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RESEARCH PAPER

Forensic significance of dried seminal and vaginal fluid, biochemical parameters in the corroboration of the occurrence of rape

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ABSTRACT

***Background and aims**: Rape as a crime is on the increase despite various punitive measures put in place by governments. The major handicaps to the containment of rape are due to societal discriminatory outlooks, poor investigative tools, and expensive empirical evidential biomarkers. Evolving cheap and measurable biochemical biomarkers to bridge these gaps is pertinent. **Method**: Fifty subjects (males and females) were recruited for the study. The subjects voluntarily had unprotected sex with their respective partners. The under wares and pants were collected, seminal and vaginal fluids stained sections were excised and eluted for biochemical analysis. The subjects were divided into four groups: Group A: Absence of sexual intercourse- Female (ASIF), Group B: Occurrence of sexual intercourse- Female (OSIF), Group C- Occurrence of sexual intercourse, bathed and washed- Female (OSIBWF), Group D- Occurrence of sexual intercourse, bathed and not washed- Female (OSIBNWF). The above categorizations were replicated for the male counterparts designed as ASIM, OSIM, OSIBWM, and OSIBNWM, respectively. The eluted fluids extracted from both dried seminal and vaginal fluids were estimated for prostate-specific antigen (PSA), acid phosphatase (ACP), gamma-glutamyl transferase (GGT), magnesium, zinc, and phosphorus concentrations. **Results**: The result revealed a significant increase ($p<0.05$) in PSA and ACP both in the seminal and vaginal fluid stained and stained-washed fabrics compared to the unstained. **Conclusion**: The findings of the study could be applied to the inclusion and exclusion of alleged culprits of rape cases. Furthermore, the use dry seminal and/or vaginal biochemistry could be used as a preliminary test before DNA fingerprinting and profiling.*

***Keywords**: Rape; seminal fluid; vaginal fluid; electrolytes; prostate antigen, acid phosphatase.*

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INTRODUCTION

The definition of rape varies from nation to nation, and the diverse views are based on individual nation's constitutions and perspectives. It is defined in many jurisdictions as sexual intercourse, or other forms of sexual penetration, of one person by another person without the consent of the victim.[1] The United Nations Office on Drugs and Crime defines it as "sexual intercourse without valid consent."

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The World Health Organization defined it in 2002 as "physically forced or otherwise coerced penetration – even if slight – of the vulva or anus, using a penis, other body parts or an object".[2] Nigeria's 1999 constitution, chapter 30, sub-section 357 captured it as "any person who has unlawful carnal knowledge of a woman or girl, without her consent, or with her consent, if the consent is obtained by force or using threats or intimidation of any kind, or by fear of harm, or utilizing false and fraudulent representation as to the nature of the act, or, in the case of a married woman, by personating her husband, is guilty of an offense which is called rape".

The term rape is sometimes used interchangeably with that of sexual assault. Rape is one of the oldest crimes and is geometrically on the increase globally. It is a serious crime that affects millions of people around the globe. According to the World Health Organization (WHO), approximately 1 in 3 women will experience sexual violence in their lifetime, with 7% of those women having experienced rape or attempted rape. The ten countries with the highest recorded rape statistics in the world are Botswana, Lesotho, Grenada, Sweden, Eswatini, South Africa, Saint Kitts and Nevis, Panama, Iceland, and Suriname.[3] This data is not entirely correct due to the absence of data from many nations due to antithetical factors. The yearly prevalence of rape in Nigeria, as reported, put the rates at 0.47, 0.96, and 0.5 for 2014, 2015, and 2016 respectively.[4]

The two major fluids spontaneously produced during sexual intercourse are seminal and vaginal fluids. These fluids are pivotal and evidential in affirming the occurrence of sexual intercourse and rape in particular. Seminal fluid, also known as semen, is a blend of cells, sperm, and a combination of inorganic and organic substances. It is a very dense and gelatinous material produced in males by seminal vesicles, prostate, and Cowper's glands.[5] An average male ejaculates approximately 3.5 ml of seminal fluid. Every millilitre can generate around 10-50 million sperm cells.[6]

In a similar vein, vaginal fluid (VF), also known as vaginal discharge, is a mixture of liquid, cells, and bacteria that lubricate and protect the vagina.[7] Vaginal fluid is a complex biological fluid consisting of water, electrolytes, low molecular weight organic compounds (glucose, amino acids, and lipids), a vast array of proteins and proteolytic enzymes arising from plasma transudate, vaginal epithelial cells and vaginal microbiota.[8-10] The vagina, uterus, and cervix produce it. The composition, amount, and quality of discharge vary between individuals and throughout the menstrual cycle and the stages of sexual reproductive development.[11]

During sexual intercourse, either consensually or non-consensually, these fluids are copiously released and could be mixed or exchanged amongst partners. A study conducted by Woolf-King et al.[12] affirmed the fact that biochemical exchange between seminal and vaginal fluids as per prostate-specific antigen (PSA) was detected in vaginal fluids.

