Journal of Oncological Sciences 3 (2017) 12–17

Contents lists available at ScienceDirect

Journal of Oncological Sciences

journal homepage: https://www.elsevier.com/locate/jons

The preponderance and dye-tissue receptive variability analyses of malignant and benign lesions of the female genitalia

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ARTICLE INFO

Article history:
Received 22 October 2016
Received in revised form
20 February 2017
Accepted 27 February 2017
Available online 14 March 2017

Keywords:
Benign
Malignant
Female genitalia
Histochemical dyes

ABSTRACT

Background: Benign and malignant lesions of the female genitalia are of great concern worldwide. The roles of dyes to aid identification of diagnosis in these two classes of lesions are of importance. The aim of this research was to determine the prevalence of malignant and benign lesions in the female genitalia and their receptivities to seven histochemical dyes.

Materials and Methods: Six hundred and thirty two (n = 632) gynaecological malignant and benign lesions data collected from the archives of the Histopathology Laboratory of Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Nigeria between 2010 and 2014 were used for this study. The representative tissues were sectioned and stained with Haematoxylin and Eosin (H&E), Masson's Trichrome (MT), Periodic Acid Schiff (PAS), Phosphotungstic Acid Haematoxylin (PTAH), Southgate Mucincamine (SGM), Alcian Blue (AB) and Verhoeff Van Gieson (VVG) dyes.

Results: We identified 601 (95.1%) benign and 31 (4.9%) malignant lesions during the 5-year period. The mean and standard deviation (±SD) of patients' age associated with the malignant and benign tissues were 47.7 ± 16.7 and 37.3 ± 11.2 years. There were significant ($p < 0.05$) associations in the distribution of lesions by age category, reproductive status, region, and origin of tissue, but not by year of diagnosis and developmental stage ($p > 0.05$). Stain analyses revealed significant variations in the receptivity of the seven dye-tissues with the mean % area for benign lesions ranging from 38.94 ± 10.60% in SGM to 64.51 ± 12.04% in MT and those of malignant lesions ranging from 37.64 ± 17.71% in AB to 63.95 ± 8.94% in MT. Similarly, intensity measurements for benign lesions ranged from 81.76 ± 13.96 points (pts) in MT to 161.39 ± 17.23 pts in AB compared to malignant lesions, which ranged from 78.04 ± 25.73 pts in MT and 167.75 ± 12.62 pts in AB.

Conclusion: Our study reported the preponderance of benign lesions than malignant lesions in the sample population. Comparatively, MT exhibited the best dye-tissue receptivity in both benign and malignant lesions than the baseline dye (H&E) and remains a valuable tool for the diagnosis of gynecological lesions.

1. Introduction

The frequent occurrence of lesions in the female genital tract[1] have been of serious concern worldwide, especially in developing countries,[2] resulting in high rate of gynecologic morbidity and mortality.[3] According to Rao[4] and Philippi et al.[5] lesions are classified into benign and malignant. Benign lesions are non-cancerous mass of cells that lacks the ability to metastasize and its growth rate is usually slow, whereas malignant lesions are cancerous, has the ability to metastasize and grow faster than benign lesions. Risk factors associated with malignant lesions include chemical carcinogens, age, life style, radiation, weak immune system, inheritance and infection.[6] Persistent Human papilloma virus (HPV) infection is also a notable risk factor for cancers.[7–9]

Histological staining is a commonly used medical process in

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Peer review under responsibility of Turkish Society of Medical Oncology.

http://dx.doi.org/10.1016/j.jons.2017.02.002

pathological diagnosis and forensic studies. There have been great changes in the techniques used for histological staining through chemical, molecular biology assays and immunological techniques, which has facilitated greatly in the study of organs and tissues.[10] At the same time, research and knowledge relating to anatomy and tissues of the human body also increased, resulting in the need to further research into new histological techniques for the study of diseased tissue.[11]

