AJMAS
https://journal.utripoli.edu.ly/index.php/Alqalam/indexeISSN 2707-7179

Original Article

Rape: The Role of Histocytopathologist in Nigeria

Victor Ekundina, Chiamaka Ejelue, Emmanuel Omon*

Department of Medical Laboratory Science, College of Health and Medical Sciences, Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria

ARTICLE INFO

Corresponding Email. *omonea@pg.abuad.edu.ng*

Received*: 04-02-2023*
Accepted*: 17-03-2023*
Published*: 22-03-2023*

Keywords*. Rape, Sexual Assault, Histocytopathologist, Forensic science, Histochemistry*

ABSTRACT

Background and aim. *Sexual violence refers to a specific constellation of crimes including sexual harassment, sexual assault, and rape. This study was carried out to establish the role of histocytopathologist in establishing rape cases in Nigeria.* ***Methods.*** *One of the first interventions is the macroscopic analysis that consists of evaluating evidence/garments collected (from the victim, corpse, aggressor, and crime scene) to the laboratory in order to perform a search for blood, semen, hair, saliva, sweat, tissues, fibers, and other elements through meticulous and sequential observation, evaluating and establishing strategies to find biological spots. Coloscopic analysis was done using the histological stain; Toluidine blue.* ***Results.*** *Identification by microscopy (e.g., spermatozoa), comparison microscopy (e.g., hairs and fibers), serological analyses (e.g. conventional ABO grouping or species identification), and biochemical analyses (e.g. phosphoglucomutase) played a fundamental role in the investigation of crime for many years and are still used today in some circumstances. In the forensic analysis of male on male rape, microscopic examination for spermatozoa is initially undertaken, followed by DNA analysis if any body fluids are identified.* ***Conclusion.*** *The role of a histocytopathologist in rape diagnosis include detection of biological evidence using microscopic techniques, assessment of a crime scene using Alternative Light Sources, use of fluorescent contrast techniques and use of histochemistry and staining methods to detect and analyze biological evidence.*

Cite this article. *Ekundina V, Ejelue C, Omon E. Rape: The Role of Histocytopathologist in Nigeria. Alq J Med App Sci. 2023;6(1):113-126. https://doi.org/10.5281/zenodo.77612206*

INTRODUCTION

Sexual harassment ranges from degrading remarks, gestures, and jokes to indecent exposure. Sexual assault is an act in which one intentionally sexually touches another person without the person's consent, or coerces or the use of physical force to engage a person in a sexual act against their will especially when the victim cannot consent because of age, disability, or under the influence of alcohol or drugs. It is a form of sexual violence, which includes child sexual abuse, groping, rape (forced vaginal, anal, or oral penetration or a drug facilitated sexual assault), or the torture of the person in a sexual manner [1].

The definitions of rape vary by state and in response to legislative advocacy. Most statutes currently define rape as nonconsensual oral, anal, or vaginal penetration of the victim by body parts or objects using force, threats of bodily harm, or by taking advantage of a victim who is incapacitated or otherwise incapable of giving consent. Incapacitation may include mental or cognitive disability, self-induced or forced intoxication, status as minor, or any other condition defined by law that voids an individual's ability to give consent [2]. Sexual assault and rape are generally defined as felonies. During the past 30 years, states have enacted rape shield laws to protect victims and criminal and civil legal remedies to punish perpetrators. The effectiveness of these laws in accomplishing their goals is a topic of concern [2].

Biological evidence with forensic interest may be found in several cases of assault, being particularly relevant for sexually related ones. Sexual aggression constitutes a serious social and public health problem that calls for an urgent

forensic medical examination (FME), particularly in acute cases, that is, when the elapsed time between the assault and the FME is less than 72 hours, in the generality of cases [3]. In these cases, a large number of forensic areas are involved (e.g., clinical forensic medicine, genetics, and toxicology) aiming to obtain the proof and elaboration of a final forensic report [4].

From the forensic intervention perspective, despite some published protocols and guidelines, few countries have officially adopted guidelines for evidence management, namely, in acute sexual assault (ASA) cases. Even when guidelines are adopted they may vary within the same country, between different regions and different institutions. However, to standardize the FME of ASA victims and the credibility of forensic practices, which are essential during judicial proceedings, clear guidelines developed by the scientific community are required [3]. These guidelines will aid in optimizing forensic intervention and reduce unnecessary variations in the procedures, as well as improving collaboration among several entities and professionals, while enabling a well-timed and comprehensive forensic evaluation. An essential part of these guidelines should concern management of biological evidence for DNA analytical studies [5]. This study was carried out to establish the role of histocytopathologist in establishing rape cases in Nigeria.

Nigeria and Rape

In Nigeria, the criminal and the penal codes view rape as a serious offense based on how they defined the crime. Thus rape is defined under Section 357 of the Nigerian criminal code which is applicable to the southern part of Nigeria and its environs (East and West) as: "Any person who has unlawful carnal knowledge of a woman or girl without her consent, if the consent is obtained by force or by means of threats or intimidation of any kind or by fear of harm, or by means of 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 rape" [6].

In the Northern part of Nigeria, rape is defined under Section 282 of the penal code as:

1. "A man is said to commit rape who… has sexual intercourse With a woman in any of the following circumstances: (a) against Her will; (b) obtained by putting her in fear of death or of hurt; (c) With her consent when the man knows that he is not her husband And that her consent is given because she believes that he is the Man to whom she is or believes herself to be lawfully married; (d) With or without her consent when she is under fourteen years Of age or of unsound mind."
2. "Sexual intercourse by a man with his own wife is not considered as rape, if she has attained puberty" [6].

These two definitions show that in Nigeria, the conception of rape is seen as being perpetuated only by men against women and not vice versa. In other places, this is not the case because it has also been recognized that a man can also be victim to rape and it may be perpetuated by any person including a woman or a man. The definitions also never factored in non-penetrative rape such as oral sex and use of objects or other instrument aside from the organs as rape. This puts into context what could be adjudicated upon in Nigeria owing to existing laws. Rape remains a serious problem as there is no comprehensive national law for combating violence against women. Rape is a crime in Nigeria, but sentences for persons convicted of rape and sexual assault are inconsistent and often minor. According to the Violence against Persons Prohibition (VAPP) Act, currently applicable only in the Federal Capital Territory (Abuja) until adopted by the states, rape is punishable by 12 years to life imprisonment for offenders older than 14, and a maximum of 14 years' imprisonment for all others. The VAPP Act also addresses sexual violence, physical violence, psychological violence, harmful traditional practices, and socioeconomic violence [6].