Dried seminal and vaginal biochemical parameters are biochemical elements, compounds, metabolites, or enzymes that are inherent in the semen or vaginal discharge. Biochemical parameters such as gamma-glutamyl transferase (GGT), prostate-specific antigen (PSA), acid phosphatase (ACP), zinc, phosphorus, and magnesium are known to be present in seminal and vaginal fluids and other body fluids.[13] Similar studies have also utilized these parameters to advance the occurrence of sexual intercourse.[14-17]

A lot of rape cases are swept under the carpet due to several factors. Top of the list are societal denigration, poor investigation tools, and the cost of litigation. Investigation tools such as DNA fingerprinting and/or profiling are pretty expensive. This has created difficulties in financing rape investigations and subsequent litigation. The need for a reproducible, accurate, and cheap empirical tool is apt. This forms the

bases of the use of dry seminal biochemical parameters in the inclusion and exclusion of rape.

MATERIALS AND METHODS

Study area: Federal University Otuoke was the location of choice for the study as the subjects were drawn from the student population. The university is in Otuoke, a town in the east senatorial district of Bayelsa State, Nigeria. In a similar vein, the laboratory analysis aspect of the study was conducted at the Eni-yimini Laboratories LTD, Yenezue-Gene Epie, Yenagoa, Bayelsa State.

Study population: The sample size was determined using Araoye's method.[18] Fifty subjects were recruited for the study and divided into equal numbers of males and females.

Ethical approval: The Directorate of Research and Quality Assurance of Federal University Otuoke approved the ethical clearance. Aside from the ethical clearance, consents were sorted and obtained individually from the subjects before recruitment into the study group. This was done voluntarily without any iota of compulsion or enticement.

Selection criteria: All the subjects used for the study were confirmed healthy and void of any chronic diseases associated with the reproductive system. The University Medical Centre health team carried out the medical and laboratory screening. Any subject that failed the laydown criteria for the research was excluded from the study, and those who were confirmed suitable were recruited into the study. The age brackets of the subjects were pegged at 18 to 30 years old due to the student age grade and population.

Collection of samples: The study population was divided into four groups consisting of fifty healthy female and male students. Fifty females' and males' pants and underwear were purchased from Tombia market, Bayelsa State. The underwear and pants were distributed to the subjects. The study was grouped into four groups;

Group A: Absence of sexual intercourse-Female **(ASIF)**

Group B: Occurrence of sexual intercourse- Female **(OSIF)**

Group C: Occurrence of sexual intercourse, bathed and washed- Female **(OSIBWF)**

Group D: Occurrence of sexual intercourse, bathed and not washed- Female **(OSIBNWF)**

Sample collection and preparation: The area of the pant or underwear stained with vagina fluid or seminal fluid was marked with water-insoluble inks and then cut off from the pant or underwear. The cut-off parts were soaked in distilled water inside a beaker. The top layer of the water was decanted, and the sediments were transferred into plain bottles, spun, and the supernatant decanted again. The sediments were used for laboratory analysis.

Laboratory analysis: Prostate-specific antigen (PSA) was estimated using the ELISA method (Accubind kit-). Acid phosphatase (Randox kit) and gamma-glutamyl transferase (Randox kit) were assayed on CONTEC SEMI-AUTO Analyzer (China). Similarly, inorganic phosphate, magnesium, and zinc concentrations were estimated spectrophotometrically (Agappe Diagnostics, Switzerland).

Statistical analysis: Data were analyzed using the statistical package for social science (SPSS) program version 22 (SPSS Inc., Chicago, IL, USA; Version 18-12). One-way ANOVA (Pos hoc-LSD) was the chosen statistical tool. The level of significance of the study was fixed at ≤0.05.

RESULTS

Table 1 shows a statistically significant increase ($p<0.05$) in vaginal PSA concentration in the experimental group compared to the controls.