The gold standard for the study and diagnosis of both malignant and non-malignant (benign) lesions of the female genital tract are primarily based on dye-tissue interaction to aid visibility of tissue components using histochemical dyes or the more recently immunohistochemical antibodies. The major classes of interaction (bonds) are ionic, covalent, and hydrophobic.[12–14] The histochemical process enhances the binding of dyes to specific cellular organelles or extracellular features thereby revealing the different sizes of tissue structures and components. The effectiveness of a staining procedure lies in its ability to bind dye selected structures, highlighting these structures in contrast with the rest of the section.[12]

It has been established that most histopathological processes could be studied using the Haematoxylin and Eosin procedures.[15] Although this staining method is quick to execute, cheap and can be easily altered, Haematoxylin and Eosin are inefficient because not all features of a substance can be received and special stains must be used.[16] There has been a rising need for efficient, accurate and less complex staining procedures.[17] Additionally, the complexity of stains has been enhanced for the purpose of efficient and consistent staining processes that show fine and differentiated tissues.[18]

The dwindling economy of low and middle income countries, have put enormous strain on the importation of immunohistochemical antibodies used in the analyses of tissue samples, thereby causing many histopathologists to explore alternative histochemical dyes, which are by far cheaper and readily available. In modern histology, several stains have been modified and combined with other stains to improve their effectiveness. Background study on commonly used histological staining techniques and stains indicate that some fixatives and techniques used in the histological processes are effective.[10] However, some stains and processes are ineffective leading to denaturalization of tissues and cells, which inhibit effective histological studies.[10]

The aim of this study was to determine the preponderance of malignant and benign lesions in the female genitalia among patients who received medical care at the Braithwaite Memorial Specialist Hospital (BMSH) in Port Harcourt, Nigeria during a five-year period; and the receptivity of the lesions to seven histochemical dyes.

2. Materials and methods

2.1. Tissue collection

Six hundred and thirty two (632) gynecological lesion representative tissues collected between 2010 and 2014 were retrieved from the archives of the Histopathology laboratory of Braithwaite Memorial Specialist Hospital (BMSH) in Port Harcourt, Nigeria and used for the current study.

2.2. Staining methods

Following tissue processing, the representative tissue blocks were sectioned at 5 μm thickness using the rotary microtome and prepared for staining according to the method of *Suvarna* et al.[19] Seven staining methods namely: Haematoxylin and Eosin (H&E),[20] Masson's Trichrome (MT),[21] Periodic Acid Schiff (PAS),[22] Phosphotungstic Acid Haematoxylin (PTAH),[23] Southgate Mucincamine (SGM),[24] Alcian Blue (AB),[25] and Verhoeff Van Gieson (VVG)[26] were used to stain the tissue samples. The H&E served as the baseline dye for comparison of the other six dyes. Fundamentally, the procedure involved the application of these stains/dyes onto the tissue sections, which in turn through either a chemical or physical method of adsorption, absorption, solubility, osmotic pressure or capillary attraction produced visible characteristic peculiarities of shape and structure that were observed through the microscope. The type and nature of the uptake differed from one tissue to another, as well as by the techniques employed.

2.3. Microscopy and data acquisition from photomicrographs

The stained tissue slides were viewed using OMAX 40X-2000X built-in 3.0 MP digital camera compound LED Binocular Microscope. The stain intensity and percentage area stained were analyzed using ImageJ 1.48 version (National Institute of Health, USA).

2.4. Percentage (%) area and intensity measurement

Imported RGB images are converted to gray scale images on ImageJ. ImageJ analyses the % area as a measure of the portion of tissue covered by dye. The software quantifies the staining intensity by measuring the pixel value of each pixel in grayscale images following a threshold of areas of staining activity, and converting the pixel value to brightness value or gray value, in a scale of 0–255 points (pts) from the less bright (that is lower points and greater intensity) to brighter (that is higher points and reduced intensity).

2.5. Statistical analysis

Non-parametric statistical analyses of the frequency of occurrence of demographic and gynecological lesions' (benign and malignant) characteristics of study participants were carried out using the Chi-square test of independence. The measurement system analysis was performed to compare the staining methods and the associated interactions between the staining methods and lesion types. All data management and statistical analyses were conducted using the JMP statistical discovery™ software, version 12.1 (SAS Institute, Cary, NC, USA). For all tests performed, the probability value of 0.05 was used as a threshold for determining statistical significance level.

