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ORIGINAL ARTICLE

Imprint Cytology of Gastrointestinal Endoscopic Tissue Biopsies at Kenyatta National Hospital, East Africa, Kenya

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ABSTRACT

Background: Endoscopic biopsy is the gold standard for the diagnosis of gastrointestinal pathology. However, imprint cytology of endoscopic biopsies which is a rapid and inexpensive method has gained less attention. This study intended to examine the performance of imprint cytology of endoscopic biopsies for rapid diagnosis of malignant gastrointestinal lesions at Kenyatta National Hospital, Kenya.

Methodology: A cross-sectional descriptive study was carried out on 124 consecutive patients in Endoscopy Unit at Kenyatta National Hospital, within a period of 3 months. Endoscopic biopsies were gently rolled on two microscopic slides to make imprint smears prior to formalin fixation. Both slides were air-dried and subsequently stained with Papanicolaou and Giemsa stains. Cytological findings were compared with those of histology to determine the diagnostic performance of imprint cytology in endoscopic specimens. Representative photomicrographs were used to describe and display morphological features.

Results: Imprint cytology revealed that 37 (29.83%) were positive for malignancy. For cases where both histology and imprint cytology were used as diagnostic methods for malignancy detection, the percentage of agreement between the two methods was 94.3% (Kappa =0.857, $P<.001$)

Conclusion: The performance of imprint cytology in this study, underscores the need to embrace the technique in our health care settings as it can provide results in a short period of time for proper patients' management.

BACKGROUND

Globally, over 53.5 million people have cancer of which almost 20 million are new cases, and close to 10 million being cancer deaths that occurred in the year 2022.[1] In Africa, the ratio of cancer deaths is higher than the ratio of cancer incidences (7.2 % versus 5.7 %).[2] The available data show that East Africa had 11.4% cumulative risk of dying from cancer among women in 2018, this is higher than the corresponding risks which is estimated in North America (8.6%), Northern Europe (9.1%), and Australia/New Zealand (8.1%).[3] Cancer is really a challenge for low and middle income countries where there are other competing issues like infections, poverty and hunger.[4] This makes cancer not being the priority number one, yet it is a silent killer.[5]

According to Global Cancer Statistics 2022, gastrointestinal tract (GIT) cancers are among top ten in cancer related deaths worldwide.[1] Colorectal cancer is the second leading cause of cancer death with 904,019 in 2022, stomach ranks fifth with 660,175 whereas esophagus is number seven with 445,391.[1]

In sub-Saharan Africa, data which are specific to gastrointestinal tract cancers are few and mostly not representative, partly due to lack of national cancer registries in many countries, secondary due to underdiagnosis of gastric cancer and lack of endoscopes in different areas.[5,6] In Africa, particularly in Kenya, the diagnosis of gastric cancer is limited to the traditional histology and usually of tumors of progressed stage.[7,8] In Kenya, there were 44,726 new cancer cases and 29,317 cancer deaths in the year 2022.[1] With regards to GIT cancers, esophageal cancer ranks fourth as the most diagnosed cancer with 3,340, colorectal cancer comes fifth with 3,091 while stomach is sixth with 1,899 cases.[1] With projections indicating that low and middle income countries will have 80% or even more of the global cancer burden by 2030,[9] diversifying methods of cancer diagnosis will be vital for case detection and therefore improve on the survival outcome and minimizing incidences.[10]

Diagnosis of gastrointestinal cancers relies upon history, physical examination, endoscopy, radiology, and laboratory features. In anatomic pathology, tissue

diagnosis is the gold standard in this case and relies upon the identification of specific histological patterns, cells, cell products and etiological agents.[11] Cytology has emerged as a valuable diagnostic tool and an adjunct to histopathology. This relies upon the identification of cells, cellular patterns, and cell products such as mucin, and etiological agents. Gastrointestinal cytology is performed on specimens obtained using the following techniques: brush cytology, crush preparation, and endoscopic fine needle aspiration.[11] Touch preparation of endoscopically obtained biopsies can also serve as an adjunct in the diagnosis of gastrointestinal lesions.[12]

The use of imprint cytological preparations in the diagnosis of GIT cancers has the potential for major cost savings. A previous study showed that touch preparation cytology slides were highly cheaper to prepare than histology slides of formalin-fixed tissues.[13] Glass slides and relevant stains are the basic tools needed for touch preparation slides, while various processing equipment are additional requirements for histologic samples.[13] The significantly lower cost for cytological assessment can be a very useful advantage in the provision of health care, particularly in tertiary and remote health care facilities in Africa.