Prevalence of Rape in Nigeria

The prevalence of rape ranges between 15 and 40% in sub-Saharan Africa, and between 11 and 55% in Nigeria. In Nigeria, 15% of rape victims are under the age of 12 years, 29% are age 12–17 years, 44% are under age 18 years, and 80% are under age 30 years. By gender, 88.7% of rape victims are women, the other 11.3% being men. Only one in ten victims who had been raped report the offence to the police. Recently, the Nigerian police recorded 717 rape cases between January and May, 2019 (CLEEN Foundation, 2020) [7].

The United Nations Women said a total of 11,200 rape cases, including children who were raped to death, were reported in Nigeria in 2020. In 2020, a total of 11,200 rape cases were reported; some of these included children who were raped to death. Violence against women continues to occur at an alarming rate [8]. United Nations International Children's Emergency Fund (UNICEF) reported that one in four girls and one in ten boys in Nigeria had experienced sexual violence before the age of 18. According to a survey by Positive Action for Treatment Access, over 31.4 percent of girls there said that their first sexual encounter had been rape or forced sex of some kind. According to UNICEF, six out of ten children in Nigeria experience emotional, physical or sexual abuse before the age of 18, with half experiencing physical violence [9].

Findings from a National Survey carried out in 2014 on Violence Against Children in Nigeria confirmed one in four females reported experiencing sexual violence in childhood with approximately 70% reporting more than one incident of sexual violence. In the same study, it was found that 24.8% of females' ages 18 to 24 years experienced sexual abuse prior to age 18 of which 5.0% sought help, with only 3.5% receiving any services. Data reported from the Women Against Rape International Foundation(WARIF) Centre indicates that over 1,100 girls in less than 3 years have been attended to at the WARIF Centre, of which 759 (accounts for 69%) were minors between the ages of 0-18 years old [10].

METHODS

Analyzing Cases of Rape in the Laboratory

One of the first interventions is the macroscopic analysis that consists of evaluating evidence/garments collected (from the victim, corpse, aggressor, and crime scene) the laboratory in order to perform a search for blood, semen, hair, saliva, sweat, tissues, fibers, and other elements through meticulous and sequential observation, evaluating and establishing strategies to find biological spots. When biological evidence is not visible to the naked eye, it is then necessary to use technological help: the forensic light sources with specific wavelengths for its detection [11].

In daily forensic practice, the latent spots of some biological fluids such as semen, saliva, urine, and sweat require the application of light radiation with specific wavelengths for detection by fluorescence depending on their emission properties or absorption of light; although fibers and hairs are elements that can be observed without instruments. Once identified, the biological evidence on the area—depending of surface or support of the fluid—is taken with moistened swabs with sterile water, or a portion is cut to perform a presumptive or confirmatory analysis of the evidence. In the case of trace evidence, it should be kept in its original support (textile) and analyzed ensuring sufficient evidence is left for subsequent trials [12].

Some forensic laboratories analyze semen through optic microscopes, aiming to identify the sperm cells. There is controversy regarding this procedure since a portion of the sample is separated from the original support, making it difficult to apply other analyses, even though it is important to consider it as minimal evidence for obtaining genetic profiles. On the other hand, laboratories use fluorescence microscopy for cytological preparations to apply fluorescent techniques that allow increasing the sensitivity in the detection of spermatozoa, confirming the presence of these cells in the analyzed fluids [13].

Analysis of Biological Evidence

Identification by microscopy (e.g., spermatozoa), comparison microscopy (e.g., hairs and fibers), serological analyses (e.g. conventional ABO grouping or species identification), and biochemical analyses (e.g. phosphoglucomutase) have played a fundamental role in the investigation of crime for many years and are still used today in some circumstances. However, discovery of the specificity of an individual's DNA profile has considerably enhanced the information that can be for connecting a person to an offense and linking offenses to each other [14].

Blood

Blood is the most common body fluid encountered at crime scenes. There are several presumptive tests to identify blood as well as confirmatory tests. The simplest test that crime scene investigators use to detect bloodstains that are not clearly visible is an alternate light source (ALS) such as ultraviolet light. This method is especially helpful when the stain is on a dark background [15]. A versatile light source product known as Polilight1 contains a range of wavelengths and can even reveal stains covered by paint. These light sources must be used with caution, however, since certain ultraviolet wavelength scan damage the DNA evidence in a sample [15].

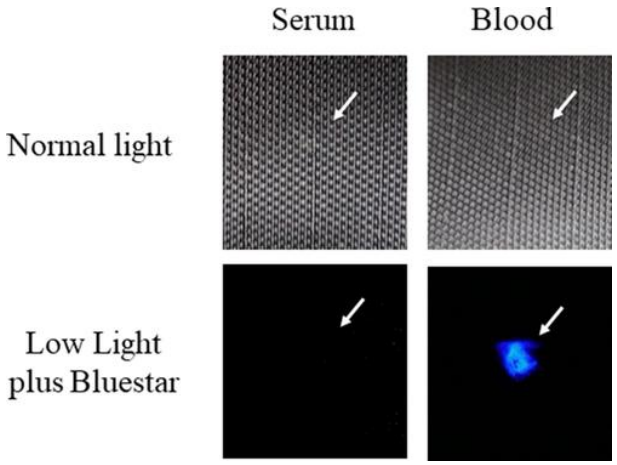

Figure 1. Detection of blood and serum using BLUESTAR®FORENSIC. Approximately 5 µL of blood or serum was added to a black nylon backpack and evaluated using normal light (untreated) or under low light following addition of BLUESTAR®FORENSIC. Arrow indicates the position of serum (left) or blood (right) [16]

The luminol test is one of the first presumptive blood tests that investigators often use at a crime scene. It is based on the ability of hemoglobin and derivatives in blood to enhance the oxidation of luminol in the presence of an alkaline solution and involves spraying a suspected area with an aqueous solution of luminol and an oxidant. It is known to be the most sensitive of the current presumptive tests being used. It can even be used on an area that has been cleaned by a suspect. One study found that a certain popular form of the luminol test known as the Grodsky formulation can have detrimental effects on subsequent DNA analysis when compared to the Weber, Weber II, and Bluestar1 alternatives. The luminol test remains popular due to the lack of false positives and false negatives in comparison with other screening tests as well as the fact that luminol is not as hazardous as other reagents. However, it is limited to use in dark environments [17].

