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The Role of Histopathology Laboratory in Post-Mortem Examination of COVID-19 Cases: Challenges and Way Forward

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Abstract

The Coronavirus Disease-2019 (COVID-19) outbreak which started about a year ago (late December, 2019) in Wuhan-China has escalated to 220 countries and territories of the world, resulting in the current pandemic with more than 70 million confirmed cases and over 1.5 million fatalities recorded so far as of December 11, 2020. The disease as we now know is a syndrome with more than 100 signs and symptoms on the Centre for Disease Control and Prevention (CDC)'s list. Considering the novelty of the disease, significant research efforts are being made globally by Histopathologists through autopsy of suspected COVID-19 cases to help understand its pathogenesis, dynamics and other clinical findings. The so many acclaimed COVID-19 deaths need to be verified to ascertain the true cause of deaths. To this end, efforts are being made to differentiate those that died from and with the disease. Unfortunately, very little has been achieved through verbal autopsy. Hence, the role of the Histopathology laboratory in the post mortem examination (autopsy) of COVID-19 cases. Post mortem involves a systematic examination of human or animal body after death with the aim of determining and establishing the cause of death, character and extent of changes produced by the disease. It has become a major game changer providing valuable information on deaths resulting from not so well understood disease conditions such as COVID-19. At the onset of the outbreak, there are no enough clinical information on COVID-19 available to clinicians and other healthcare professionals. Hence the need for autopsy to reveal post mortem changes and pattern of the disease spread in the body. This current review therefore seeks to examine the role of the Histopathology laboratory in post-mortem examination of COVID-19 cases, the associated challenges in Nigeria and the way-forward.

Keywords: COVID-19; Post-mortem; Histopathology laboratory; Challenges; Nigeria

Introduction

The Coronavirus Disease-2019 (COVID-19) outbreak which started in late December, 2019 in Wuhan-China has so far escalated to 220 countries and territories of the world resulting in the current pandemic. The World Health Organization officially declared the outbreak a pandemic, on 11 March, 2020. Barely a year later, as of December 11, 2020, more than 70 million confirmed cases and over 1.5 million deaths have been recorded in 220 countries and territories of the world [1-4].

The Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2), the etiology of COVID-19 has been considered highly infectious with an incubation period of about 14 days on the average [5,6]. Most COVID-19 patients do experience mild to moderate respiratory illness and do recover without requiring special treatment. Only about 20%, particularly the elderly and those having underlying conditions such as diabetes, chronic respiratory diseases, cardiovascular disease, cancer and other immune suppressing illnesses are at greater risk of suffering complications from the infection [7,8]. As a novel infection, significant research efforts are being made globally by scientists through autopsy of suspected COVID-19 cases to help understand the pathogenesis, dynamics and other clinical findings about this emerging viral disease [9,10].

COVID-19 as we now know is a syndrome with more than 100 signs and symptoms on the CDC's list. This warrants the need to establish the cause of death due to COVID-19. The so many acclaimed COVID-19 related deaths need to be verified to ascertain the true cause of deaths. To this end, efforts are being made to differentiate those that died from and with the disease. Unfortunately, very little has been achieved with verbal autopsy because of the obvious inherent limitations. It is said that the dead will teach the living and one sure way of such is through post mortem examination (autopsy) done in the Histopathology Laboratory. Post mortem has been defined as an

examination of human or animal body after death with the aim of determining and establishing the cause of death, character and extent of changes produced by the disease [11].

Post mortem, overtime, has become a major game changer providing valuable information on deaths resulting from not so well understood disease conditions such as COVID-19. Since the emergence of SARS-CoV-2 infection, as a novel virus, there are no enough clinical information available to clinicians, medical laboratory scientists and other stakeholder in the diagnosis and management of COVID-19. Hence the crucial role of autopsy in revealing post mortem changes and pattern of the disease spread in the body [10,12]. This current review therefore seeks to examine the role of Histopathology laboratory in post-mortem examination of COVID-19 cases, associated challenges and way-forward.

The Role of Histopathology Laboratory in Post-mortem Examination of COVID-19 Cases

Histopathology is a branch of medical science that study diseased tissues, cells and components. The histopathology laboratory therefore plays a crucial role in defining the cause of death, diagnosed lesion and diseases involving tissues and cells like cancers. The laboratory is also in charge of autopsy examination [13]. In the case of a suspected COVID-19 death, the choice of either to carry out full or limited autopsy has to be done with the aim of harvesting organs and tissues necessary to establish and confirm COVID-19 infection. However; the choice has to be made according to the individual medical history [14].

