DOI: 10.7860/JCDR/2022/57944.16834

Original Article

Pathology Section

Formalin Sediment Cytology of Small Biopsy Samples: An Adjunct to Histopathology

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ABSTRACT

Introduction: Cytological evaluation of malignant neoplasms forms an integral part of diagnostic work-up in any malignant disorders. Sediment cytology or more aptly called as biopsy sediment cytology is a technique that involves study of smears prepared from sediment of biopsy specimen fixatives. The preliminary diagnosis on sediment cytology helps in planning and early initiation of treatment.

Aim: To evaluate the cytomorphological features of sediment cytology of small biopsy samples and compare the same with histopathological diagnosis.

Materials and Methods: A cross-sectional study was conducted over a period of two months from July to August 2021 at ESIC Medical College and PGIMSR, Bengaluru, Karnataka, India. A total of 51 samples were included for the study. The sediments of biopsy specimens were collected in a test tube, centrifuged at 2500 rpm for 10 minutes and smears were prepared. Stained smears were evaluated for presence and morphology of cells. The morphology was compared with histopathological diagnosis of biopsy specimens. The concordance rate was assessed by Pearson's correlation coefficient in non neoplastic/benign lesions and malignant lesions separately. A p value of <0.05 was taken to be statistically significant.

Results: Biopsies from gastrointestinal system accounted for the maximum number of 16 (31.4%) cases, followed by biopsies from oral cavity 15 (29.4%) cases and female genital tract 11 (21.5%) cases. Sediment cytology yielded diagnostic material in 46 (90.2%) cases. The diagnostic yield was 90.2% with an overall concordance of 60.8%. The sensitivity, specificity and diagnostic accuracy was 65.12%, 100% and 60.8%, respectively. The concordance rate in non neoplastic/benign lesions was r=0.99, with p=0.0001, whereas with respect to malignant lesions, concordance rate was r=0.86 with p=0.0003.

Conclusion: Cytological evaluation plays an important role in early and effective planning of appropriate treatment. Biopsy sediment cytology although an adjunct to histopathology has practical utility and fulfills the desired role of any cytological sample in providing a provisional diagnosis.

Keywords: Cytomorphological evaluation, Diagnostic yield, Exfoliated cells, Malignant neoplasms, Preliminary diagnosis, Technique

INTRODUCTION

Cytological evaluation of malignant neoplasms forms an integral part of diagnostic work-up in any malignant disorder. Fine Needle Aspiration Cytology (FNAC), fluid cytology, cervical smear cytology, lavage, imprint smears are some of the domains where a rapid cytological diagnosis is possible [1,2]. Sediment cytology or more aptly called as biopsy sediment cytology is a technique that involves study of smears prepared from sediment of biopsy specimen fixatives [3,4]. The fixative in which the biopsy is received contains exfoliated cells from the cut surface of the biopsy specimens. The cytological examination of fixative fluid has distinct advantages, as it is a rich source for cytological material and a preliminary diagnosis can probably be established by the time the final histopathological diagnosis is formulated. Studies have shown that sediment cytology is a good complementary method to histopathology in biopsy material in various lesions of gastrointestinal, breast, cervix, bone, urinary bladder, ovarian neoplasms and oral cavity neoplasms [1,5-9]. Diagnosis on sediment cytology acts as an ancillary presumptive diagnostic test which helps in the formulation of final diagnosis on histopathology. With this background, this study was conducted with an objective to evaluate the cytomorphological features of sediment cytology of small biopsy samples and compare the same with histopathological diagnosis. Hitherto, only a few studies were conducted and that too on specific organ systems, however the present study includes biopsy samples from various organ systems which adds novelty to this study.

