University of Khartoum  
Faculty of medical laboratory sciences

Study on:

Comparison between Papanicolaou and May-Grunwald Giemsa stains in thyroid fine needle aspiration.
By:

Zeinab Abdallah Ibrahim
Supervisor:

Dr. Elgizouli Omer Musa

A thesis Submitted to the University of Khartoum in partial fulfillment of the requirement for the degree of B.Sc. (honors) in Histopathology and Cytology Department.

University of Khartoum  
August 2004
Dedication

To My Mother ..................

My Father ...................

My Lovely Family ..........

To all whom supported me with their advice.
Acknowledgement

This work was supported by faculty of medical laboratory science of Khartoum university.
Thanks to Histopathology and Cytology department in radiation & isotopes center Khartoum especially Dr. Husam and saria for their helping in collection of samples. Thanks to ustaz Abdallah Hasab Elnabi for his comment. The ultimate thanks to ustaz Abobuker Adam for helping in analysis of data.
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Abstract
This a descriptive study conducted in Khartoum to evaluate MGG stain compared to Pap stain in thyroid fine needle aspiration smears. Moreover to evaluate air-dried Pap stained smears after rehydrated in normal saline for 30 seconds.
Three samples were obtained from each patient and the material was smeared into three labeled glass slides, wet fixed Pap stained smears (WP), air-dried Pap stained smears (DP) and dry MGG stained smears (DMGG).

The staining quality of all samples were assessed, and the T test was used to compare between MGG and Pap stains and to evaluate the effect of rehydrated air-dried smears on Pap stain.
The result of comparison between MGG and Pap stain revealed that there was no significance difference between them (P>0.05).

while the comparison between wet fixed and air-dried Pap stained smears showed that was significance difference between them (P<0.05).

The staining quality which produced by MGG stained smears considered as good as Pap stained smears.

The effect of rehydrated air-dried smears on Pap stain was produced bad staining quality compared to wet smears.
A study was conducted in Khartoum to evaluate the staining of (MGG) compared to (Pap) in thyroid smears obtained using fine-needle aspiration. In addition, the stained dry (Pap) slides were rehydrated in salt for 30 seconds before staining. Three samples were taken from each patient and three glass slides were prepared: wet fixed stained (Pap), dry (MGG) stained slides, and dry (MGG) stained slides. The staining efficiency was evaluated in all samples.

The comparison result between (MGG) and (Pap) showed no difference between the two values (P > 0.05). However, the comparison result between (Pap) dry slides and rehydrated (Pap) slides showed a difference (P < 0.05). The (MGG) stained dry slides showed a worse result compared to the (Pap) wet fixed stained slides.

Introduction And Literature Review

Cytology is a branch of diagnostic medicine which deals with study of individual cells or tissue fragments (Sood, 1994). It includes exfoliative cytology which relies on the presence of cells that are shed spontaneously into body fluids and secretions such as urine and effusion obtained from
body cavities. Collection of cells found in these fluids is considered only a minimally invasive procedure with little risk of complication. Cellular degeneration may be a problem if the specimen is not collected, fixed, transported and prepared in the appropriate manner. In addition cytology includes Fine Needle Aspiration (FNA) perhaps the most powerful technique used to study those cells which pulled from such surfaces. Since FNA introduced, the role of diagnostic cytology has expanded and covers different parts of the body. The availability of variety of rapid, safe, and cost effective techniques and stains often places cytopathology in the forefront of diagnostic evaluation (Poewrs, 1998).

1.1 Fine Needle Aspiration Cytology (FNAC):

It was first introduced during the 1930s, by Martin and Ellis at the Memorial Sloan Kettering Cancer center in New York (Chaturvedi, 2000).

At present, many situations that previously required open biopsy or surgical removal of a mass lesion for diagnosis can now be diagnosed safely and accurately with fine needle aspiration biopsy (Jackson et al, 1986). FNA biopsy is quick, safe and inexpensive method for obtaining material for pathological diagnosis particularly diagnosis of malignant tumor (Zakowski, 1994). Another prerequisite for the use of aspiration biopsy cytology is specially interested cytopathologist who is familiar with the methodological aspects, as well as the interpretation of cells in aspirates (Willems, 1981). The method is applicable to lesions that are easily palpable, for example superficial growth of the skin, subcutaneous and soft tissue and organs such as the thyroid, breast, salivary gland and superficial lymph nodes. For internal organs and lesions in sites not easily accessible to surgical biopsy there were radiological techniques opened opportunities for FNA cytology of deeper structures (Chaturvedi, 2000). Although aspiration cytology is widely used but it is not substitute for
conventional surgical histopathology, it regarded as an extremely valuable complement to it (Chaturvedi, 2000). FNA biopsy is a relative simple procedure, provided significant advancement in the diagnosis of a number of pathological conditions of the head and neck as well as other areas (Jackson et al, 1986).

