PROSTATE-SPECIFIC ANTIGEN (PSA) RAPID DIAGNOSTIC TESTS COMPARED WITH SRY GENE FOR DETECTING MALE COMPONENT IN VAGINAL SWABS

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ABSTRACT

Proving intercourse signs on sexual assault victims still become a major challenge for forensic physicians in Indonesia. Many cases unsolved due to minimal evidences. One of the difficulties is evincing ejaculation in vagina. Most of forensic laboratories only depend on spermatozoa examination to find ejaculate. Surely, it is difficult to find spermatozoa if the perpetrators are azoospermia. Moreover, this examination may give false negative results as well as low sensitivity, especially in women who have washed their vagina. However, nowadays, there is a rapid test to detect PSA in seminal fluid which is very practical, quick, and inexpensive. This study will show the performance of PSA rapid test to detect male component in vaginal swabs taken from sexually assaulted victims.

A cross sectional study was conducted between October 2012 and December 2012. Sixteen vaginal swabs had been collected consecutively from raped women who were examined in gynecologic emergency ward Sanglah Public Hospital. The vaginal swabs were tested with PSA rapid test and extracted for SRY gene analysis as a gold standard to confirm male genetic material. The result of this study shows that PSA rapid test diagnostic values to detect male component in vaginal swabs are sensitivity 84.62%, specificity 100%, PPV 100%, NPV 60%, LR (+) 100%, LR (-) ~, and accuracy 87.5%. These values are better than spermatozoa examination. Based on this study, PSA rapid test is highly recommended to take the place of spermatozoa examination as a new standard for proving sexual intercourse in Indonesia.

Keywords: PSA – SRY – Sexual Assault

INTRODUCTION

In the world wide, the prevalence of sexual violence, particularly rape, is extremely high. In 2010, 373,476 women were sexually assaulted throughout the world while about 52% (193,124 women) of them were raped. There was a wide variety of sexual assault rate per 100,000 population among countries from 2,2 in Azerbajian to 183,0 in Sweden [1]. Moreover, in Indonesia, the National Commission on Violence against Women had documented 93,960 sexual harrashment cases over a 13-year period, from 1998 to 2011. This mean, on average, 20 women experienced sexual violence daily [2]. Furthermore, in 2012, the number of sexually assaulted women who examined in Sanglah Public Hospital was seven people per month [3].

However, the conviction rate of rape cases is poor. In UK and India, the conviction rate stand at 40% and 26% respectively [4, 5]. Meanwhile, in Indonesia, it accounts for just 49% [6]. Many rape cases lost at police stages as a result of insufficient evidences [2]. In these cases, forensic evidences.

Practically, most rape cases require evidence of semen through forensic examinations. Unfortunately, lots of forensic laboratories rely on spermatozoa examination solely to prove it. To our knowledge, this examination may give false negative results as well as low sensitivity, especially in women who has washed the vagina. Furthermore, it is vitally important to consider the impossibility of spermatozoa finding in azoospermia perpetrators. Moreover, National Family Planning Coordinating Agency has made a target to increase the percentage of male sterilization for controlling population growth in 2014 [7]. In fact, the number of men who had undergone vasectomy in Bali reached 373 people or made up 93.25% of local target in 2012 [8]. In addition, other seminal fluid examinations such as Florence, Berberio, Acid Phosphatase, and Zinc are not specific for seminal fluid and impractical [9,10].

Graves (1985) has verified that the most specific component in seminal fluid is Prostate-Specific Antigen (PSA). It is a protein secreted from prostate gland which is found only in males. Therefore, the presence of PSA in female genitalia, suggest strong evidence of sexual intercourse [10,11].

However, PSA examination on sexual assault victims had never been done before in Indonesia until Henky (2011) conducted an experiment on PSA rapid test which normally used for detecting PSA in blood qualitatively. The purpose of this research was to determine whether the rapid test could be used to detect PSA in seminal fluid. Even though the result was plausible, it could not be applied yet in real cases because this research only used ejaculate which had been taken directly from male subject. Hence, further research must be performed in actual condition to utilize the PSA rapid test as a standard to confirm sexual intercourse in sexual assault cases [12].

SRY gene is one of the molecular tests which can be used to detect male component in vaginal swabs

assuring sexual intercourse has happened. SRY gene is located on the short arm of Y-chromosome at 11.3. This gene does not have intron and fall in the size about 3.8 kb. It produces protein consisting 204 amino acids which initiates a fetus to differentiate as a male. Thus, SRY gene can only be found in men, not women [13].

This research will flesh out the previous study on PSA rapid test by comparing its result with SRY gene analysis on vaginal swabs taken from sexual assault victims in order to detect male component which leads to the confirmation of sexual intercourse sign.

