

The use of special stains at two dermatopathology laboratories in East Africa

Background: Histopathology is often essential to establish an accurate diagnosis. Pathology laboratories are scarce in most Sub-Saharan Africa where dermatopathology is a developing field. In resource-poor countries, most specimens are analyzed only after hematoxylin and eosin staining. The availability of special stains is very limited and restricted to only few centers. The aim of this study is to analyze the extent of dermatopathological cases which can be adequately diagnosed after hematoxylin and eosin alone. Secondly, to investigate which cases required further special stains.

Methods: All skin specimens submitted to two University Hospitals (Tanzania and Kenya) were included in this study. All specimens were first analyzed with hematoxylin and eosin and a diagnosis established when possible. All cases in which an accurate diagnosis after hematoxylin and eosin only was not possible, were registered and evaluated after further special stains.

Results: A total of 386 specimens were examined. A proper histopathologic diagnosis with hematoxylin and eosin alone was possible in 344 (89.1%) samples. In 45 (11.6%) cases, mostly skin infections, further special stains were necessary.

Conclusion: A proper histopathologic diagnosis was possible after hematoxylin and eosin alone in almost 90% of the specimens submitted to the two laboratories in Sub-Saharan Africa.

Keywords: dermatopathology, developing countries, laboratories, special stains, Sub-Saharan Africa

Kiprono S, Muchunu J, Beltraminelli H. The use of special stains at two dermatopathology laboratories in East Africa.

J Cutan Pathol 2016; 43: 242–245. © 2015 John Wiley & Sons A/S.
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Accepted for publication October 7, 2015

Histopathologic analysis and clinical–pathological correlation are often essential to establish an accurate diagnosis of skin diseases. Taking a skin biopsy also depends on the suspected clinical diagnosis, the experience of the clinician, the economic situation of the patient, the availability of a pathology laboratory and the availability of a pathologist/dermatopathologist.¹ Diagnostic

pathology services are not yet routinely used by most dermatologists practicing in Sub-Saharan Africa; instead, clinicians rely on visual (clinical) diagnosis alone.² In developing countries, histopathologic diagnosis is still frequently hampered by the scarce availability of histopathology laboratories, poorly developed infrastructure, poor quality of specimens and shortage of

well-trained and experienced pathologists.^{3–6} Frequent diagnostic delays and misdiagnoses are also factors influencing the decision whether a skin biopsy is necessary.

Hematoxylin and eosin staining is routinely used worldwide for examining the majority of histopathologic specimens including skin biopsies. Other histochemical stains (special stains) such as periodic acid-Schiff (PAS), Gram, Ziehl–Neelsen, mucin, and Giemsa are routinely used when particular tissue characteristics cannot be identified after hematoxylin and eosin alone. The use of special stains at histopathology laboratories in Sub-Saharan Africa is scarcely reported,⁷ probably because such stains are only available in a few centers.⁸ We offered dermatopathology services to two clinics in Kenya and Tanzania. The goal of this study was to characterize the use of special stains in dermatopathology in this resource-poor setting.

Materials and methods

Dermatopathology is an emerging field of medicine limited to a few countries in Sub-Saharan Africa^{2,8} where there are no dermatopathology fellowships at all. Trying to fill this gap we have been running a project to develop dermatopathology supported by the European Academy of Dermatology and Venereology (EADV) for the last 4 years. The aim is to develop dermatopathology in Sub-Saharan Africa by offering dermatopathology training to young and motivated African dermatologists/pathologists who are interested in dermatopathology. As a part of this project, we are interested to study the number of skin specimens that can be evaluated accurately after hematoxylin and eosin alone in routine cases from Sub-Saharan Africa. Two dermatopathologists and one pathologist are involved in the project. One dermatopathologist (KS) is working on the ground full time. Telepathology is available in Moshi and frequently used to discuss difficult cases.