1.1.1 **FNA Sampling:**

The experience aspirator pathologist wears gloves and prepare patient in setting position, without local anesthesia. Then the skin is cleaned with alcoholic antiseptic, the mass is fixed with one hand and the other hand aspirates the material using conventional plastic disposable syringe. For adequate sampling the needle may be moved in three to four different direction. The needle is disconnected and after filling the syringe With air, it’s reconnected. Content is expressed on clean glass slides then smears are made by applying gentle pressure with another glass slide and fixed while it’s wet for Papanicolaou staining or allowed to air dry for May-Grunwald-Giemsa staining (Sood, 1994).

1.1.2 **Advantages of FNA:**

FNA has many advantages; cost effective, safe, local anesthesia is not required, quick with rapid report and highly sensitive and specific for the diagnosis of malignancy. Further more it helps in the evaluation of the stage of disease prior to surgery or treatment and the aspirated material can be used for immunological, cytochemical, and microbiological studies. In addition it requires simple and few equipment, no problem in wound healing and readily repeatable (Chaturvedi, 2000).

1.1.3 **Disadvantages of FNA:**

FNA has many pitfalls; it needs practice and skill in technical steps that involved palpation, aspiration, smear making, fixation and staining
which are critical for good result. Experience in diagnosis is required also for accurate interpretation and diagnostic role is limited must always be correlated with other investigation (Chaturvedi,2000).

The knowledgement of limitation of any diagnostic procedure is most important and is result in increasing experience and understanding of the use of the technique. The two fundamental requirements on which the success of FNA cytology depend are representativeness of the Sample and high quality of smear preparation (Chaturvedi,2000).

For palpable lesion of the thyroid, FNA is a diagnostic method routinely used to evaluate and diagnose thyroid enlargement and nodules in several medical centers, but still relatively new in others (Willems,1981). The increasing popularity of FNA as a primary diagnostic procedure has introduced another stains such as the Romanowsky stains and H&E with routine Papanicolaou stain (Poewrs,1998).

1.2 The Papanicolaou Stain(Pap):

It was introduced by Dr. George N. Papanicolaou in 1942, and modified by him in 1954 and 1960. It is used today universally throughout cytology and known as Pap stain (Gill,1999). Papanicolaou stain is recommended for routine diagnostic cytology because it characterized by good nuclear detail, differential counter staining and cytoplasm transparency (Koss,1979). The Pap stain is a highly developed polychrome stain that demonstrate nuclei blue / black and cytoplasmic staining show a broad spectrum of colors ranging from yellow in highly keratinized cells through ranges of orange /pink in superficial cells and green /blue in intermediate and Parabasal cells, Metaplastic cells may show amphophilia and will often stain green and pink together. A well
stained Pap shows variation in colors and shades, reflecting the dynamic cell population (Proe, 2000).

The material in the needle spread on slide should immediately wet fixed for Papanicolaou staining (Drury et al, 1980). The traditional fixative for cytological specimens is alcohol usually 95% ethanol, but 100% methanol or 80% isopropanol may be used (Powers, 1998). Alcohol is a coagulative fixative that result in a sharp nuclear detail, causes cells to shrink by removing intracellular water so, cytoplasmic features may be less well defined (Powers, 1998).

For Pap stain if there any short delay in fixation results in drying that may render specimen uninterpretable, this the most common problem faced technologist particularly when the specimen is obtained by inexperience personnel. Another common problem related with FNA specimen, is excessive blood; fibrin in clotting blood entraps cells, result in heavy and uneven staining and distortion of smear pattern, even clotting of small volume it greatly interferes with good smear preparation and subsequent interpretation (Powers, 1998).