Figure 2. ***Above left: a footwear impression in blood that has been enhanced with luminol reagent. Above right: by applying luminol to a linoleum floor, an attempt to clean up blood is apparent*** **[18].**

The simplest method of identifying blood is the use of microscope tests which involves the identification of blood cells by directly visualizing them in liquid blood. It is believed that visually identifying the red and white blood cells along with fibrin is definite proof that blood is present **[19]**

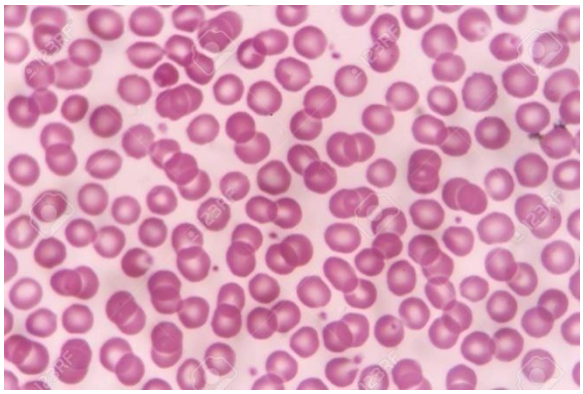

Figure 3. ***Blood Cells under the light microscope.*** **[19]**

An expansion on this method involves the use of a scanning electron microscope (SEM) which allows scientists to study the morphology of an unknown stain and to analyze its chemical composition using an energy dispersive X-ray (EDX) analyzer. Very small or dilute stains can be detected with this method [20].

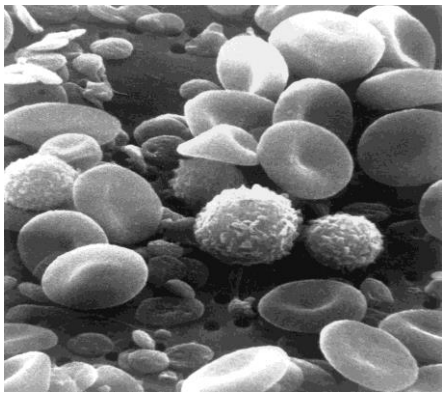

Figure 4. Blood under the electron microscope [19].

Crystal tests are the most common confirmatory tests for blood that are used, and the Teichman and Takayama crystal tests are the two most popular. The Teichman test is based on the formation of hematin by heating a dried stain in the presence of ahalide and glacial acetic acid. This test forms brown, rhombiccrystals and is very sensitive to under- and over-heating [21].

Figure 5. Teichmann Test *[21].*

The Takayama test is based on the formation of hemochromogen by heating a dried stain in the presence of pyridine and glucose under alkaline conditions, although acidic conditions have also been used. A positive result yields needle-shaped crystal, and this test is preferred due to advantages such as less heat sensitivity, ease of use, and a wider variety of stain compatibility [21]. Both crystal tests were improved upon in another experiment so that they became easier to use and more sensitive.

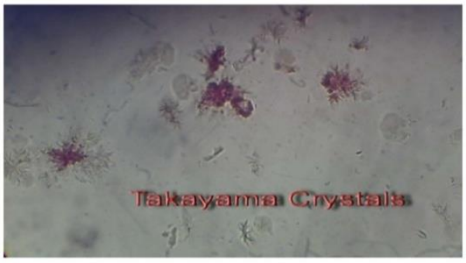

Figure 6. Takayama crystal *[21]*

Semen

Semen is one of the other most encountered body fluids at crime scenes. Like with blood; semen can also be detected using an ALS such as ultraviolet light. It is routine procedure to search a crime scene for semen and other fluids using this simple and non-destructive method [22]

Figure 7. The images below show a shirt as viewed in normal lighting (left) and a semen stain on the pocket (right), just under the logo, as seen using the ALS *[22].*

A commercial ALS, the BluemaxxTM (BM500), was tested and was 100% sensitive to semen stains. Physicians using the ALS were able to distinguish semen from other products 83% of the time after receiving training on how to use the device. Another ALS that has been used on several fluids including semen is Polilight1 which has a wavelength range of 415–650 nm as well as white and ultraviolet light. Many alternative light sources (ALS) have been developed for use in the screening of biological fluids. The excitation spectrum for semen has been determined at between approximately 300–480 nm, and the emission spectrum stretches out over 400–700 nm. Correspondingly, many procedures have been proposed that utilize a combination of specific excitation bands and colored filters, with the user wearing different colored goggles, to visualize semen stains [23].

The most popular and accepted presumptive test for the presence of semen is the test for seminal acid phosphatase (SAP). This enzyme can catalyze the hydrolysis of organic phosphates which forms a product that will react with a diazonium salt chromogen to cause a color change. This is the basic principle behind all the variations of the SAP test. One popular substrate/colour developer combination is alpha-naphthyl phosphate with Brentamine Fast Blue. Other combinations that have been successful are beta-naphthol with Fast Garnet B, and alpha-naphthol with Fast Red. There are false positives such as some plant materials and even vaginal acid phosphatase (VAP), so this technique cannot be considered confirmatory. One way to avoid a potential false positive for VAP is to observe a color change that only occurs between 5 and 30 s since VAP has never given a positive result that quickly. Other methods that have been developed to distinguish between SAP and VAP involve the separation of the two acid phosphatases using isoelectric focusing and by using acrylamide gel electrophoresis further disadvantages of SAP tests are that the enzyme can degrade when exposed to heat, mold, putrefaction, or chemicals [24].

The most reliable and widely accepted confirmatory technique for the detection of semen is the microscopic identification of sperm cells. Semen is the only body fluid which possesses sperm cells, and the large amount of DNA in the heads can be treated with a stain to make the sperm visible. The most popular stain used is the Christmas tree stain, and it is known for its characteristic colors which stain the heads red and the tails green. Other stains that are less effective than the Christmas tree stain that have been tested are Gram modified Christmas tree, hematoxylin and eosin, Papanicolaou and Wright stain respectively. The greatest disadvantage of this microscope technique is if the suspect is azoospermic due to natural causes or by vasectomy; for this reason, other chemical tests have been developed [6].