The results of imprint cytology study show that it is an important diagnostic technique with significant diagnostic accuracy. It is easy to perform in limited time and even at centers with low medical facilities while considering African set-up, it can be performed at the level of district hospitals, where many surgeries are being conducted. If a cytologist is available in the hospital, it can be reported in a limited time easily. The lack of artifact imposed by Frozen sections and decreased cost has made imprint cytology to be the most common method of analysis in intraoperative diagnosis of tumor in non-African settings.[14] In another study, it was concluded that touch smear cytology may improve upon pathological diagnosis of malignancies when used in conjunction with biopsy.[15]

Imprint cytology is currently not practiced in the endoscopy clinic in Kenyatta National Hospital, partly because studies documenting the use of touch imprint cytology in the diagnosis of gastrointestinal tract endoscopic biopsies in Africa are scarce. Secondly, according to a recent study, pathology services in Kenya have an estimated 55% gap in pathologist staffing.[16] With a population of over 54 million in 2022,[17] the reports indicate that practicing pathologists in Kenya are in the ratio of 1 to >725,000.[18] In the year 2016, the total case load for pathologists was estimated at 26,472 interpretations (histology, fine needle aspirations and bone marrow aspirations).[16] Assuming that all those interpretations lead to a new cancer case, which is probably not the case, this would have been equivalent to 59.2% of all estimated new cancer cases (44,726) in Kenya for the year 2022.[1] The preliminary assessment of imprint cytology cases can be performed by a trained clinical cytologists who are still few in numbers in African settings particularly in Kenya,[16] and this would ease the pathologists' workload. Therefore, this study intended to evaluate the diagnostic performance of imprint cytology on gastrointestinal endoscopic biopsies at the endoscopy unit of a referral hospital in Kenya in order to provide grounds for improving the cancer detection rate by training more clinical cytologists who would work hand in hand with pathologists.

MATERIALS AND METHODS

Study Setting

The present study was carried out at the endoscopy unit of Kenyatta National Hospital (KNH), Nairobi, Kenya. The endoscopy unit is located at clinic 23. It opens every day from Monday to Friday and serves an average number of 13 patients per day. In this unit, diagnostic and therapeutic endoscopies are performed for upper and lower gastrointestinal tract. Endoscopic biopsies are taken by consultant gastroenterologists assisted with nurses. Endoscopic biopsies are normally fixed in formalin and taken to the histology laboratory for processing.

Study Design and Sampling

A cross-sectional descriptive study was carried out from October 2015 to December 2015. Using a convenient sampling method, the study was carried out on a total number of 124 patients (67 females and 57 males) who were consecutively received at the endoscopic clinic of the Kenyatta National Hospital, Kenya. This study was carried out on patients whom endoscopy of the esophagus, stomach, duodenum, and colon was indicated. During the examination, endoscopy biopsies were taken from all patients referred to endoscopy clinic and who appeared to have GIT lesions upon examination.

Sample Size Estimation

The number of samples (n = 124) for this study was calculated using the prevalence of gastrointestinal malignancy of 8.8% obtained in the study done in Lusaka-Zambia from 2,132 upper gastrointestinal tract endoscopic records examined in the year between 1999-2005.[19] The sample size was calculated using the Fisher's formula:

Sample size (n) = [DEFF × Np (1 - p)] / [(d^2 / $Z^2_{1-\alpha/2}$x (N - 1) + p (1 - p)].

In the formula: "n" = sample size, "N" is an estimate of patients' size served by Kenyatta National Hospital per month that corresponds to 120,816. As for "*P*" is the known prevalence, "Z" is the normal standard deviate that correspond to 95% confidence interval, "d" is the margin of error for degree of precision set at +/- 5% and DEFF is the design effect which equals to 1.

Data Collection and Laboratory Analysis

Specimen Collection Procedures

Cytological slides were given identification numbers before sample collection. From biopsies taken by the physician, a minimum of 2 imprint smears were prepared by the cytologist and the assisting nurse in theatre, this was done from fresh biopsy by rolling the tissue on glass slides using a needle while applying a gentle pressure; both smears were air-dried; one smear was rehydrated in 0.9% ethanol for 3 minutes, fixed in 95% ethanol and stained with Papanicolaou stain. The second slide was stained with Giemsa stain for *Helicobacter pylori* (HP) detection. Tissue biopsies were fixed in 10% formalin and processed in the usual manner for histological diagnosis. Samples were processed from the University of Nairobi's anatomic pathology core laboratory.