A recently developed rapid Pap stain method helps to overcome problems with fixation and cell loss. This method has the advantage of being performed on air-dried smears, so that rapid preparation and fixation are less critical. Air drying also enhances the adherence of the cells to the slides, increasing cell recovery. Smears are rehydrated briefly in normal saline, which tends to lyse erythrocytes by reducing the obscuring effect of particularly bloody smears. In addition to excellent preservative of cell structure, this rapid method still preserves the nuclear detail expected with any Pap stain (Powers, 1998).

In 2001 Shidham had collected fine needle aspiration cytology (FNAC) smears from 118 cases to evaluate the possibility of routine use of air-dried smears (ADS) instead of wet-fixed smears (WFS). The ADS
processed with their protocol for haematoxylin and eosin (H&E) and Pap staining after saline rehydration and post fixation in 95% ethanol. The study found that ADS were easy to prepare without air drying artifact in the H&E and Pap stained smears, and were highly cellular than WFS. Erythrocyte interference was frequent in WFS. All ADS show results comparable to or better than WFS, offers the flexibility of selection a variety of staining methods and is a practical alternative to WFS (Shidham et al,2001).

Another study made comparison between wet fixed and air-dried Pap stained material. Air-dried material after rehydration was showed superior nuclear definition compared to wet fixed material, the removal of erythrocytes enhanced the staining of the remaining epithelial cells (Jones,1999).

In 1988 Chan and Kung found that in fine needle aspiration nuclear features were often better assessed in H&E or Pap stain than Romanowsky stained smears. Pap stain requires immediate wet fixed smears for cytomorphology preservation, so some degree of air drying is usually inevitable. The quality of the rehydrated smears is superior or identical to that of wet fixed smears provided that the period of drying did not exceed 30 minutes (Chan et al,1988).

1.3 May-Grunwald-Giemsa Stain:

The term Romanowsky stain is a variety of stains derived from the Giemsa stain, including the May-Grunwald-Giemsa (MGG), Wright-Giemsa, and Diff-Quik stains. May-Grunwald-Giemsa used as routine stain for air-dried cytological preparation involved aspiration biopsy. MGG stain reduced the effects of poor technique and increase cell yield.
Justification

Thyroid fine needle aspiration has been used widely in recent days as diagnostic tool for thyroid disorders, for this above mentioned reason quality of stains play great role in interpretation. In order to obtain reliable result, this study is going to evaluate MGG stain which used in most laboratories compared to Pap stain.
Objectives

The aims of this study are to:
1. Compare between Papanicolaou and May-Grunwald Giemsa stains in thyroid fine needle aspiration.
2. Evaluate the effect of rehydrated air-dried smears on Papanicolaou stain in thyroid fine needle aspiration.
Materials and Methods

3.1 Study Design:
This is a descriptive study was carried out in Radiation & Isotopes center Khartoum to compare between Pap and MGG stains in thyroid fine needle aspiration.

3.2 Study Population:
Thyroid fine needle aspiration from Sudanese patients have thyroid disorders at different level of age and sex.

3.3 Sample Size:
Twenty thyroid FNA was collected conveniently during three months from April to June in order to compare between Pap and MGG stains.

3.4 Lab Procedure:
3.4.1 Sample Collection:
The experience aspirator pathologist wearied gloves and prepared patient in sitting position, without local anesthesia. Then the skin was cleaned with alcoholic antiseptic, the mass was fixed with one hand and the other hand aspirated the material using conventional plastic disposable syringe. For adequate sampling the needle might be moved in three to four different direction. The needle was disconnected and after filling the syringe with air, it was reconnected. Content was expressed on clean, free grease glass slides then, smears were made by applying gentle pressure with another glass slide and fixed while it was wet for Papanicolaou staining or allowed to air dry for may-Grunwald -Giemsa staining (Sood, 1994).
3.4.2 Staining Methods:

(1) Pap Stain:

Wet fixed smears (WP) and rehydrated air-dried smears (DP) were stained using Pap. Wet smears fixed in 95% ethyl alcohol for at least 30 minutes while air dried smears were rehydrated after 24 hours in normal saline for 30 seconds followed by fixation in 95% ethanol. Both (WP) and (DP) smears hydrated through descending grades of alcohol 90% and 70% until reach distilled water, 2 minutes for each. Then stained nucleus with regressive Harris’s haematoxylin for 3 minutes, differentiated in aqueous hydrolytic acid for few seconds and bluing in running tap water for 10 minutes. The cytoplasmic stain used was EA50 with out O.G.6 which was omitted in this procedure. EA50 stained for 3 minutes after dehydrated in ascending grades of alcohol 70%, 90%, 95%. Dehydrated, cleared and mounted (Drury, 1980).