The most popular confirmatory test for semen beyond looking for sperm cells is the test for prostate-specific antigen (PSA). The semen from azoospermic males will still contain this antigen which is present in the seminal plasma; other body fluids contain a very low level of PSA and do not interfere. These low levels require that PSA tests are not too sensitive so that there are no false positives. An important aspect of detecting PSA involves the ability to detect it on contaminated or scarce samples including laundered fabrics and decomposed cadavers. The original methods involved immunoelectrophoresis or ELISA, and some specific techniques involved a dot blot immunoassay with a radio-labeled Protein as well as a dot-immunobinding method called the membrane aspiration test (MAT) [25].

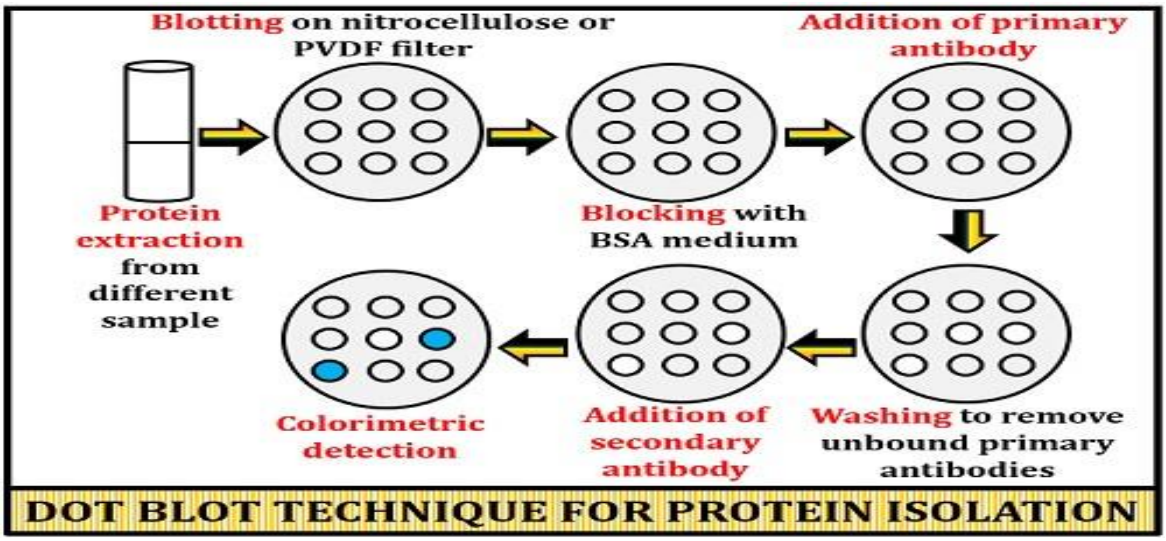

Figure 8. DOT Blot technique for protein isolation [26]

PCR Amplification using Y-Chromosomal Polymorphisms

PCR amplification uses Y-chromosomal STR polymorphisms in specimens from female victims of sexual assault with negative cytology to assay its reliability and utility. One hundred and four swabs without spermatozoa detected by cytology were collected from 79 alleged sexually assaulted female victims and amplification of Y-STR and of amelogenin were performed [27].

Swabs were rehydrated for 2 h in H_2O at room temperature, centrifuged in Forensic tubes for 10 min at 10,000 g. The cell pellet was washed once in H_2O, centrifuged as above and digested for 2 h at 56 8C in the presence of proteinase K (20 mg/0.15 ml final), DTT 4 mM final to allow digestion of sperm nuclear proteins and Chelex resin (4% final) to chelate divalent ions inhibiting the PCR. The tubes were then incubated at 100^0C for 8 min, centrifuged for 3 min and the supernatants were used for amplification. Additional controls were performed with sterile swabs (two in each extraction series) and known positive swabs at cytological examination (four to eight in each series), known negative swabs from the female staff (four in each series). A total of 12 different extractions were carried out in separate series. Amplifications were performed in a 2400 or 9700 thermocycler after an initial denaturation step of 3 min at 94^0C, for 40 cycles of 30 s at 94^0C, 30 s at 60^0C and 60 s at 72^0C, followed by a final elongation step of 7 min at 72^0C. The PCR mix was as follows: 0.25 mM of amelogenin primers, or 0.1 mM of DYS389 primers, 1IU Taq polymerase 0.2 mM of each dNTP, 1.5 mM MgCl2, 50 mMKCl and 10 mMTris–HClpH 8.3 for 25 ml reactions. The PCR products were detected on an ABI 310 instrument [27].

Overall, Y-chromosome was detected and evidenced sexual penetration in 28.8% of swabs. In the population of victims examined more than 48 h after the sexual assault, Y-STR was still evidenced in 30% of the cases. These results show that swabs should be taken from victims for Y-chromosome DNA typing even after long delays between sexual assault and medical examination. Identification of spermatozoa is the biological evidence most often sought in

specimens from rape victims. Absence of spermatozoa usually terminates biological investigations, and the victim's testimony can be contested [28].

Saliva

Like blood and semen, saliva can also be located using an ALS. Saliva stains will appear bluish-white when being viewed under an ultraviolet light, though this will not distinguish it from another body fluid stain. Saliva is also harder to detect than semen due to the lack of solid particles in the saliva sample. One study that compared two different argon laser light sources to a high intensity quartz arc tube found the quartz arc tube to be superior based on portability, cost, sensitivity, and power output. The lifetime of the quartz tubes was found to be the largest disadvantage, though they were cheaper to replace than the laser light sources [29].

The most popular and widely accepted technique to test presumptively for saliva is based on the activity of amylase. Two different forms are found in the human body. Amylase found in saliva, breast milk, and perspiration is coded by the AMY1 locus on chromosome 1, while amylase found in the pancreas, semen, and vaginal secretions is coded by the AMY2 locus. Although AMY1 is found more in saliva than any other fluid; it can still only give presumptive information since it is not exclusive to saliva. A radial diffusion assay has been used to distinguish sources of AMY1 and AMY2. The starch–iodine test is because starch appears blue when in the presence of iodine, and salivary amylase will break down the starch to cause a color change. However, competing proteins such as albumin and gamma-globulin in blood and semen will also break down iodine to form a false positive result [29].

Vaginal fluid

Although not as common at crime scenes as blood, semen, and saliva, vaginal fluid evidence can play an important role in sexual assault cases. However, there are not very many tests available to test for the presence of this fluid mainly because it is not very well defined. The constituents can change based on the menstrual cycle of the female, and this makes testing for specific components very difficult.