2.6. Ethical approval

The study was approved by the ethics committee of BMSH and the Hospital Management Board of Rivers State, Nigeria.

3. Results

3.1. Preponderance of malignant and benign lesions

Table 1, shows the distribution of malignant and benign lesions by gynecological and histopathological characteristics. The majority of lesions were benign (n = 601, 95.1%), with only 4.9% (n = 31) identified as malignant lesions. The overall mean (± standard deviation) age of study participants was 39.1 ± 12.8 (benign = 37.3 ± 11.2; malignant = 47.7 ± 16.7) years (Fig. 1). There were significant ($p < 0.05$) associations in the distribution of lesions by age category, reproductive status, region, and origin of tissue, but not by year of diagnosis and developmental stage ($p > 0.05$). Most of the benign lesions (n = 490, 81.1%) occurred among females

Table 1
Distribution of benign and malignant lesions by gynecological and histopathological characteristics of study participants (n = 632).

Characteristic	N (%)	Benign	Malignant	Test statistics	
		n (%)	n (%)	χ^2 Value (df)	*P*-value
Overall	632 (100)	601 (95.1)	31 (4.9)		
Year					
2010	140 (22.4)	128 (20.5)	12 (1.9)		
2011	130 (20.8)	123 (19.7)	7 (1.1)		
2012	144 (23.0)	139 (22.2)	5 (0.8)		
2013	176 (28.1)	169 (27.0)	7 (1.1)		
2014	36 (5.8)	36 (5.8)	0 (0.0)	6.85 (4)	0.144 ^{ns}
Developmental Stage					
Infant	2 (0.3)	2 (0.3)	0 (0.0)		
Teenager	5 (0.8)	5 (0.8)	0 (0.0)		
Adult	597 (98.8)	566 (93.7)	31 (5.1)	0.38(2)	0.826 ^{ns}
Age Category (Years)					
0–19	12 (2.0)	12 (2.0)	0 (0.0)		
20–29	120 (19.9)	116 (19.2)	4 (0.7)		
30–39	248 (41.1)	241 (39.9)	7 (1.2)		
40–49	141 (23.3)	133 (22.0)	8 (1.3)		
50–59	35 (5.8)	35 (5.8)	0 (0.0)		
60–69	35.(5.8)	27 (4.5)	8 (1.3)		
70+	13 (2.2)	9 (1.5)	4 (0.7)	46.27 (6)	<0.0001****
Mean ± SD	39.1 ± 12.8	37.3 ± 11.2	47.7 ± 16.7		
Reproductive Status					
Menopausal	115 (19.0)	103 (17.1)	12 (2.0)		
Premenarchal	3 (0.5)	3 (0.5)	0 (0.0)		
Postmenarchal	486 (80.5)	467 (77.3)	19 (3.2)	8.30 (2)	0.016*
Region					
Cervix	24 (3.8)	11 (1.7)	13 (2.1)		
Endometrium	517 (81.8)	506 (80.1)	11 (1.7)		
Fallopian Tube	2 (0.3)	2 (0.3)	0 (0.0)		
Ovary	79 (12.5)	74 (11.7)	5 (0.8)		
Vulva	10 (1.6)	8 (1.3)	2 (0.3)	138.74 (4)	<0.0001****
Origin of Tissue					
Blood Vessels	2 (0.3)	2 (0.3)	0 (0.0)		
Connective Tissue	1 (0.2)	1 (0.2)	0 (0.0)		
Epithelial Tissue	102 (16.1)	75 (11.9)	27 (4.3)		
Muscle	456 (72.2)	453 (71.7)	3 (0.5)		
Sex Cord/Stroma	71 (11.2)	70 (11.1)	1 (0.2)	121.35 (4)	<0.0001****

Note: The percentages may not add up to 100 due to rounding; SD=Standard Deviation.
Significance Level: * = $p < 0.05$; **** = $p < 0.0001$; ns = Not significant ($p > 0.05$).

