in BPH and cancer groups, respectively. The mean difference of age between the two groups was significantly higher in the cancer group ($P < .01$). The mean serum level of EPCA-2 and PSA was significantly higher in the cancer group ($P < .001$). Other variables which were significantly higher in the BPH group included hemoglobin and platelet count (Hb: 14.99 versus 14.2; and for Platelet: 23.46 versus 21.32 in controls and cases, respectively). **Table 1** shows other clinical characteristics of the patients according to their final diagnosis. The multivariable analysis of the association between different independent variables and cancer diagnosis showed that age and EPCA-2 have a significant association with cancer diagnosis ($P < 0.001$ and $P = 0.21$, respectively). **Table 2** shows the result of the logistic regression for variables remaining in the model using backward method with Wald test. The ROC curve of EPCA-2, PSA and age was calculated for cancer diagnosis and demonstrated that the association between these three factors and the outcome is statistically significant ($P = .001$ for PSA and $P < .001$for EPCA-2 and age). The frequency of cancer and BPH was calculated following stratification of the patients based on PSA (PSA < 10, and PSA > 10). About half of the patients with PSA levels lower than 10(50.06%) were found with

Table 3: Test validity indices for differentiating cancer from BPH according to different cut-off points of EPCA-2 when PSA>10

Cut-Off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
3.23	100	0	49.33	-
30.1	89.2	23.7	53.23	69.26
43.79	75.7	39.5	54.92	62.53
55.62	70.3	50	57.78	63.35
119.41	51.4	86.8	79.12	64.71
133.98	40.5	94.7	88.15	62.04
164.54	35.1	97.4	92.93	60.65
375.38	10.8	100	100	53.51

BPH diagnosis. In this group, cancer diagnosis was not associated with EPCA -2 values. However, the relation between EPCA-2 and cancer diagnosis showed that EPCA-2 and cancer diagnosis are significantly related when PSA > 10 ($P < .001$). In this group (patients with PSA > 10) higher EPCA-2 levels were significantly associated with a higher Gleason score (mean EPCA was 196.6 for Gleason score >= 7 in comparison to 90.52 for Gleason score < 7; $P = .034$) while it was not the same in the other group of the patients (PSA < 10). Area under the curve (AUC) was calculated for EPCA-2 and cancer diagnosis (**Figure 2**) for those with PSA > 10. According to the results of AUC, different validity indices (sensitivity, specificity, positive and negative predictive values) were calculated for different cut-off points of EPCA-2 for cancer diagnosis in this PSA stratum. **Table 3** shows the estimated validity indices for EPCA2.

DISCUSSION

PSA was detected in serum in 1980 and revolutionized PCa management. But soon, hopes disappeared, since it was found that PSA is a tissue marker rather than tumor marker and some conditions like benign prostatic hyperplasia, infection and manipulation will affect the serum levels of PSA[13]. On the other hand, racial and geographical variations in serum PSA level was another problem in interpreting it and defining a definitive cut off point for cancer diagnosis; for example, in USA and Europe, a cutoff point of 2.5 ng/ml is offered to diagnose cancer while in Iran, it was estimated to be 7.85 ng/ml in one report[14]. When PSA is elevated, several factors propose the need to seek other cancer specific biomarkers to diagnose PCa and reduce unnecessary biopsies. It has been shown that EPCA is a nuclear matrix protein known to be expressed by PCa cells showing 84% and 92% sensitivity versus 85% and 94%specificity for PCa detection when assayed by immunohistochemical or ELIZA methods respectively[4,15,16]. It was observed that age, PSA and EPCA2.22 level are associated with PCa diagnosis. In the current study, EPCA 2.22 was evaluated by ELIZA method to investigate if it is useful to diagnose prostate cancer and prevent unnecessary biopsies. When sensitivity and specificity for different cutoff points were calculated at 28.55ng/ml, EPCA2.22 diagnosed cancer with 74.1% sensitivity, 50% specificity, and 49.69% PPV; however, it was previously observed that serum levels higher than 30ng/ml have 94% sensitivity and 92% specificity for PCa diagnosis[8, 9]. In this study, EPCA2.22 did not predict PCa diagnosis as good as previous reports, especially for the specificity index. To define the best diagnostic effect of EPCA2.22, patients were stratified into two groups (PSA=< 10, PSA>10). It was observed that EPCA2.22 can predict cancer diagnosis only when PSA>10 (89.2% sensi-