Based on the detection of glycogenated epithelial cells using a periodic Acid-Schiff (PAS) reagent is recommended. This reagent will stain glycogen in the cytoplasm a magenta color, and the intensity of the color is rated to determine the concentration of cells. However, since glycogenation varies based on the menstrual cycle, this test is not very reliable. Also, some females will likely show no glycogenated cells if they are pre-pubescent or postmenopausal, so this technique can easily have false negative results. False positive results can emerge from the mouth or urethral tract in males in cases of female on male rape. Finally, the test uses a large amount of sample and will destroy valuable DNA evidence therefore testing for vaginal fluid is foregone in most cases [30].

Cytopathologic Staining of Biological Evidence

Sperm cell staining method can help separate sperm cells from forensic samples. This method reduces sperm cell loss during sample separation. This method facilitates genetic identification of perpetrators in sexual assault cases [31]. Seminal evidence obtained from a sexual crime scene provides clues for solving a case through forensic analysis. However, most evidence collected from sexual crime scenes is a mixture of sperm cells, vaginal discharge and other contaminants from the crime scene. Therefore, separating the sperm cells from the seminal evidence is particularly important [32].

In a study carried out by Kim *et al.* [31], a separation method for effectively separating sperm cells using differential extraction with commercially available sperm staining reagents such as hematoxylin and nigrosin was developed. Hematoxylin (0.03% v/v) effectively stained the sperm cells in ATL and TNE lysis buffer, while nigrosin did not. The loss of sperm cells during washing of the specimen was minimized using the differential extraction method. Subsequently, genomic DNA was extracted from the hematoxylin-stained sperm cells and subjected to short tandem repeat genotyping. No interference from hematoxylin was observed. These results indicate that hematoxylin can be used to stain sperm cells and thus facilitate subsequent genetic identification [31].

Sperm detection can be an important factor in confirming sexual assault in cases of rape. Semen samples were collected from 62 healthy men of reproductive age group at the central laboratory attached to the Department of Pathology, Sri Adichuchanagiri Institute of Medical sciences. Initially, wet mount preparations of the semen samples were made and studied, followed by assessment of sperm count on improved Neubauer's chamber. Four smears were prepared from the collected samples, three of which were dried at room temperature for staining by H&E, Giemsa and Eosin-Nigrosin stains. The fourth smear was fixed in ethyl alcohol for staining by rapid Papanicolaou stain. All the smears were analyzed by four independent observers [33].

RESULTS AND DISCUSSION

Table 1: Results of the 62 subjects by four independent observers using the scoring system formulated by the research team

Stain used	Head				Acrosome				Middle Piece				Tail			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Hematoxylin & Eosin	186	184	186	185	186	186	185	185	61	62	62	61	124	123	123	123
Giemsa	186	186	185	185	124	123	124	123	62	61	61	62	62	62	61	61
Eosin-Nigrosin	123	124	124	123	124	123	124	123	15	16	15	14	14	16	15	16
Rapid Papanicolaou	186	185	186	185	185	186	186	185	124	123	123	122	124	123	123	123

Table 2: Scoring system formulated and utilized by the research team

CLARITY	SCORE
Very clear	3
Clear	2
Not clear	1
Pale/poorly stained	0

Table 3: Results of consensus of sperm morphology assessment using various staining techniques

Staining Technique	Acrosome	Head	Middle Piece	Tail
Hematoxylin& Eosin	Very clear	Very clear	Not clear	Clear
Giemsa	Very clear	Clear	Not clear	Not clear
Eosin-Nigrosin	Clear	Clear	Pale	Pale
Rapid Papanicolaou	Very clear	Very clear	Clear	Clear

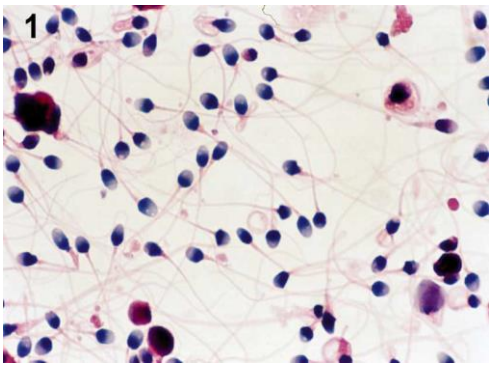

Figure 9: Spermatozoa stained by Haematoxylin& Eosin, showing very clear morphology of the head of sperms (1000X)

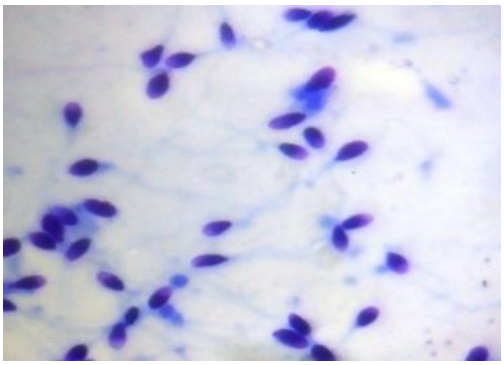

Figure 10: Spermatozoa stained by Giemsa showing very clear morphology of the head, but middle piece & tail are not visible (1000X)

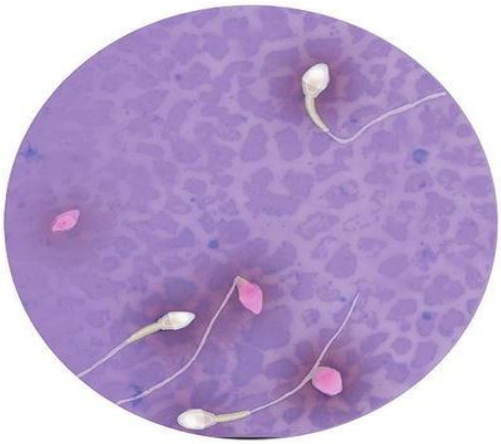

Figure 11: Spermatozoa stained by eosin-nigrosin showing clear morphology of the head and acrosome, but the middle piece and tail are barely visible (1000x)

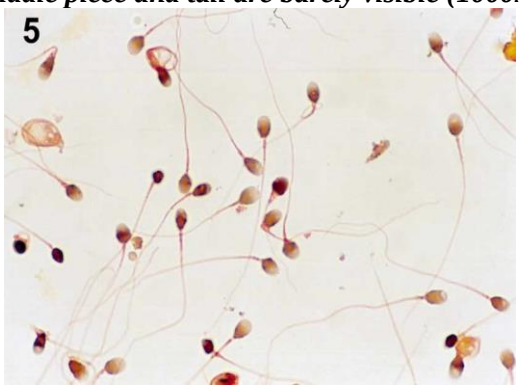

Figure 12: Rapid Papanicolaou stain showing overall clear morphology of sperms (1000X)

In a paper by Allery *et al.* [34], three of the most used staining methods cited in the scientific literature: Christmas tree, hematoxylin-eosin, and alkaline fuchsin were compared in effectiveness for sperm detection. The population studied was composed of 174 consenting women. The date of their last sexual intercourse was accurately known. Alkaline fuchsin did not seem effective in detecting spermatozoa in vaginal samples. Compared with hematoxylin-eosin, Christmas tree stain appeared to be the most useful test in the first 72 h. Two external factors were associated with decreased detection of spermatozoa: time since intercourse and sperm volume [34].