MATERIAL AND METHODS

A cross sectional study was conducted in Department of Forensic Medicine, Gynecology Emergency Ward Sanglah Public Hospital Denpasar, and Department of Histology Faculty of Medicine Udayana University from October 2012 to December 2012. Vaginal swabs samples were taken consecutively from 16 sexually assaulted women who came to Sanglah Public Hospital Denpasar less than three days before the incident. Both written and verbal informed consent had been obtained from them. Furthermore, Research Ethics Committee Sanglah Hospital already gave permission to perform this study based on Ethical Clearance number: 861/UN.14.2/Litbang/2012.

Table 1. Participant Characteristics

Age(years)	25 (13–39)
≤ 18	7 (43.75%)
> 18	9 (56.25%)
Nationality	
Indonesian	15 (93.75%)
Foreigners	1 (6.25%)
Hymen	
Recent tear	2 (12.50%)
Old tear	14 (87.50%)

Overall, there were four main stages in this research. First of all, obtaining two vaginal swabs from each subject. The samples were taken with cotton swabs around genitalia by twisting them for 5 – 10 times. Secondly, one of the cotton swabs was smeared on an object glass for microscopic examination to find spermatozoa. After that, thirdly, it was inserted into test tube containing 2 cc of distilled water. Then the fluid was taken from the test tube using pipette. Subsequently, two drops of samples were put on PSA rapid test device and the result was interpreted by observing the appearance of pink lines in test (T), reference (R), and control (C) area.

Finally, another cotton swab was brought to DNA laboratory for SRY gene analysis. The DNA was isolated from a vaginal swab using QIAamp DNA Blood Mini Kit (Qiagen, Hilden) according to manufacture protocol.

Table 2. Spermatozoa Examination Results in Vaginal Swabs

	SRY (+)	SRY (-)	Total
Spermatozoa (+)	3	0	3
Spermatozoa (-)	10	3	13
	13	3	16

Amplification was performed in a total volume of 25 µl, consisting 5 µl DNA template, 0.3 µM forward primer 5'-TGGCGATTAAGTCAAATTCGC-3' (First Base, Singapore), 0.3 µM reverse primer 5'-CCCCCTAGTACCCTGACAATGTATT-3' (First Base, Singapore), 0.4 mM dNTPs (Applied Biosystems), 4 mM $MgCl_2$ (Applied Biosystems), 1X PCR buffer (Applied Biosystems), 2.5 U AmpliTaq Gold® (Applied Biosystems), 1 µl AmpErase® Uracil N-Glycosylase (Applied Biosystems) and 10.4 µl distilled water. Amplification was performed in Biometra Personal Cycler with 50° C for 2 minutes, initial denaturation at 95° C for 10 minutes, denaturation at 95° C for 15 seconds, annealing at 60° C for 1 minutes, extension at 72° C for 30 seconds and final extension at 72° C for 10 minutes. The protocol was done for 40 cycles.

Amplification product was visualized using EZ-Vision® One Dye (Amresco, Solon, Ohio) in agarose 1% gel electrophoresis. Visualization was done using UV Transilluminator (UVP, Upland, CA) at 365 nm.

Table 3. PSA Rapid Test Results in Vaginal Swabs

	SRY (+)	SRY (-)	Total
PSA (+)	11	0	11
PSA (-)	2	3	5
	13	3	16

After obtaining the results of spermatozoa, PSA, and DNA examinations, these outcomes were analyzed by STATA 10. The performance of PSA rapid test device and spermatozoa examination were calculated by performing diagnostic test analysis with SRY, male determining gene, as a gold standard. Afterwards, the diagnostic value between PSA rapid test and spermatozoa examination were compared.

RESULTS AND DISCUSSION

About 43,75% of respondents is children (under 18 years of age). The youngest subject was 13 years old while the eldest was 39 years old. Most of them (87,5%) showed old hymeneal tears. One of the participants was foreigner. Details of participant characteristics are illustrated in table 1. The results of PSA rapid test and SRY gene analysis on 16 vaginal swabs are shown in figure 1 and 2. The diagnostic value of spermatozoa examination to detect seminal fluid in vaginal swabs token from sexually assaulted victims are sensitivity 23.08%, specificity 100%, PPV 100%, NPV 23.08%, LR (+) ~, LR(-) 0.77, and accuracy 37.5% as shown in table 2. Table 3 presents the performance of PSA rapid test to detect semen in

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vaginal swabs obtained from sexual assault women which are sensitivity 84.62%, specificity 100%, PPV 100%, NPV 60%, LR (+) ~, LR(-) 0.16, and accuracy 87.5%.

The results show that the specificity of spermatozoa examination and PSA rapid test has a similar level of 100%. Thus, both tests are very specific to detect semen or male component in female genitalia. Therefore, the positive result of spermatozoa examination and PSA rapid test ensures that sexual intercourse has occurred.

On the other hand, spermatozoa examination has low sensitivity (23.08%) compared with PSA rapid test PSA (84.62%). These data show that the possibility of false negative results on spermatozoa examination is considerably high, particularly on victims who have washed their genitals. Meanwhile, the probability of false negative results on PSA rapid test is smaller than spermatozoa examination since it is quite sensitive to detect low concentration of PSA [12].

