

## Comparison of Leishman Staining With H&E Staining Technique in the Study of FNAC Smears

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### Abstract

**Introduction:** Fine Needle Aspiration Cytology (FNAC) is used widely for the preliminary pre-operative diagnosis of all superficial palpable lesions and for deep seated lesions under ultrasound guidance. Though Pap stain, May GrunwaldGiemsa (MGG) and Haematoxylin and Eosin (H & E) stains are used commonly, Leishman stain provides good cytoplasmic and background details. Hence, it can be used in adjunct with other stains.

**Aims:** The aims of this study are to compare the FNAC smears stained with Leishman with those stained with H&E stain and to analyze the better staining technique for specific smears by comparing the characteristics of nuclear, cytoplasmic and background staining.

**Materials and Methods:** A comparative study was carried out with 200 cases in the surgical OPD, 4 smears were prepared for each case, two stained with Leishman and the other two with H&E stain. A scoring system was created in which 4 grades (1 to 4) were allotted to nucleus, cytoplasm and background staining, and quality index is calculated by dividing the score obtained with the total score possible.

**Results:** H&E stained slides were excellent in nuclear morphology, cytoplasmic color and background while the Leishman stained slides varied in the parameters. Cases of colloid goiter with cystic change (QI=1.0), ganglion (QI=1.0), colloid goiter (QI=0.78), lipoma (QI=0.77) and benign cystic lesion (QI=0.74) were among the top to be viewed well in Leishman stain while that of fibroadenoma (QI=0.53) and fibrocystic disease (QI=0.55) were the ones which failed to give an excellent quality picture.

**Conclusion:** Though Leishman stain has lesser mean quality index compared with H&E stain, it can be combined with other stains thus making the staining procedure simplified and staining time shortened, thereby saving manpower which can prove economical.

**Key words:** FNAC, Leishman stain, H & E stain.

### I. Introduction

Fine Needle Aspiration Cytology (FNAC) is the preferred method of diagnosis and is most often the first investigation for a wide spectrum of lesions. FNAC is widely used today for the preliminary pre-operative diagnosis of all suspicious neoplastic lesions<sup>6</sup>. Though mainly used in malignancies, it is also used in variable inflammatory, infectious and degenerative conditions and in the monitoring of graft rejection<sup>1</sup>. It can also be used for diagnosing many deep seated lesions<sup>7,8,9,10,11,12,13</sup>. Other potential areas where FNAC can be adapted include screening the high risk BRCA1/BRCA2 mutation carrying population for breast pathology<sup>14</sup>.

The drawbacks of FNAC are mainly due to lack of tissue architecture<sup>18</sup>. Repeat aspirations are common, especially in thyroid<sup>15</sup>. The necrosis in tumors obscures the cell details and hence warranting for repeat FNAC<sup>17</sup>. The satisfactory rate can be increased by about 20% by adapting the 3-pass method<sup>16</sup>. Chances for obtaining representative sample are increased to 94% by simple angle modification in experienced hands<sup>8</sup>.

Various stains such as the Romanowsky and Hematoxylin & Eosin are being tried in FNAC<sup>2</sup>. Leishman stain provides superior staining quality in peripheral blood smears<sup>3</sup>, semen smear<sup>4</sup> and in leukemia<sup>23</sup>. Leishman stain can also be used intraoperatively in ovarian neoplasms<sup>24</sup>, in equine tracheal aspirates and bronchoalveolar lavage<sup>20</sup> and in the diagnosis of Pneumocystis carinii infection in AIDS patients<sup>21</sup>. The specific count of cerebrospinal fluid cells can be done employing Leishman stain<sup>22</sup>. The LeishmanGiemsa (LG) cocktail<sup>5</sup> provides

comparable cytoplasmic staining and better nuclear staining to Pap in exfoliative cytology and also has potential application in the screening of oral cancer.

In most cases of cytology, interpretation of the smear becomes difficult due to the blind technique and inadequate sampling. In this scenario, quality of the stain is vital. Leishman has the potential to reduce the cost, the staining time thus saving critical manpower. However, there appears to be very little literature comparing the quality of these two stains. Leishman stain can be of value when the outpatient load is heavy and even in screening intraoperative smears. The study aims at measuring the extent to which this potential can be extracted. FNAC smears stained with Leishman stain and those stained with H&E stains are studied and their characteristics are observed, compared and analyzed.

