UTILITY OF AGAROSE CELL BLOCK TECHNIQUE FOR FINE NEEDLE ASPIRATION, URINE AND SEROUS EFFUSIONS CYTOLOGY, IN GEZIRA STATE.

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ABSTRACT

Objective: To assess utility of the agarose cell block technique based on specimen adequacy and diagnostic accuracy. Material and Methods: 63 study subjects were selected for this study, among them 12(19%) were males and 51(81%) were females with age range between 4-77 years and the mean age was 36.97 ± 1.9. One cell block section and one smear were made from each case, the study cases were divided into 4 groups according to the type of specimens including thyroid 23 (36.5%), breast 15(23.8%), lymph nodes 6 (9.5%) and soft tissue masses 9 (14.28). The rest include three (4.76 %) effusion (Two Ascetic fluids and One pleural fluid), and 7(11.11%) urine samples. The soft tissue masses include face lipoma, post auricular, arm, back, right mandible, foot, ankle, neck, and abdominal masses. Result: When comparing the final diagnosis of cell block sections and cytological smears according to the background, cellularity, the nuclear and cytoplasm preservation, 21(33.3%) of samples were better by cell block technique, 27(42.9%) showed same results in both cellblock technique and smearing technique and 15(23.8%) were found to be less using cell block technique. Conclusion: The study concludes that different cellblock sections should be used for, histochemical, immunohistochemical (IHC), and Immunocytochemical (ICC) investigations in diagnostic cytology laboratories. Molecular studies such as, fluorescent /chromogenic in-situ hybridization (FISH/CISH) and in-situ PCR, could also be applied on cell block sections.

KEYWORDS: Agarose cell block, Fine Needle Aspiration (FNA), Effusion.

INTRODUCTION

Cytopathology is commonly used to investigate precancerous cervical lesions, thyroid lesions, diseases involving sterile body cavities (peritoneal, pleural, and cerebrospinal), and a wide range of other body sites. It is used to aid in the diagnosis of cancer, certain infectious diseases and various inflammatory conditions, Cytopathology is generally used on samples of free cells or tissue fragments, in contrast to histopathology, which studies whole tissues. Cytopathologic tests are some times called smear tests because the samples may be smeared across a glass microscope slide for subsequent staining and microscopic examination (e.g. Pap smear). However, cytology samples may be prepared in other ways, including cytocentrifugation. Different types of smear tests (cytologic smears) may be used for cancer diagnosis (Abstracts, 36th European Congress of Cytology,(2011).

Preparation of conventional centrifuge smears is a well established method for the cytological examination of serous fluids. Cell blocks prepared from residual fluid are a useful adjunct to smears for establishing a more definitive cytopathological diagnosis. They are particularly useful for categorization of tumors when not possible from smears alone.

The effectiveness of the cell block lies in the availability of diagnostic material for further histological examination, histochemistry and immunohistochemistry (IHC) for precise classification of tumors and for identification of infectious agents with microbiological stains (Nathan et al., 2000). Provision of IHC staining as an adjunct to improve the accuracy of the cytological diagnosis of body cavity fluids is well known (Li et al., 1989). One study reported that for IHC, cell blocks provide the best material for morphological interpretation, with less background staining than cytospin or thin Preparation samples techniques (Fetsch
et al., 2002). In serous fluids, IHC staining is most often needed for distinguishing reactive mesothelial cells from metastatic malignant cells (Shield et al., 1996).

In routine cytological practice, the cell morphologic changes in smear are not always obvious and judgment is sometimes difficult. Therefore the application of cell block for better presentation of detailed cytological architectural features, histochemical or immunocytochemical staining has been a useful adjunct for establishing a more definitive cytopathological diagnosis (Nathan et al., 2000). Furthermore, cell block is also a source of archival material for cytological research (Wen et al., 2007). For many years, cell block preparation techniques have been widely applied in many cytology specimens, including; body fluids, fine needle aspirations, and liquid –based specimens. The techniques used for cell block preparation vary in different institutions. Several cell block preparation techniques have been introduced such as: ethanol formalin fixative (Nathan et al., 2000), bacterial agar (Yeoh and Chan, 1999, Kulkarni et al., 2000) plasma thrombin clot (Karnauchow and Bonin, 1982), albumin inverted filter sedimentation (Nigro et al., 2007), and simple sedimentation techniques (Zito et al., 1995, Richard et al., 1999). Sometimes cytology does not provide sufficient information and the risk of false negative or undetermined diagnosis exist, (Kulkarni et al., 2000). The cell block technique increases the chance to achieve positive results and to demonstrate better architectural patterns which could be of great value for approaching the correct diagnosis (Mansy et al., 2006, Thapar et al., 2009).

