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Evaluate the fPSA for Discriminate between Prostate Cancer Patients (PCa) and Benign Prostatic Hyperplasia (BPH) Depending on PSA Fucosylation by Using Nano-Lectin Immunoassay

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ABSTRACT

Background: The objective was to evaluate the fPSA for discriminate between prostate cancer patients (PCa) and benign prostatic hyperplasia (BPH) depend on the variations in PSA fucosylation (Fucα1-6/3GlcNAc carbohydrates) by using Aleuria aurantia lectin (AAL) .

Materials and Methods: The new method lectin-immunoassays were established for the investigation of PSA fucosylation by measuring the fluorescence of Eu(III)-chelate-labeled nanoparticles by microplate flourometer. To the identification of Fucα1-6/3GlcNAc carbohydrates on PSA surface , the lectin-immunoassays using free PSA specific monoclonal antibody as acapture and AAL coupled with Eu(III)-chelate-labeled nanoparticles using as probe for detecting fPSA . these methods compared with conventional immunoassay ELISA

Result and discussion: The nanoparticle-based lectin-immunoassay method we showed a statistical significant ($p < 0.001$) in fPSA level depend on the fucosylation from PCa patients compared to healthy control, and also clear significant ($p < 0.001$) when comparing HBP patients to PCa patients but HBP compared with healthy control there is non-significant the ($p=0.086$) in the nanoparticle-based lectin-immunoassay methods. while in the immunoasssy ELISA was showed deffrent result .

Keywords: Eu-chelat; nanoparticle-based lectin-immunoassay; discrimination of fPSA from benign prostatic hyperplasia BPH and malignant prostate cancer PCa source

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INTRODUCTION

The second diagnosed malignancy is prostatic carcinoma (PCa) as well as the sixth cause of cancer death in men. Incidence rates globally vary more than 25 times, with the highest rates in the developed countries this may be due to the widespread use of prostatespecific antigen screening and prostatic tissue biopsy (Jemal *et al*.,2011). Overall the complex morphology, histologic heterogeneity, and the early metastatic ability of localized PCa show the requirement for extra clinical and pathologic tests for the evaluation of PCa and its early mdiagnosis(Mohammed and Helal,2017). Benign prostatic hyperplasia (BPH) is one of the most common chronic conditions in aging men. BPH is defined histologically as epithelial and stromal cell proliferation, which leads to prostate enlargement, Prevalence rates of BPH increase with age, with 50% of men over 50 years of age and 90% of men over 80 years having the condition. Like most chronic diseases, BPH is progressive (Yuan,2011).Both benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are frequent diseases in middle-aged to elderly men worldwide. While both diseases are linked to abnormal growth of the prostate, the epidemiological and pathological features of these two prostate diseases are different. BPH nodules typically arise from the transitional zone, and, in contrast, PCa arises from the peripheral zone. Androgen deprivation therapy alone may not be sufficient to cure these two prostatic diseases due to its undesirable side effects (Izumi and Chang,2014). The present review explored a portion of current and future biomarkers used in the detection of prostate cancer, PSA, also known as hK3, was discovered and today it remains the most widely used, and controversial, biomarker in prostate cancer. The search for a more rapid, specific marker for the detection of prostate cancer has led to numerous laboratories examining biomarkers(Pentyala *et al*.,2016).Free prostate-specific antigen (fPSA) is another common prostate tumour marker, and the ratio of the serum levels of fPSA and PSA (fPSA/PSA) is also frequently used as a clinical indicator for diagnosing PCa, However, an increased serum level of PSA can also be caused by benign prostate hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN), acute or chronic prostatitis, and other non-malignant diseases of the prostate (Zhou,2018)

MATERIAL AND METHODS

Fluoro-Max carboxylate-Modified dye nanoparticles from Thermo Fisher (USA), *Aluria Aurantia* Lectin (AAL) from Vector (USA) and DELFIA Eu-labelling Kit from Perkin Elmer (Finland)

biotinylation of antibodies and preparation of solid-phase surfaces

A- Immobilizing of streptavidin in solid-phase surface of microtitration plate

Streptavidin lyophilized protein was dissolved in D.D.water 10 mg/mL and stored frozen in aliquots. Maxisorp™ microtitration plates in C12-strips or single well formats were from (Perkin-Elmer Life Sciences, Turku, Finland). Streptavidin was diluted in the coating buffer (100 mM Na2HPO4/50 mM citric acid, pH 5.0) to the final concentration 5.0 μg/mL. Then 200 μL of the coating solution was dispensed into each well, giving 1 μg streptavidin per well. The plates were closed in a humidified box and incubated overnight at +35 °C. Then the plates were washed in a DELFIA Platewash (Perkin-Elmer Life Sciences,

