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Pilot Study of PCA3 Quantitative Analysis in Peripheral Blood with Real Time PCR and Non-Contrast Multiparametrics MRI for Prostate Cancer Detection

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Abstract

Prostate cancer is one of major health concerns worldwide. Early detection for prostate cancer is very important since effective treatment is very limited for advanced prostate cancer. Molecular alteration happen prior to any morphological change, and Prostate Cancer Antigen 3 (PCA3) as the most specific marker for prostate cancer had been studied and proved its usefulness in detecting prostate cancer.

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However, the PCA3 level in peripheral blood remains unclear and only few research focused in the topic. Multiparametric Magnetic Resonance Imaging (mp-MRI) has superiorities in viewing prostate gland, without radiation exposure, also can be used without contrast injection. Early detection should avoid unnecessary medical examinations or interventions that will add up patients' psychological burden, not to mention health care cost and logistic burdens. This pilot study will quantify PCA3 in blood, and the use of non-contrast mp-MRI for prostate cancer detection. There were 19 patients who met inclusion and exclusion criteria. Evaluation with non-contrast mp-MRI used T2 Weighted Image (T2WI), and Diffusion Weighted Image (DWI). T2WI and DWI image category were based on PI-RADS version 2 criteria, while Apparent Diffusion Coefficient (ADC) value was acquired by defining lesion Region of Interest (ROI) in DWI Sequence. Summation of T2WI and DWI score result in total score. Peripheral blood samples were drawn and analyzed with Real Time Polymerase Chain Reaction (RT-PCR). Distribution table also independent t-test and Mann Whitney test were done, with Receiver Operating Curve (ROC) to find out the cut off value as secondary endpoints. Results revealed that, PCA3 mean level in prostate cancer is 10.996±1.901, mp-MRI median score in prostate cancer is 9 (8:10) with ADC median value is0.542 (0.339:0.768).There is statistically significant difference ($p<0.05$)in mp-MRI score and ADC value between prostate malignancy and non-malignancy ($p=0.005$, $p=0.022$), but there is no significant difference in PCA3 mean level for prostate malignancy and non-malignancy ($p=0.851$).In conclusion, PCA3 can be quantified from blood sample with mean level in prostate cancer is 10.996±1.901. Total score of non-contrast mp-MRI and ADC value can be used to differentiate prostate malignancy with non-malignancy for prostate cancer detection. Further studies with more samples are needed to validate this study.

Keywords: PCA3; PCR; quantitative test; non contrast mp; MRI; prostate cancer.

1. Introduction

Prostate cancer is the second common cancer in men, and fifth mortality cause due to cancer. Prostate cancer incidence will become 1.4 million in the world population, increase from 1.1 million in 2012 [1]. This condition is very concerning, moreover with the fact that there are limited therapy options available for advanced prostate cancer. Early detection is an important key factor to lower mortality and to avoid morbidity events related which will result in quality of life decrease. However, early detection should not cause unnecessary examination or treatment which could bring more burden to patients' life aspects. Abnormal Digital Rectal Examination (DRE) and elevated Prostate Specific Antigen (PSA), followed by biopsy for histopathology evaluation is the standard prostate cancer diagnostic procedure in several guidelines commonly used by clinicians worldwide, that are National Institute for Health and Clinical Excellence (NICE), European Association of Urology (EAU), American Urological Association (AUA) and National Comprehensive Cancer Network (NCCN) [2-5]. Elevated PSA > 4 ng/ml has 80.6% sensitivity, 19.7% specificity and DRE for PSA > 4 ng/ml has 98% sensitivity, 9% specificity. Despite of good sensitivity, both DRE and PSA has low specificity that can lead to unnecessary biopsy recommendation [6,7]. Emerging modalities, mp-MRI with DWI and PCA3 might be used as early and reliable prostate cancer detection. Both modalities utilize molecular changes' as an important parameter for prostate gland abnormalities. High resolution MRI imaging is best imaging modality without radiation effect that can improve prostate cancer detection; also to define its characterization, staging and treatment follow up. Multi-parametric MRI consists of several sequences with at least one more sequence apart

from the anatomical T2-weighted imaging [8]. PCA3 is a promising biomarker, since it is the most specific marker for prostate cancer [9,11]. There were previous studies regarding PCA3 showed positive result and support the roles of PCA3 in diagnosis of prostate cancer. Increased RNA levels in prostatic cancer cells can be detected in prostatic tissue, urine after prostatic massage and peripheral blood sample [10] Data from Sardareh HM and his colleagues showed PCA3 blood had 94.74% sensitivity with 81.82% specificity in detecting prostate cancer [12]. However, there were not much data about PCA3 from blood samples, yet we found it is the most practical method to do in daily practice for screening also to avoid patients' discomfort because of prostate massage that is done before urine sample collection. Through this research, we aim to prove our hypothesis that mp-MRI score, ADC value and PCA3 mRNA level could differentiate between prostate malignancy and non-malignancy. This research also has goal to find out the level of PCA3 in prostate cancer peripheral blood. Cut off values are secondary endpoints.