A prospective observational study was done between January and December 2013. All skin biopsies received for analysis at the Regional Dermatology Training Center, Moshi, Tanzania and the County Teaching and Referral Hospital, Kakamega, Kenya were included. All samples were registered consecutively, and none was excluded. The laboratories at these hospitals received skin biopsies or excisions from dermatologists, dermato-venereology officers and general practitioners in neighboring hospitals.

Formalin 10% was used in fixing all samples. The samples were wax-embedded and routinely processed. All slides were stained with hematoxylin and eosin and evaluated by a board-certified dermatopathologist (KS) (ICDP – UEMS International Board Certification in Dermatopathology, Frankfurt, Germany) in conjunction with the clinical information mentioned on the request form. Additional special stains (PAS, Gram, Ziehl–Neelsen, colloidal iron and Giemsa) were performed if a correct diagnosis was not possible after hematoxylin and eosin alone. Fite Faraco stain was not available in this centers for diagnosis of leprosy. In some cases, deeper cuts were necessary. Other special stains, immunohistochemistry and immunofluorescence techniques were not available at these two centers. Nevertheless, all instances that required the use of further techniques for establishing a correct diagnosis were registered and sent to a reference center in Europe for evaluation.

A specific diagnosis was made when possible. If not possible, the cases were classified according to the following reaction patterns: (A) Inflammatory: inflammatory not otherwise specified (n.o.s.), spongiotic, psoriasiform, lichenoid, bullous, vasculopathic, panniculitis, granulomatous. (B) Tumors: malignant tumors, benign tumors. (C) Infections: infection n.o.s., viral, bacterial, fungal, and protozoan. (D) Other.

This study was ethically cleared by Kakamega Hospital Research and Ethics Committee and permission for research was obtained from Regional Dermatology Training Center. Statistical analysis were done with SPSS version 16 (IBM SPSS Statistics).

Results

We examined a total of 389 skin specimens. The most frequent diagnoses were squamous cell carcinoma, Kaposi sarcoma, lichen planus, and psoriasis vulgaris (Table 1). Inflammatory conditions (51.2%) and tumors (38.0%) represent the vast majority of the cases (Table 2).

A histopathologic diagnosis was possible after hematoxylin and eosin alone in 344 (88.4%) specimens. Deeper cuts were performed on 53 (13.6%) specimens. Special stains were necessary in 45 (11.6%) specimens (Table 3). The most frequent diagnoses using special stains were dermatophytosis ($n = 8$) and leprosy ($n = 4$) (Table 4). Immunohistochemistry ($n = 13$) and immunofluorescence ($n = 7$) analysis was necessary for the correct diagnosis of 20 specimens although it was not available.

Table 1. Top 11 specific histological diagnoses

Histological diagnosis	Frequency	%
Squamous cell carcinoma	26	6.7
Kaposi sarcoma	23	6.0
Lichen planus	21	5.4
Psoriasis vulgaris	15	3.9
Basal cell carcinoma	10	2.6
Bullous pemphigoid	9	2.3
Cutaneous lymphoma	9	2.3
Pemphigus vulgaris	8	2.1
Cutaneous lupus erythematosus	8	2.1
Molluscum contagiosum	8	2.1
Pityriasis rosea	8	2.1

Table 2. The distribution of all specimens (n=389) according to histological patterns

Histological pattern	Frequency	%
Inflammatory	199	51.2
Spongiotic	81	20.8
Lichenoid	33	8.5
Psoriasiform	30	7.7
Vesicobullous	26	6.7
Inflammatory n.o.s.	23	5.9
Vasculopathic	6	1.5
Tumors	148	38.0
Malignant tumors	89	22.9
Benign tumors	59	15.2
Infections	30	7.7
Viral	14	3.6
Fungal	11	2.8
Bacterial	5	1.3
Other	12	3.1

Table 3. Frequency of all stains and deeper cuts in 389 specimens

Stain	Frequency	%
Hematoxylin and eosin only	344	88.4
Periodic acid-Schiff	22	5.7
Gram	9	2.3
Mucin	7	1.8
Ziehl-Neelsen	6	1.5
Giemsa	1	0.3
Deeper cuts	53	13.6

Discussion

In this prospective study, we used skin biopsy specimens submitted to two East African tertiary institutions with developed dermatology and pathology/dermatopathology services to describe how often a histopathologic diagnosis could be obtained after hematoxylin and eosin staining alone, vs. requiring special stains to establish a diagnosis.