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Department of Pathology, ESIC Medical College and PGIMSR, Bengaluru, Karnataka, India. Ethical clearance was obtained from Institutional Ethical Committee (IEC) vide no: 532/L/11/12/Ethics/ESICMC&PGIMSR/EsttVol.IV dated 22.06.2021. Since, this study was done on routine samples, prior informed consent taken at the time of surgical procedure was deemed to be sufficient. The study was conducted over a period of two months from July to August 2021.

Sample size calculation: Based on the laboratory data of the proportion of small biopsy samples in institution, the sample size was calculated with a relative precision of 13%, 95% confidence interval and 80% power. The minimum sample size was found to be 48 for this study. As all the consecutive small biopsy samples received during the study period at the laboratory were included for the study, the final sample size was 51.

Inclusion criteria: As the study involved purposive sampling, all small biopsy specimens received in the Department of Pathology were included in the study.

Exclusion criteria: Small biopsy samples sent without formalin fixative and without clinical details were excluded from the study.

Study Procedure

Upon receipt of the sample in the container with the fixative, the sample was gently shaken to loosen the cells. The sediment was collected in a test tube, centrifuged at 2500 rpm for 10 minutes

and smears were prepared. Smears were stained with Leishman stain and Haematoxylin and Eosin (H&E) stain. Fresh formalin was put in the biopsy container and the biopsy was kept for fixation and further processing as per standard operating protocol of histopathology. Stained smears were evaluated for presence and morphology of cells. Based on the presence of cellular material, cases were categorised as those with diagnostic yield and those with no diagnostic yield. Further based on cellular morphology, the lesions were categorised as non neoplastic/benign and malignant. The morphology of sediment cytology slides was seen by two pathologists who were blinded to clinical details and specimen type (tissue/organ). The morphology was compared with histopathological diagnosis and histopathological diagnosis was considered as gold standard.

STATISTICAL ANALYSIS

Data was entered in Microsoft excel and tabulated. Percentages for each variables were calculated. The diagnostic yield for each of the biopsies of various organs was calculated. Cohen's kappa was used to assess the agreement between the two pathologists with respect to the diagnostic yield and categorisation of cases into benign/non neoplastic and malignant. The Cohen's kappa value with respect to diagnostic yield was k=0.85 (% of agreement=96%); whereas with respect to categorisation of cases into non neoplastic/benign and malignant was k=1 (% of agreement=100%) and k=0.89 (% of agreement=95.8%) respectively. The concordance of findings on sediment cytology with histopathology was calculated organ wise. The concordance rate was assessed by Pearson's correlation coefficient in non neoplastic/benign lesions and malignant lesions separately. The 2 tailed p-value was used to test if the correlation obtained was significant or not. Statplus statistical analysis software for Mac (version 8.0) was used for computation of correlation coefficient and p-value. A p-value of <0.05 was taken to be statistically significant.

RESULTS

This study was conducted on a total of 51 small biopsy specimens. The biopsy samples received from various organs/tissues is detailed in [Table/Fig-1]. Biopsies from gastrointestinal system accounted for the maximum number of 16 (31.4%) cases, followed by biopsies from oral cavity 15 (29.4%) cases and female genital tract 11 (21.5%) cases.

Sl. No.	Organ systems/organs	Number (n)	Percentage (%)
1	**Gastrointestinal system**	16	31.4
	• Oesophagus	02	3.9
	• Stomach	03	5.9
	• Duodenum	04	7.8
	• Colon	06	11.8
	• Rectum	01	2.0
2	**Oral cavity**	15	29.4
3	**Female genital tract**	11	21.5
	• Endometrium	02	3.9
	• Cervix	09	17.6
4	**Breast**	04	7.8
5	**Skin**	03	5.9
6	**Bone**	01	2.0
7	**Prostate**	01	2.0
	Total	51	100%

[Table/Fig-1]: Sites of biopsy of sediment cytology samples.

