TREATMENT OF CYTOLOGY SPECIMENS BY THE PARAFFIN WAX TECHNIQUE

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ABSTRACT

Fifty specimens each of urine, cerebrospinal, pleural and ascitic fluids were centrifuged slowly and the sediments were separately fixed in 10% formol saline, Bouin’s fluid, 95% alcohol and ether-alcohol. The sediments were rinsed in distilled water and concentrated by centrifugation. The sediments were subsequently suspended in pooled plasma. A clot was immediately formed when calcium chloride and brain thromboplastin were added to the plasma. The resulting clot containing the cells was treated like a tissue and processed by the paraffin wax technique through alcohol and xylene. Sections were cut at 6µm thick and stained by the haematoxylin and eosin and by the Papanicolaou methods. When the sections were compared with Papanicolaou smears, the cells had reduced considerably in size with little cytoplasmic detail. Nuclei were also contracted but they still retained diagnostic values as malignant cells could still be recognized. However, for diagnostic purposes, this method is not superior to smears stained by the Papanicolaou technique. It is recommended only when the cells are abundant in the specimen and if there is a need to keep the specimen for a very long time for subsequent analysis.

Key words: Papanicolaou, H&E, paraffin wax, cytology, fixative

1. INTRODUCTION

The paraffin wax tissue processing method is the most used and most important tissue processing method in histology. It enables serial sections to be cut and it does not distort tissue architecture. It is also compatible with several staining techniques enabling staining of different intracellular and extracellular substances (Avwioro 2010). Its uniqueness in histology is similar to the Papanicolaou staining method in cytology where several intracellular and extracellular substances including acidophilic and basophilic substances are stained (Avwioro 2010). One of the major problems facing cytologists is the inability to examine all the cells contained in an aspirate so that when tumour cells are scanty, they are often not detected except when many smears are examined. However, the use of liquid based cytology (Siebers et al., 2010) has helped to resolve many problems arising from inadequate specimen, although Confortini (Confortini et al., 2010) has helped to agree with this statement. When cytological results were compared with histological findings variations of 36.5% and 22.4% were detected (Kline 1962; Mayor 1964), while Gordons and King (Gordons and King 1976) detected only 55.6% of metastatic cerebrospinal fluid tumour cells. Opinions vary on the recommended method of concentrating cells. Gordons and King (1976) support the use of membrane filter while others (Dyken et al., 1980) found cytocentrifugation most valuable for routine quantification of cells. However, in a recent work (Confortini et al., 2010) studied by independent assessors no difference in the accuracy of liquid-based cytology was observed. It is with a view to finding a more purposeful method suitable for class work that the cascade coagulation theory of Mc Farlene (1964) was revisited. This theory was corroborated (Quick 1966) with the introduction of calcium chloride and brain thromboplastin into plasma leading to the formation of a clot. Cells in aspires and body fluids added into these preparations are trapped in the clot and can be processed and embedded in wax and stained by haematoxylin and eosin. The work reported here was designed to evaluate the usefulness of the cell clot technique in aspirate cell examination and to determine the usefulness of a viable concentration method for cytology specimens which will not only be durable but will enable several cytochemical methods to be used for the recognition of cellular contents.