## **II. Materials And Methodology**

The study was a comparative study conducted in the surgical OP for a period of 2 months-from May 2014 to July 2014. A total of 200 samples were collected for examination and 800 smears were prepared, two with Leishman stain and the other two with H&E stain.

### **Materials used:**

- Sample- An appropriate aspirate obtained by Fine Needle Aspiration
- Fixative- Isopropyl Alcohol
- Stain- Leishman's stain and Hematoxylin&Eosin stain
- Mountant- DPX

### **Sample collection and test procedure:**

The aspirates were obtained from the patients following all the necessary precautions. The lesion is fixed between the thumb and index finger of one hand while the needle with the syringe is introduced with the other. 5mL plastic disposable syringe with 22-27 gauge fine needles were used depending on the lesion. Once arrived at the lesion, suction was applied by moving the syringe. Thus, the tissue was pulled up against the margins of the needle and into it. 4 samples were taken from each patient to enable comparative study.

The material from needle was expelled onto a clean slide and a good thin smear was made on it. Smears were made by placing another slide on top of the material and performing a firm and quick swiping movement. Fixing was done by air drying for the Leishman stain smears while the H&E stain smears were fixed with Isopropyl Alcohol. Then, the smears were stained.

In the case of Leishman staining, the amount of stain sufficient to cover the smear was added. After about 2 minutes, double the amount of buffered water was added and mixed with the stain present. A proper time is elapsed according to the stock used (about 8-10 minutes) and the slide was washed off.

In case of H&E staining, after about 10-15 minutes of fixing with isopropyl alcohol, the slide was stained first with Hematoxylin for 15-20 minutes and then washed. Then, 1% acid alcohol was added for differentiation and the slide washed in running tap water. The slide was then placed in water for 5 minutes for blueing. Finally, the slide was dipped in Eosin for 3-4 dips for counterstaining and washed off.

Hence, we can compare the staining time of both the procedures. It is about 10 to 12 minutes for Leishman stain while it is about 30 to 40 minutes for Hematoxylin and Eosin stain.

The slides were viewed and compared on the basis of nuclear morphology, color of the cytoplasm and the background. In order to enable better understanding of the results, a scoring system was created in which 4 grades were allotted to each parameter.

Parameter	Grade 1 (1 point)	Grade 2 (2 points)	Grade 3 (3 points)	Grade 4 (4 points)
Nuclear Morphology	Not very clear	Satisfactory	Good	Excellent
Color of the cytoplasm	Not very clear	Satisfactory	Good	Excellent
Background	Not very clear	Satisfactory	Good	Excellent

Thus the maximum score a slide can get is 12 including all the 3 parameters. The maximum possible score for the study is arrived at by multiplying the number of cases by 12 for each of the 2 stains.

Also for better comparison of both stains, a 'quality index' was calculated by dividing the actual score obtained by the maximum score possible.

$$\text{Quality index} = \text{Actual score obtained} / \text{Maximum score possible}$$

## **III. Observations And Results**

All the 800 slides were viewed and graded according to the grades mentioned above. Of the 200 samples obtained, 53 were males and 147 were females. Breast lesions were the commonest followed by lymphnode and thyroid lesions (Table 1).

Among the 68 breast lesions, 2 smears were inconclusive with very poor cellularity and hence could not be compared. Of the rest, 12 were suppurative lesions, 6 were granulomatous, 16 were fibroadenoma, 22 fibrocystic disease and 10 were positive for malignancy. The Quality index of Leishman stain for suppurative lesions is 0.72, Granulomatous lesions is 0.66, Fibroadenoma is 0.53, Fibrocystic disease is 0.55, malignancy is 0.71 whereas the quality index for all the lesions in H&E is 1.0 (Table 2).

There were 29 thyroid lesions of which 3 were of very low cellularity and inconclusive hence could not be compared. Of the rest, 16 were colloid goiter, 6 were nodular goiter with cystic change and rest 4 Hashimoto's. The Quality index of Leishman stain for colloid goiter is 0.78, Nodular colloid goiter with cystic change is 1.0, Hashimoto thyroiditis is 0.5 in comparison with the quality index of 1.0 for all lesions in H&E stain (Table 3).

