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Study (Prostatic Specific Antigen P30) in Females Vaginal and Rectal Swabs Without Sexual Assault

Ahmed Kadhim Mohammed*

Department of DNA Fingerprint, Ministry of Health, Medical Legal Directorate Baghdad, Iraq

***Corresponding author:** Ahmed Kadhim Mohammed, Department of DNA Fingerprint, Ministry of Health, Medical Legal Directorate Baghdad, Iraq, Tel: 07901707159; Email: ahmed.mohammed@mizan.edu.iq; geneticforensic@yahoo.com; forensicgenetic@gmail.com

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Abstract

Globally, the incidence of sexual assault cases, especially rape, is increasing. sexual aggression is a serious social and public health issue that requires an urgent forensic medical examination. The ability to detect seminal fluid is vital in forensic cases involving sexual assaults and Sodomy Crimes Prostate specific antigen (PSA, P30) a glycoprotein produced by the prostatic gland and secreted into seminal plasma, is now accepted as a marker for detecting semen in criminal cases involving vasectomised or azoospermia males. Many studies approved that PSA present in variety of other body fluids, the greatest concentrations of PSA outside of semen have been in breast milk and amniotic fluid. Since no cross reactivity has been reported to date, this supports the hypothesis that P30 is a male-specific protein. For this reason, the detection of the P30 antigen in a forensic stain is strong evidence that the stain is seminal in nature. We do not have studies about the presence of P30 in female body fluid in normal condition; very little global studies revealed that female body fluid contain very little concentration of PSA.

Objectives

The objective includes the evaluation of the presence of P30 in vaginal and rectal swabs using P30 test from different female ages, race, referred to clinical forensic medicine Department in Medical Legal Directorate / Baghdad as cases not for sexual assault, Child sexual abuse (CSA), Adult sexual Offences (ASO) but for age determination, examination of torture, domestic violence., Pre-prison examination and. Drink-Driving Assessment

Materials and Methods

The material and methods were used SERATEC® P30 kits for detection prostate specific protein, the principle of test is **one-step chromatographic sandwich immunoassay**. Total samples (124) swabs (vagina and rectum) were investigated for semen detection.

Results

The results show that the **one-step chromatographic sandwich immunoassay** method for P30 detection is useful for the identification of seminal fluid (Plasma) in sexual assault because it is evidence saved, highly sensitivity, specificity for human semen detection.

Conclusion

The results show that the female vaginal and rectal swabs P30 detection are less than 1 ng/ml PSA because all cases are negative, the less sensitivity for SERATEC® P30 is 1 ng/ml PS. Female vaginal and rectal swabs may have P30 concentration less than 1 ng/ml PSA, we recommend to enhance manufactures to produce new kits for P30 with sensitivity less than 1 ng/ml PSA.

Keywords: Forensic Science, Prostate Specific Antigen (PSA), Biological Evidence.

Introduction

Prostatic specific antigen (PSA) or p30, was discovered in the 1970's independently by three groups. After antisera to the protein was developed, detection of p30 in forensic samples quickly became the method of choice in determining the presence of semen in the absence of sperm [1,2].

Many studies approved that PSA present in variety of other body fluids Table - 1 represents PSA in different body fluids, the greatest concentrations of PSA outside of semen have been in breast milk and amniotic fluid. Generally, the forensic biologist doses not encounter these fluids; however one unusual case of the detection of PSA in diaper originating from the colostrum in breast milk from a nursing child has been reported by Yu and Diamand is [4;10]. Table 1 - represents PSA in different body fluids.