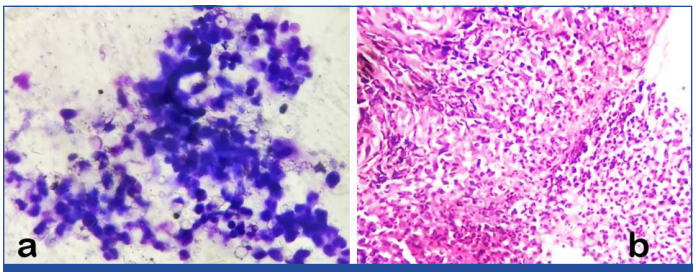
Sediment cytology yielded diagnostic material in 46 (90.2%) cases [Table/Fig-2]. In the samples which did not yield diagnostic material on sediment cytology, two were from oral cavity and one each were from stomach, skin and oesophagus.

With respect to 16 cases from gastrointestinal tract, 14 (87.5%) cases had diagnostic yield. Among them, biopsy samples from

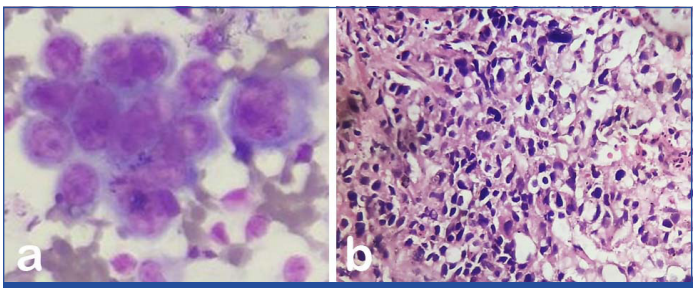
Sl. No.	Organs	Total no. of cases	Diagnostic yield		Non neoplastic/Benign lesions		Malignant lesions	
			Obtained	No yield obtained	Number of cases	SH concordance	Number of cases	SH concordance
			(Percentages mentioned in parenthesis)					
1	Gastrointestinal system	16	14 (87.5)	02 (12.5)	08	7 (87.5)	06	02 (33)
	• Oesophagus	02	01 (50)	01 (6.2)	-	-	01	-
	• Stomach	03	02 (66.7)	01 (6.2)	-	-	02	-
	• Duodenum	04	04 (100)	-	03	03 (100)	01	01 (100)
	• Colon	06	06 (100)	-	05	04 (80)	01	-
	• Rectum	01	01 (100)	-	00	-	01	01 (100)
2	Oral cavity	15	13 (86.7)	02 (13.3)	03	03 (100)	10	04 (40)
3	Female genital system	11	11 (100)	-	09	09 (100)	02	01 (50)
	• Endometrium	02	02 (100)	-	02	02 (100)	00	-
	• Cervix	09	09 (100)	-	07	07 (100)	02	01 (50)
4	Breast	04	04 (100)	-	00	-	04	-
5	Skin	03	02 (66.7)	01 (33.3)	01	01 (100)	01	-
6	Bone	01	01 (100)	-	01	01 (100)	00	-
7	Prostate	01	01 (100)	-	00	-	01	-
	Total	51	46 (90.2)	05 (9.9)	22	21 (95.5)	24	07 (29.1)
	r value				0.99		0.86	
	p value (2-tailed)				0.0001		0.0003	
	Overall concordance	51	46 (90.2)	05 (9.9)	28/46 (60.8%)			
	Diagnostic yield	46/51 (90.2%)						
	Sensitivity	65.12%						
	Specificity	100%						
	Diagnostic accuracy	60.8%						

[Table/Fig-2]: Diagnostic yield and concordance and discordance rate of sediment cytology with histopathology.
*SH: Sediment Cytology-Histopathology