Another uncommon method for detecting vaginocervical injury in cases of sexual assault is the use of colposcopy (females) and anoscopy (males). A colposcope is a free-standing, binocular microscope on wheels that is most used for direct visualization of the cervix (using a bivalve speculum) after the detection of abnormal cervical cytology. The colposcope undoubtedly provides considerable advantages over gross visualization. First, it provides magnification (5–30 times) and greater illumination, enabling detection of more abnormalities. Colposcopy and anoscopy, where available, may have considerable value in documentation because it allows photographic recording of injuries [35]. The coloscopic analysis is done using the histological stain; Toluidine blue which stains the nuclei and has been used

on the posterior fourchette to identify lacerations of the keratinized squamous epithelium that were not apparent on gross visualization [36].

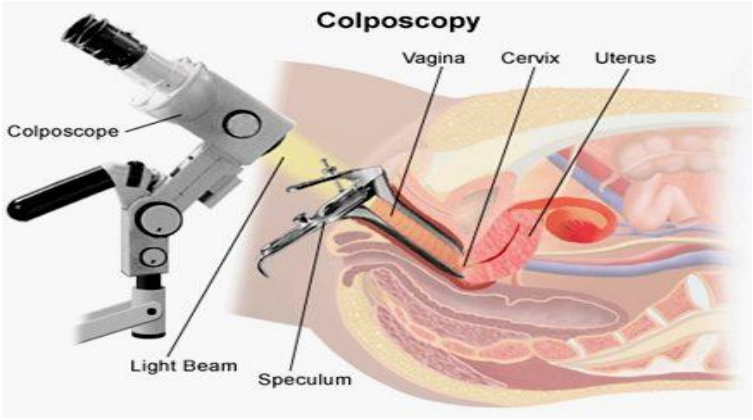

Figure 13. Colposcopy [36]

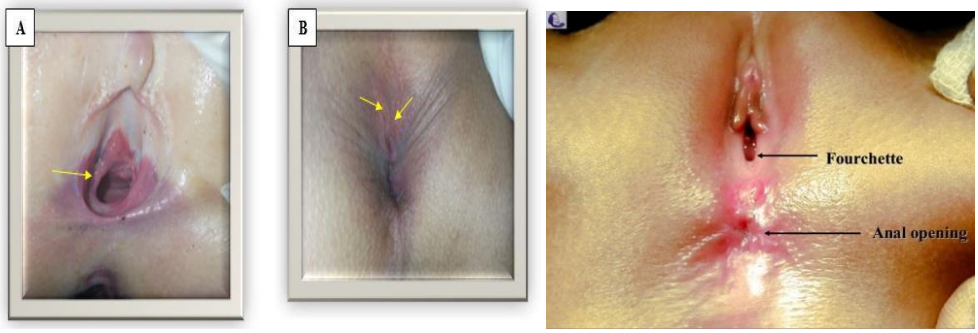

Figure 14: ***Colposcopic photographs of a 3 years female child victim of sexual assault showed: A- A trial to penetrate the hymen with bruises. B- The same case with two recent anal fissures at 12, 1 o'clock in the knee-chest position. C-colposcopic photograph of a normal 3 year female*** **[37].**

Use of toluidine blue increased the detection rate of posterior fourchette lacerations from 4 to 58% in adult (older than 19 years) complainants of non-consensual vaginal intercourse, from 4 to28% in sexually abused adolescents (11–18 years old), and from 16.5 to 33% in pediatric sexually abused patients (0–10 years old). Evidence would suggest that if speculum examination is performed before toluidine blue application to the posterior fourchette (to enhance small lesions that may occur during forceful genital penetration), the speculum itself may cause small lesions that will take up the dye. These iatrogenic lesions will be seen on colposcopy. Clinicians should consider deferring speculum examination until after external colposcopy if toluidine blue is to be used [37].

At the American College of Obstetricians and Gynecologist Forty-four women were examined for evidence of traumatic intercourse. Toluidine blue dye was employed as an objective adjunct in the evaluation because of its sensitivity for exposed superficial nuclei. Seventy percent of nulliparas and 40% of the total number of patients examined within 48 hours after complaint of sexual assault demonstrated toluidine blue-positive lacerations. Only 1 of 22 patients presenting after consenting intercourse demonstrated toluidine blue-positive lacerations. This new use of toluidine blue may prove of value in examining of the victim of sexual assault because of the dye's ability, independent of the examiner's skill, to indicate injury in collection of evidence for court proceedings [38].

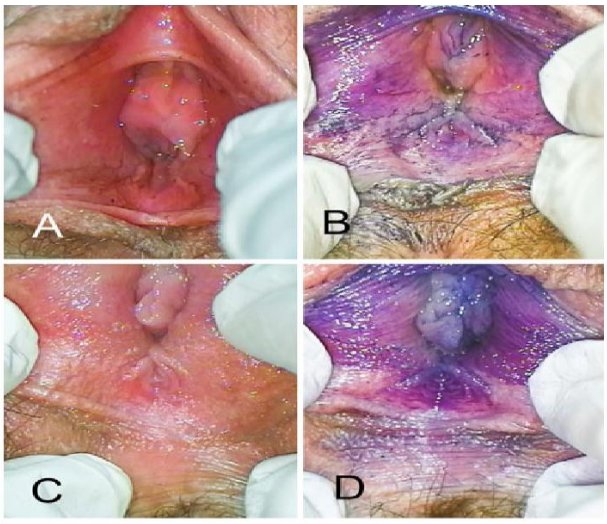

Figure 15: ***Image: Colposcopic photographs; A and B are the same woman and C and D are the same woman from a different group. Two investigators diagnosed abrasions on all four pictures and two diagnosed redness and excessive toluidine blue dye uptake due to irritation/inflammation of the mucosa, but not a lesion per se*** **[39]**

In another study by Jones *et al*.[40] to determine the incidence of toluidine blue positive findings after speculum examination of sexual assault victims, a prospective before and after study of 27 female patients presenting after sexual assault to a free-standing nurse examiner clinic was performed. Before the insertion of a speculum, a 1% aqueous solution of toluidine blue was applied to the posterior fourchette and photographs were taken using colposcopy with digital imaging. After the forensic examination was completed, dye was reapplied. Photographs taken before and after speculum examination were reviewed for superficial lacerations or abrasions. Before speculum examination, genital injuries from sexual assault were documented in 67% of the patients (mean number of genital injuries, 1.4). After speculum examination, one patient (3.7%) demonstrated a new genital injury—an abrasion to the labia [40].