Turku, Finland) with DELFIA Wash Solution supplemented with Tween 20 to the final concentration 0.05%. After washing, 250 μL of saturation solution (50 mM Tris-HCl, pH 7.0; 150 mM NaCl; 0.05% NaN3; 0.2% bovine serum albumin and 6% D-sorbitol) was added per well. The plates were saturated overnight at +25 °C. The saturation solution was aspirated and the plates were dried (+35 °C, relative humidity < 5%) for 2 h. Finally the plates were packed with moisture adsorbent and stored dry at +4 °C(Välimaa *et al*., 2003)

B- biotinylation of antibodies

Sulfosuccinimidobiotin was added at a 30-fold molar excess over IgG (about 90 mg/mg lectin). The solution was mixed well, and incubated at room temperature for 30 min with gentle shaking. The solution was dialyzed extensively against several changes (>100-fold dilution) of phosphate-buffered saline to remove uncoupled biotin. The solution was centrifuged dialysate (8,000 × *g*, 10 min, 4 °C) to remove any precipitate formed during dialysis. the IgG concentration was determined by measuring the absorbance at 280 nm (measured using nanodrop spectrophotometer). the conjugate was stored at 4 °C in the presence of 0.02% sodium azide final concentration (Javois, 1994).

Nanoparticle – lectins bioconjugates

a- Pre-activation of carboxylate modified nanoparticle

about 1ml nanoparticle was added to microcentrifuge appendrof tube. It was centrifuged at 13000 rpm, the supernatant was decanted. the pellet particles was dissolved in o.5 ml phosphate buffer saline 25 mM was mixed by vortex and particle were resuspended by probe solicitor (10 s, 80-W power level)

b- Activation of carboxylate modified nanoparticle

the suspension nanoparticles in eppendorf tube was added 500ml. the EDAC solution was prepared immediately 0.75 mmol/L then it was added to the reaction , it was mixed rapidly by syringing repeatedly with pipettor. sulfo- NHS solution was prepared immediately 10mmol/L then it was added to the reaction , it was mixed rapidly by syringing repeatedly with pipettor.the reaction solution was completed with waster . eppendrof tube was mixed at room temperature on amixing wheel for 30 min the carboxylate-modified particles were centrifuged at 13000 rpm, the supernatant was decanted. plellet particles were resuspended with 1ml 25mM PBS by by probe solicitor (10 s, 80-W power level)

c- Nanoparticle-AAL bioconjugates

The AAL was dissolved in 1ml of 10Mm phosphate buffer PH 7.5 , About 200 µl from AAL was added to the active carboxylate-modified nanoparticles, the solution reaction was mixed rapidly by syringing repeatedly with pipettor.The coupling reaction was incubated Tris-based buffer (10mmol/l Tris HCL,0.5 g/l NaN_3,PH 8.5) , tube was mixed at room temperature on amixing wheel for one hour.The unbound AAL was removed by centrifugation at 13000 rpm, the supernatant was decanted.The nanoparticles-AAL was washed with fresh Tris-based buffer to remove EDAC and unbound AAL. The nanoparticles-AAL were pelleted by centrifugation at 13000 rpm, the supernatant was decanted. The nanoparticles-AAL were resuspended between washes by probe solicitor (10 s, 80-W power level).The above steps were repeated three time. The storage buffer was used to block the remaining active groups , it was contained (10 mmol/l Tris HCL, o.1% tween 40 , 50mM salicylic acid , .1% bovine serum albumin ,0.1 gelatin)(Soukka *et al*., 2001)

Nanoparticle-based lectin-immunoassay

The developed research assay was conducted in three-step sandwich-type format, where AAL coupled to Eu(III)-chelate-dyed nanoparticles was used as a tracer. All subsequent steps were undertaken at RT and incubations done with slow shaking. 50 µl of TSM buffer and 50 µl of either sample or calibrator, in two replicas, were added and incubated for 1 hour. The wells were washed four times and nanoparticles-AAL Eu chelate dye (100 ng/well) was added in 150 µl of TSM buffer and incubated for 1 h. The plates were washed six times before adding the enhancement solution (200 µL/well), and incubated for 10min before measuring by microplate flourometer of Europium signal the bound nanoparticle bioconjugates was directly measured fromthe surface of thewell.

RESULT AND DISCUSSION

Study Design, Setting and Data Collection Time

A case – control study was conducted between August (2018) to April (2019) and it was carried out at Babylon oncology center . The study subjects comprised three groups. The malignant tumor is first group prostate adenocarcinoma PCA (n=24), randomly selected from patients admitted to the Babylon oncology center Hospital/iraq, were diagnostic by physician . The benign tumor is the second group benign prostatic hyperplasia BPH (n=21) from margan hospital/iraq , in addition to third group as a control group that included 15 healthy individual, men is (n=15) ,The last group matched with the age of patients group

The results

The descriptive statistics for fPSA, total number of subjects participated in the study is n= 60, 21 subjects for BPH who undergone all method, Control is 15 subjects, PCA is 24 subjects, the mean and st.dv. Is discussed below, further to establish significant difference among the groups, Kruskal Wallis test is conducted.

