THE IMPORTANCE OF ADEQUATE PRE-ANALYTICAL MANAGEMENT FOR HISTOLOGICAL AND MOLECULAR EXAMINATION FOR DIAGNOSTIC PURPOSES

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ABSTRACT

Almost all Anatomic Pathology laboratories have experienced that tissue patient who comes to these laboratories without adequate fixation and causes complicated patient diagnosis. In addition, adequate fixation also makes advanced procedures such as immunohistochemical staining or DNA and RNA extraction difficult because protein, DNA, and RNA become damaged due to inadequate fixation. The fixation procedure should be the responsibility of the clinicians, specifically surgeons and team. If this procedure is not carried out appropriately resulting in damage to the tissue due to the natural process, namely autolysis, which the fixation procedure can prevent. This autolysis will damage the tissue. The laboratory process results are not optimal, complicating the evaluation of patient tissues, resulting in difficulty and delay of diagnosis. If further examination is needed, such as immunohistochemistry and other molecular examinations related to the extraction of DNA or RNA, the examination results will also be not optimal.
Moreover, patient therapy becomes delayed or difficult. For this reason, we conducted extension activities for health workers, some of whom may be involved in a team of surgeons. Knowledge of pre-analytic procedures can improve the quality of pre-analytical handling, which will improve the quality of tissue and cytology specimens; hence, a precise and optimal diagnosis can be obtained. This will help the clinicians in determining the patient's therapy. Therefore, morbidity and mortality rates of patients can be reduced.

Keywords: pre-analytical management, histological and molecular examination, DNA and RNA extraction.

INTRODUCTION

The histopathological and cytological preparations technique is divided into 3 phases, namely pre-analytic, analytical and post-analytic phases. The principle of the procedures is how to maintain cells and tissues naturally. The cells or tissue obtained from the body must be immediately subjected to a fixation technique to achieve this purpose. Fixation is a complex series of chemical events.[1]

Cells or tissues are composed of cells and extracellular components which consist of peptide and protein elements, lipids and phospholipids (membranes), carbohydrates and complex carbohydrates, various types of RNA and DNA. These elements will react during the fixation process and depend on the type of fixation used and whether it will be removed or retained. Some of these elements will react chemically with the fixative, stabilized by the "crosslinking" mechanism. Some elements are not affected by the fixation solution. They are "trapped" in cells or tissues due to the formation of new elements.[2]

The general goal of tissue fixation is to maintain cell and tissue components when the cell is still alive. In the fixation process and subsequent steps in the manufacture of tissue preparations, there are undoubtedly substantial changes in the composition and appearance of cell and tissue components.[3]

Technically, fixation aims to prevent or restrain the degenerative process that causes. It begins as soon as the tissue loses control of the body and loses its blood supply. This degenerative process is sometimes referred to as a metabolic depletion process or cessation of metabolism leading to cell death and cell destruction. Besides degenerative processes, the loss and diffusion of solutes within the cell should be avoided as much as possible by the deposition or coagulation of these components with a "cross linked" mechanism with other insoluble structural components. The network must be protected from damage caused by tissue maturation processes, including infiltration of high temperature in liquid paraffin. Apart from structural damage, the most important thing is to defend the tissue from damage that can eliminate (false negative) or elicit reactivity (false positive) to stains and reagents. Others include antibodies and nucleic acid probes.[4] It is important to realize that the fixative will produce several changes to the network at the beginning of the fixative. These changes include shrinkage, swelling, and hardening various components. However, changes will occur again when the network is processed next. For example, when the tissue is placed in a 10% formalin fixation solution, the tissue will experience a little shrink, but when the tissue enters into maturation tissue, the specimen is likely to shrink Back up to 20% - 30% of the volume. The fixative process carried out on certain tissues can also affect the elements to be stained with various histochemical and immunohistochemical reagents. From various roles and effects of fixation, it is necessary to pay attention to

the ultimate goal of the network to be processed, cut, and colored, whether the structure or chemical components.[5]

METHODS

Procedures of fixative handling

I. Handling of Tissue Specimen

Tissue specimens can be obtained from the body by excision/incision/biopsy removed by the surgeon. The tissues or fluid are immediately sent to the Anatomical Pathology diagnostic laboratory for examination and diagnosis. Before starting all laboratory activities, personal protective equipment (PPE) must be prepared in advance. [2]