2. Materials and Method

Inclusion criteria are PSA level ≥ 4 ng/ml with or without nodular palpable prostate from DRE. Exclusion criteria are hypoglycemia, hyponatremia, deviant temperature, not cooperative/ fail to do MRI procedure, patients with MRI contra indications, claustrophobia and fail to provide biopsy result. There were 19 patients who met inclusion and exclusion criteria with characteristics defined in table 1 below

Table 1: Patients Characteristics

No	Initial	Age	PSA	Nodule (DRE)	Glucose	Natrium	Temperature (^{0} C)	Biopsy finding
1	JLA	83	25.28	-	175	138	36.8	benign
2	WSA	65	100	+	107	140	37	malignant
3	RGU	68	10.76	-	102	142	36.6	malignant
4	RHA	67	5.22	-	82	140	36.9	benign
5	IRU	72	10.99	-	205	137	37.1	benign
6	RSO	63	38.81	-	142	141	37.2	malignant
7	CAK	69	10.11	-	94	140	37	benign
8	JIS	67	5.3	-	91	145	36.9	malignant
9	RTU	77	28.6	-	89	143	36.9	malignant
10	BSU	68	4.49	-	157	138	36.7	malignant
11	JKH	65	64.26	-	144	136	37.2	malignant
12	SUH	74	5.77	-	97	134	37.2	malignant
13	CHA	68	32.02	-	143	140	37.1	malignant
14	BAT	59	10.98	-	99	139	36.8	malignant
15	HTO	73	11.3	-	107	132	37	malignant
16	RUD	73	100	-	107	139	36.6	malignant
17	HNA	70	110.69	-	106	138	36.8	malignant
18	FBO	75	9.5	-	121	140	36.9	malignant
19	JPA	65	8.95	-	149	138	36.6	benign

All patients underwent mp-MRI procedure using single calibrated MRI unit 1.5 Tesla (Avanto Fit), gradient strength 45 mT/m, slew rate 200 T/m/s, with parameter and specifications as described in the table.

No	Sequence and imaging plane	Sequence type	TR (msec)	TE (msec)	Field of View (mm)	Matrix	In-Plane Resolution (mm2)	Slice Thickness (mm)	Flip Angle (degree)	Average / b value	Slice Spacing (mm)	Phase Encoding Direction	Scan Duration (min:sec)
1	**T2-weighted**												
	Axial	Turbo Spin Echo	7000	112	160	330 x 384	0.4 x 0.4	4	160	2	0	R-L	3:39
	Coronal	Turbo Spin Echo	7000	98	160	298 x 320	0.5 x 0.5	4	160	2	0	R-L	2:57
	Sagital	Turbo Spin Echo	7000	119	160	266 x 320	0.5 x 0.5	4	160	2	0	H-F	2:57
2	**DWI**												
	Axial	Echo Planar Spin Echo	3600	86	200	48 x 140	1.4 x 1.4	4	-	0, 50, 500, 800, 1000, 1500, 2000	0	R-L	9:16

TR, repetition time; TE, echo time; DWI, Diffusion Weighted Imaged; msec, millisecond; mm; millimeter; min, minute; sec, second; R, Right; L, Left; H, Head; F, Feet

Figure 1: Parameter of mp-MRI

An endorectal prostate coil (Sentinelle Medical, Siemens AG) was inserted using analgesic gel prior to the exam to avoid pain and rectal spasm. Evaluation of mp-MRI used T2WI and DWI. Imaging category of T2WI and DWI based on Prostate Imaging-Reporting and Data System (PIRADS) version 2. Summation of both score resulted in total score of mp-MRI. ADC value was acquired by setting Region of Interest (ROI) in the specific lesion/area in the DWI sequence. ADC value would automatically generated by the MRI software, the error in placing the ROI would cause an error for the value as well.

Peripheral blood was drawn and directly put in EDTA tube fornucleotide extraction followed by DNA amplification with primers Forward 5'-TATTCTGAAGTCAGAGTGTTCCAG-3' and primer Reverse:5'-CTTATTTCTCACCTCTGTATCATCAGG-3' with housekeeping gene b2MF:5-GTCTTTCTATCTCTTGTACTACACTGAA-3 and b2MR: 5 – AACTATCTTGGGCTGTGACAAAG -3 using Qiagen SYBR-GREEN qPCR Mastermix (QIAGEN, Germany). Each reaction contained 7.5 ml of qPCR mastermix, 5 pM of each forward and reverse primer and 5 ml of the diluted cDNA template.

The following cycling conditions were applied: 95^{o}C for 15 minutes, followed by 45 cycles of 95^{o}C for each 20 seconds period, 58–59^{o}C for 20 seconds and 72^{o}C for 20 seconds. Data for each cycle was acquired at the elongation step and each reaction was carried out in triplicate. Relative gene expression levels were calculated using methodology described in Pfaffl [13,14].