Staining Assessment:

- Nuclei dark blue.
- Cytoplasm of superficial cells pink.
- Cytoplasm of intermediate cells pale greenish-blue.
- Cytoplasm of parabasal cells deep greenish-blue.
- Red blood cells bright red.

(2) May- Grunwald Giemsa:

Air-dried smears (DM) were fixed in methanol for 15 minutes, rinsed in buffer PH 6.8, stained with May-Grunwald for 5 minutes and stained with Giemsa for 10 minutes, differentiated in buffer, smears allowed to air dry, cleared and mounted (Drury, 1980).
Staining Assessment:

- Nuclei blue.
- Cytoplasm pink.

The result of staining smears assessed as followed:

Excellent 8-10
Very good 6-8
Satisfactory 4-6
Bad less than 4

3.5 Data Analysis:

Data analysis was done using computer SPSS programme
Result

Twenty specimens of thyroid fine needle aspiration were collected from patients with thyroid disorders. Two different stains included Pap and MGG were applied to each patient samples.

Table (1) showing the result of staining assessment of Pap and MGG stained smears. The excellent stained smears in pap were 6 while 4 in MGG stain, very good stained smears were 7 equal in both Pap and MGG, satisfactory stained smears were 6 equal in both Pap and MGG stains, bad stained smears in pap was 1 compared to 3 in MGG stain.

Table (2) showing the result of staining assessment of wet fixed and air-dried Pap stained smears. The excellent stained smears revealed only in wet fixed smears, very good stained smears in wet fixed were 7 compared to 1 in air-dried smears, satisfactory stained smears in wet fixed were 6 compared to 7 in air-dried smears, bad stained smears in wet fixed was 1 compared to 12 in air-dried smears.

Table (3) showing the result of comparison between Pap and MGG stains. T test used to compare between Pap and MGG and to find out whether the two stains were differed or not. The result produced no statistically significance difference between them (P>0.05).

Table (4) showing the result of comparison between wet fixed and air-dried Pap stained smears. T test was performed to compare between them and find out whether there was difference or not. The result showed statistically significance difference between wet and air-dried Pap stained smears (P<0.05).
Table(1) shows the result of staining assessment of Pap and MGG stained smears.

<table>
<thead>
<tr>
<th>assessment</th>
<th>PAP(W)</th>
<th>DMGG</th>
</tr>
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<tbody>
<tr>
<td>Excellent</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Very good</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Satisfactory</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Bad</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>20</td>
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Table (2) shows the staining assessment of wet fixed and air-dried Pap stained smears.

<table>
<thead>
<tr>
<th>assessment</th>
<th>Pap(w)</th>
<th>Pap(d)</th>
</tr>
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<tbody>
<tr>
<td>Excellent</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Very good</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Satisfactory</td>
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<td>Bad</td>
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</tr>
<tr>
<td>Total</td>
<td>20</td>
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Table (3) shows the result of comparison between Pap and MGG stains

<table>
<thead>
<tr>
<th>Type of Stain</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap (w)</td>
<td>6.55</td>
<td>1.669</td>
<td>0.373</td>
<td>0.292</td>
</tr>
<tr>
<td>MGG</td>
<td>5.95</td>
<td>1.877</td>
<td>0.420</td>
<td></td>
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</table>
Table (4) shows the result of comparison Between wet and air-dried Pap Stained Smears.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap(w)</td>
<td>6.55</td>
<td>1.669</td>
<td>0.373</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pap(d)</td>
<td>2.60</td>
<td>2.280</td>
<td>0.510</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: shows the result of comparison between Pap and MGG stains.
Figure 2: shows the result of comparison of between wet and air-dried Pap stained smears.
Discussion