The initial procedure is to prepare a suitable container for tissue fixation with the appropriate size. Patient identity written on the container according to the patient's identity as written on the request form (name, age, tissue origin). The tissues are laid on a cutting board, measured using a ruler of the length, width, and height in centimeters, then determine the consistency, color, and abnormalities in the tissue (such as necrosis and bleeding). After that, tissues are lamellated with a distance of 0.5-1 centimeters parallel (Figure 1), with one side is left uncut. Then laid a piece of tissue paper in between the slices. Hence, the formalin buffer can be adequately absorbed. Then the tissues are dipped into the formalin until the tissue is completely submerged. In this case volume of formalin is 10-20 X volume of the tissue volume. Then the container is closed tightly to prevent the liquid from spilling out. The next step is sending the tissue to the Anatomical Pathology laboratory along with the request form.[6]

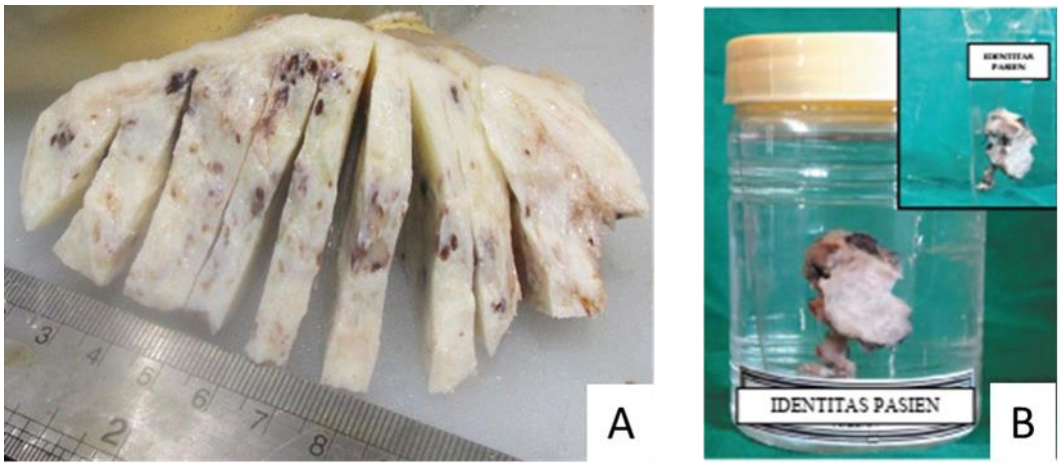

Figure 1. Tissue lamellation and fixation. The tissues are lamellated with a distance of 0.5-1 centimeters parallelly, with one side is left uncut. Then the tissues are dipped into the formalin until the tissue is completely submerged.

II. Cytology Specimen Handling (Smear Preparation)

Cytological specimens are all non-tissue materials removed from the human body in body fluids, urine, tumor products/pathological processes, and feces. Specimens that are pathological conditions must be sent to the Anatomical Pathology Diagnostic Center for histopathological examination and cytopathological diagnosis. Cytology specimens consist of:

A. Gynecological cytology: cervical/vaginal mucus smear specimen (pap's smear)

B. Non-gynecological cytology

- Respiratory tracts
  - Sputum
  - Bronchial washing (rinsing bronchi)
  - Transthoracic needle aspiration (TTNA)
- Digestive tracts: endoscopy results in the form of gastric fluid etc
- Urinary tracts: urine
- Cavities: pleural fluid, pericardial fluid, synovial, abdominal (ascites)
- Cerebrospinal fluid
- Fine needle aspiration cytology (FNAC)[1,7]

Techniques of smear and fixation of the cytologic specimen

There are 2 (two) fixation techniques in the cytological process that are in principle very different, namely:

1. Dry technique

The dry technique is a technique in which the specimen that is smeared on a slide is fixed by drying in the air until completely dry, followed by a Diff-Quick or May Grunwald-Giemsa (MGG) staining. This technique is important to examine general cell morphology.

Materials and tools needed, including specimens (aspirate/sedimentation from centrifuge), slides (slide size 7.5x2.5 cm), toothpick, tissue, label identity (name, age, gender), pen and pencil, slide holder, request form.

After cleaning hands and using appropriate protection, prepare two glasses with the patient's identity (name/age) written on glass slides using a pencil. The specimen is taken slightly using a toothpick placed on the object-glass. By using another object-glass, the specimen is smeared. After drying, the slides are packed in special boxes and shipped along with the request form to the Anatomic Pathology Diagnostic Center.

2. Wet technique

The wet technique is a technique in which the specimen after being smeared on a slide and immediately fixed in 96% alcohol for 30 minutes or fixed directly with hair spray or special spray for cytological fixation—followed by a Papaniculaou or Hematoxylin and Eosin (HE) staining. This technique is important to examine the morphology of the core clearly and in detail.