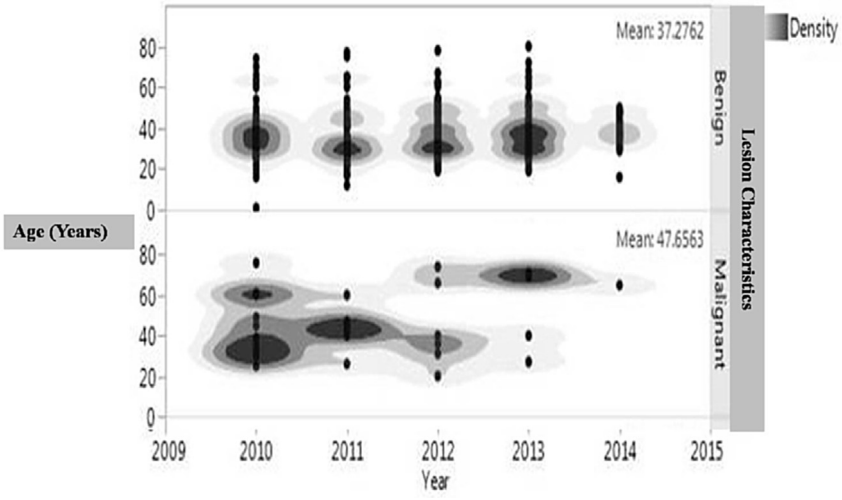

Fig. 1. Density distribution of lesion classification by patients age and year of diagnosis.

who were aged between 20 and 49 years old compared to malignant lesions that were majorly reported among females who were aged 30 and above years old (n = 27, 4.5%).

The endometrial region was reported as the location where

most of the benign lesions were found (n = 506, 80.1%), followed by the ovary (n = 74, 11.7%). In contrast, the malignant lesions were most common in the cervix (n = 13,2.1%), followed by endometrium (n = 11, 1.7%). The fallopian tube and vulva were the least affected organs by both benign and malignant lesions. Muscle was the most tissue origin for benign lesions (n = 453, 71.7%) while epithelial tissue (n = 27, 4.3%) was the origin of most malignant lesions.

Figs. 2 and 3 show the distribution of types of benign and malignant lesions identified in our study population. Among benign lesion, leiomyoma (n = 391, 65%) was the most occurring tumor, followed by ovarian cyst (n = 72, 12%) and product of conception (benign variant) (n = 54, 9%). Squamous cell carcinoma (n = 13, 42%) was the most occurring malignant lesion reported in our study population followed by adenocarcinoma (n = 9, 29%), (Fig. 3).

3.2. Variability of histochemical dyes on malignant and benign lesions

3.2.1. Percent (%) area

Fig. 4 presents the variability chart for % area of benign and malignant lesions by the seven stain methods with an overall mean of 47.88 ± 15.25%. Benign and malignant tissues stained with MT had the most coverage with a mean area of 64.38 ± 11.05% followed by PTAH (54.18 ± 11.20%) and VVG (53.65 ± 11.66%). However, tissues stained with H&E (42.64 ± 11.06%), AB (41.26 ± 11.63%), PAS (40.72 ± 17.32%) and SGM (38.30 ± 9.38%) were generally lower than the overall mean % area of the seven dyes used in the present study.

Using the % area of malignant and benign lesions in H&E (42.70 ± 18.85% versus (vs.) 42.62 ± 9.17%) as a baseline, the % area of malignant and benign lesions in MT (63.95 ± 8.94% vs. 64.51 ± 12.04%), PTAH (44.20 ± 5.75 vs. 57.17 ± 10.81%) and VVG (49.759 ± 8.65% vs. 54.82 ± 12.57%) were higher. Malignant lesions in PAS (43.12 ± 33.99%) and benign lesions in AB (42.62 ± 9.17%) were within the same range with the baseline (H&E). On the other hand, malignant and benign in SGM (36.19 ± 3.42% vs. 38.9421 ± 10.60%), benign in PAS (40.00 ± 12.47%) and malignant lesions in AB (37.64 ± 17.71%) were generally lower than the baseline values.