Table 4. Most frequent diagnoses with special stains

Disease	Frequency
Dermatopytosis (PAS)	8
Leprosy (Ziehl-Neelsen)	4
Cutaneous lupus erythematosus (Mucin)	3
Pityriasis versicolor (PAS)	3
Cutaneous tuberculosis (Ziehl-Neelsen)	2
Cutaneous histoplasmosis (PAS)	2

PAS, periodic acid-Schiff.

The majority of the histopathologic diagnoses were inflammatory conditions (51%), followed by tumors (38.3%) and infectious conditions (7.8%) (Tables 1 and 2). Similar data were published in other dermatopathological studies from Sub-Saharan Africa.^{7,9} Clinical studies from Sub-Saharan Africa reported higher skin infections (50–85%)^{10,11} suggesting that infectious skin diseases were not often biopsied.

In our study, the majority (89.1%) of the specimens were analyzed after hematoxylin and eosin alone, nevertheless in 13.7% of cases deeper cuts were necessary. The rate of deeper sections reported in the dermatopathology literature has varied from 7% to 37.3% of cases.¹² There are several reasons for the necessity of deeper cuts such as sampling error during macroscopy, small biopsy, unspecific findings, financial reasons, time constraints, and inexperienced laboratory staff or poor quality of laboratory facilities.^{1,12} In this study, as in many other laboratories in Sub-Saharan Africa, the necessity for deeper cuts was because of the poor quality of the original slides. It is frequently a direct consequence of insufficiently trained laboratory personnel and poor quality of laboratory facilities and materials. Therefore, there is need to improve the quality of training of the laboratory personnel and the quality of processing the specimens.

Considering the most frequent specific diagnoses (Table 1) and histopathologic patterns (Table 2), which shows several tumors and few infections, one can imagine that most of the diagnoses can be established after hematoxylin and eosin alone. In 11.6% of cases where special stains were necessary (Table 4), we found frequently infections (86.3%), mostly dermatophytosis, leprosy, and tuberculosis. Maingi et al.¹ reported similar findings where most of the special stains were necessary to diagnose an infection.

Immunohistochemistry and immunofluorescence would have been necessary for a correct diagnosis in 3.3% and 1.8%, respectively, of all

specimens. Most of the cases requiring immunohistochemical stains were tumors, whereas most of the cases requiring immunofluorescence analyses were inflammatory blistering disorders. We believe that these more costly techniques should be available at least in one large public (University) hospital in each country. The specimens should be transported from peripherally situated pathology units to well-equipped and staffed referral laboratories for quality processing. The current small number of cases do not justify the introduction of these expensive procedures in all pathology units.

We are conscious of the following limitations of our study: small number of samples, only two specialized centers in East Africa. This study was conducted at two tertiary institutions with developed dermatology and pathology/dermatopathology services and therefore, cannot be generalized to all health facilities in East Africa. Despite

the clinicopathologic correlation we had a high number of nonspecific reaction patterns due to inadequate clinical information in the histology request forms and no clinical pictures.

Conclusion

A correct histopathologic diagnosis was possible in the majority (up to 90%) of specimens after hematoxylin and eosin only. In about 10% of the cases, further special stains were needed, mostly PAS to establish or confirm the diagnosis of cutaneous infections. Since special stains are affordable in Sub-Saharan Africa, they should be introduced to all histopathology laboratories.

Acknowledgements

We thank Prof. Jean Bolognia for contributing to the concept of this research and Dr Bob Tank for the English review.

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