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Table 1 Comparison of mean concentrations of selected vaginal fluid enzymes and antigen

Parameters	Group A	Group B	GroupC	GroupD	F-value	P-value
GGT(U/L)	0.0000±0.00	0.0040±0.008	0.0030±0.008	0.0170±0.014	1.281	0.395
ACP(U/L)	0.0030±0.08	0.0020±0.008	0.0035±0.0021	0.0360±0.0269	2.967	0.160
PSA(U/L)	0.0085±0.007	5.9815±0.069[a]	2.8065±3.331[a]	3.0660±0.8457[a]	3.797	0.115

Legends: Group A: Absence of sexual intercourse- Female **(ASIF**); **Group B:** Occurrence of sexual intercourse- Female **(OSIF); Group C-** Occurrence of sexual intercourse, bathed and washed- Female **(OSIBWF); Group D-** Occurrence of sexual intercourse, bathed and not washed- Female **(OSIBNWF);** Symbols- *a*: P < 0.05 vs Group B, C, & D, *b*: P < 0.05 vs Group C, &D; c: P < 0.05 vs Group D. Data are expressed as mean ± SD; Significant at 0.05 Confidence (p < 0.05); ±- Plus-Minus Sign.

Table 2 shows no significant difference (p>0.05) in vaginal fluid electrolyte concentrations (magnesium, zinc and inorganic phosphate) when compared among the four groups.

Table 2 Comparison of mean concentrations of selected vaginal fluid electrolytes

Parameters	Group A	Group B	Group C	Group D	F-Test	P-Value
Mg (mmol/L)	0.2590± 0.1386	0.2250± 0.0989	0.4700± 0.3338	0.2595± 0.13336	0.637	0.630
Zn (mmol/L)	57.6595±15.8385	158.5115±175.3519	119.9930±30.0478	62.8285±15.3279	0.581	0.658
Pi (mmol/L)	0.0705±0.0092	0.0565±0.0459	0.1140±0.0325	0.3400±0.4752	0.610	0.643

Legends: Group A: Absence of sexual intercourse- Female **(ASIF); Group B:** Occurrence of sexual intercourse- Female **(OSIF); Group C-** Occurrence of sexual intercourse, bathed and washed- Female **(OSIBWF); Group D-** Occurrence of sexual intercourse, bathed and not washed- Female **(OSIBNWF);** Mg- Magnesium, Zn- Zinc, P_i-Inorganic phosphate

Symbols- *a*: P < 0.05 vs Group B, C, & D, *b*: P < 0.05 vs Group C, &D; c: P < 0.05 vs Group D. Data are expressed as mean ± SD; Significant at 0.05 Confidence (p < 0.05), ±- Plus-Minus Sign.

Table 3 shows a significant increase (p<0.05) in the experimental groups' ACP and PSA seminal concentrations compared to the controls. Similarly, the concentrations of PSA and ACP in group B were significantly higher than those of groups C and D.

Table 3 Comparison of mean concentrations of seminal ACP and PSA

Parameters	Group A	Group B	Group C	Group D	F-Test	P-Value
ACP (U/L)	0.0000±0.0000	0.0560±0.0401[a]	0.0073±0.0006[a,b]	0.0093±0.0085[a,b]	0.956	0.04
PSA (U/L)	0.0033±0.0041	7.9367±10.2629[a]	41.9407±67.9971[a,b]	42.0393±67.8235[a,b]	0.633	0.03

Legends: Group A: Absence of sexual intercourse- Male **(ASIM); Group B:** Occurrence of sexual intercourse- Male **(OSIM); Group C-** Occurrence of sexual intercourse, bathed and washed- Male **(OSIBWM); Group D-** Occurrence of sexual intercourse, bathed and not washed- Male **(OSIBNWM);** ACP**:** Acid phosphatase, PSA: Prostate-specific antigen

Symbols- *a*: P < 0.05 vs Group B, C, & D, *b*: P < 0.05 vs Group C, & D; c: P < 0.05 vs Group D. Data are expressed as mean ± SD; Significant at 0.05 Confidence (p < 0.05), ±- Plus-Minus Sign
Source: Agoro & Emmanuel.[19]

Table 4 shows no statistically significant difference in the selected electrolyte concentrations among the four groups.