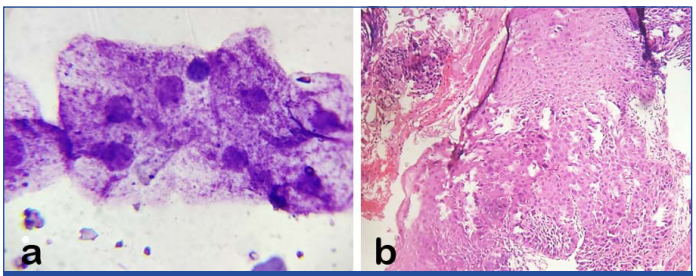
duodenum, colon and rectum yielded 100% diagnostic yield whereas biopsy samples from oesophagus and stomach had a diagnostic yield of 50% and 66.7% respectively. Total 7 (87.5%) out of eight nonneoplastic/benign lesions showed concordance with histopathology. Out of these 7 cases, all 3 (100%) cases of duodenal biopsies and 4 (80%) out of 5 cases of colon biopsies showed concordance with histopathology. The [Table/Fig-3] shows one of the non neoplastic lesions from biopsy of colon showing concordance between sediment cytology and histopathology. Among the 6 malignant lesions of GIT, only two (one each from duodenum and rectum) (33%) showed concordance with histopathology. [Table/Fig-4] shows biopsy of malignant rectal lesion showing concordance between sediment cytology and histopathology. Sediment cytology samples from malignant lesions of stomach, oesophagus and colon did not yield the desired cells. The [Table/Fig-5] shows biopsy from gastric junction. There was a disconcordance between sediment cytology and histopathology.



[Table/Fig-3]: a) Sediment cytology smears from biopsy of colon showing plenty of neutrophils against a background of red blood cells (Leishman, 1000X); b) Histopathology of the same diagnosed as acute suppurative lesion of ascending colon (H&E, 400X).



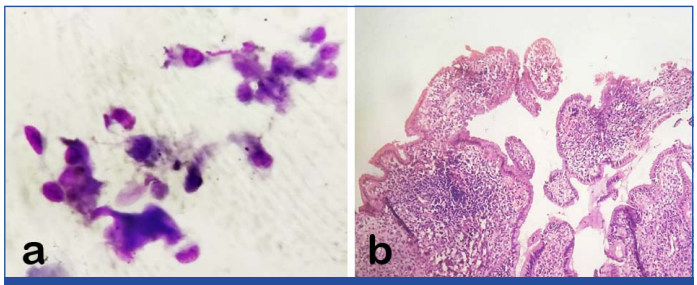
[Table/Fig-4]: a) Sediment cytology smears from biopsy of rectal lesion showing malignant glandular cells (Leishman, 1000X); b) Histopathology of the same showing adenocarcinoma of rectum (H&E, 400X).



[Table/Fig-5]: a) Sediment cytology smears from biopsy of gastric junction showing benign nucleated squamous cells (Leishman, 1000X); b) Histopathology of the same showing squamous cell carcinoma, moderately differentiated type (H&E, 400X). The findings of sediment cytology and histopathology were discordant.

Biopsies from the oral cavity yielded diagnostic material in 13 (86.6%) out of 15 cases on sediment cytology. All 3 (100%) non neoplastic/benign lesions showed concordance with histopathology. Only 4 (40%) out of 10 biopsies with a diagnosis of malignancy showed concordance with histopathology.

All 11 (100%) cases pertaining to female genital tract (nine from cervix and two from endometrium) showed diagnostic yield. All the (100%) non neoplastic/benign conditions on sediment cytology showed concordance with histopathology. The [Table/Fig-6] shows biopsy from cervix of a non neoplastic lesion showing concordance between sediment cytology and histopathology. The concordance with histopathology with respect to malignant lesions was 50%.



[Table/Fig-6]: a) Sediment cytology smears from biopsy of cervix showing endocervical cell clusters (non neoplastic) (Leishman, 1000X); b) The same was diagnosed on histopathology as chronic cervicitis (H&E, 400X).

All 4 (100%) breast lesions did not have any diagnostic yield on cytology but were malignant on histopathology. Biopsies from skin yielded diagnostic material in 2 (66.7%) out of three cases. One non neoplastic/benign lesion showed concordance with histopathological examination whereas one malignant case did not yield malignant cells on sediment cytology.