MATERIALS AND METHODS

Type of Study
This was a descriptive prospective; cross-sectional, study carried out in Gezira state, Medical laboratory, University of Gezira, during the period (April - September 2012) to assess utility of the agarose cell block technique as compared to conventional cytology.

Study population
All patients attending to the Medical laboratory, University of Gezira for different cytological investigations during a period April - September 2012.

Sample size, collection and processing
Sample size
Sixty- three study subjects were selected for this study by simple random method. From each sample taken smears were prepared for conventional cytology (controls), and the rest was used for preparing agarose cell block (cases).

Samples collections
Cytological specimens including fine needle aspirations (breast, thyroid and soft tissue masses, in addition to effusions and urine samples) were used in this study.

SAMPLE PROCESSING
Smearing technique
The FNAC specimens were obtained using syringes and needle and 2 _4 smears were prepared from each sample using coated –slides and immediately fix in 95% ethanol then H&E stained. Effusions and urine specimens were centrifuged at 4000 rpm for 6 minutes, and smears were prepared from centrifuged deposit. All smears were fixed in 95% ethanol and H&E stained.

Cell blocking
Following smear preparations for cytology, the needles and syringes used to obtain fine-needle aspirates were rinsed in 10 mL of 10% formalin in a specimen container. Any residual clot or tissue in the hub of needles was removed carefully with the aid of another needle and rinsed in 10% formalin. The entire material was centrifuged in a 10-mL disposable centrifuge tube at 2500 rpm for 3 minutes to create cell pellets. The supernatant fluid was decanted then equal amount of 10% formalin was added to the cell sediment and mixed for 5 sec, followed by centrifuging again at 2500 rpm for 3 min, the supernatant fluid was decanted and 6 drops of molten 2% agarose gel was added and allowed to stand for 3-5 min to solidify and form cell block. The agarose cell block was removed from the tube and placed on a filter paper, then cut into 1-2 pieces; each piece was wrapped in filter paper and then placed in a tissue cassette.

All tissue cassettes were immersed in10% formalin before processing in an automatic tissue processor. The cell blocks were embedded in paraffin and 4-6 µm sections were cut using standard rotary microtome. The sections were stained with Haematoxylin and eosin (H&E) stain and covered with cover slips using DPX mounting media.

Staining
Routine H&E (Mayer's) staining was used on all cell block sections and smears according to. Bancroft (1999), as follow.
- Section to water.
- Stain with Haematoxylin for 8 min
- Wash briefly in water and differentiate in acid-alcohol.
- Wash well in water and blue for 8 min
- Wash in water and stain in eosin solution for 3 min.
- Wash quickly in water, differentiate and dehydrate in alcohol.
- Clear and mount in DPX.

RESULTS INTERPRETATION
All Haematoxylin and Eosin stained cell block sections and smears were reviewed by a histopathologist and cytotecnologist to evaluate the following parameters: background, cellularity, nuclear and cytoplasmic preservation. The final results of the cell block sections and that cytological smears were compared.
Statistical analysis
All data were statistically analysis using SPSS (Statistical Package for the Social Sciences) program version 16, kappa test was used and the results were presented mainly as frequencies and percentage.

Ethical considerations
All participants were informed about the aim of the study and asked for their approval before taken the samples.

RESULTS

Particulars of study subjects
Sixty-three subjects were included in this study; 12 (19%) were males and 51 (81%) were females. Their age ranged between 4-77 years with mean age of 36.97 ±1.9 years (Table 1 and Fig. 1). As can be seen from the table only five subjects were under 18 years old and the number of subjects in the other age groups was not largely different.

Table (4.1) Age group and sex of the study subjects, No = 63.

<table>
<thead>
<tr>
<th>Years</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>18-30</td>
<td>4.8%</td>
<td>3.2%</td>
<td>7.9%</td>
</tr>
<tr>
<td>31-45</td>
<td>7.9%</td>
<td>25.4%</td>
<td>33.3%</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>3.2%</td>
<td>23.8%</td>
<td>27%</td>
</tr>
<tr>
<td>Total</td>
<td>19%</td>
<td>81%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure (4.1): Age group and sex of the study subjects, No = 63.