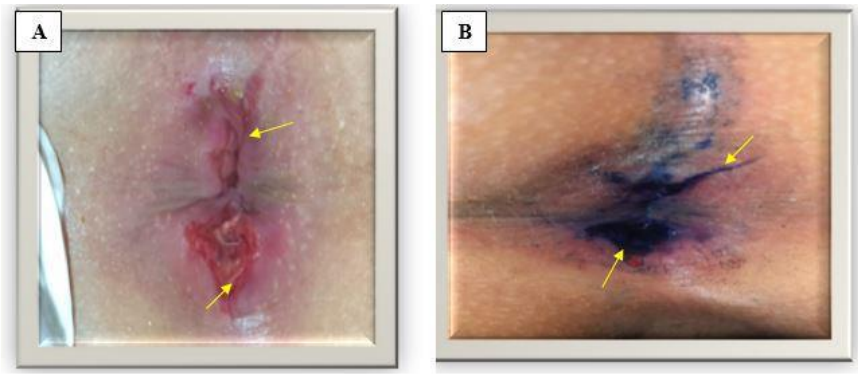

Figure 16: Colposcopic photographs of a child victim of sexual assault showed: A- Severe deep anal fissures at 1 and 6 o'clock. B- The same case after using toluidine blue dye in confirmation of injuries sites showing the absorption of injured areas of the anal mucosa **[41]**

Until further studies are performed, use of a speculum should be delayed until after toluidine dye application [40].

Limitation

We cannot state confidently how often male rape occurs in the Nigerian society because there are no statistics to back this up and as a society, this is an issue we purposely sweep under the rug. There is no research body that explicates on the issue of male rape in Nigeria and this stigma is an issue that must be corrected as a society in the 21st century.

CONCLUSION

Sexual assault is a complex crime that involves medical and psychological attention for the victim. During investigation, the identification, collecting and packing of biological fluids in the crime scene and the analysis of

evidence in labs are fundamental since errors during this stage would affect the rest of the investigation. The analysis of evidence in the laboratory continues with the macroscopic examination of biological spots. The methods used by crime laboratories are presumptive screening tests, and some of them have confirmatory tests that will conclusively identify their presence. A disadvantage of most of these current methods is that they are designed to detect a specific body fluid. Microscopic identification of sperm cells continues to be used in some forensic laboratories; its usefulness continues to be controversial due to the fact that the use of this technique in cases in which the evidence is minimal leads to the loss of such evidence besides making sperm cell identification difficult due to the lack of contrast. Fluorescent contrast techniques (FISH and immunolabeling) and LMD solve the problem of microscopic identification by allowing to separate cell mixtures from more than one contributor and producing genetic autosomal profiles free from DNA contamination. Therefore, the role of a histocytopathologist in rape diagnosis is; the detection of biological evidence using microscopic techniques, assessment of a crime scene using Alternative Light Sources, use of fluorescent contrast techniques and use of histochemistry and staining methods to detect and analyze biological evidence.

Disclaimer

The article has not been previously presented or published, and it not part of a thesis project.

Conflict of Interest

There are no financial personal, or professional conflicts of interest to declare.

REFERENCES

1. Peter C, George J, Kelly A, Anthony FTB, Mark L. Textbook of Adult Emergency Medicine E-Book. Elsevier Health Sciences. 2011, p. 658.
2. National Institute of Justice. Overview of Rape and Sexual Violence. [Aug 2019; cited 2023 Jan]. Available at: https://nij.ojp.gov/topics/articles/overview-rape-and-sexual-violence
3. Johnson D, Peterson J, Sommers I, Baskin D. Use of forensic science in investigating crimes of sexual violence: contrasting its theoretical potential with empirical realities. Violence Against Women, 2012;18(2):193–222.
4. Gomes V, Jardim P, Taveira F, Dinis-Oliveira R, Magalhaes T. Alleged biological father incest: a forensic approach. Journal of Forensic Science, 2014; 59(1): 255–259.
5. Magalhães T, Dinis-Oliveira RJ, Silva B, Corte-Real F, Nuno-Vieira D. Biological Evidence Management for DNA Analysis in Cases of Sexual Assault. Scientific World Journal, 2015; 36(5): 674-680.
6. Michael J, Toluwani B. Rape under the Nigerian laws and the need for amendment. [2019 April, cited 2023 Jan] Available at: https://legalpediaonline.com/rape-under-the-nigerian-laws/
7. Statista Research Department. Prevalence of sexual violence in Nigeria in 2018, by state. [Dec 2018, cited 2023 Jan] Available from: https://www.statista.com/statistics/1261242/prevalence-of-sexual-violence-in-nigeria-by-state/
8. Punch. 11,200 Nigerian women, children raped in 2020 – UN. [Jun 2020, cited 2023 Jan] Available at: https://punchng.com/11200-nigerian-women-children-raped-in-2020-un/
9. UNICEF. Rate of Rape in Nigeria. [Feb 2020, cited 2023 Jan] Available at: https://www.unicef.org/nigeria/child-protection
10. WARIF. Rape Statistics in Nigeria. [2020 Aug cited 2023 Jan] Available at: https://warifng.org/rape-stats-in-nigeria/
11. González B, Mercado M, Salas O, Reyes J, Ramos M, Esquivel E, Aguilar G, Torres P. Biological Evidence Analysis in Cases of Sexual Assault. In: Boldura CB and Awwad NS (Eds.), Biochemical Analysis Tools - Methods for Bio-Molecules Studies, 2020. Available at: https://doi.org/10.5772/intechopen.821
12. Sabine H, Haas C. Recovery of trace dna on clothing: A comparison of mini-tape lifting and three other forensic evidence collection techniques. Journal of Forensic Science, 2017; 62(2):187-191.
13. Westring C, Morten W, Nielsen S, Fogleman J, Old J, Lenz C. Sperm Hy-litertm for the identification of spermatozoa from sexual assault evidence. Forensic Science International, 2014; 12(1): 161-167.
14. Suyama H, Ohya I, Fukae T, Imai T. Identification of blood groups and isoenzymic phenotypes from blood stains, heart blood and nervous tissues after transfusion. Forensic Science, 2006; 8(3): 277-80.
15. Virkler K, Lednev I. Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic Science International, 2009;18(8):1-17.
16. Dilbeck L. Use of Bluestar Forensic in lieu of Luminol at Crime Scenes. Journal of Forensic Identification, 2006; 56(1): 706-729.
17. Webb J, Creamer J, Quickenden T. A comparison of the presumptive luminol test for blood with four non-chemiluminescent forensic techniques. The Journal of Biological and Chemical Luminescence, 2006; 21(1): 214-220.
18. Lincoln A. Collection and Preservation of Blood Evidence from Crime Scenes. [2019 Jan cited 2023 May] Available at: https://www.crime-scene-investigator.net/print/blood-print.html