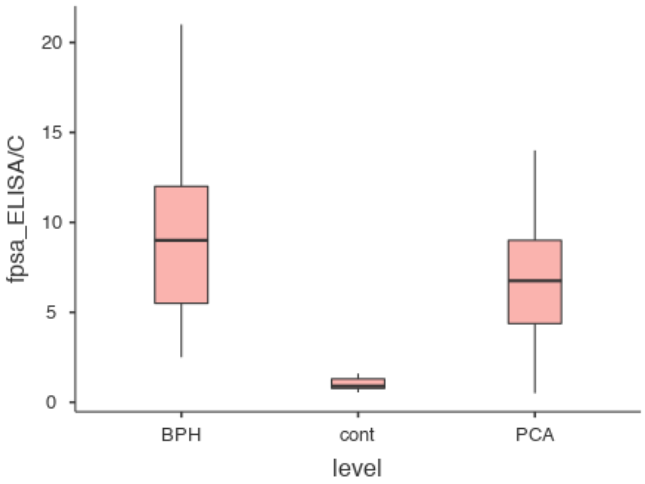

Figure 1: The concentration of fPSA by ELISA method : x-axis the groups of diseases ; y-axis the concentration of fPSA

From the above graph, it is understood that, on average, BPH attained higher value compared to rest, and control group attained lowest level.

On BPH with ELISA/C is 9.310 (±4.5), Cont with ELISA/C is 1.03 (±.330), PCA with ELISA/C is 6.958 (±3.5),

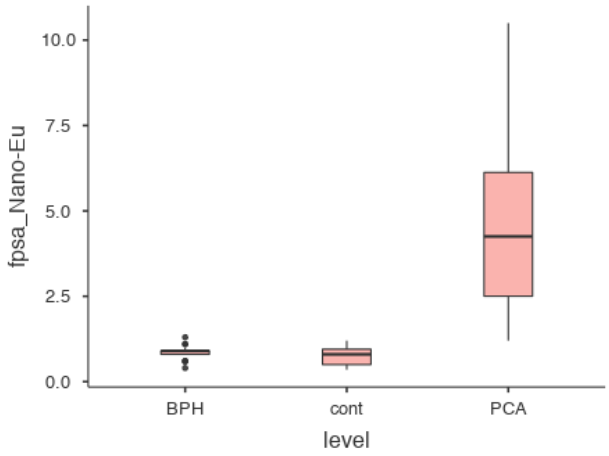

Figure 2: Values of BPH, Cont. and PCA

It is understood that, on average, BPH attained higher value compared to rest, and control group attained lowest level.

On BPH with Nano-Eu is .852 (±.197), Cont with Nano-Eu is .757 (±.284), PCA with Nano-Eu is 4.75 (±2.74), from the graph it is understood that, PCA obtained higher score and control group is least.

Based on the normality test, for all the 3 data seems to non-normally distributed, as per statistics guidelines, non-parametric test is more suitable than parametric, since response variable is continuous and independent variable is factor, Kruskal Walli is most suitable test Chi square(DF) for ELISA/C is 32.35(2), P<.001, for Nano-Eu, 42.54(2) and P<.001 in all the 2 test p value is less than .05, hence significant difference among Control, BPH an PCA is clearly established statistically at least 5% level.

On pairwise comparison among 2on ELISA/C, there is no difference between BPH and PCA and other two combinations are statistically significant in the convential ELISA mwthod

On fpsa_Nano-Eu, there is no different between BPH and cont but other two combination established significant difference in new mthod Nanoparticle-based lectin-immunoassay

The diagnostic test evaluation by the MEDCALC program The result showed the accuracy 56.67% depended on the sensitivity and specificity rate that mean the number of False Positive sample is raised in ELISA method

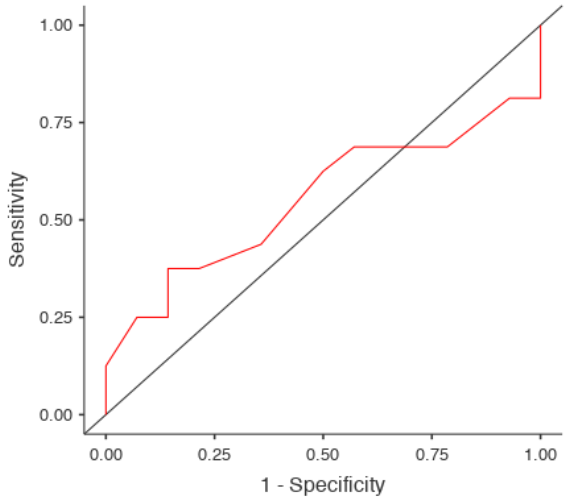

Figure 3: ROC Curve between specificity and sensitivity of ELISA method for fPSA diagnostic