There were 31 cases of lymph nodes of which one had very poor cellularity. As for the rest, there were 14 cases of reactive lymphadenitis and 16 cases of tuberculous lymphadenitis. The quality index of Leishman for cases of reactive lymphadenitis is 0.70 and that for TB lymphadenitis is 0.54 while the mean quality index for lymph node lesions is 0.62 (Table 4).

There were 22 cases of epidermoid cyst for which the quality index of Leishman is 0.70 (Table 5). There were 28 cases of benign lipomatous lesions whose quality index of Leishman is 0.77 (Table 5). There were 12 cases of benign cystic lesions whose quality index of Leishman is 0.74 (Table 5). There were 4 cases of ganglions and all the 4 appeared excellent on Leishman stain. Thus, ganglion in Leishman stain had a quality index of 1 (Table 5).

As far as the other cases are considered, there were 2 cases of pleomorphic adenoma with a mean quality index of 0.83, 1 case of multifocal abscess with a quality index of 1.0 and 3 cases of granulomatous abscess with a mean quality index of 0.75.

Thus, in all 200 cases, the mean quality index of Leishman stain is 0.78 (1872/2400).

#### **IV. Discussion**

Fine Needle Aspiration Cytology is a minimally invasive and cost effective outpatient procedure that is being widely used today. The two basic factors that affect the interpretation of the FNAC smears are sampling and the quality of staining. Sampling improves with the experience of the pathologist while staining depends on the stain, staining technique and the time allotted. Though this study could not prove that Leishman is a better stain than Hematoxylin and Eosin stain, it has pointed out the potential of Leishman in various lesions.

In our study, Leishman stained FNAC smears had an overall mean quality index of 0.78 with the index of 0.7 for nuclear morphology. This is in contrast with the study by Uniya et al on cytologic preparation of bone marrow aspirates where Leishman is found to have a quality index of 0.20 as opposed to the value of 0.12 in Giemsa for nuclear details<sup>3</sup>.

Lesions which have good quality index in Leishman stain include nodular goiter with cystic change (Figure 1& 2) when compared with H&E (Figure 3&4) with QI of 1.0 and colloid goiter (Figure 5&6,) with QI of 0.78. Leishman provides a good picture of colloid and lining follicular cells in these cases, especially a very good background in the cases of colloid goiter. Ganglion is also viewed pretty well in Leishman with an excellent quality index of 1.0 (Figure 7).

Benign lipomatous lesions (QI=0.77) also gave an excellent picture with adipocytes seen clearly. In fact, in certain cases of lipoma in the study where there was slight admixture of blood in the aspirate, Leishman stained slide was found to be better than that of H&E, particularly in visualizing the adipocytes (Figure 8&9).

Benign cystic lesions (QI=0.74) was another high ranking entity with cyst macrophages being visualized clearly (Figure 10&11). At the outset, Leishman slides give a better picture in cystic and hypocellular lesions when compared to the solid lesions. This is in contrast to the study of bone marrow aspirates by Uniya et al where H&E was found to be the better stain for hypocellular aspirates<sup>3</sup>. Epidermoid cysts were also visualized well in Leishman stain (Figure 12&13) with a quality index of 0.7.

However, the less clearly visualized cases in Leishman include that of the breast lesions (mean QI=0.63), especially fibroadenoma (Figure 14&15). This can be contrasted with the study on Breast lumps by Idris et al where Pap was found to be the better stain with a quality index of 0.87<sup>2</sup>. However, in 2 cases of fibrocystic disease of the breast, the Leishman stained slide was better than the H&E stained slide which might most likely be due to the cystic nature of the lesion. However, among the breast lesions, malignancies of ductal origin came out pretty good in Leishman stain (Figure 16&17) with a quality index of 0.71.

Leishman, like other Romanowsky stains is air dried causing cellular swelling and unclear nuclear details. In fact, 4 cases of fibroadenoma in the study were most likely to be diagnosed as proliferative breast disease with atypia on Leishman stain. Though the cell size is increased, the nuclear cytoplasmic ratio is preserved thus preventing misdiagnosis. Similar findings were also reported by Idris et al in their study on breast lumps in Sudanese females<sup>2</sup> and also by Shinde et al<sup>26</sup>.