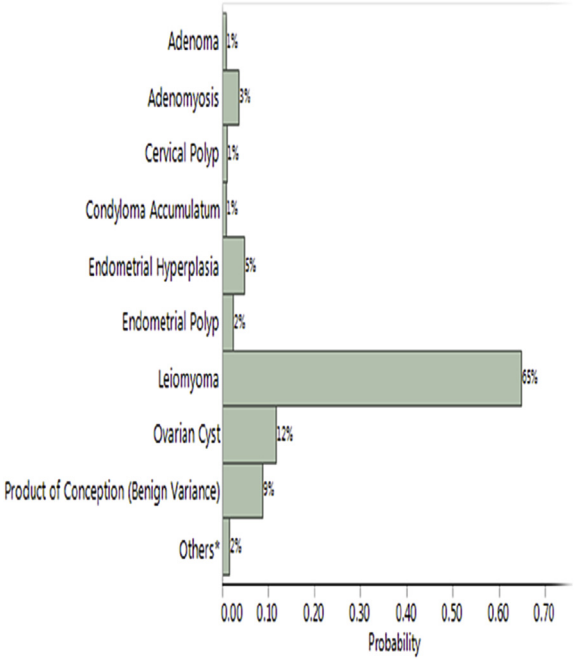

Fig. 2. Distribution of types of benign lesion identified in the study population.

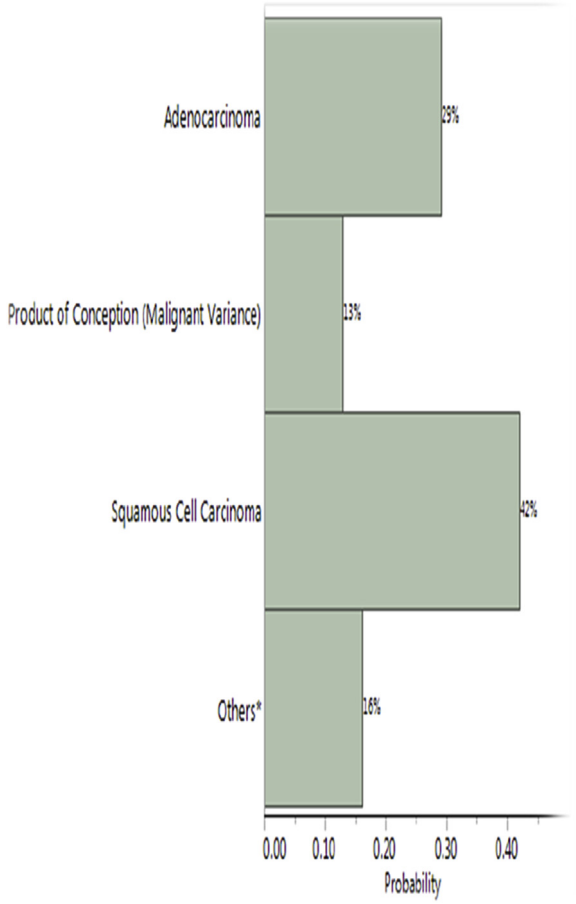

Fig. 3. Distribution of types of malignant lesion identified in the study population.

3.2.2. Intensity measurement

Fig. 5 shows the variability chart for intensity measurement of benign and malignant lesions by the seven stain methods used. The overall mean intensity measurement for the seven different dyes was 122.23 ± 34.09 pts. Malignant and benign lesions in H&E (122.11 ± 21.23 pts) recorded the same intensity as the general mean intensity measurement. Tissue samples stained with MT (80.90 ± 16.10 pts) and PTAH (87.27 ± 16.47 pts) were the most intense, while AB (162.85 ± 16.03 pts), PAS (144.68 ± 33.35), SGM (131.16 ± 14.90 pts) and VVG (126.62 ± 23.06 pts) were generally less intense, being slightly above the overall mean intensity measurement.