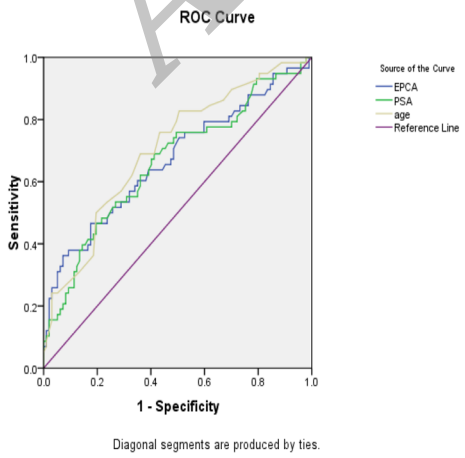

Figure 1: ROC curve of EPCA-2, PSA and Age for cancer diagnosis

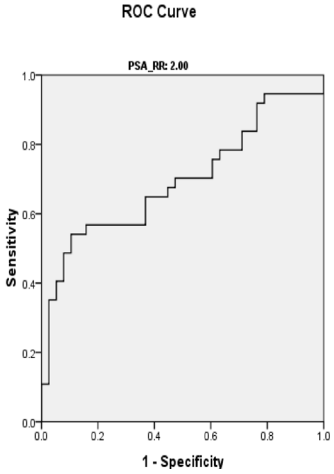

Figure2: ROC curve of EPSA-2 for prostate cancer diagnosis when PSA>10

tivity, 23.7% specificity, 53.23% PPV and 69.26% NPV in the cut-off point 30.1), and in these patients, higher EPCA2.22 level is associated with a higher Gleason score (Gleason score >=7 in comparison to Gleason score < 7; P = .034). This may be rooted in the lower diagnostic power of EPCA2.22 in lower PSA levels and probably localized and low-risk PCa. It was documented that EPCA 2.22 in contrast to PSA was highly accurate in differentiating localized from extra-capsular disease[17]. We observed that in patients with PSA>10, higher EPCA 2.22 levels are associated with a higher Gleason score (Gleason score >=7) which expresses its promising role in defining high risk patients; however, further studies are needed. Although the specificity of EPCA and predictive values are not proofs recommending EPCA as a diagnostic measure, high sensitivity values for EPCA, that are almost the same in different studies show that it could be a good measure for ruling out cancer diagnosed-patients without biopsy.

CONCLUSIONS

EPCA-2 has a notable power of differentiating BPH from cancer in prostate cancer suspects. However, its result must be considered in combination with PSA result and patient's age. This suggests that EPCA-2 can be helpful in diagnosing PCa and can be a preventive test to avoid unnecessary biopsies in patients who are supposed to do biopsy because of the high value of PSA like when PSA>10.

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REFERENCES

1. Center MM, Jemal A, Lortet-Tieulent J, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol. 2012;61:1079-92.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013;49:1374–1403.
3. Salem S, Salahi M, Mohseni M, et al. Major dietary factors and prostate cancer risk: A prospective multicenter case-control study. Nutr Cancer. 2011;63:21-7.
4. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64:9-29.
5. Dhir R, Vietmeier B, Arlotti J, et.al. Early identification of individuals with prostate cancer in negative biopsies. J Urol. 2004;171:1419-23.
6. Uetsuki H, Tsunemori H, Taoka R, Haba R, Ishikawa M, Kakehi Y. Expression of a novel biomarker, EPCA, in adenocarcinomas and precancerous lesions in the prostate. J Urol. 2005;174:514-8.
7. Pal RP, Maitra NU, Mellon JK, Khan MA. R. Defining prostate cancer risk before prostate biopsy. Urol Oncol. 2013;31:1408-18.
8. Uetsuki H, Tsunemori H, Taoka R, Haba R, Ishikawa M, Kakehi Y. Expression of a novel biomarker, EPCA, in adenocarcinomas and precancerous lesions in the prostate. J Urol. 2005;174:514-8.
9. Steuber T, O'Brien MF, Lilja H. Serum Markers for Prostate Cancer: A Rational Approach to the Literature. Eur Urol. 2008;54:31-40.
10. You J, Cozzi P, Walsh B, et al. Critical Reviews in Innovative biomarkers for prostate cancer early diagnosis and progression. Crit Rev Oncol Hematol. 2010;73:10-22.
11. Leman ES1, Cannon GW, Trock BJ, et.al. EPCA-2: a highly specific serum marker for prostate cancer. Urology. 2007;69:714-20.
12. OteroJR, GomezBG, JuanateyFC, et.al; Prostate cancer biomarkers:AnUpdate, Oncology: Seminar sand Original Investigations. 32 (2014)252-260.
13. Diamandis EP. POINT: EPCA-2: a promising new serum biomarker for prostatic carcinoma? Clin.Biochem. 2007; 40:1437–9.
14. Stephan C, Ralla B, Jung K. Review: Prostate-specific antigen and other serum and urine markers in prostate cancer. Biochim Biophys Acta. 2014;1846:99-112.
15. Pourmand G, Ramezani R, Sabahgoulian B, et.al. Preventing Unnecessary Invasive Cancer-Diagnostic Tests: Changing the Cut-off Points. Iran J Public Health. 2012;41:47-52.
16. You J, Cozzi P, Walsh B, et.al, Innovative biomarkers for prostate cancer early diagnosis and progression. Crit Rev Oncol Hematol. 2010;73:10-22.
17. Paul B, Dhir R, Landsittel D, Hitchens MR, Getzenberg RH. Detection of prostate cancer with a blood-based assay for early prostate cancer antigen. Cancer Res. 2005; 65:4097-100.
18. Leman ES1, Magheli A, Cannon GW, Mangold L, Partin AW, Getzenberg RH. Analysis of second EPCA-2 epitope as serum test for prostate cancer. Prostate. 2009;69:1188-94.