Original Articles/นิพนธ์ต้นฉบับ

Prostate Specific Antigen Test Kit as a New Innovation in Forensic Investigation for the Detection of Semen in Suspected Evidences in The Kingdom of Thailand.

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Abstract

Introduction: Recently, routine forensic investigations of suspected evidences in rape cases are microscopic identification of the spermatozoa and acid phosphatase test for detection of the seminal fluid.

Objective: Our article aimed to study the application of prostate specific antigen (PSA) test kit (PSA immunochromatographic assay) as a new method for the detection of semen in focus on the accuracy of test compared with the routinely used method, acid phosphatase test by using the sperm test as a gold standard method.

Methods: Five hundred vaginal swab and stain specimens from health care centers in Thailand submitted to Department of Pathology, Ramathibodi hospital were selected to this study. PSA immunochromatographic assay test and acid phosphatase test were performed in all specimens with compared to sperm test as a reference gold standard method. Statistical analysis was done to analyze the results.

Results: Our study results were correlated with many previous international studies that the PSA method has more accuracy than the acid phosphatase method in all parameters of test, including sensitivity, specificity, positive predictive value, and negative predictive value with statistical significance ($p<0.05$).

Conclusion: We had confirmed that the PSA test kit would be an interesting innovation for forensic investigation in sexual assault cases in The Kingdom of Thailand and all over the world.

Keywords: Prostate specific antigen (PSA), PSA test kit, ELISA for PSA, acid phosphatase, sperm, rape, sexual assault, medicolegal case, forensic investigation

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Introduction

Sexual assault is usually the un-witness crime, therefore forensic investigation to identify the spermatozoa or seminal fluid on the specimen collected from vagina of the female victims plays an important role to confirm recent episode of sexual intercourse to provide the court testimony.

As a matter of fact, microscopic identification of the spermatozoa is a gold standard method to proof that the female victims had recently been involved with sexual intercourse episode. But if the male offender is a vasectomized or an azoospermic individual, it is not possible to proof the recent sexual assault episode by spermatozoa identification alone because it usually shows negative result and therefore may let the offender free. For these cases, investigation of seminal fluid is very essential. In The United States, the reported frequency of azoospermia is 1-9% in seminal stains or swabs examined in sexual assault cases and the reported frequency of contraceptive vasectomy has been estimated 750,000 to 1,000,000 per year[1,2].

In the recent decades, acid phosphatase test is generally used as a routine method for identification of the seminal fluid in fabric stain or vaginal swab specimens. But according to the scientific background, acid phosphatase test is only a presumptive or a screening test and it also degrades rapidly in normal vaginal environment, therefore could be detected in only a short duration after sexual intercourse episode [3-8]. In addition, acid phosphatase test also give false positive result to many body fluids and many chemical products used in daily life[9]. Interpretation of positive result in the routine qualitative acid phosphatase testing method by the laboratory personnel is also an important problem and false positive report could lead to misinterpretation by the forensic physicians and finally lead to false legal testimony.

Nowadays, prostate specific antigen (PSA) test is generally accepted for its application in forensic investigation of seminal fluid in the evidences of sexual assault cases. According to the scientific background, PSA test is a confirmatory or a diagnostic test for identification of the seminal fluid. Regards to previous study, PSA test has better accuracy than acid phosphatase test in all parameters of test (sensitivity, specificity, positive predictive value, and negative predictive value)[5]. In addition, PSA also degrades slower than acid phosphatase in the normal vaginal condition[3-8].

According to the technical method, Enzyme Linked Immunosorbent assay (ELISA) is the conventional technique for PSA analysis and values as a quantitative test[10]. But in the recent years, PSA test kit has been widely studied for its substitution of the conventional ELISA testing method. The PSA test kit provides a less-expensive cost, less time consumption, and more convenience than the ELISA method. PSA test kit has been proved by many studies that its accuracy is not different from the ELISA method.[11-13] Therefore, PSA test kit could benefit as a rapid technique for detection of the seminal fluid. The PSA test kit is a semi-quantitative analyzing method by immunochromatographic principle.[14-16]

In the recent years, PSA test kit becomes more popular among forensic personnel as a new innovation for detection of seminal fluid in sexual assault related evidences according to its better accuracy than routinely used acid phosphatase method, the slower intra-vaginal degradation time, and better specificity for identification of seminal fluid.