Types and numbers of samples taken
The types of specimens taken from the study subjects included: thyroid, 23 (36.5%); breast, 15 (23.8%); lymph nodes, 6 (9.5%); and soft tissue masses, 9 (14.28%); in addition to effusions (2 Ascetic fluids +1 pleural fluid), and urine (7 samples). The soft tissue masses comprised face lipoma, post auricular swelling, arm, back, right mandible, foot, ankle, neck, and abdomen (Table 4.2).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Numbers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle biopsies</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>23 (36.5)</td>
</tr>
<tr>
<td>Breast</td>
<td>15 (23.8)</td>
</tr>
<tr>
<td>L.N</td>
<td>6 (9.5)</td>
</tr>
<tr>
<td>Soft tissue masses</td>
<td>9 (14.28)</td>
</tr>
<tr>
<td>Effusions</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
<td>2 (3.17)</td>
</tr>
<tr>
<td>pleural</td>
<td>1 (1.59)</td>
</tr>
<tr>
<td>Urine</td>
<td>7 (11.11)</td>
</tr>
<tr>
<td>Total</td>
<td>63 (100%)</td>
</tr>
</tbody>
</table>

Table (4.2): Types and numbers of samples taken.

Figure (4.2): Types and numbers of samples taken.

Data analysis
The level of agreement (concordance) between cellblock sections and conventional smear as standard technique was measured using Cohen's kappa coefficient (κ). As a rule of thumb values of Kappa (κ) of < 0 indicate no agreement and 0 - 0.20 considered as slight agreement, 0.21 - 0.40 as fair agreement, 0.41 - 0.60 as moderate agreement, 0.61 - 0.80 as substantial agreement, and 0.81 - 1 as almost perfect agreement. When comparing the two techniques, considering the background, cellularity and nuclear and cytoplasm preservation, the results were as follows.

Slight concordance was found between the cell block sections and the cytological smears considering background (κ = 0.1) Table (4.3.1) and cellularity (κ = 0.06) Table (4.3.2) while fair agreement was found when comparing nuclear and cytoplasmic preservation (κ = 0.27) Table (4.3.3) and total score evaluation (κ = 0.32) Table (4.3.4) of the two techniques.

Comparison between cytological smears and cellblock sections for some of the investigated cases can be seen in photomicrographs (1, A&B; 2, A&B; 3, A&B and 4, A&B).
Total score
When comparing the total score, which grades from 2 to 9, the level of agreement (concordance) between cell blocks sections and conventional smears was found to be fair with kappa p. value (κ = .32) as shown in Table 4.4.

Evaluation of cell blocks sections
Seven (11.1%) of sections contained no cells and were of no diagnostic value. Fifty-five (87.3%) of sections showed normal cells appearance and were diagnosed as benign gross. Only one case (1.6%) had cellular changes suggestive of malignancy (Fig 4.5).

Comparison between cellblock sections and cytological smears in case diagnosis
When comparing the final diagnosis of cellblock sections and cytological smears according to the background, cellularity and the nuclear and cytoplasm preservation in each type of specimens, there is a significant association between the type of specimens and final diagnosis, Pearson test chi-square = 23.3, p-value = 0.001. It was found that 3/23, 4/15 and 2/6 of thyroid, breast and L.N fine needle aspiration (FNA) samples respectively, prepared as cellblock sections had better diagnostic value than cytological smears, while 16/23 of thyroid and 8/15 of breast samples prepared by both technique had similar quality.

On the other hand 4/23, 3/15 and 4/6 of the respective organ samples prepared as cellblock sections had lower value as compared to cytological smears. Similarly better results were obtained from cellblock sections of other specimens (soft tissue masses, urine and effusions) when compared to cytological smears (12/19); 4/19 sections were of less quality and 3/19 had comparable quality. Table 4.7.

<table>
<thead>
<tr>
<th>Types of specimens</th>
<th>Final diagnosis comparing of cellblock and smear</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>better</td>
<td>similar</td>
</tr>
<tr>
<td>thyroid</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>percent</td>
<td>13.0%</td>
<td>69.6%</td>
</tr>
<tr>
<td>breast</td>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>percent</td>
<td>26.7%</td>
<td>53.3%</td>
</tr>
<tr>
<td>L.N</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>percent</td>
<td>33.3%</td>
<td>.0%</td>
</tr>
<tr>
<td>others</td>
<td>No</td>
<td>12</td>
</tr>
<tr>
<td>percent</td>
<td>63.2%</td>
<td>15.8%</td>
</tr>
<tr>
<td>Total</td>
<td>No</td>
<td>21</td>
</tr>
<tr>
<td>percent</td>
<td>33.3%</td>
<td>42.9%</td>
</tr>
</tbody>
</table>

Table (4.3): Comparison of cellblocks sections and cytological smears according to type of specimens, No = 63.
DISCUSSION

The objective of this study was to assess sections prepared by cell block technique compare to gold standard smearing technique. A wide range of histological fixatives has been used for cell blocks, e.g. neutral buffered formaldehyde solution, bouin’s solution, picric acid fixatives, carnoy fixative, and ethanol (Zito et al., 1995) Formalin an acceptable tissue fixative which has been used widely for cell blocks by most researchers (Nathan et al., 2000). So, in this study we used 10% formalin as a common purpose fixative for cell blocks fixation, and 95% ethanol for smears fixation and both were satisfactory.