The materials needed include specimens (aspirate/sedimentation results) centrifuge), glass object (slide size 7.5x2.5 cm), toothpick, tissue, identity label (name, age, gender), pen and pencil, slide holder, and request form. [7,1]

Smear Techniques

There are three types of cytological smear techniques: the blood film method direct methods and squash methods (Figure. 2). Every technician working in the laboratory may choose a technique that is convenient for each person. These techniques also apply to wet and dry smears.

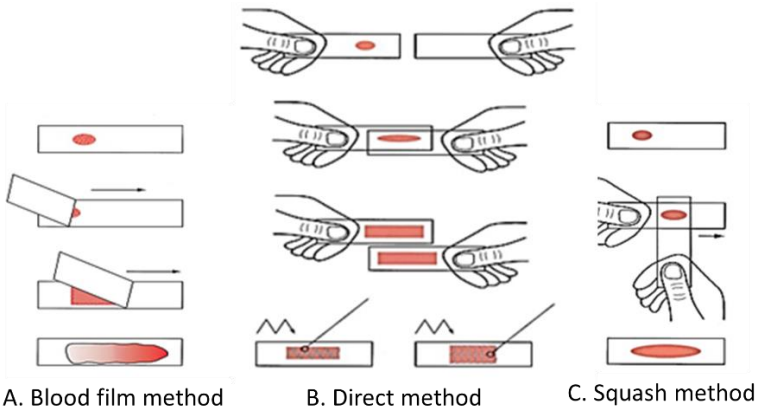

Figure 2. Cytological smear techniques. A. Blood film method. After placing the specimen on a glass object, set another glass with an angle 45° touching the specimen and remove it by pulling or pushing it in the opposite direction. B. Direct method. After dripping the specimen on the slide, place another glass slide above parallelly and pull it in the opposite direction. This method gives us two slides containing the specimen. C. Squash method. After dripping the specimen on the slide, place another glass slide above it, forming a 90° angle, then pulling towards the opposite.

III. Fixation of liquid specimen

Fixation for body fluids is aimed to prevent the degeneration of exfoliative cells in fluids such as pleural fluid, pericardial fluid, peritoneum, ascites, and urine. The materials needed for this activity include a liquid sample of urine, 50% alcohol in a tightly closed container, 5 cc disposable syringe/pipette, sample pot with lid, label, ballpoint.[5]

Initially, the patient's identity is written on the label and affixed to the sample pot. The sample is placed by a disposable syringe into a sample container. Then, a fixation solution (50% alcohol) was added with a syringe, with as same as sample volume (aa), next to both components are mixed homogeneously. The container was then tightly closed and sealed and sent to the Anatomical Pathology Diagnostic Center and request form.[8]

For this period, two hospitals were visited, namely, Siti Khodijah Hospital and Dr. Rivai Abdullah Hospital. At these sites, all participants were enthusiastic and actively discussed the lecture. The questioner was distributed before and after lectures. The booklets were distributed after the activity.

DISCUSSION

This activity was carried out in counseling and assistance periodically and followed by monitoring the tissue brought to the Department of Anatomic Pathology Faculty of Medicine Universitas Sriwijaya and a private Anatomical Pathology laboratory within three months. Evaluation is performed by observing the appearance of the tissue macroscopically if there are still errors in the pre-analytic procedure.

When the tissue is evaluated microscopically, we know whether the pre-analytic procedures are appropriately performed from microscopic appearances. Several conditions follow inadequate fixation, such as difficulty in detachment of tissue to the slides. Under microscopic examination, in the middle of the section, the cells are smaller compared to those in the peripheral tissue section.[9]

Immunohistochemistry procedures and results are also affected by the pre-analytic procedure. The better the pre-analytic procedure, the better the results of immunohistochemistry. The worse impact of the pre-analytic procedure is a false negative of immunostaining, resulting in inadequate or wrong treatment of patients.[8]

The other point is the better quality of block paraffin as an intermediate product which is also utilized for research purposes. Block paraffins are an important component as research samples used by Anatomic Pathology students and staff, other students from different departments and faculties, and other people who conducted their research in our departments and use block paraffin as the samples. [5],[10]

From the given questioner, we know that participants' knowledge is improved after lectures and active discussion. Therefore, as expected, much improvement is observed in the pre-analytic procedure. This yields better diagnosis procedures and improves immunohistochemistry results. Finally, the best treatment approach for patients can be performed.

CONCLUSION

Periodic counseling and assistance are ideal activities for these aims. This activity is effective in facilitating the improvement of pre-analytic procedures. The positive impact is achieved during diagnosis and the immunohistochemical examination procedure and interpretation. Furthermore, visiting and counseling can be carried out again. Counseling comprehensively accompanied by interactive discussions positively impacts pre-analytic procedures and is supposed to be performed in the future.

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