The authors considered that there was no research study about application of PSA test kit in forensic works in The Kingdom of Thailand and most of previous international articles had studied from well controlled semen samples from volunteer subjects, not from real rape victims. This may not represent the practical application in real victims. So our study intend to perform the experiment from submitted specimens collected from real sexual assault victims

from all over the country. We purposed to study the accuracy of PSA test kit compared with the routinely used acid phosphatase method and reference with gold standard method (spermatozoa identification by microscopic exam). We also expect to be the pioneer in studying about the application of PSA test kit in forensic works for detection of seminal fluid in sexual assault related evidences in The Kingdom of Thailand.

Materials and methods

Study groups

Over 500 specimens of vaginal swabs and filter-papers stained with vaginal secretion submitted from medical institutes from all over the country to Department of Pathology, Ramathibodi Hospital requested for the acid phosphatase and sperm test during July 2006 to January 2007 were selected to this study. Only specimens collected from the rape victim's vagina were included in our study. The specimens collected from penile swab or other part of male offenders, specimens collected from other source of female victims such as oral, anal, and specimens collected from stained objects at crime scene were excluded from our study. The specimens contaminated with blood appeared as red or brown color which may interfered with assessment of color change in acid phosphatase test were also excluded from the study. The specimens collected from dead bodies either during crime scene investigation or autopsy and specimens inadequately collected such as small stains at tip of swabs and filter papers were also excluded from the study.

After excluded by mentioned criteria, selected specimens from 500 subject cases consist of 429 vaginal swabs (86%), 62 filter papers stained with vaginal secretion (12%), and 9 cases submitted both vaginal swabs and filter papers (2%). In the last category, we had tested for both swabs and filter papers.

All specimens were examined for acid phosphatase test and spermatozoa analysis as the submitter request. The left-over specimens from routine acid phosphatase and sperm test were tested for PSA immunochromatographic assay.

Demographic data of the victim's subject from reviewing the laboratory request form revealed 211 out of total 500 cases (42%) were the girl age under 15-year-old and 289 cases (58%) were female age over 15-year-old. We could not categorize the age group more specifically because many of the laboratory request forms are filled incompletely. So we can only interpreted by the article data, which are change at the age of 15 in Thailand. The time of specimen collection after onset of sexual assault event could not be interpreted due to incomplete laboratory request form data also. Only two out of 500 cases recorded time of specimen collection after episode of sexual assault, so this data could not be evaluated.

Laboratory Procedure

All 500 selected specimens were tested within 48 hours after received (All specimens were stored at 4°C until tested.) following these procedures :-

1. Review the laboratory request form to verify the submitted specimens.

2. Label the Specimen Identification Number (SID) for each cases.

3. Evaluate the adequacy of specimen then isolate the stained swabs and filter papers.

4. Put the isolated stained part of the specimen in to the centrifugal tube together with 250 μL of buffered saline (consist of 1 ml. of NSS and 1 ml. of NH_3OH) then centrifuge for 10 minutes to isolated the stained secretion from the specimen to maximize extraction of the samples.

5. Isolate the 300 μL of centrifuged sediments to the micro-tube labeled with SID number.

6. The centrifuged sediments were divided for 3 tests :-

   6.1 Acid phosphatase test

6.2 Microscopic visualization for the spermatozoa

6.3 PSA immunochromatographic assay

7. Acid phosphatase test working methods :-

7.1 Preparation of the reagent

Solution A :-

- Brentamine fast blue B 1 gm. (O-Dianisidine Tetrazolized)
- Sodium acetate 20 gm.
- Glacial acetic acid 10 ml.
- Distilled water 100 ml.

Solution B :-

- Sodium napthyl PO_4 0.08 gm. («-Napthyl acid phosphate)
- Distilled water 90 ml.

7.2 Mixed solution A : solution B in 1:9 ratio and filtered for the reagent

7.3 Test the 100 ml. of centrifuged sediments from the specimen with the prepared reagent

7.4 Positive test was interpreted by visualization of purplish discoloration within 40 seconds and negative test was interpreted by absence of discoloration within 40 seconds.