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Table 4 Comparison of the mean concentrations of studied seminal electrolytes

Parameters	Group A	Group B	Group C	Group D	F-value	P-value
Mg(mmol/L)	0.6863± 0.1150	0.3683±0.1564	1.7057± 1.2165	0.5320±0.3321	2.676	0.118
Zn(mmol/L)	300.7897±353.9380	576.9473±603.0632	2209.9113±2091.9064	698.9330± 509.4425	1.726	0.239
P_i(mmol/L)	0.3347± 0.2482	1.6103± 0.640	2.4467± 2.0525	0.4167±0.2555	2.565	0.127

Legends: Group A: Absence of sexual intercourse- Male **(ASIM); Group B:** Occurrence of sexual intercourse- Male **(OSIM); Group C-** Occurrence of sexual intercourse, bathed and washed- Male **(OSIBWM); Group D-** Occurrence of sexual intercourse, bathed and not washed- Male **(OSIBNWM);** Mg- Magnesium, Zn- Zinc, P_i-Inorganic phosphate, Symbols- *a*: P < 0.05 vs Group B, C, & D, *b*: P < 0.05 vs Group C, &D; c: P < 0.05 vs Group D. Data are expressed as mean ± SD; Significant at 0.05 Confidence (p < 0.05), ±- Plus-Minus Sign. **Source**: Agoro & Emmanuel.[19]

DISCUSSION

Rape is as old as the existence of a man with a lot of encumbrances in its investigation and jurisprudence. This study was therefore designed to interrogate the use of some biochemical parameters in the inclusion and/or exclusion of rape suspects in a pool of suspects.

The study revealed a statistically significant difference in prostate-specific antigen (PSA) concentration found in vagina fluid, while others were not significant (**Table 1**). Vagina fluid does not contain PSA; however, it could be transferred from seminal fluids to vagina fluids during sex.[12] Prostate-specific antigens are found in seminal fluid and produced by the prostate gland. The finding is, therefore, suggestive that PSA can be used to affirm the occurrence of sexual intercourse, which is indicative of rape. Prostate Specific Antigen has been validated as a reliable marker of semen exposure in studies of vaginal specimens in detecting an alleged occurrence of sexual intercourse and rape.[14-17] On the contrary, a study has shown detectable amounts of PSA in the breast milk and urine of females.[20] Therefore, caution should be taken when interpreting such a result singly with vaginal fluids PSA.

Furthermore, the mean comparison of the selected vaginal fluid electrolytes of the various groups showed no significant difference (**Table 2**). This depicts that the concentrations of vaginal fluid electrolytes eluted from vagina fluid-stained pants are of no importance in rape occurrence inclusion and exclusion. The difficulties associated with the use of vagina fluid electrolytes in rape discrimination could be due to the presence of electrolytes in the various matrices used for the study. Electrolytes are found in vaginal fluids, clothing, soaps, detergents, water, etc.

The permissible concentration of inorganic phosphate found in water is 1mg/L, while the average concentration of zinc is between 5-10 ppb, and that of magnesium is between 40mg\L and 50mg\L.[21] As postulated by the WHO on the concentrations of these electrolytes in water, coupled with that of detergents, the utilization of the electrolytes in rape inclusion or exclusion is still quite remote considering the combinational propensity. The need for seminal electrolyte tagging could be the only solution to the problem and should be advanced in similar studies.

Moreover, the concentration or activity of PSA and ACP were significant when compared among the studied groups, as posited in a study by Agoro and Eric (Table 3).[19] The adult male prostate gland and the urethral epithelium's surrounding cells produce a serine protease known as PSA. It is a protein only found in males and secreted by the prostate gland. The presence of PSA and/or ACP on the underwear of a male could be indicative of an occurrence of sexual intercourse, which points to a rape.

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A suspect alleged denial to rape could be corroborated if PSA and/or ACP is detected on the under ware. In such a situation, PSA/ACP could be used to determine the culprit's inclusion/exclusion before advancing to DNA sequencing/fingerprinting. This finding concurs with some recent publications.[22,23] The dilemma with this finding is factors that can affect the viability of seminal PSA and ACP upon exposure to harsh environments. Similar concerns are found in some publications.[24,25]

Comparison of the studied seminal electrolytes between the various groups exhibited non-significant differences (Table 4).[19] Irrespective of the non-significant stance, the non-detection of these seminal electrolytes on a male under wares is of futuristic advantage as identified in the discourse in **Table 2**.

The application of vaginal and seminal fluids' biochemical parameters in the affirmation of the occurrence of rape is an emerging concept that needs further interrogation and empirical validation. Vaginal and seminal PSA, and that of seminal ACP, are promising tools that could shape rape investigation, especially at the preliminary investigation stage.

CONCLUSION

This study highlighted the scientific approaches to detecting the occurrence of rape. Seminal and vaginal electrolytes, PSA, and ACP, formed the parameters of interest in interrogating the emerging concept. Summarily, the study revealed that seminal and vaginal fluid PSA and ACP could constitute a supporting tool in the inclusion or exclusion of rape suspects.

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