19. Bagnell C. Darkfield Microscopy Chapt. 9 Pathology 464 – Light microscopy. [2008 Feb, cited 2023 July] Available from https://www.chem.uci.edu/~dmitryf/manuals/Fundamentals/Dark%20Field%20microscopy.pdf
20. Beane R. Using the Scanning Electron Microscope for Discovery Based Learning in Undergraduate Courses. Journal of Geoscience Education, 2004;52(3): 250-253.
21. Priyanka V, Deepika B, Harpreet K. Detection and Comparison of Normal and Menstrual Blood Samples Found At Crime Scene. Journal of Forensic Science & Criminal Investigation, 2018; 9(2):555-560
22. Lincoln C, McBride P, Turbett G, Garbin C, MacDonald E. The use of an alternative light source to detect semen in clinical forensic medical practice. Journal of Clinical Forensic Medicine, 2006;13(4): 215-218
23. Su J. Seminal stain fluorescence using three alternate light source-barrier filter combinations on six different colors of cotton fabrics. Fluorescence Journal, 2005; 5(1):145-156.
24. Ajay S, Priyanka V, Hiren V, Priyanka M. Study on Acid Phosphatase Enzyme Activity in Semen Mixed with Various Body Fluids. Journal of Forensic Science & Criminal Investigation, 2019;11(5): 555-558.
25. Sensabaugh G. Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification. Journal of Forensic Science, 1978; 23(1): 106–115.
26. Supriya N. DOT Blot technique for protein isolation. 2020. Available at: https://biologyreader.com/dot-blot-technique.html
27. Sibille I, Duverneuil C, Grandmaison G, Guerrouache K, Teissière F, Durigon M, et al. Y-STR-DNA Amplification as Biological Evidence in Sexually Assaulted Female Victims with No Cytological Detection of Spermatozoa. Forensic Science International, 2022; 12(5): 212-216.
28. Collins K, Rao P, Hayworth R, Schnell S, Tap M, Lantz PE, et al. Identification of sperm and non-sperm male cells in cervicovaginal smears using fluorescence in situ hybridization: applications in alleged sexual assault cases. Journal of Forensic Science, 1994; 39(4): 1347-1355.
29. An J, Shin K, Yang W, Lee HY. Body fluid identification in forensics. BMB Reports, 2012; 45(1): 545-553.
30. Zambrano F, Melo A, Rivera-Concha R, Schulz M, Uribe P, Fonseca-Salamanca F, et al. High Presence of NETotic Cells and Neutrophil Extracellular Traps in Vaginal Discharges of Women with Vaginitis: An Exploratory Study. Cells, 2022; 111(8): 3185-3189
31. Kim J, Kim M, Hye H, Hye L, Eun-Jung L, Yang-Han L, et al. Application of hematoxylin reagent for sperm cell separation in sexual crime evidence. Forensic Science International, 2020;30(7):110-114
32. Duenhoelter J, Stone I, Santos-Ramos R, Scott DE. Detection of Seminal fluid constituents after alleged Sexual Assault. Journal of Forensic Sciences, 1978; 23(4): 824-829
33. Lingappa H, Govindashetty A, Krishnamurthy A, Puttaveerachary A, Manchaiah S, Shimoga I, et al. Quest for an Ideal, Simple and Cost-Effective Stain for Morphological Assessment of Sperms. Journal of clinical and Diagnostic Research, 2015; 9(1):1-4.
34. Allery JP, Telmon N, Mieusset R, Blanc A, Rougé D. Cytological detection of spermatozoa: comparison of three staining methods. Journal of Forensic Science, 2001; 46(2):349-51.
35. Zink T, Fargo JD, Baker RB, Buschur C, Fisher BS, Sommers MS. Comparison of methods for identifying ano-genital injury after consensual intercourse. Journal of Emergency Medicine, 2015; 39(1):113-118.
36. Wentzensen N, Massad LS, Mayeaux EJ. Evidence-based consensus recommendations for colposcopy practice for cervical cancer prevention in the United States. Journal of Low Genital Tract Disease, 2017; 21(4):216-222.
37. Kaur S, Kaur S, Rawat B. Medico-legal evidence collection in child sexual assault cases: a forensic significance. Egyptian Journal of Forensic Sciences, 2021; 11(4):361-365
38. Krivokhat'ko A. Evaluation of extragenital injuries in female children with sexual abuse. Russian Journal of Forensic Medicine, 2020; 13 (5):254-259
39. Astrup B, Lauritsen J, Thomsen J. Colposcopic photography of genital injury following sexual intercourse in adults. Forensic Science Medical Pathology, 2013; 9(1):24–30.
40. Jeffrey J, Chris-Dunnuck L, Barbara N, Nelson-Horan C. Significance of toluidine blue positive findings after speculum examination for sexual assault. American Journal of Emergency Medicine, 2004; 22(3):201-203.
41. Myhre A, Adams J, Kaufhold M, Davis J, Suresh P, Kuelbs C. Anal findings in children with and without probable anal penetration: A retrospective study of 1115 children referred for suspected sexual abuse. Child Abuse & Neglect, 2013; 3(7):110 -114.