Lymph nodes were only reasonably good in Leishman stain with Reactive nodes (Figure 18&19) (QI=0.70) better than tuberculous lymph nodes (QI=0.54) as granulomas failed to appear well in Leishman. Even normal cells tend to appear epithelioid in Leishman stain. Also, many instances of reactive lymphadenitis in the study tend to mimic lymphoproliferative changes due to the increased size in Leishman stain. This point should be borne in mind while examining the Leishman stained smears. Thus, pathologists who are used to H&E stain require familiarization with Leishman stain and the various characters observed in it.

## V. Conclusion

Thus, in this study comparing the Leishman and H&E stained fine needle aspiration smears, Leishman was found to have appreciably good staining quality with an overall quality index of 0.78 as opposed to the excellent value of 1.0 of H&E stain. Studies with a cocktail combining Leishman with some other stain, preferably Giemsa might reveal some interesting details and Leishman can be adopted as an effective alternative for FNAC smears. Such an attempt would help in saving time and manpower thus proving economical. The cases of colloid goiter, benign cystic lesions, lipoma, ganglion and epidermoid cyst were well visualized in Leishman while that of breast lesions especially that of fibroadenoma were not as good as their H&E counterpart. In other words, it can be stated that cystic and low cellular lesions are better visualized with Leishman stain

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Lesion	No of cases	Percentage (%)
Breast	68	34
Thyroid	29	14.5
Lymph node	31	15.5
Epidermoid cyst	22	11
Benign cystic lesion	12	6
Ganglion	4	2
Lipoma	28	14
Others	6	3

Table 1: Distribution of the cases

Parameter	Suppurative Breast lesions (12 cases)		Granulomatous Breast lesions (6 cases)		Fibroadenoma (16 cases)		Fibrocystic disease (22 cases)		Malignancy of Breast (10 cases)	
	Leishman	H&E	Leishman	H&E	Leishman	H&E	Leishman	H&E	Leishman	H&E
<b>Nuclear Morphology</b>										
Not very clear	-	-	1	-	4	-	10	-	-	-
Satisfactory	12	-	2	-	12	-	8	-	4	-
Good	6	-	9	-	18	-	-	-	18	-
Excellent	16	48	4	24	-	64	32	88	8	40
<b>Color of the cytoplasm</b>										
Not very clear	-	-	1	-	4	-	10	-	-	-
Satisfactory	12	-	2	-	12	-	8	-	4	-
Good	6	-	9	-	18	-	-	-	18	-
Excellent	16	48	4	24	-	64	32	88	8	40
<b>Background</b>										
Not very clear	-	-	1	-	4	-	14	-	2	-
Satisfactory	8	-	2	-	12	-	-	-	4	-
Good	12	-	9	-	18	-	-	-	12	-
Excellent	16	48	4	24	-	64	32	88	8	40
<b>Actual score obtained</b>	104	144	48	72	102	192	146	264	86	120
<b>Maximum score possible</b>	144	144	72	72	192	192	264	264	120	120
<b>Quality index</b>	<b>0.72</b>	<b>1.0</b>	<b>0.66</b>	<b>1.0</b>	<b>0.53</b>	<b>1.0</b>	<b>0.55</b>	<b>1.0</b>	<b>0.71</b>	<b>1.0</b>

Table2: Score for Breast lesions

Parameter	Colloid goiter (16 cases)		Nodular colloid goiter with cystic change (6 cases)		Hashimoto thyroiditis (4 cases)	
	Leishman	H&E	Leishman	H&E	Leishman	H&E
<b>Nuclear Morphology</b>						
Not very clear	4	-	-	-	2	-
Satisfactory	4	-	-	-	-	-
Good	18	-	-	-	6	-
Excellent	16	64	24	24	-	16
<b>Color of the cytoplasm</b>						
Not very clear	-	-	-	-	2	-
Satisfactory	4	-	-	-	-	-
Good	24	-	-	-	6	-
Excellent	24	64	24	24	-	16
<b>Background</b>						
Not very clear	-	-	-	-	2	-
Satisfactory	2	-	-	-	-	-