On the whole, PSA rapid test has an excellent diagnostic value based on the value of likelihood ratio (+) and likelihood ratio (-) (LR(+) > 10 and LR(-) < 0.2). In contrast, the LR(-) of spermatozoa examination is 0.77. Therefore, if spermatozoa examination in vaginal swab shows negative result, it cannot be concluded that there is no sign of sexual intercourse.

Furthermore, PSA rapid test is very accurate. This study demonstrates that it has 87.5% accuracy to detect male component. Similar result is shown by Chomon, et al which discover the accuracy of PSA rapid test reaches 81% [14]. Hence, the PSA rapid test is recommended to replace spermatozoa examination which only have accuracy rate for just 37.5%.

One of the weaknesses in this study that should be considered is the precision of subjects' selection who have sexual intercourse less than three days. These data are obtained from history taking which is very subjective. This is a privacy question, so no one can guarantee the reliability of victims' answer. Unfortunately, the timing of intercourse is one of critical variables which must be controlled in this research because PSA will disappear around 3 days after sexual act, while SRY still can be discovered from 7 days [15] to 15 days post coitus [16]. When the subjects have intercourse more than 3 days after the examination, the accuracy of PSA rapid test will decrease.

Moreover, there are several conditions which might reduce the diagnostic value of PSA rapid test. For examples, in some cases, sexual intercourse is not followed by ejaculation. Another example is when the perpetrators only inserting their fingers into victims' vagina. It means a contact between male body part and female genital has already happened. These conditions will leave the traces of male leucocytes and epithelial in female genital which can be detected by DNA analysis. As a consequence, SRY gene shows positive result, while PSA rapid test shows negative result because PSA can only be found in ejaculat [13].

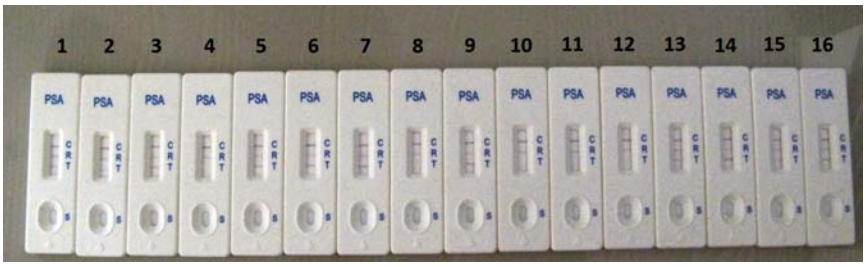

Figure 1. PSA Rapid Test Results

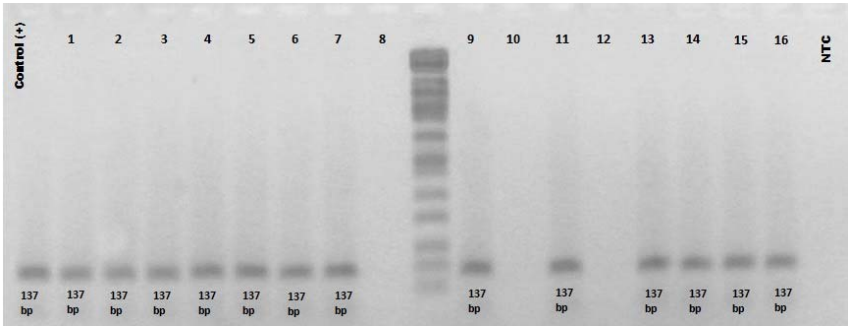

Figure 2. SRY Gene Analysis Results

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Even though SRY gene is believed only found in men, few studies in persisting fetal microchimerism should be considered by forensic practitioners. Bianchi, et al prove that male fetal cells migrate to the mother's body during pregnancy. They can last out in her bloodstream until 27 years after giving birth [17]. Thus, Y-chromosome DNA analysis might also produce false positive results, especially in women who have delivered a baby boy in their life, as there is a SRY gene in their blood and secretions. However, Klintschar, et al demonstrate that persisting fetal microchimerism will not interfere the Y-chromosome DNA analysis if using nested primers and up to 60 PCR cycles (high sensitivity PCR protocols) [18].

CONCLUSION

To sum up, PSA rapid test can be used as forensic evidence in sexual assault cases. According to the specificity and LR (-) value, the positive result of PSA test confirms intercourse sign. However, the sensitivity value is not enough to achieve the percentage of "beyond reasonable doubt" proofing in criminal court which must be above 90%. Therefore, negative result does not rule out the absence of sexual intercourse.

Similar research is highly recommended to be conducted throughout Indonesia to obtain more samples which lead to representativeness of Indonesian population. In addition, post-coital time variable must be controlled. Furthermore, using Y-chromosome DNA analysis with high sensitivity PCR protocol is advisable to eliminate the phenomenon of persisting fetal microchimerism.

Overall, PSA the rapid test is strongly recommended to replace spermatozoa examination as a new standard for evincing medical proof from sexual violence victims in Indonesia.

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