Alternatively, a staged post mortem may be of help where specific samples needed to diagnosed COVID-19 after which full autopsy is done with clarity of hazardous nature of such patients. On autopsy table, physical features of COVID-19 may likely be in the chest and could include: pericarditis, pleurisy, lungs consolidation and pulmonary edema. The weight of the lungs may be abnormal (Osborne et al., 2020). Whereas under microscopy, non-specific findings in lungs include pneumocytes hyperplasia, adenocarcinoma, focal inflammation and multinucleated giant cell formation [15]. In addition, diffuse alveolar damage characterized by thick densely eosinophilic hyaline membrane formation on the alveolar walls, type II pneumocyte hyperplasia with sloughing, and pulmonary edema have also been previously reported [16-18]. These findings are comparable to those of SARS as earlier reported by Ding et al. [14].

In the autopsy room, sufficient and well ventilation is required where autopsies for COVID-19 is to be done. [19]. Electric bone saws to be used should have vacuum that separates aerosolized particles. Every useful instrument required for the autopsy such as sample bottles should be brought in the process, this will reduce the tendencies of moving in and out of the premises [20]. On performance, personnel should be adequately trained. Sharp injuries may be reduced when blunt end equipment are deployed such as the use of round end scissors, blunt ended PM40 blades and having only one person per time on the body cavity. Organ and tissues freshly harvested should be sliced while being stabilized with a sponge on a solid surface. Personal protective equipment (PPE) is crucial and be appropriately used along face shield [12].

With regards to diagnosis, the same samples required to diagnose COVID-19 in life time is same in post mortem examination. This may include 5 ml sample in a plain bottle to be used for serology, swabs from nose and throats, sputum and bronchoalveolar lavage. Respiratory tract swabs can be sent to the histopathology, microbiology and molecular laboratories for differential and confirmatory diagnosis. Complete histological examination is of utmost importance alongside other specific investigation. It should be noted that formalin fixation inactivates the COVID-19 virus [21].

The Royal College of Pathology [22] provided a guide for histopathology laboratory that will perform autopsy on patients who died of COVID-19 diseases. This template provides sufficient guide for global community having histopathology laboratory investigating COVID-19 [23]. To conduct autopsy on a patient suspected to have died of COVID-19, risk assessment, understanding of the pathology that may be found, universal standard precaution and any standard operating procedures for specific HG3 pathology, must be well understood. Workers in histopathology laboratory performing autopsies must perform risk assessment in every post mortem examination for their own safety first and that of other healthcare professionals [22].

Routine post mortem examination includes: documentation of patient's biodata, epidemiological history, categorization of patients, personal protection, sample collection, sample testing, as well as wastes disposal amongst others.

Documentation of Patient's Biodata

Information involving the patient whose sample is sent to the laboratory must be well collected and documented. If necessary, clinicians are consulted to determine whether the sample is from a suspected case or confirmed infection [24,25].

Epidemiological History

Epidemiological History include: (1) recent travel history or residence history in high risk community or surrounding communities reporting confirmed case(s); (2) close contacts of persons with COVID-19 (with positive results using molecular diagnostic method like the Polymerase Chain Reaction-PCR); (3) exposure history to patients with fever or respiratory symptoms who came from high risk communities or surrounding communities with confirmed case(s) and (4) evidence of clustering [26].

Clinical Features

Clinical features include: (1) High temperature and/or respiratory symptoms like dry cough, running nose and loss of sense of smell; (2) In early manifestations; leukopenia and or lymphopenia may be present; and (3) X-ray of the lungs may reveal early small patchy shadows and interstitial changes, especially in the lateral lung. Ground-glass opacities and infiltrates are seen subsequently in bilateral lungs. Lung consolidation might occur in severe cases [27].

Categorization of Patients

Patients may be categorized as follows: suspected cases, confirmed cases or latent cases

Suspected Cases

A suspected COVID-19 patient must have one of the epidemiological history features and meet any two of the clinical features, or meet three clinical features and none of the epidemiological features.