Good	6	-	-	-	6	-
Excellent	48	64	24	24	-	16
Actual score obtained	150	192	72	72	24	48
Maximum score possible	192	192	72	72	48	48
Quality index	0.78	1.0	1.0	1.0	0.5	1.0

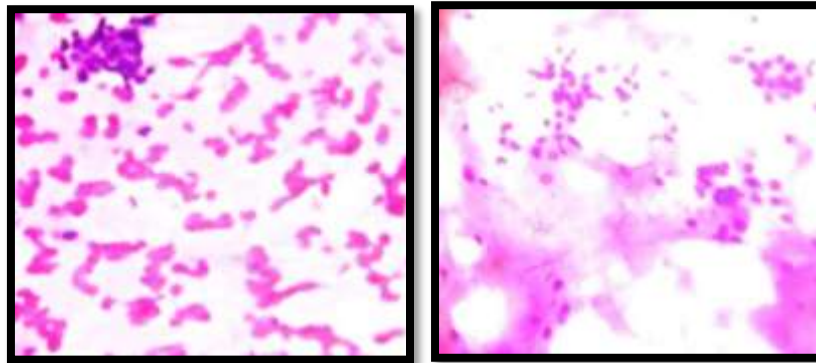
**Table 3: Score for thyroid lesions**

Parameter	Reactive lymphadenitis (14 cases)		TB Lymphadenitis(16 cases)	
	Score			
	Leishman	H&E	Leishman	H&E
<b>Nuclear Morphology</b>				
Not very clear	2	-	4	-
Satisfactory	-	-	8	-
Good	24	-	24	-
Excellent	16	56	-	64
<b>Color of the cytoplasm</b>				
Not very clear	2	-	4	-
Satisfactory	-	-	8	-
Good	24	-	24	-
Excellent	16	56	-	64
<b>Background</b>				
Not very clear	2	-	6	-
Satisfactory	8	-	8	-
Good	24	-	18	-
Excellent	-	56	-	64
Actual score obtained	118	168	104	192
Maximum score possible	168	168	192	192
Quality index	0.70	1.0	0.54	1.0

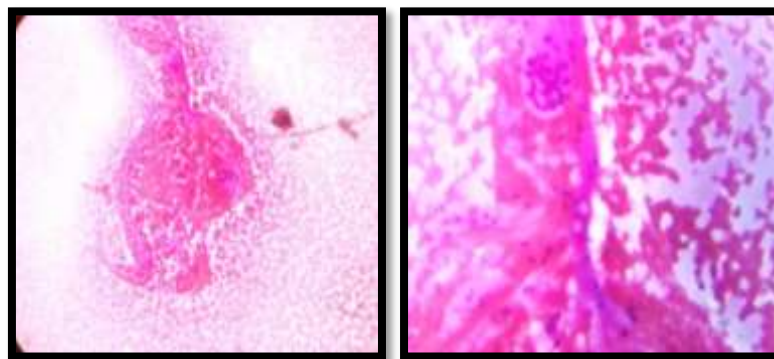
**Table 4: Score for Lymph node lesions**

Parameter	Epidermoid cyst (66 cases)		Lipoma (84 cases)		Benign cystic lesion (36 cases)		Ganglion (12 cases)	
	Score							
	Leishman	H&E	Leishman	H&E	Leishman	H&E	Leishman	H&E
<b>Nuclear Morphology`</b>								
Not very clear	4	-	4	-	-	-	-	-
Satisfactory	8	-	8	-	4	-	-	-
Good	24	-	18	-	12	-	-	-
Excellent	24	88	56	112	24	48	16	16
<b>Color of the cytoplasm</b>								
Not very clear	4	-	4	-	-	-	-	-
Satisfactory	8	-	8	-	4	-	-	-
Good	24	-	18	-	12	-	-	-
Excellent	24	88	56	112	24	48	16	16
<b>Background</b>								
Not very clear	3	-	4	-	4	-	-	-
Satisfactory	6	-	8	-	2	-	-	-
Good	24	-	18	-	12	-	-	-
Excellent	32	88	56	112	8	48	16	16
<b>Actual score obtained</b>	185	264	258	336	106	144	48	48
<b>Maximum score possible</b>	264	264	336	336	144	144	48	48
<b>Quality index</b>	<b>0.70</b>	<b>1.0</b>	<b>0.77</b>	<b>1.0</b>	<b>0.74</b>	<b>1.0</b>	<b>1.0</b>	<b>1.0</b>