Various methods for preparing paraffin embedded cell blocks from FNA have been reported, centrifuged cellular material wrapped in lens paper or embedding in plasma or agar and then processed as routine histological specimens. The main problem with the cell block technique is the risk of losing the cellular material during preparation. Therefore, a critical point during preparation of cell block is the collection of sediments without loss of material. (Kulkarni et al., 2000) used 3% molten agar which bound the sediment cells and tissue particles and thus avoided the loss of material. In this study we used 2% molten agar for binding the sediment cells and tissue particles as used by Wen et al. (2011).

It is important to mention that cellblock components may be missed during sectioning due to low cellularity, especially when using body fluids and effusions. (Kulkarni et al., 2000), reported that, the main problem with the cell block technique is the risk of losing the cellular material during preparation; this may be resolved by using tumor marker or Indian ink for demarcation line for the area to be sectioned.

many studies have compared the value of cell block with cytological smears: (Keyhani-Rofagha et al., 1990) reported that in 55% of 85 studied cases the smear diagnoses was improved after the cell block sections were examined. The sensitivity of cell block varies from 60% to 80% depending on sampling method, size, type of specimens and aspiration technique used.(Axe et al., 1986) showed that the sensitivity of papanicolauo stained smears (79%) was slightly superior to that of cell block (73%).(Kern and Haber, 1986) studied 393 cases of cell block preparation; in 237 (60.3 %) the finding were confirmatory cytological smears, and in 103 cases (26.2 %) cell block provided additional information for diagnosis,(Wojcik and Selvaggi, 1991) showed that 84% of the cases had identical results on both smears and cell blocks. (Leung and Bedard, 1996) found that all cases with adequate material could be diagnosed on a cell block preparation.

In this study we compared the final diagnosis of cellblock sections and smears according to the background, cellularity and the nuclear and cytoplasm preservation and we found that 21cases (33.3%) of samples were better by cellblock technique. This result similar to that of(Keyhani-Rofagha et al., 1990) and (Kern and Haber, 1986) who concluded that cellblock technique improve diagnosis, studied 393 cases of cell block preparation and they found that 103 cases (26.2 %) cell block provided additional information for diagnosis, in contrast, in the present study 27cases (42.9%) showed similar results using both cellblock and smearing techniques. Cellblock sections may be used as confirmatory (Kern and Haber, 1986).we also found in 15(23.8%) cases cytological smears gave better results than cellblock sections and this seems to agree with (Axe et al., 1986).

(Kulkarni et al., 2000). Studied 27 FNA biopsies and found that cell block sections added more information to the cytology smears in 12 cases, gave similar results in 14 cases while was inconclusive in one case. It was concluded that cellblock technique is very useful to utilize available material when re aspiration is difficult.

Concerning the results of FNA specimens of thyroid, breast and L.N currently investigated it appear that cellblock sections added little information to the cytological smears, but when evaluating the total results including soft tissue masses, effusions and urine, the cellblock preparation gave better results to conventional cytology smears (12/19 versus 4/19).This makes the overall evaluations in favor of cellblock preparation. However cellblock preparations may be as good as or superior to conventional cytological smears for cytological investigations especially those involving fluids and both techniques may be adopted in diagnostic laboratories.

CONCLUSION AND RECOMMENDATIONS

- Cell block preparations and conventional cytological smears are both reliable for cytological investigation.
- Cellblock technique may have advantages over cytological smears when investigating soft tissue aspirations, and body fluids.
- Cytological smears have the advantage of being easy to prepare within a shorter time compared to cellblock sections.
- While cytological smears are used once but cellblocks can be preserved for further use.

RECOMMENDATIONS

- Both procedures cytological smears and cellblock sections should be adopted for cytological investigations and the cellblock preparations used for confirmatory diagnosis.
- For precise classification of tumors and identification of infectious agents’ histochemistry, immunohistochemistry and Immunocytochemistry should better be done on cell block sections.
Molecular studies such as, fluorescent/chromogenic in-situ hybridization (FISH/ISH) and in-situ PCR, could be applied on cell block sections.

REFERENCES