Confirmed Cases

A confirmed case must have one of the following two etiological evidences:

1. Nucleic acid-positive by real-time fluorescent quantitative polymerase chain reaction testing or

2. A high homology to SARS-CoV-2, as indicated by viral gene sequencing [5].

Latent Cases

Since patients in the incubation period, as well as those that are symptom-free constitute significant sources of infection; therefore, identifying latent cases is the most critical task. People who do not meet the diagnostic criteria for suspected cases, but possess any of the following features, are recommended to undergo screening as possible latent cases:

1. Meet some of the items stated for "suspected COVID-19" but have not reached the diagnostic thresholds for suspected cases yet;

2. Have the following epidemiological history: recent travel history or residence history in high risk communities or surrounding communities reporting confirmed cases, close contacts of persons with COVID-19 (with positive PCR test results) and/or exposure history to patients coming from high risk communities or surrounding communities with confirmed cases;

3. Have the following clinical features: high temperature and/or respiratory symptoms, leukopenia or lymphopenia; and (4) radiographic features of SARS-CoV-2 infection.

Personnel Protection

SARS-CoV-2 falls into the Risk Group 4 category, just like it counterparts: SARS-CoV-1 and MERS. The pathogens in this group are deadly and offer a high risk to the laboratory worker and to the community [28,29]. A significant quantity of SARS-CoV-2 has been reported to remain in the dead body of COVID-19 patients, and may survive for a longer period in refrigerated corpses [30]. To this end, personnel protection is very critical and must be taken very seriously. In resource limited countries, where the Maximum Containment laboratory, Level 4 is lacking, it is required that any work on COVID-19 virus must be carried out in Biosafety Level 3 (BSL-3) facility or at least a BSL-2 as required by International Standard Organization [31]. BSL-3 protection requires health workers to wear a one-piece protective suit, grade-N95 protective mask, goggles or protective eye mask, protective shoes, covers and latex gloves (at least two layers). A respirator should be worn, and replaced immediately once contaminated If liquid splash is possible. The Histopathologists are expected to stand on the windward side of the room where the post-mortem examination is to be conducted to reduce the risk of potential exposure to SARS-CoV-2 [32,33].

Environmental Disinfection

Workbench and surfaces

Contaminated and semi-contaminated areas should be isolated before examination. During the examination, appropriate disinfectant (e.g those containing a 500 mg/L chlorine) can be sprayed on the floors, walls and frequently contacted surfaces. After the examination, the cut up room should be thoroughly sprayed and disinfected with a 1000 mg/L chlorine-containing disinfectant [34].

Apparatus and instrument

Before the examination, appropriate disinfectant should be used to wipe tools and instruments, for the purpose of disinfection. After the examination, a 1000 mg/L chlorine-containing disinfectant can be used for wiping and soaking for 30 min (or boiling in water for 30 min), and then clean water is used to rinse three times, followed by wiping clean for later use. Other surfaces like the computer mouse and keyboards, cameras, telephones etc. can be wiped clean with 75% ethanol solution [34].

Air

The indoor air can be sterilized using the ultraviolet ray disinfection lamps for one (1) hour. Otherwise, ultra-low capacity sprays can be used, examples include the 5000 mg/L Peroxyacetic acid, 500 mg/L chlorine dioxide disinfectant (20-30 mL/m^3) and 3% hydrogen peroxide for two (2) hours. It should be ensured that the doors and windows are closed during the air sterilization procedure, to be followed by full ventilation after the procedure is completed [32,34].

Operation Protection

Personnel working in the Histopathology Laboratory must perform careful and gentle operations, with clear sense of specialization and professionalism. Dissecting instruments must be handled skillfully to avoid any form of laboratory hazards such as puncturing or injury. If gloves are torn, they should be disinfected and replaced immediately [33,35].

Sample Collection and Testing

Prior to histopathological examination, a sample collection plan can be made by consulting with the physicians, pathologists, virologists and other important stakeholders. To reduce the risk of infection, samples should be taken immediately after the body cavity is opened, and organ and tissue cuttings should be limited to the barest minimum [25]. Samples for etiological and electron microscopy testing and cryopreservation should be extracted first. Second, samples (secretions and tissue blocks) for etiological gene testing should be stored in Hanks' solution. Third, tissues requiring freezing can be cut into blocks (≈1.5 cm in length, width and height) and put into a plastic bottle with a screw top before freezing although laboratories are advised to refrain from performing frozen sections on possible cases of COVID-19, unless the laboratory is confident in containing the associated aerosols in the cryostat. The same consideration should be applied to the grossing of partially fixed specimens [36]. Fourth, tissues for electron microscopy examination can be cut into blocks (≈0.3 cm in length, width and height) and fixed in 3% glutaraldehyde. Finally, for specimens used for conventional paraffin embedding, entire pathological organs, tissues or tissue blocks with a length, width and height of 3-5 cm can be fixed in 4% paraformaldehyde solution for 48-72 h, and can then be examined, dehydrated, embedded and sectioned.