Fluid	Concentrations of PSA(ng/ml)	References
Semen	200,000 to 5.5million	[2]
Semen	820,000 (mean)	[5]
Amniotic fluid	0.60 (average)8.98 in one case	[5]
Breast milk	1(average) 2100 in one case	[5]
Breast milk	Majority <1.0 > ;100 in one case	[6]
Breast milk	0.47 (median)	[7]
Saliva	Non	[5]
Female urine	3.72 (mean)	[8]
Female urine	1.73 (mean)	[9]
Female urine	0.12 – 1.06 ; 0.29 mean	[10]
Female urine	0.53 (mean)	[8]
Female urine	Majority <0.01	[7]
Female urine	Majority < 0.1	[11]

Biological evidence in most cases is the only one to prove the occurrence of sexual contact and to identify the perpetrator and is most important for legal proof in courts of law now a days. Contrary to other offenses, where major effort is invested upon investigating the crime scene, in sexual assault cases the victim his/herself constitutes the crime scene [3].

Because spermatozoa production in males can be affected by aspermia, vasectomy or other psychiatric conditions, forensic scientists have recognized the need for seminal fluid diagnostic tests which did not rely upon sperm cell presence. P30 is a 30,000 Dalton semen glycoprotein of prostatic origin. The range of PSA is 200,000 to 5.5 million ng/ml of semen. Since no cross reactivity has been reported to date, this supports the hypothesis that P30 is a male-specific protein. For this reason, the detection of the P30 antigen in a forensic stain is strong evidence that the stain is seminal in nature [1-3]. Spermatozoa are usually found in the vagina up to 3 days after intercourse and occasionally up to 6 days later. Tail are frequently found attached to spermatozoa on swabs taken within one hour of intercourse [4]. They are commonly found up to 16 hours and rarely up to 72 hours [5,6].

Various antigen specific membrane tests are currently used in clinical setting to screen a patients serum for the presence of PSA in levels > 4 ng/ml indicating either benign prostatic hyperplasia or prostatic cancer [7,8].

The P30 can be detected in seminal fluid without spermatozoa (e.g. seminal fluid of vasectomized or sterile man) it shows high stability and could be detected in 30 years old semen stain (dried) , it is possible to detect PSA from vomit samples at least up to 4 hours using simulated gastric juice also its shows PSA is a more specific marker than acidic phosphatase , many studies of PSA in vaginal swabs is more reliable than the detection of seminogeline [9].

In Iraq, the laboratory of Medico-Legal Directorate (**MLD**) was using the conventional methods like UV light for flavin detection (as a source of illumination) or direct microscopically detection of head or tail of the sperm using special stains. However, since 2011 the laboratory of the MLD has switched to use **one-step chromatographic sandwich immunoassay** as efficient technique for seminal fluid investigation [**2**]. we use SERATEC® kit made in Germany which have sensitivity as low as 1ng / ml while before that we were used ABA card® made in United State of America and had minimum detection limit of P30 is 4 ng/ml this let as to study female swabs in non-sexual assault cases to prove that no false positive are found from natural body of female fluid.

Material and Methods

Setting and Study design

This is descriptive cross sectional study conducted in Iraqi Medical Legal Directorate DNA Fingerprint department forensic Serology Division, which serves as the referral center for all Iraqi Provinces sexual assault victims and Sodomy crimes. From first of Jan 2015 to 31 of Dec 2015 about 124 swabs (vagina and rectum) were investigated for semen detection.

Ethical Consideration

This study was approved form scientific and ethical council in the Iraq medical legal directorate / Ministry of Health and Environment (MOH) according to the CODE of ethics in research. This research was conducted based on Article 2 of the Iraqi Forensic Medicine Law of 2013.

Case Definition Inclusion and Exclusion Criteria

Any adult woman over age 18 or over is referred officially from police offices and investigation bureaus to the clinical forensic medicine department to be investigated for reasons other than sexual assault like age determination, examination of torture, domestic violence, and Pre-prison examination. Vaginal and rectal swabs are taken from each female by a nurse who has special training and a forensic physician. Any married females who have had sexual intercourse for the past six days (consensual coitus) were excluded, also virgin females giving high vaginal swabs.

Sampling

During the one year prior to the implementation totally (124) swabs were investigated for present of p30 protein in vaginal and rectal swabs. Because the resource is limited and research staff not found in all the work week we selected study samples by convenient style to reduce the bas.