8. Microscopic identification of the spermatozoa working methods :-

8.1 Use a drop of prepared centrifuge-sediment onto two prepared label-glass slides.

8.2 Incubation at 60°C

8.3 Stained with Hematoxyline-Eosin

8.4 Microscopic visualization for spermatozoa by two experienced technicians

8.5 Positive results were obtained by visualization of at least one spermatozoa under low-magnification.

9. PSA immunochromatographic assay

9.1 Use 100 µL. of prepared centrifuge-sediment drop into sample well of "ONE STEP Prostate specific antigen (PSA) test kit" produced by In Tec Product, Inc. (XIAMEN).

9.2 Read the test result between 5 to 10 minutes after test.

9.3 Interpretation of test results :-

Positive: In addition to the control band, a distinct colored band also appears in the test region

Negative: Only one colored band appears in the control region

Invalid: Neither test band nor control band appears

10. Enzyme Linked Immuno-Sorbent Assay (ELISA) for Prostate specific antigen (PSA) was also performed as a confirmatory test for all problem cases, which consist of 2 categories :-

10.1 PSA test kit positive but sperm test negative

10.2 PSA test kit negative but sperm test positive

The ELISA method for PSA was done using the sandwich principle (PSA antigen + Anti-PSA monoclonal antibody-BIOTIN / Anti-PSA monoclonal antibody-ruthenium + Strptavidin-coated micropeptides) using 20 µL of prepared centrifuged sediments analyzed by "ElecSys 2010 Roche Diagnostics" automated machine.

Data analysis

The positive and negative laboratory testing results of acid phosphatase test and PSA immunochromatographic assay from all 500 cases were statistically calculated for the accuracy of test in 4 parameters; sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) compared with sperm identification method as a gold standard reference for the detection of semen in forensic evidences. All data were analyzed by program "Intercooled STATA 8.0". Chi-Square test for the correlation between PSA test kit with reference spermatozoa identification method and 95% confidence interval were also analyzed by the same program.

Results

From total specimen of 500 cases tested, 123 cases (25%), 133 cases (27%), and 151 cases (30%) give positive result for acid phosphatase (AP) test, PSA test, and sperm identification method respectively. Eighty seven cases (17%) give positive results for all 3 tests and 320 cases (64%) give negative results for all 3 tests.

Twelve cases (2% of total cases or 9% of the positive PSA cases or 10% of the positive AP cases) revealed positive results for both AP and PSA tests but give negative result for sperm test.

Eight cases (2% of total specimen) give positive result for sperm test alone but give negative result for both AP and PSA tests.

The accuracy of test were calculated in 4 parameters (sensitivity, specificity, positive predictive value - PPV, and negative predictive value - NPV) compared between the routinely used AP method and the new PSA test kit analyzing method using sperm test as a gold standard method for reference.

The correlation between AP test and reference sperm method versus PSA test and reference sperm method are calculated with statistical chi^2 method and demonstrated in Table 4.

In problem cases (the case that sperm test positive but negative for both AP and PSA tests and the case that sperm test negative but positive for both AP and PSA test) which consists of 76 cases out of the total 500 cases (15.20%), ELIZA for PSA was done to verify the interpretation of PSA test kit method. The result of PSA test kit compared with PSA ELISA method are showed as table 5.

The negative control for PSA ELISA was also done by testing the samples of distilled water and 0.9% NaCl (NSS) and the results are showed as table 6.

Discussion and Conclusion

The strength and unique of our study from the previous literatures is that we studied from the real specimens from sexual assault victims which represent the practical situation and could provide high value of external validity.

In practical situation, sexual assault victims in Thailand usually had late visit to the physicians (usually later than 7 days). This could explained why there are so many negative results in our study [320 cases out of total 500 cases (64%) were negative all 3 tests]

Table 1 The comparison between the number of positive and negative cases between Acid Phosphatase (AP) test and gold standard sperm identification method.