Using the intensity measurement of malignant and benign lesions in H&E (103.92 ± 10.34 pts vs. 127.57 ± 20.83 pts) as a baseline, the intensity measurement of malignant and benign lesion tissues in MT (78.04 ± 25.73 pts vs. 81.76 ± 13.96 pts) and PTAH (92.30 ± 25.29 pts vs. 85.76 ± 14.44 pts) stained more intensely than the baseline values. However, the intensity measurement of malignant and benign lesions in AB (167.75 ± 12.62 pts vs. 161.39 ± 17.23 pts), PAS (114.10 ± 39.42 vs. 153.85 ± 27.07 pts), SGM (131.66 ± 17.54 pts vs. 131.00 ± 15.08 pts) and VVG (111.53 ± 31.62 pts vs. 131.15 ± 19.71 pts) were less intense when compared to the baseline values.

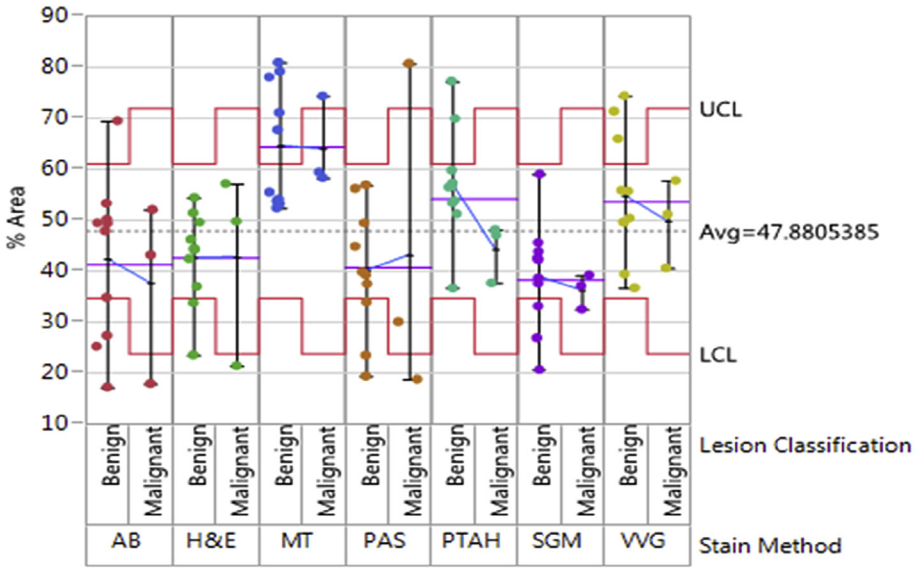

Fig. 4. Variability chart for % area measurement of benign and malignant lesions by stain methods.

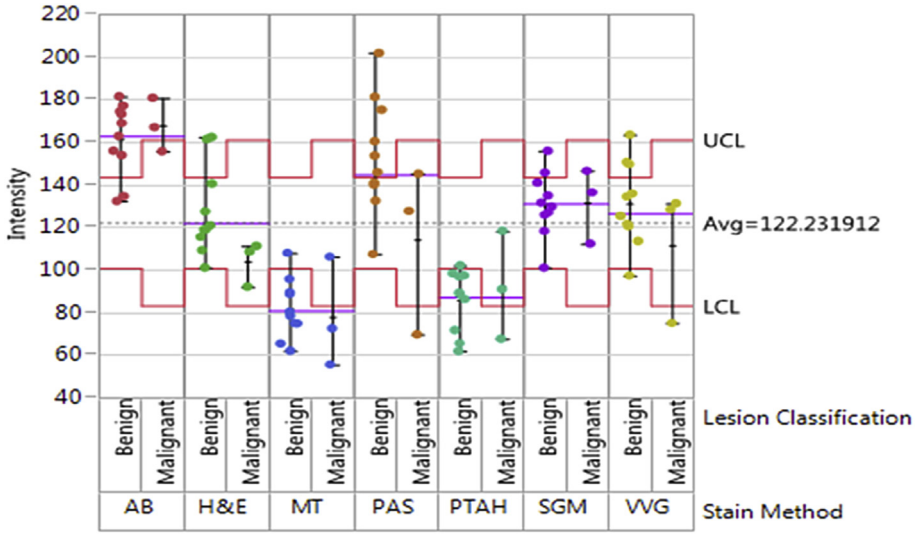

Fig. 5. Variability chart for intensity measurementof benign and malignant lesions by stain methods.