Outcome

SERATEC® PSA SEMIQUANT test kit is has the ability to detect minimum concentration 1 ng/ml.PSA kits was used is a chromatographic immunoassay for rapid semi-quantitative detection of PSA in forensic samples. Vaginal and rectal swabs were used to detect P30 protein, Positive and Negative results are recorded for all swabs. The kit contains 40 test cards and one transfer pipette (sealed and desiccated in a foil pouch) and 50 ml of standard solution tubes of extraction buffer.

Note: Each new lot number of kits must be validated using a positive and negative control before using it in casework.

Membrane test assay (**one-step chromatographic sandwich immunoassay**).
Swabs let to dry in room temperature for three hours than put in swabs cover and storage in room temperature inside paper envelope.

1. All samples and test components are allowed to warm to room temperature before starting the test.

2. A small section of the swab approximately (1/4) was cut and extract with 4 to 6 drops (approximately 300µl) of specific buffer at room temperature for 2 hours.

3. The device and the dropper from the sealed pouch were removed.

4. The SERATEC® PSA SEMIQUANT test P30 was labeled with case and item, exhibit numbers, data and initials.

5. 120 µl of sample was added to the sample well (S) of the test device.

6. Result at 10 minutes was read, positive results could be seen as early as 1 minute depending on the p30 concentration. for negative results, one might wait for the full 10 minutes.

Statistical Analysis

The database was examined for errors using range and logical data cleaning methods, and inconsistencies were remedied. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 21 computer software (**statistical package for social sciences**) in association with Microsoft Excel 2013.

Results

During the time prior to the implementation of the SERATEC® p30 test. one hundred twentyfour females were evaluated for non-sexual assault referred to clinical forensic medicine Department in Medical Legal Directorate / Baghdad as cases not for sexual assault. Total 248 swabs (Vaginal and Rectal) were investigated all of them are negative for semen protein P30 as show in table- 2.

Table 2 represent total results for P30 test in vaginal and rectal swabs.

	Vaginal Swabs	Rectal Swabs	Age	Non Married	Married	High vaginal swabs	P30 detection Result
124 Female	62	62	18 - 55 year	50	74	50	248 Swabs Negative
Total	248			248			

Discussion

Initially believed to be a prostate specific protein, it is now known to be found in many different fluids and tissues including breast tissue and tumors [10,12], periurethral glands [12,13] breast milk (9), amniotic fluid (10), and female urine (11).

PSA is a glycoprotein produced in the prostate and secreted into the seminal fluid. PSA is one of the major protein in seminal fluid with concentration between 0.2 and 3.0 mg/ml. Its main function is to liquefy the seminal fluid. The high concentration of PSA in seminal fluid and its very low concentration in female vaginal fluid (0.4 – 0.9 ng/ml and 0.0 – 1.25 respectively) makes PSA a suitable marker in forensic casework for identifying even small amounts of seminal fluid, particularly, these are the benefits of PSA determination in forensic biology [14,15]**.** In most of the crime, laboratories serological tests are used to screen evidence material for the presence of biological fluid of human origin. The key issue in serological analysis is the human specificity and sensitivity [16].

PSA can be detected in seminal fluid without spermatozoa (vasectomized), PSA is a more specific marker than acidic phosphatase [17]. Macaluso *et al* demonstrate that the detection of PSA in vaginal swabs is more reliable than the detection of seminogelin [18]. As PSA is protein this may be degraded by many physical, chemical and with extreme environmental factors lead to lose their three-dimensional conformation. It is possible that the monoclonal antibodies used in the kits lose their ability to bind the partially degraded PSA [19].

Conclusion

In conclusion that female body fluid may not have P30 in their body or have concentration less than 1 ng/ml, the results of this study indicate that the forensic biologist can extract material from vaginal and rectal swabs and be confident that a positive result is due to the presence of semen with concentration at as low as 1 ng/ml PSA.

Recommendation

We enhance researcher for study p30 protein in females body fluids especially vaginal and rectal to improve this study as a first study in Iraq.

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