Test	Sperm positive (cases)	Sperm negative (cases)	Total
AP positive (cases)	102	21	123
AP negative (cases)	49	328	377
Total	151	349	500

Table 2 The comparison between number of positive and negative cases between PSA test and gold standard sperm identification method.

Test	Sperm positive (cases)	Sperm negative (cases)	Total
PSA positive (cases)	117	16	133
PSA negative (cases)	34	333	367
Total	151	349	500

Table 3 The accuracy of test in 4 parameters compared between AP and PSA test with the reference gold standard method (sperm test).

	AP	95% CI	PSA	95% CI
Sensitivity	67.55%	63.45 - 71.65	77.48%	73.82 - 81.14
Specificity	93.98%	91.90 - 96.07	94.27%	92.23 -96.31
PPV	82.93%	79.63 - 86.22	85.40%	82.31 - 88.50
NPV	87.00%	84.06 - 89.95	90.63%	88.08 - 93.19

Table 4 Chi-square test for correlation between AP and reference sperm method versus PSA test and reference sperm method.

	Standard Error	95% Confidence Interval[a]
AP	0.0201	0.768 - 0.847
PSA	0.0182	0.823 - 0.894

[a]p-value = 0.0159

Table 5 Average and range of PSA level by ELISA for cases of negative and positive PSA test kit.

PSA test kit	Average ELISA (ng/ml)	Range ELISA (ng/ml)	Cases (N)
Negative (-)	0.049	0.016 - 0.363	41
Positive (+)	8.819	0.107 - over 100	35

Table 6 Average and range of PSA level by ELISA method using negative control specimens (distilled water and NSS).

Negative Control Sample	Average ELISA (ng/ml)	Range ELISA (ng/ml)	Cases (N)
Distilled water	0.0354	0.034 - 0.039	10
0.9% NaCl (NSS)	0.0342	0.031 - 0.038	10

Our results had shown that 8 cases (2% of the total cases) were positive only for sperm test but negative for both AP and PSA. This could be also according to late examination after sexual assault episode which cause degradation of the AP and PSA while spermatozoa still remain.

According to the previous studies 8-10% of general male population have low or absent sperm counts. The reported frequency of "azoospermia" is 1-9% in seminal stains or swabs examined in sexual assault cases[1,2]. Our study revealed approximately the same prevalence that 12 cases (2% of total cases, 10% of the positive AP case, or 9% of the positive PSA test) had positive result for both AP and PSA but absent for sperm (Possible vasectomized and azoospermic offender cases)

Comparing the lab results between our study and previous study, our results showed that the PSA test has more accuracy than the AP test in all parameters of test as the previous study had mentioned.

The lower sensitivity results for both AP and PSA tests in our study compared with the previous studies were probable due to late visit of the sexual assault victims to the physicians in Thailand than in the well-developed country.

The weak point of our study was that we had

the limitation about determining time period between sexual assault episode and the time that the victims had visited to physicians. This was according to inadequate information filling of the laboratory request form.

Our statistical data of chi^2 method calculated by program "Intercooled STATA 8.0" revealed significant correlation between the PSA test kit and gold standard spermatozoa identification method of 0.0182 which was higher than the correlation between the AP test and sperm test (0.0201)

Our confirmatory PSA ELISA method for problem cases revealed the highest value of PSA of 0.0362 ng/ml in PSA test kit negative cases and the lowest value of PSA of 0.107 ng/ml in PSA test kit positive cases compared to the highest value of the negative control samples 0.039 for distilled water and 0.038 for NSS. These data assured us that our positive and negative PSA test kit results could be reliable.

According to the previous studies, the PSA has slower degradation time than acid phosphatase in the normal vaginal condition.

In conclusion, we suggest that PSA test has more practical benefit than routinely used acid phosphatase test in the investigation of sexual assault cases according to these following reasons :-

1. PSA test has more accuracy than AP test in all parameters of test.
2. PSA test has less false positive result than AP and has less problem with interpretation of positive result as the AP do.
3. PSA has slower vaginal decay time than the AP.
4. PSA is a confirmatory test but AP is only a screening test according to the scientific principle.