The choice to select an appropriate stain for FNA smears consider the basic entry to obtain reliable and adequate information result. Further more decrease the rates of false negative and positive diagnoses. There are two factors that affect interpretation of FNA smears the first one is sampling and the second is quality of stains. The most stain used for FNA smears in most laboratories was MGG stain, so evaluation of MGG have been performed compared it to Pap stain. The result of comparison between Pap and MGG stains revealed that there was no significance difference between them and the MGG stain as good as Pap stain. Although staining deposit was frequent in MGG stain, but it was rapid and easily prepared compared to Pap stain. There were many problems associate with Pap stain involved dryness of smears before fixed and preparation of EA50. In addition to thick bloody smears result in uneven staining with dirty back ground. MGG stain used in staining of blood components, it more useful than Pap stain for FNA smears particularly bloody smears. Previous study regarded that the nuclear features of FNA were often better assessed in H&E or Pap stains than Romanowsky stain (Chan et al, 1988). There is disagreement between our result and the above consideration. The result of this study showed that MGG stain gave the same result obtained by using Pap stain. On the other hand rehydration technique introduced to solve the problems of wet fixed Pap stained smears which included ; dryness, erythrocyte interference especially in bloody smears. Air-dried smears rehydrated after 24 hours by using normal saline for 30 seconds result in
less frequent erythrocyte. The result of comparison between wet and air-
dried Pap stained smears revealed that there was significance difference
between them, and wet fixed smears was better than air-dried one.
Previous study found that the result which obtained from air-dried
smears were better than wet fixed smears. The air-dried smears were
easily prepared with out air drying artifact, highly cellular and
erythrocyte interference was less frequent(Shidham et al, 2001).
The quality of rehydrated air-dried Pap stained smears was superior
compared to wet fixed smears, in terms of good nuclear definition and
clean back ground(Jones, 1999).
All the previous studies showed there is disagreement between our result
and the above consideration.
Conclusion and recommendations

From this study we conclude that:

- MGG stain consider as good as Pap stain.
- MGG stain is rapid, easily prepared and can be used for rapid report instead of Pap stain.
- The effect of rehydrated air-dried smears on Pap stain indicates that it can not used as alternative to wet fixed smears.
- Further researches are needed to confirm this work with large samples size.
References


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Proe D. Sub optimal staining, a recipe for disaster. scan. 2000; 11(1).

Shidham VB, Kampalath B, England J. Routine air drying of all smears prepared during fine needle aspiration and intraoperative cytology studies, an opportunity to practice a unified protocol offering the flexibility of choose a variety of staining methods. Acta cytol.2001; 45(1):60-8.

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Appendices

❖ Pap Stain:

1. Harris haematoxylin:
   haematoxylin    1g
   absolute alcohol 10cm³
   ammonium alum 20g
   distilled water 200cm³
   mercuric oxide 0.5g

   Dissolve the haematoxylin in alcohol and add to the alum, previously dissolved in hot water. Bring quickly to boil, then add the mercuric oxide. When the solution will turn dark purple, cool rapidly under the tap. Filter before use.

2. 0.5% Aqueous hydrochloric acid:
   0.5 ml of hydrochloric acid added to 95.5 ml of distilled water.

3. Eosin-Azure 50 (EA50):
   Light green SF (yellowish) 0.1 percent solution in 45 percent ethyl alcohol 45cm³
   • Bismarck brown 0.5 percent solution in 95 percent ethyl alcohol 10cm³
   • Eosin yellowish 0.5 percent in 95 percent ethyl alcohol 45cm³
   • phosphotungestic acid .2g
   • lithium carbonate (aqueous solution) 1 drop

   Mixed respectively.
4. Normal Saline:
   sodium chloride    0.85g
   distilled water    100ml

   **May-Grunwald-Giemsa (MGG):**

   1. phosphate buffer (sørensen):
      - phosphate Na$_2$Hpo$_4$ (mw:142) dissolve 9.465g in distilled water
        and make up to one liter.
      - potassium acid phosphate KH$_2$po$_4$ (mw:136) dissolve 9.08g in
        distilled water and make up to one liter.

      Take equal volume from each solution and mix to give 6.8 pH.

   2. May-Grunwald Stain:
      Weight 0.3g in conical flask, add 100ml of methanol, warm the
      mixture to 50°C. Allow flask to cool to 20°C and shake several times
      during day, after 24hr standing filter solution and then ready to be used.
      Equal parts of May Grunwald and phosphate buffer filter into coplin jar.

   3. Giemsa stain:
      - Weight 1g in conical flask 200 –250ml, add 100ml of methanol,
        warm mixture to 50°C, keep temperature for 15 min with
        occasional shaking and then filter the solution.
      - Giemsa        5cm³
      - phosphate buffer 45cm³

      Mix and filter in coplin jar.