4. Discussion

Our study recorded a high preponderance of benign lesions (95.1%) than malignant lesions (4.9%) among women who received medical care at BMSH. Findings from our study agree with those of Shoail,[27] and Ajani et al.,[28] who reported that benign lesions are far more common than malignant lesions. In Enugu, Nigeria, Ozumba et al.[29] reported that benign lesions were 82.6% as against 17.4% for malignant lesions among women with average age of 38.2 years. Nwachokor and Forae[30] in Warri, Nigeria also reported that benign lesions (56.3%) were more common than malignant lesions (43.7%) in women who were 20 years or older. Although the cause of these diseases is unclear, they are thought to be linked to frequent exposure to factors such as environmental toxin, genetic influence, diet, emotional stress, local trauma and inflammation.[31,32]

We noted that benign lesions tend to occur more among females of younger age (mean = 37.3 ± 11.2 years), while malignant lesions were more common among females of older age (mean = 47.7 ± 16.7 years). Our findings are similar to that of Rahman et al.,[33] who reported a peak age range of 35–55 years and mean age 46.64 ± 10 years for malignant tumors of the cervix uteri. Wasim et al., [34] also reported a mean age of 49.07 ± 18.5 years and 36.96 ± 8.2 years for females diagnosed with malignant and benign lesions. Advancing age have been identified as one of the greatest risk factor for developing cancer with about 60% of people aged 65 years or older having cancer, and 70% of those accounting for cancer death.[35] The mechanism underlying age-related diseases is also unclear.[36] Our study identified cervical and endometrial regions as the main sites associated with malignant and benign lesions. These findings are in line with earlier research studies.[37,38]

The intensity measurement, as well as the % area of the tissue covered is dependent on the type of stain and tissue. Stain uptake is often due to dye-tissue or reagent-tissue affinities, permeability and poresize.[13] In the current study, we observed that the mean % area for both benign and malignant lesions in H&E was approximately 43% each. This validates its wide use as routine stain for

general tissue architecture in histopathology laboratories. Despite this, our study revealed that the % area covered by MT, PTAH and VVG in both benign and malignant lesions were higher than that of the H&E as well as the intensity in MT and PTAH, especially in malignant tissues. These indicate greater affinity between the tissue structures of gynaecological lesions and the three indicated dyes with MT exhibiting the strongest affinity. This finding corresponds with an earlier report by onyije et al.,[13] where they documented that squamous cell carcinoma (malignant lesion) had the most intense measurement. The British Association for the Advancement of Science similarly noted that malignant cells tend to take up stains two to three times more than the normal cells.[12] The increased intensity of malignant cells has been attributed to the metachromatic nature exhibited by the nuclei of malignant cells.[13,39] The low affinity exhibited in some of the dye-tissue interactions (AB, PAS and SGM in benign and malignant lesions) may be associated with the acid-base principle of staining or Coulombic attraction, referred to as salt links or electrostatic bonds. Van der Waals forces including intermolecular attractions, hydrogen bonding, covalent bonding, and the hydrophobic effect are among other known contributing factors.[19,40,41]

5. Conclusion

Our study recorded a high preponderance of benign lesions than malignant lesions and concludes that the cervix is the most regions of the female genitalia associated with malignant lesions, while the endometrium is more associated with benign lesions. MT proved to be the best staining method by producing greater dye-tissue receptivity for both benign and malignant tissues compared to other staining methods including the baseline dye. PTAH and VVG were considered to be the next class of staining methods that performed better in benign than malignant tissues. It is important for women especially those residing in the oil producing and exploration areas, where environmental pollutions are common to undertake annual medical checkup as this could help with early diagnosis and treatment of gynecological lesions. However, further in-depth research is needed to uncover the risk factors that may be associated with benign and malignant lesions among the women. The outcomes of such research may be effective in developing comprehensive intervention programs for residents of the oil and gas producing areas of the Niger Delta region of Nigeria.

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