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Original Articles/นิพนธ์ต้นฉบับ

ชุดตรวจแบบรวดเร็วหาสารจำเพาะจากต่อมลูกหมาก (PSA) นวัตกรรมใหม่ในการตรวจพิสูจน์น้ำอสุจิจากวัตถุพยานทางนิติเวช ในราชอาณาจักรไทย

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ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

บทคัดย่อ

บทนำ: การตรวจพิสูจน์ทางห้องปฏิบัติการทางนิติเวชในผู้ป่วยคดีหรือศพคดีที่สงสัยว่าถูกกระทำชำเราในปัจจุบันโดยทั่วไปจะทำการตรวจด้วยเทคนิค 2 วิธีคือ การตรวจหาตัวอสุจิด้วยวิธีการดูด้วยกล้องจุลทรรศน์ และการตรวจหาส่วนประกอบของน้ำอสุจิทางเคมีด้วยวิธี แอซิดฟอสฟาเตส

วัตถุประสงค์: งานศึกษานี้มีวัตถุประสงค์ในการศึกษาถึงการประยุกต์ใช้ชุดตรวจแบบรวดเร็วหาสารจากต่อมลูกหมากหรือ พี เอส เอ (PSA) ในการนำมาใช้ตรวจพิสูจน์หาน้ำอสุจิจากวัตถุพยานทางนิติเวชในคดีที่เกี่ยวข้องกับการกระทำชำเราในประเทศไทย

วิธีการวิจัย: ทำการศึกษาวิจัยจากสิ่งส่งตรวจจำนวน 500 ตัวอย่าง ที่ส่งตรวจ ณ ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี ซึ่งส่งมาจากสถานพยาบาลต่างๆ ทั่วราชอาณาจักรไทย โดยทำการตรวจวิเคราะห์ด้วยวิธีการตรวจหาสารจำเพาะจากต่อมลูกหมากหรือ พี เอส เอ (PSA) เปรียบเทียบกับการตรวจวิธีทางเคมีแอซิดฟอสฟาเตส ซึ่งเป็นที่นิยมใช้กันแพร่หลายอยู่ในปัจจุบัน โดยใช้วิธีการตรวจพิสูจน์ตัวอสุจิด้วยกล้องจุลทรรศน์เป็นวิธีมาตรฐานในการเปรียบเทียบ และนำผลที่ได้มาวิเคราะห์ทางสถิติ

ผลการศึกษา: ผลการศึกษาที่ได้มีความสอดคล้องกับการศึกษาหลายๆรายของต่างประเทศก่อนหน้านี้ ซึ่งยืนยันว่าการตรวจหาน้ำอสุจิด้วยวิธีการตรวจหาสาร PSA มีค่าความแม่นยำของผลการตรวจ (Accuracy of test) ที่ดีกว่าวิธีแอซิดฟอสฟาเตส ทั้ง sensitivity, specificity, positive predictive value (PPV), และ negative predictive value (NPV) อย่างมีนัยสำคัญ ($p<0.05$)

สรุปผลการศึกษา: งานวิจัยนี้ได้ผลสนับสนุนว่าการตรวจพิสูจน์น้ำอสุจิด้วยวิธีการตรวจหาสาร PSA เป็นนวัตกรรมใหม่ที่มีความแม่นยำและน่าเชื่อถือสูงในการตรวจพิสูจน์หาน้ำอสุจิจากวัตถุพยานทางนิติเวชเพื่อใช้เป็นหลักฐานยืนยันการกระทำชำเรา ซึ่งจะเป็นประโยชน์ต่อการพัฒนาทางนิติเวชศาสตร์และเป็นประโยชน์ต่อกระบวนการยุติธรรมในราชอาณาจักรไทยสืบต่อไปในอนาคต

คำหลัก: สารจำเพาะจากต่อมลูกหมาก พีเอสเอ ชุดตรวจลำเร็จรูป แอซิด ฟอสฟาเตส น้ำอสุจิ ตัวอสุจิ ล่วงละเมิดทางเพศ ข่มขืนกระทำชำเรา นิติเวชศาสตร์

ติดต่อบทนิพนธ์: วิชาญ เปี้ยวนิ่ม หน่วยนิติเวช ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ ร.พ.รามาธิบดี กทม. 10400
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