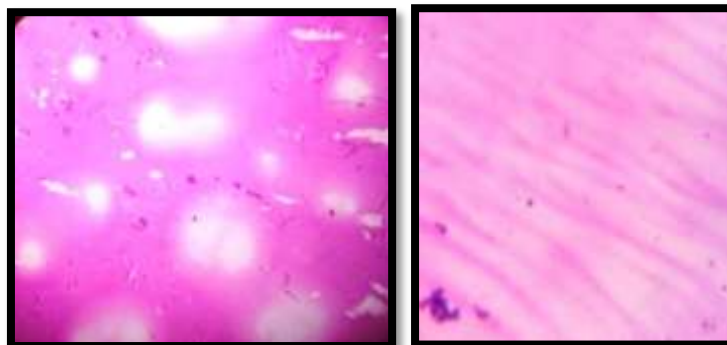
**Table 5: Score for Epidermoid cyst, Lipoma, Benign cystic lesions and Ganglion**



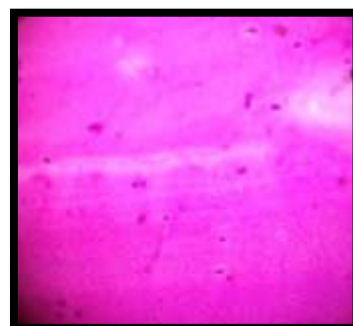
**Figure 1 & 2: Nodular colloid goiter in Leishman stain in low power (a) and high power (b) showing follicular cells and colloid spaces**



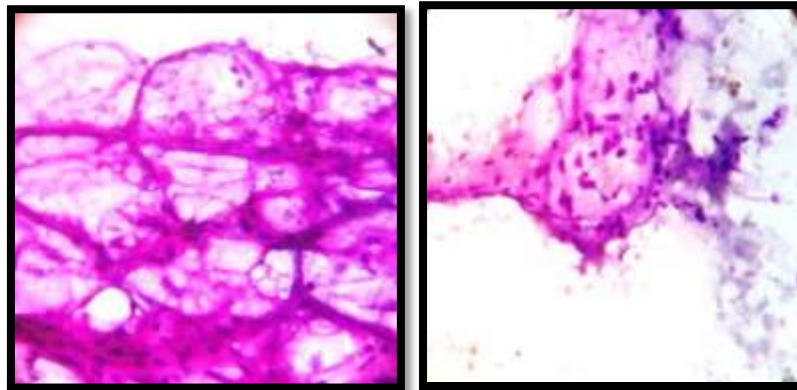
**Figure 3 & 4: Nodular colloid goiter in H&E stain in low power (a) and high power (b)**



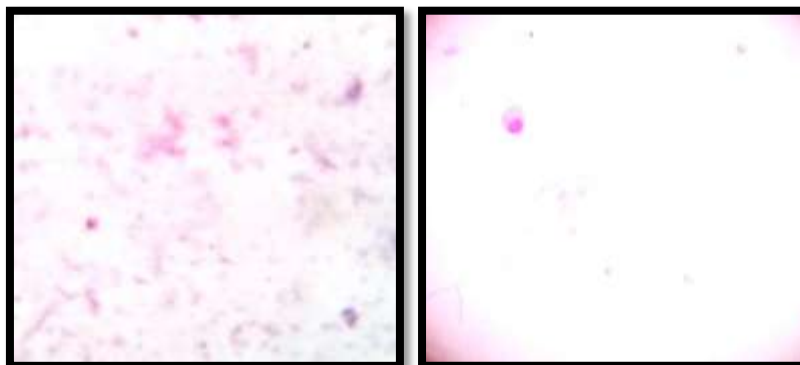
**Figure 5 & 6: Colloid goiter as seen in H&E (a) and Leishman (b) with the colloid background**