Cytopathology Evaluation of Specimen

Imprint smear preparation for GIT lesions was previously described by different authors.[12,15] Microscopic examination was done by the clinical cytologist and the pathologist. Discrepant findings were evaluated by a third pathologist. Histology sections were reported by pathologists blinded to the findings of imprint cytology. Cytology results were either interpreted as positive, suspicious, and negative for malignancy. Cytology slides with atypical cells, suspicious but not confirmatory for malignancy would be classified as suspicious for malignancy. Cytology slides with unequivocally negative or atypical cells consistent with an inflammatory or reparative process were considered negative. On histology, lesions were categorized as negative for any pathology, dysplasia and positive for malignancy.

Diagnostic Performance of Imprint Cytology

In this study, histology was considered as a gold standard for imprint cytology. In all instances, cytology specimens were first assessed by the trained clinical cytologist and the final report was made by consultant pathologists. In case of discrepant findings, a third examiner (pathologist) was required in order to reach a consensus. Histology sections were reported by consultant pathologists blinded to the findings of imprint cytology and later the results were shared to assess the level of agreement between imprint cytology and histology reports. Using a computer software [Statistical analysis was performed by using SPSS version 23.0 software (SPSS, Inc., Chicago, IL, USA)], a 2 × 2 contingency table was used to determine sensitivity and specificity of imprint cytology compared to histology. During the process, all positive smears and 10% of randomly selected negatives smears, were re-examined by an independent pathologist for quality assurance purposes.

Data Analysis

Statistical analysis was performed by using SPSS version 23.0 software (SPSS, Inc., Chicago, IL, USA). A 2x2 contingency table was used to determine the sensitivity, specificity, Negative Predictive Values, Positive Predictive Values, and the overall accuracy of imprint cytology compared to histology. *Kappa* statistics test was used to calculate the degree of agreement between the two diagnostic methods.

Ethical Considerations

Before commencement of the study, ethical clearance was obtained from KNH/UoN Ethics and Research Committee; and permission to conduct research in the unit was sought from the management of endoscopic clinic at Kenyatta National Hospital. Written informed consent was obtained from all the participants in the study. The physician took consent for the study at the same time of taking consent for the endoscopy procedure. All tissue biopsy samples were carefully used to make imprints smears to avoid risks such as crush artifacts that can be caused by repeat of procedure. Patient privacy and confidentiality was strictly observed, in place of names, unique identification numbers were used. All results of imprint cytology were communicated to the attending physician. The study did not involve any extra procedure to obtain a separate sample other than the endoscopy already planned, therefore no added risk or harm from the study was foreseen. All data collected in hard copy was kept in a lockable cabinet where the researcher only could access to maintain confidentiality. Information stored in soft copies was protected from access from unauthorized persons by password which was being changed periodically.

RESULTS

Clinicopathological Characteristics of Study Participants

The present study was carried out on 124 participants, with age between 20 and 87 years. The overall mean age was 55.2 ± 15.2. Our study participants were mainly female accounting for 67 (54.03%), males were 57 (45.96%). With regard to admission status, 76 (61.3%) were outpatients. Patients presented with a wide range of symptoms including dysphagia 37 (29.8%), anaemia 14 (11.3%), upper GI bleeding 14 (11.3%), epigastric pain 11 (8.69%) and others.

The most common site of endoscopic biopsies was the stomach with 84 (67.7%) followed by oesophagus with 31 (25.0%) cases. At least two biopsies were taken from each patient with the maximum number of biopsies being eight. The assessment of *Helicobacter pylori* (HP) revealed a positivity of 29 (23.4%) while HP status from 19 (15.3%) patients was not available.

The histology results from 105 patients revealed that 29 (27.6%) were positive for malignancy, of those 18 cases were from the oesophagus while 11 cases were from the stomach. No positive case was noted from the duodenum and the colon (Table 1).

