Histopathological Tissue Demonstration of Common Microorganisms in Resource-Limited Centre

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Abstract: Microorganisms are important in histopathology because vast majority of pathological basis of diseases are either direct or indirect effects of pathogens. Therefore demonstration of microorganisms in tissue sections is essential in complementing clinical findings, identification of the infecting organism, cost effectiveness and administering prompt treatment regimen where applicable. Tissue detection of microorganisms using molecular techniques is highly specific and sensitive; however, they are expensive to procure and maintain in a resource-limited laboratory mostly in the developing countries. Hence, the use of conventional light microscope along with the use of general and different special histopathological stains are essential to assist the Histopathologist in providing an opinion as to the type of microorganism causing the disease condition.

Index Terms: histopathology, tissue demonstration, resource-limited centre, microorganisms

I. INTRODUCTION

Microorganisms are living organism that can only be observed with the aid of microscopes and they are capable of causing disease.[1] They are broadly classified into bacteria, viruses, parasites and fungi.[1]

Demonstration of microorganisms in tissue sections, without the application of microbiological processes, is important in histopathology because it is relatively accurate in making a diagnosis.[1, 2] This can be achieved most especially by using the special staining techniques, examples of which include; modified Giemsa, Ziehl-Neelsen, Grocott/Gomori methenamine silver, Periodic acid Schiff.[1, 2]

The general staining technique in histopathology, Haematoxylin and eosin (H&E) stain, can demonstrate vast majority of microorganisms.[2] It can also demonstrate specific inflammatory cell response as a result of specific microorganisms present in the tissue.[2, 3] The specific inflammatory cells response of diagnostic importance in tissue samples include; increase number of eosinophils in parasitic/helminthic infections, neutrophils in acute bacterial infections, lymphocytes in viral infections, multinucleated giant cells in chronic granulomatous inflammation (Langhans cell in tuberculosis, Touton cells in xanthogranulomas).[2, 3, 4]

The special staining technique is employed, in most cases, to take the advantage of the properties of the microorganisms.[1, 5] These include poor demonstration of the microorganism by the general H&E stain, relatively small organism size (seen with *Treponema spp*), few number of organisms present in the tissue that can be visualised, high lipid content of cell wall (seen with *Mycobacteria spp*), primarily intracellular organism (seen with *Legionella spp, Chlamydia spp*) and absence of cell wall (seen with *Mycoplasma spp*).[1, 5]

II. DETECTION AND IDENTIFICATION TECHNIQUES OF MICROORGANISMS IN TISSUE

A. Bacteria

Giemsa stain (modified) is the most commonly used bacteria stain in histopathology.[6] It demonstrates *Helicobacter pylori* in gastric tissue biopsies as purple rod-like structures within the gastric glands crypts.[6] *Helicobacter pylori* is the causative agent for peptic ulcer disease and the most common type of gastric cancer.

Histopathological demonstration of bacteria in tissue using the Gram stain divides bacteria into 2 major categories; Gram Positive and Gram-negative bacteria.[7] The Gram staining method (mostly Brown-Brenn method) is characterised by the affinity of the organism's cell wall for the dye used (crystal violet-iodine complex). Gram-positive organisms stain blue-black as opposed to Gram-negative organisms that stain pink to red.[7, 8]

Atypical bacteria like *Legionella spp*, because of their intracellular nature, are best demonstrated using the Dieterle silver stain.[9] *Bartonella henselae* can also be demonstrated using Dieterle sliver stain.[1, 9] *Mycobacteria spp*, with a high lipid cell wall (hydrophobic mycolic acid) requires a acid and alcohol-fast stain which is provided by the Ziehl Neelsen procedure (which has carbolfuchsin as the principal dye) and demonstrate the bacilli as red rod-shaped organism in a background of blue or green counter stain.[10]

B. Fungi

These are found in nature in three forms; spores (e.g. *Histoplama spp*), yeast (e.g. *Candida spp, Cryptococcus spp*) and mould (e.g. *Aspergillus spp*).[11] Most fungi infections are cutaneous; hence the use of potassium hydroxide (KOH) to demonstrate dermatophytes on skin scrapings smear preparation.[11, 12] The global increase in incidence and prevalence of human immunodeficiency virus (HIV) infection has led to increase in the occurrence of systemic and tissue mycotic infections.[13] Main examples include *Pneumocystis carinii, histoplasma spp* and *Crytococcus spp.*[13]
Demonstration of fungi in tissue is can be done using Periodic acid Schiff (because of the high polysaccharides level in the fungi cell wall) and most importantly, Gomori/Grocott methenamine silver stains (polysaccharide cell wall forms aldehyde following oxidation and upon reduction, forms black coloured silver precipitates).[11,14] Giemsa stain can also be used for *Aspergillus spp* identification in tissue.[15]
The Haematoxylin & Eosin stain can also demonstrate fungi organisms in tissue because of their relatively large size.[14]

C. Viruses

The viruses are the smallest of the entire microorganisms that are demonstrated in histopathology laboratory; hence, the molecular methods are desirable.[2] However, due to high cost in resource limited centre, the viral effects on host cells and tissue, the cytopathic and cytoproliferative effects, can be demonstrated in tissue sections.[16] In some cases like measles, rabies, cytomegalovirus and herpes, there is formation of intracellular inclusion bodies.[16, 17] These inclusion bodies can either be intranuclear or intracytoplamic, or both, and they stain readily with haematoxylin and eosin stain most of the times.[12,17] In addition, various staining techniques have been developed and modified over the years to distinguish these inclusion bodies.[12,17] These methods include; Mann's methyl blue-eosin method stains intracytoplasmic Negri bodies of Rabies as red structures.[17] Lendrum's phloxine-tartrazine and Macchiavello techniques stain viral inclusion bodies (intranuclear or intracytoplasmic) as red structures within the affected cell.[12, 17] Modified orcein stain is used for the detection of hepatitis B surface antigen in affected hepatocytes which stain brown-black (due to prominent staining of the rough endoplasmic reticulum).[18]
The cytopathic effects of some viruses can be visualized on cells or tissues affected with H&E stain; example include koliocytic atypia of squamous cell in Human papilloma virus infection.[3,12]

D. Parasites / Protozoa

They can easily be visualised with the haematoxylin and eosin stain because of their relatively large size.[12] Periodic acid Schiff and Giemsa stains can also be applied to the majority of the organisms in this group to demonstrate them clearly in tissue sections.[12,19]
Ascaris lumbricoides, Trichuris trichiura, Taenia spp (larva), Schistosoma spp (egg), Onchocerca volvulos and *Strongyloides stercoralis* all stain well with either the combination of haematoxylin and eosin and PAS or using either stain individually.[18]
Dieterle silver stain can be used to stain spirochetes and Donovan bodies (Leishmaniasis).[3,12] Macchiavello techniques can also be used for detection of Rickettsia in tissue.[3,12]

III. CONCLUSION

The identification methods mentioned above are mainly to make quick, safe and complementary diagnosis in histopathology in a resource-limited centre. Where resources are abundant, the newer and more specific molecular methods are preferred.[20, 21] This include use of fluorescent stains with fluorescence microscope, immunohistochemistry, In-situ Hybridization, flow cytometry and polymerase chain reaction.[20, 21]
The microbiological method, however, remains the gold standard in differentiation of specific species of microorganism causing diseases.[1, 3, 12]

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