Journal of Oncological Sciences xxx (2017) 1–6

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 xxx

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

Please cite this article in press as: Onyije FM, et al., The preponderance and dye-tissue receptive variability analyses of malignant and benign lesions of the female genitalia, Journal of Oncological Sciences (2017), http://dx.doi.org/10.1016/j.jons.2017.02.002