The result showed the accuracy 56.67% depended on the sensitivity and specificity rate that mean the number of False Positive sample is raised in ELISA method

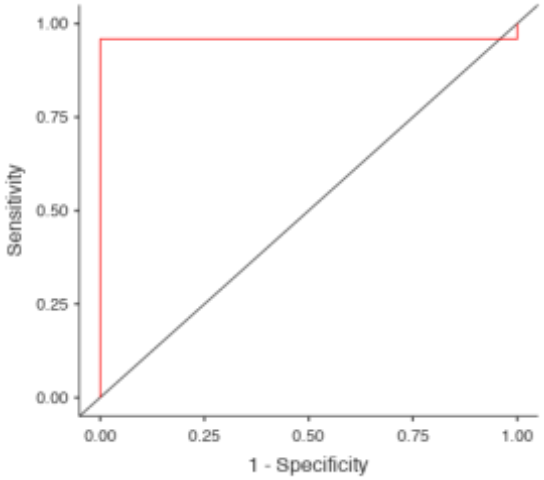

Figure 4: ROC Curve between specificity and sensitivity of Nano-Eu method for fPSA diagnostic

The result above showed the accuracy 92.86% depended on the sensitivity and specificity rate that mean the number of False Positive sample is very reduce in Nano-Eu method compared with another two method ELISA and Eu-chelate and this method is more specificity to discrimination the source of PSA tumor marker from the benign and malignant

DISCUSSION

We are proposing a test for analytically sensitive and quantit ative aberrant fucosylation detection n fPSA and the aberrant fucosylation on fPSA from the prostate carcinoma source that enhanced preference for antigen cancer related isofom, For the first capture, we was used a MAb to detect the protein epitope,Monoclonal antibodies are a concern in order to capture the protein of interest the suitable lectins were used for detection and recognize the fucosylation on the surface of antigen tumor markers, the Aleuria aurantia lectin (AAL) is a fucose-based lectin, which was used for the analysis of PSA fucosylation modification in Pca with glycoprotein core fucα1-6/3GlcNac(Yoshioka et al,2016 ; Kekki et al, 2017),

The highly improved analytical performance of the method applying lectin NPs is due to the signal amplification by the 30000 Eu3+-chelates packed within the 100 nm NPs, and of the avidity effect created by the high density of immobilized lectins on the particle, this new protocol suitable for sensitive glycan profiling of fucose profiling of PSA from different sources derived plasma (Soukka et al,2001; Gidwani, 2019)

Clinically, tests with enhanced medical specificity the rise fPSA values in the diagnostic routin constitute a common problem in the distinction between PCa and benign conditions, in particular HBP, and can cause unnecessary interventions and costs (Kekki el al,2017)

prevalent prostate cancer (PCa), especially in men above 60 years, PSA is elevated early in the disease and a result above

a selected cut-off indicating enhanced cancer risk can be seen by magnetic resonance imaging (MRI) and tested with a relatively safely performed routine biopsy procedure. However, besides being elevated in common and age related benign prostatic conditions, a more serious circumstance is that many cases of latent, non-aggressive lesions are found in the biopsy specimen leading to over diagnosis and subsequent overtreatment (Schröder et al, 2012; Bruns and Burtis, 2014)

Using the conventional immunoassay ELISA we detected a statistically significant increase ($p < 0.001$) in the fPSA level depend on the immune attachment in PCa patients and benign patients BPH compared to the healthy control, while the detected a statistically no significant ($p = 0.198$) in the fPSA fucosylation in PCa patients compared to the benign patients HBP, With the nanoparticle-based lectin immunoassay methods we showed a greater discrimination ($p < 0.001$) when comparing BPH patients to PCa patients with. The BPH compared with healthy control there is non-significant the ($p=0.631$) in the nanoparticle-based lectin-immunoassay method.

in this two methods the accuracy was differed depend on the specificity and the false positive (value of fPSA in benign condition) in the conventional immunoassay ELISA was reduced to 56.67% and specificity 40.54% while increased in nanoparticle-Eu was more specificity was 88.24% and more accuracy 92.86% because of the improved method be the alternate strategy of interaction captured antibody, enzymatic method was nonspecific for recognize the source of fPSA and the glycovariant resulted from the Changes in the glycosylation of malignant cells has long been recognized as a hallmark of cancer this agree with (Fuster and Esko,2015)

For recognize the glycovariant was used the more specific lectin AAL give more interactions and linked with florescent,it has been shown to discriminate of fPSA (Varki et al,2017).

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