2. MATERIALS AND METHODS

Two hundred specimens made up of 50 each from urine, cerebrospinal fluid, pleural and ascitic fluids were used for the experiment. Each specimen was divided into five and centrifuged slowly for 5 minutes. The supernatants were decanted and the sediments were then fixed in 10% formol saline (10 ml formalin, 0.85g sodium chloride and 90 ml water), Bouin’s fluid (75 ml saturated aqueous picric acid, 25 ml formalin and 5 ml acetic acid), 95% alcohol and ether-alcohol (equal parts diethyl ether and
alcohol) for about 45 minutes by pouring the fixatives into the centrifuge tubes containing the deposits. Smears were made from the 5th group and fixed in ether-alcohol. The deposits from the 1st, 2nd, 3rd and 4th groups were washed twice with distilled water and centrifuged. The distilled water was then decanted. Plasma from 4 voluntary donors was pooled and tested for syphilis and HIV which was negative. Two drops of plasma were added to the cell sediment at about 37ºC, mixed and allowed to settle for about 30 minutes. Brain thromboplastin was prepared by acetone extraction of the cerebellum and standardized according to standard methods. One drop of brain thromboplastin and one drop of 0.25% calcium chloride were added to the mixture and a clot which was formed was left for about 10 hours to enable retraction of the clot. The clot formed was processed by the paraffin wax technique like a biopsy by passing it through ascending grades of alcohol, two changes of xylene and two changes of liquid paraffin wax at 65ºC. The clot was then embedded in paraffin wax. The blocks obtained were subsequently cut at about 6µm with the rotary microtome and stained with haematoxylin and eosin in the following way: The sections were dewaxed in xylene, hydrated through descending grades of alcohol, stained in Erhlich’s haematoxylin for 10 minutes, rinsed in water, differentiated in 1% acid-alcohol, blued in tap water for 10 minutes and counterstained in 1% eosin for 1 minute. Sections were then rinsed in water, dehydrated through ascending grades of alcohol, cleared in xylene and finally mounted in DPX. Smears from the 5th group fixed in the ether-ethanol fixative were stained by the Papanicolaou technique in the following way: the smears were stained with Harris’s haematoxylin for 3 minutes, rinsed in water, differentiated in 1% acid-alcohol for 10 seconds and rinsed in water. Smears were then transferred to 70% alcohol, 95% alcohol, OG 6, 95% alcohol, EA 50 and 95% alcohol for 2 minutes each in that order. Smears were finally dehydrated in absolute alcohol, cleared in xylene and mounted in DPX.

3. RESULTS

Specimens fixed in alcohol gave a very poor result, while those fixed in Bouin’s fluid and 10% formol saline gave good results. In conditions were clot retraction was not allowed to take place for up to 10 hours, tiny fragments of the clot were found deposited in the dehydrating alcohol. Paraffin wax processing through alcohol, xylene and paraffin wax made the wax to contract to about 1/10th its original size to form a solid firm tissue-like structure. Sections were cut easily without crumbling. Those fixed in Bouin’s fluid retained the pinkish colour of the Bouin’s fluid. Scattering of sections during floating out on water at about 45ºC was not noticed. Good serial sections were obtained. Sections drying at 50ºC or more did not show any adverse effect on the sections. Haematoxylin and eosin staining technique gave better results than Papanicolaou stain. On microscopy, striking decreases in nuclear and cytoplasmic sizes were observed when compared with the conventional Papanicolaou technique on smears. However, nuclei and chromatin were well stained but with decreased cytoplasmic detail due to the extreme decrease in cytoplasmic size. Scanty specimens or specimens with scanty cells were difficult to examine because in all such cases, cells were not seen in the sections. However, sections prepared from specimens containing abundant cells were easy to examine.

4. DISCUSSION

Tissue processing by the paraffin wax method is obviously the most used and the best method of processing tissues for histological examination because it allows the cutting of serial and smooth sections that are not ragged. The process is not cumbersome and the blocks can be stored almost indefinitely at room temperature from where several sections can be cut (Avwioro 2010) for further cytochemical, histological and histopathological analysis. The method of preparing tissues for microscopic examination is different from the method of preparing aspirates for microscopic examination. While it is possible to obtain several sections from a paraffin wax embedded tissue, very few smears can be obtained from a specimen for cytology. For this reason, there is a limit to the number of staining techniques that can be applied onto cytology specimens. There is also a limit to its accuracy, although the introduction of liquid based cytology has greatly improved diagnosis of cytology specimens (Perez-Reyes et al., 1994). The Pap smear (Papanicolaou 1954) is a routine screening test used for the detection of cervical abnormalities and precancerous dysplastic changes of the uterine cervix. It also detects certain viral, bacterial, and fungal infections of the cervix and vagina (Donders et al., 1992). There is also epidemiological and experimental evidence that Pap smears are beneficial in detecting infections that are risk factors associated with cervical cancer, such as human papilloma virus (Schiffman et al., 1993). The Papanicolaou staining technique has also been used extensively in other cytological methods for pleural aspirates, cerebrospinal fluid, urine and other body fluids (Avwioro 2010).

REFERENCES


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