TABLE 1: Clinicopathological Characteristics of Study Participants

Clinicopathological characteristics (N=124)	Number of cases	Percent
Mean age (years)		
Mean ± SD	52.2 ±15.2	
Min-Max	20-87	
Age group		
<45	35	28.2
45-54	40	32.3
55-64	26	21.0
65-74	12	9.7
75-	11	8.9
Sex		
Male	57	46.0
Female	67	54.0
Admission status		
Inpatient	48	38.7
Outpatient	76	61.3
Anatomic site		
Esophagus	31	25.0
Stomach	84	67.7
Duodenum	8	6.5
Colon	1	0.8
Clinical diagnosis		
Abdominal pain	4	3.2

Continue

TABLE 1: Continued

Clinicopathological characteristics (N=124)	Number of cases	Percent
Abdominal tumour	4	3.2
Anemia	14	11.3
Dyspepsia	7	5.6
Dysphagia	37	29.8
Epigastric pain	11	8.9
Missing information	4	3.2
Upper GI bleeding	14	11.3
Vomiting	4	3.2
others	25	20.2
Number of biopsies per patient		
Min-Max	2-8	
Mean	3.5±1	
Helicobacter pylori status		
Negative	76	61.3
Positive	29	23.4
Missing data	19	15.3
Histology report		
Negative for Malignancy	76	61.3
Positive for Malignancy	29	23.4
Missing data	19	15.3

SD: Standard Deviation, Min-Max: Minimum-Maximum, GI: Gastro-intestinal

On imprint cytology, with the same number of patients (105), of the 29 cases diagnosed positive for malignancy on histology 3 of them turned negative on imprint cytology, leaving the total number of concordant cases to 26. However, the positivity rate was similar to that of histology 29 (27.6%), implying that, there was 3 cases that were negative of histology that turned positive on imprint cytology (Table 2, Table 3 and Figure 1).

If we consider the histology method as the gold standard (revealing the true status of the disease), the 3 cases that turned positive on cytology while negative on histology can be considered as false negative, while those turned negative on cytology while positive on histology are considered as false negative. The overall performance of these diagnostic methods has been explained in the next sections. Beside 105 cases where both histology and cytology reports were available, there are 19 cases of which only cytology results were available during data compilation. These results reveal that 8 (42.1%) were positive for malignancy while 11 (57.9) cases were negative for malignancy (Table 2 and Figure 1).

Overall, if we consider results from cases that were analysed on both histology and cytology (105/124, 29 positive), and cases (19/124, 8 positive) of which only cytology results were available, the positivity rate for cytology was 37 (29.8%).

The Diagnostic Performance of Imprint Cytology

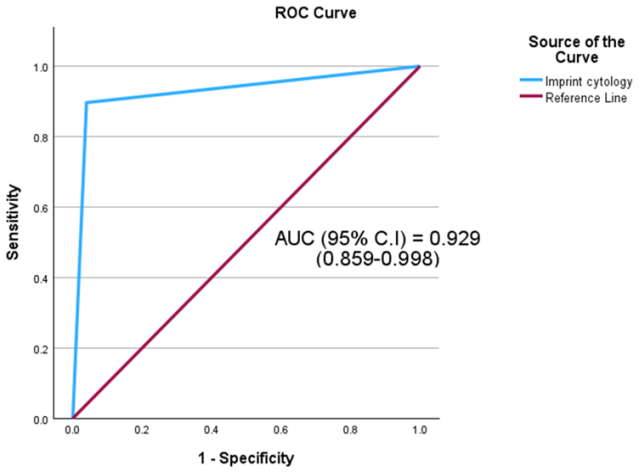
On a total number of 124 study participants, cases with available reports on both histology and cytology were 105. Thus, the results of 105 imprint cytology cases were compared against the same cases as far as histology reports were concerned. In this process, histology method was considered as the gold standard. Regarding the presence or absence of malignancy, the total number of cases that were concordantly reported by both methods is 26 for positive cases and 73 for negative cases. This brings the total number of cases agreed on to 99 (94.3%), an agreement estimated as 'almost perfect'[20,21] by Cohen's *Kappa* value = 0.857 (*P*=.001) (Table 3). When the findings were plotted and the curve generated, the Area Under the Curve (AUC) of the Receiver Operator Characteristic (ROC) for Imprint Cytology was estimated as excellent,[22] (AUC=0.929; 95% CI 0.859-0.998) (Figure 2).

Both Kappa and AUC values underscore the reliability of imprint cytology of the GI tact as a diagnostic tool. Regarding the operational characteristics of the imprint cytology, the present study revealed the sensitivity of imprint cytology as 89.7% and the specificity of 96.1% (Table 4).