Formalin-fixed specimen preparation

Preparation of formalin-fixed specimen for the purpose of post-mortem examination include: specimen collection, tissue dehydration, tissue embedding, tissue slicing and tissue staining.

Specimen collection

Fixed specimens can be retrieved in a well-ventilated laboratory in which surfaces and floors are sterilized with 2000 mg/L chlorine-containing disinfectant and air is disinfected by ultraviolet radiation [37].

Tissue dehydration

Once dehydrated, tissues are removed from the area, the surface of the dehydrator and surroundings are disinfected with 1000 mg/L chlorine containing disinfectant for 30 min, and then wiped with clean water.

Tissue embedding

As for tissue dehydration, the embedding machine, surroundings and air are disinfected in two steps.

Tissue slicing

Paraffin blocks are sterilized by immersion in 75% ethanol solution and dried before slicing [30,33], and then sealed immediately and sterilized using 75% ethanol solution after slicing. The slicer is sprayed with 75% ethanol solution as well. Other tools such as forceps and scalpel and knife blades can be disinfected in a hot-air oven at 80° C for 30 min.

Tissue staining

Instruments should be sprayed for disinfection with 75% ethanol solution or a 500 mg/L chlorine-containing disinfectant both before and after use.

Wastes disposal

Generation of wastes should be avoided as much as possible. Infectious effluent (i.e, the waste water produced during examination, should be treated by chemical or physical disinfection and discharged after complete inactivation. Solid wastes, including consumables, personal protective equipment and any remaining fixed specimens should be collected separately for processing. Consumables and personal protective equipment should be sterilized using high-pressure steam or fumigated using ethylene oxide in a timely manner before discarding them [28,33].

Required precautions

If a body with suspected or confirmed COVID-19 is selected for autopsy, the Histopathology Laboratory must ensure that safety measures are in place to protect those performing the autopsy. Autopsies must be performed in an adequately ventilated room, i.e., at least natural ventilation with at least 160 L/s/patient air flow and at least 12 air changes per hour (ACH). Only a minimum number of laboratory staff should be involved in the autopsy procedure. Histopathologists are advised to observe standard laboratory precautions when conducting autopsy on a dead body suspected or confirmed to have died of COVID-19. Appropriate Personal Protective Equipment (PPE) must be available, including a scrub suit, long sleeved fluid-resistant gown, gloves (either two pairs or one pair autopsy gloves), and face shield (preferably) or goggles, and boots. A particulate respirator (N95 mask) should be worn in the case of aerosol generating procedures [28,30].

If a person died during the infectious period of COVID-19, the lungs and other organs may still contain live virus [30], and additional respiratory protection is needed during aerosol-generating procedures (e.g. procedures that generate small-particle aerosols, such as the use of power saws or washing of intestines). Available evidence shows that SARS-CoV-2 could remain infectious on surfaces for up to 9 days. The virus has been detected 72 hours after experimental procedures. This supports the need for effective environmental cleaning, decontamination and disinfection before and after autopsy procedures. In addition, the mortuary which may serve as a breeding ground for the virus must be kept clean and properly ventilated at all times. Lighting must be adequate. Surfaces and instruments should be made of materials that can be easily disinfected and maintained between autopsies. Instruments used during the autopsy should be cleaned and disinfected immediately after the autopsy, as part of the routine procedure. Environmental surfaces, where the body was prepared, should first be cleaned with detergent and water. After cleaning, a disinfectant of 0.5% sodium hypochlorite (bleach), or 70% ethanol should be placed on a surface for at least 1 minute. All the wastes generated must be treated as highly infectious and must be appropriately disposed of [34].