One case each of bone and prostate biopsy included in the study yielded diagnostic material. The sediment cytology sample from bone biopsy showed concordance with histopathology, whereas the sediment cytology sample of prostate biopsy did not show malignant cells, thereby having a discordance with histopathology.

With an overall diagnostic yield of 90.2% and diagnostic accuracy of 60.8%, the sensitivity was 65.12% and specificity was 100%. The concordance rate assessed by Pearson's correlation coefficient in non neoplastic/benign lesions was r=0.99, with 2-tailed p=0.0001, whereas with respect to malignant lesions, concordance rate was r=0.86 with 2-tailed p=0.0003. In both these categories, sediment cytology findings showed a significant positive correlation with histopathology.

DISCUSSION

Cytological examination and interpretation of exfoliated cells has been a part of diagnostic evaluation since several decades. The variety and type of samples that can be subjected for cytological evaluation have been explored extensively. The common cytological samples that are utilised for cytomorphological evaluation are direct smears on a glass slides, study of centrifuged/cytocentrifuged samples or preparation of cell block from sediment samples [10-13]. Sediment cytology where in the centrifuged sediment deposit is used for cytological evaluation is a well-known cytological evaluation technique in body fluid evaluation and evaluation of fluid filled cystic lesions. The same principle was explored by few authors and sediment from formalin fixative of tissue biopsies was utilised to study the morphology of cells. The present study also explored the utility of this technique, the biopsy fluid cytology, which probably is a rich source for cytological material and offers a distinct advantage, where in a rapid diagnosis can be formulated based on the cytoanalysis of the formalin sediment in both non neoplastic/benign and malignant lesions. The concordance of the same with histopathological diagnosis was evaluated separately in both non neoplastic/benign and malignant lesions. Very few studies have been done to determine the role of sediment cytology as an adjunct to histopathology.

Studies conducted by Chaudhari VV et al., Shahid M et al., Shah S et al., and Nayak R et al., have documented a good over all

Authors name and year	Place of study	Number of cases	Sediment cytology			Histopathology		Concordance of sediment cytology results with histopathology
			B	M	I	B	M	
Shah S et al., [7] (2009)	Aligarh, Uttar Pradesh, India	65	29 (45%)	29 (45%)	07 (11%)	NA	NA	58 (89.2%)
Shahid M et al., [5] (2012)	Aligarh, Uttar Pradesh, India	54	22 (41%)	26 (48%)	06 (11%)	24 (44%)	30 (56%)	44 (81.5%)
Chaudhari VV et al., [4] (2014)	Nasik, Maharashtra, India	20	08 (40%)	09 (45%)	03 (15%)	08 (40%)	12 (60%)	17 (85%)
Nayak R et al., [14] (2017)	Davangere, Karnataka, India	60	38 (63%)	12 (20%)	10 (16.6%)	45 (75%)	15 (25%)	50 (83.3%)
Present study (2022)	Bengaluru, Karnataka, India	51	22 (43%)	24 (47%)	05 (10%)	21 (41%)	07 (14%)	28 (60.8%)

[Table/Fig-7]: Comparison of Diagnostic yield and Concordance of Sediment cytology with histopathology of present study with various studies [4,5,7,14].
*B- Benign, M- Malignant, I- Inconclusive, NA - Data not available

diagnostic yield using this technique [4,5,7,14]. The present study also showed a good diagnostic yield (90.2%), which in itself signifies the utility of this technique. As per the sparse available literature, authors have studied the utility of formalin sediment with a sample size of as low as 20, as in a study by Chaudhari VV et al., to an average sample size of 50-60 as studied by Shahid M et al., Shah S et al., and Nayak R et al., [4,5,7,14]. The proportion of benign cases in studies conducted by Nayak R et al., Chaudhari VV et al., Shah S et al., and Shahid M et al., were in the range of 41-63% [4,5,7,14]. In the present study, the percentage of benign cases were 43%. The proportion of malignant cases in studies conducted by Chaudhari VV et al., Shah S et al., and Shahid M et al., were in the range of 45-48% whereas the proportion of malignant cases in study conducted by Nayak R et al., was 20% [4,5,7,14]. In the present study, the proportion of malignant cases were 47%.