3. Results

Mean age for prostate cancer patients are 69.33 ± 5.348 years old. Multifocal lesions were detected in more patients (58.3%) rather than solitary lesion in mp-MRI images evaluation. There were 11 lesions out of 16

lesions (68.75%) located in peripheral zone, while 5 lesions were located in transitional zone (31.25%).

PCA3 data was normally distributed and analyzed for means comparison using independent t-test, while mp-MRI score and ADC value were analyzed using Mann Whitney test.

Table 2: Results' Statistic Analysis

	Mean	**SD**	**p**
Malignant PCA3	10.996	1.901	0.851
Benign PCA3	10.743	3.136	
	Median	**Min:Max**	**p**
Malignant mp-MRI score	9	8:10	0.005
Benign mp-MRI score	2	2:9	
Malignant ADC value	0.542	0.339:0.768	0.022
Benign ADC value	0.987	0.450:1.569	

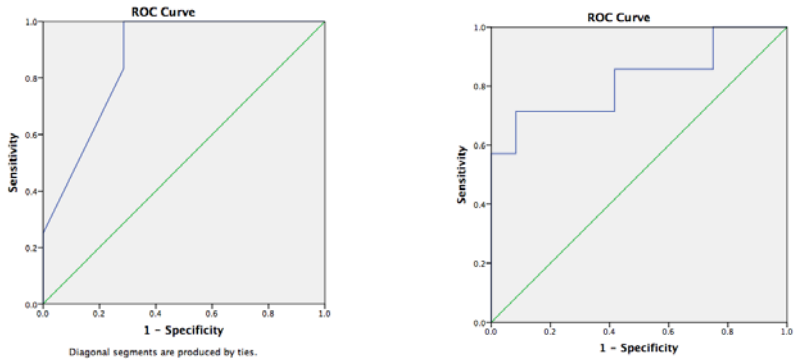

Figure 2: ROC Curve of mp-MRI Total Score (left) and ADC Value (right)

Cut off values for mp-MRI total score and ADC value were defined with ROC curve. Both ROC curve has excellent Area Under the Curve (AUC) that are 0.869 and 0.821 respectively. Best sensitivity and specificity is determined by coordinate points of the ROC. Total score of mp-MRI 7 through highest indicates malignancy with 100% sensitivity and 71.4% specificity while ADC value bigger or equal than 0.684 indicates non-malignancy with 71.4% sensitivity and 91.7% specificity.

4. Discussion

The results shown at table 2 above prove that mp-MRI total score from T2WI and DWI score also ADC value could be used to differentiate prostate cancer from other prostate benign conditions. Previous studies on mp-MRI confirmed its use to predict the malignancy probability, standardized through PI-RADS version 1 and 2 [15]. Even though the research used less sequence to avoid contrast injection, it still can be used to differentiate

prostate cancer. Previous studies also mentioned that DWI and T2WI sequences have good sensitivity and specificity in detecting prostate cancer [16,17].

Free water molecules are in constant random motion, known as Brownian motion, which the motion of water molecules within the cellular microenvironment is impeded by their interaction with cellular compartments and thermal energy. Apparent diffusion coefficient (ADC) measures diffusion rate of water molecules within a tissue. Multipoint b value analyses increase the accuracy of the calculated ADC at the expense of increased scanning time and decrease in signal to noise ratio (SNR) [18, 19]. ADC is mainly influenced by cellular/extracellular volume ratio, composition of extracellular space, and temperature [20]. The same effect on the speed of diffusion is observed in hypoglycemia andhyponatremia in which membrane depolarization makes excess extracellular water molecules move into the cells [21,22]. ADC value results in this study could not be applied instantly, because ADC is influenced by many internal (human body) factors and external (equipment setting) factors. To refer the same ADC value, there are several adjustments should be made according to this study specifications. Based on study done by Sato and his colleagues it also stated that cancerous tissue tend to have lower ADC value, similar to our study [23].

Molecular changes precede prostate cancer morphological alterations [10]. PCA3 mRNA could be detected in peripheral blood with mean level of 10.996±1.901, however it didn't have significant statistical analysis between malignancy and non-malignancy group. This result is in contrast with previous research that mentioned statistical significant difference between prostate cancer and other benign prostate conditions. Sardareh and his colleagues found the mean level of prostate cancer patients is 7.18 ±1.02 [12]). There are several factors such as epigenetic and pathway signal error that could play role in this PCA3 results [11, 24]. PCA3 is over expressed in prostate cancer, justifies the high level of PCA3 mRNA in prostate cancer blood [25-29]

There are only few patients willing to do biopsy and therefore we had lack of data. More studies with bigger sample are still needed to validate this research.

5. Conclusion

PCA3 can be quantified from blood sample with mean level in prostate cancer is 10.996±1.901. Total score of non-contrast mp-MRI and ADC value can be used to differentiate prostate malignancy with non-malignancy for prostate cancer detection.

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