Furthermore, health-care professionals saddled with the responsibility of collecting specimens for post-mortem examination must ensure they use appropriate PPE. If the specimen is collected with an aerosol-generating procedure, personnel should wear a particulate respirator at least as protective as a NIOSH certified N95 or the equivalent. All personnel who transport specimens must be trained in safe handling practices and spill decontamination procedures. Specimens should be transported in leak-proof specimen bags (secondary containers), that have a separate sealable pocket for the specimen, i.e., a plastic biohazard specimen bag, with the patient's label on the primary specimen container and a clearly written laboratory request form. The Histopathology Laboratories in health care facilities must adhere to appropriate biosafety practices and transport requirements, according to the type of organism being handled. Also, patient's full name, date of birth and suspected COVID-19 of potential concern should be clearly documented on the laboratory request form. The receiving laboratory should also be notified as soon as the specimen is dispatched [34-39].

Challenges and way forward

There are many challenges faced by many physicians as they put-in their best to manage COVID-19 patients. One of such problem involves precise determination of what caused the death in the first instance. Though the cause of death in persons that earlier tested positive to SARS-CoV-2 may be attributed to COVID-19 especially when they present with febrile conditions that leads into respiratory failure, unfortunately; it may not be that straight forward in some cases, as some patients though tested positive to COVID-19, may be suffering from other chronic illness that led to their deaths. One of the challenges is to establish both in patients who tests positive and the ones that are asymptomatic that equally died whether COVID-19 played a role or not in their deaths. In other words, do COVID-19

caused the death or not or what contribution does COVD-19 has to the cause of the death? [39,40].

Identified challenges associated with post-mortem examination which are peculiar to the Nigeria settings include: 1) Non-availability of Standard Operating Procedure (SOP) for post-mortem examination, as well as, 2) lack of basic minimum bench mark/guidelines for histopathology laboratory doing autopsy. 3) There are equally poor working unsafe environment with no biosecurity in most autopsy rooms across the country. 4) Few trained molecular Histoscientists and Histopathologists having the technical-know-how to handle the autopsy and fewer willing to take risks in performing autopsy on suspected or confirmed COVID-19 positive cases. This may be due to poor hazard allowances and insurance packages, 5) Non-availability of the required tools for autopsy [40]. 6) Another challenge is poor adherence to the approved guidelines for disposal of remains of COVID-19 patients resulting in potential exposure of both the healthcare workers and the general public to the deadly virus [41-44].

In our own opinion, the identified way forward will include: 1) Standard Operating Procedure (SOP) for post-mortem examination, as well as basic minimum bench mark/guidelines for histopathology laboratory doing autopsy must be made available and updated periodically as the need arises. 2) Personnel and working environment for COVID-19 post-mortem examination must have the required biosecurity and protection to contain escalation of the virus. 3) More molecular Histo-Scientists and Histo-Pathologists with the required expertise should be trained and re-trained according to global best practices. 4) The morale of health professionals involve in COVID-19 post-mortem examination should be renewed and sustained through improved hazard allowances and insurance packages. 5) There will be need for regulatory bodies to bring in policy documents peculiar to the Nigeria environment on procedure matters, pre- and post- autopsies of COVID-19 patients. 6) Finally, the Nigerian government should demonstrate much more political will and commitments by investing more in the health sector especially in the histopathology department saddled with role of post-mortem examination of emerging infectious disease like COVID-19.

Conclusion

The post mortem examination of COVID-19 patients is the statutory role of the Histopathology laboratory. However, in addition to the aforementioned, the histopathology laboratory can support the fight against the pandemic by: making autopsy results available in an organized, easily accessible, and timely manner, provide training/re-training on both basic and advanced techniques in histopathology for her staff, also monitor and report autopsy results for unusual findings amongst others. Amidst the COVID-19 pandemic, it is very important for the histopathology laboratory to be actively involved in all aspects of the infection control program, particularly in the hospital's infection surveillance system and in assisting the infection control program to effectively and efficiently use laboratory services for epidemiologic purposes. Finally, it is very important for personnel working in the histopathology laboratories (particularly those involve in post mortem examination of COVID-19 cases) to understand why infection control is very necessary in deescalating the spread of the virus. They must maintain good personal hygiene and take personal protection very seriously. Protective wears such as gloves, gowns/aprons masks, respirators goggle and face shields are mandatory before handling dead bodies of suspected or confirmed COVID-19 patients. In addition, they must adhere to the hospital policy on direct contact with patient and patient's clinical specimens. Adequate methods for routine cleaning, disinfecting and sterilizing must be put in place. Written policies and procedures on handling health hazards within and outside the laboratory must be developed and adhered to amidst the fight against the pandemic.

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