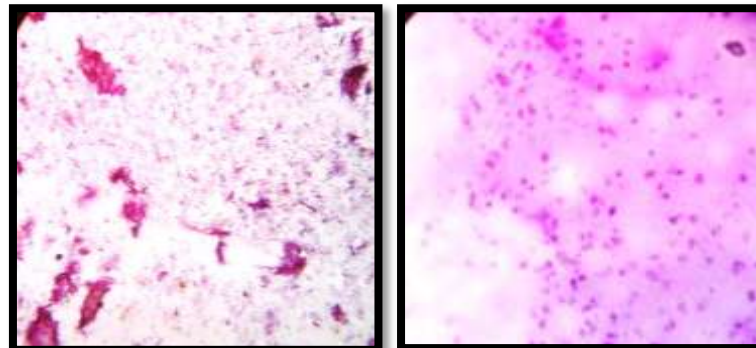
**Figure 7: Ganglion viewed in Leishman stain showing polyhedral cells in a background of myxoidstroma**



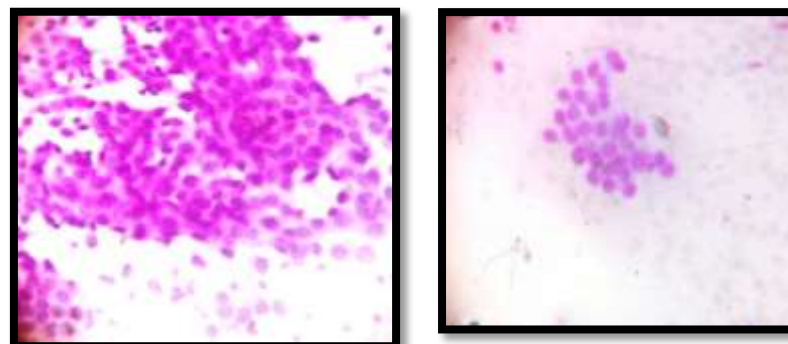
**Figure 8 & 9: Lipoma in H & E (a) and in Leishman stain (b) in high power showing adipocytes**



**Figure 10 & 11: Benign cystic lesion in H&E (a) and Leishman stain (b) both showing cyst macrophages**

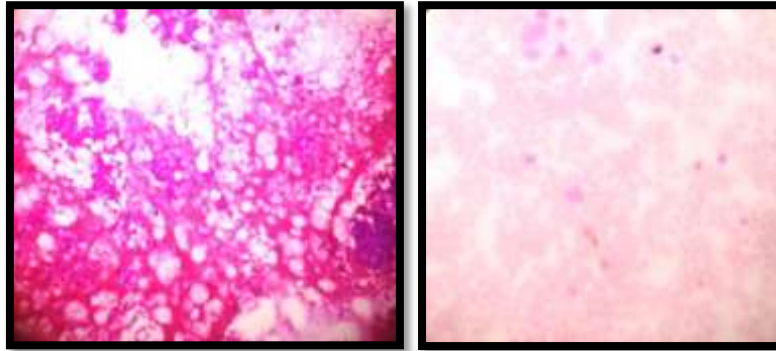


**Figure 12 & 13: Epidermoid cyst as seen in H&E (a) and Leishman stain (b)**

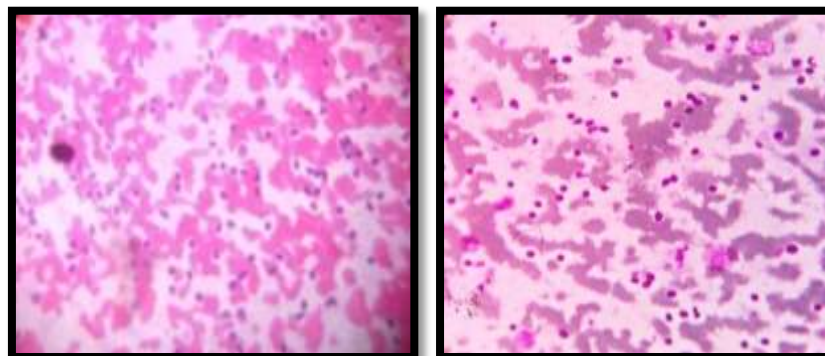


**Figure 14 & 15: H&E (a) and Leishman (b) smears of fibroadenoma on high power**





**Figure 16 & 17: H&E and Leishman smear of breast ductal malignancy in low power**



**Figure 18 & 19: Reactive lymph node as seen in H&E (a) and Leishman stain (b) with lymphocytes in various stages of development**