The proportion of sediment cytology cases which were inconclusive have been less in all the studies as documented in the literature, ranging from 11-16%. In the present study, about 10% of cases were found to be inconclusive/inadequate for opinion. The [Table/Fig-7] shows comparison of various studies [4,5,7,14].

Studies conducted by Nayak R et al., Chaudhari VV et al., Shah S et al., and Shahid M et al., had a concordance rate with histopathological diagnosis ranging from 81-89% of cases [4,5,7,14]. In the present study, although the diagnostic yield was high (90.2%), the concordance rate was 60.8%, which was lower compared to studies done by Nayak R et al., Chaudhari VV et al., Shah S et al., and Shahid M et al., [4,5,7,14]. Among the two categories of lesions i.e., non neoplastic/benign versus malignant, benign lesions showed a concordance of 95.5% with a r-value of 0.99. Malignant lesions showed a concordance of 29.1%, with a r-value of 0.86. Both the r-values were statistically significant. The low sediment cytology and histopathology concordance in malignant lesions (29.1%) compared to non neoplastic/benign lesions can be attributed to the sites of the biopsy (oral cavity and breast) and the nature and amount of desmoplastic stroma and presence of inflammatory infiltrate. In these cases, histopathological sections revealed low cellularity and high desmoplastic response.

Aishwarya KP et al., in their study highlighted the utility of sediment cytology and opined that biopsy sediment cytology is a simple and rapid tool for early diagnosis of bone lesions and also acts as a good complementary test to histopathology [6]. Shah S et al., in their study on sediment cytology of bone biopsy specimens concluded that in biopsy specimens like bone, where decalcification is a time-consuming process and often delays the diagnosis, studying sediment cytology is a rapid and effective tool for early diagnosis [7]. Similarly a study by Shahid M et al., on the role of sediment cytology in gastrointestinal lesions, obtained a sensitivity, specificity and diagnostic accuracy of 91.6%, 100% and 88.8% respectively [5]. The current study showed the sensitivity, specificity and diagnostic accuracy of 65.12%, 100% and 60.8% respectively.

Diagnostic yield was 90.2%, which was on higher side, compared to other studies. The reason for good diagnostic yield can be attributed to the technical aspects like gentle shaking of biopsy specimens prior to subjecting the formalin to centrifugation and sectioning of biopsy specimens wherever possible. The time interval between the surgery and receipt of specimen in lab along with prompt initiation of the process of sedimentation was found to be an important factor as more the delay in receipt of specimen in the lab would lead to fixing of cells and thereby causing low diagnostic yield.

Limitation(s)

The significant limitation of the study was sample size. A higher sample size representing all the organ systems/tissues would have probably resulted in an increase in the diagnostic accuracy in the present study.

CONCLUSION(S)

In the current era, where rapid diagnosis is facilitated by various techniques and with aid of various instruments, cytological evaluation plays an important role in effective planning of appropriate treatment. Biopsy sediment cytology although an adjunct to histopathology has practical utility and fulfills the desired role of any cytological sample in providing a provisional diagnosis. Hence, it is advisable to subject formalin sediment from small biopsy specimens for cytoanalysis before heading forth for routine histopathological processing.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 26, 2022
- Manual Googling: Aug 02, 2022
- iThenticate Software: Aug 26, 2022 (8%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **May 22, 2022**
Date of Peer Review: **Jun 22, 2022**
Date of Acceptance: **Aug 13, 2022**
Date of Publishing: **Sep 01, 2022**