TABLE 4: Diagnostic Performance of Imprint Cytology

Operational characteristics	Value (%)
Sensitivity (95% CI)	89.7% (75.3-97.3)
Specificity (95% CI)	96.1% (90.1-99.0)
Positive predictive value (95% CI)	89.7% (75.3-97.3)
Negative predictive value (95% CI)	96.1% (90.1-99.0)
Measure of agreement	85.7
Overall accuracy (efficiency)	94.3

FIGURE 2: Receiver Operator Characteristics for Imprint Cytology



The blue line represents the imprint cytology performance compared to Histology performance. Area under the blue line (Area Under the Curve) represent the capacity for imprint cytology to distinguish the negative cases from positive cases in keeping with histology results, in this case AUC =.929 (excellent).

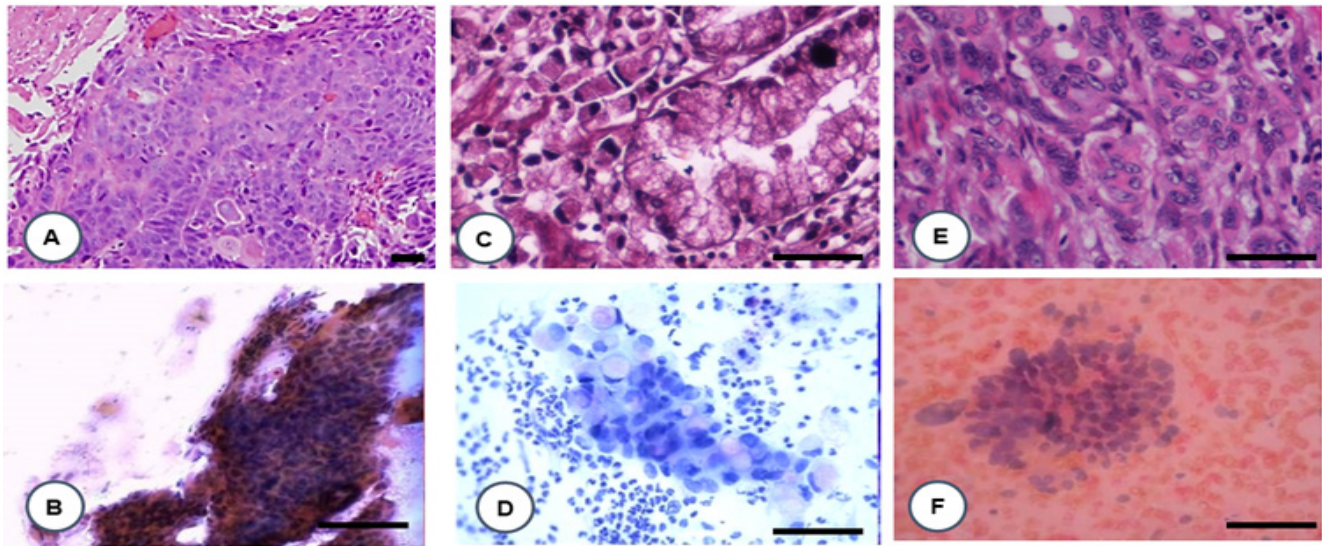
TABLE 2: Histology and Imprint Cytology Results According to Anatomic Site

Diagnostic test	Total	Anatomic site n (%) Oesophagus	Stomach	Duodenum	Colon
Cases with histology and cytology reports (n = 105)					
Histology					
Negative for Malignancy	76 (72.4)	8 (33.3)	60 (82.2)	7 (100)	1(100)
Positive for Malignancy	29 (27.6)	16 (66.7)	13 (17.8)	0 (0)	0(0)
Imprint cytology (n =105)					
Negative for malignancy	76 (72.4)	6 (25)	62 (84.9)	7 (100)	1(100)
Positive for malignancy	29 (27.6)	18 (75)	11 (15.1)	0 (0)	0(0)
Cases with no histology report (n =19)					
Imprint cytology					
Negative for malignancy	11 (57.9)	0 (0)	10 (90.9)	1 (100)	0(0)
Positive for malignancy	8 (42.1)	7 (100)	1 (9.1)	0 (0)	0(0)

TABLE 3: Agreement Between Cytology and Histology Findings (n=105)

	Histology report Positive	Negative	Total	Kappa value
Imprint cytology				
Positive	26	3	29	0.857 ($p < .001$)
Negative	3	73	76	
Total	29	76	105	

FIGURE 1: Photomicrographs of: A-B: Squamous cell carcinoma



Photomicrographs of: A-B: Squamous cell carcinoma, site: Oesophagus, A: H&E-stained section with neoplastic squamous cells arranged in a sheet with less orderly growth (10× objective), B: Sheet of pleomorphic squamous cells with orangeophilic cytoplasm (40× objective, Site: Oesophagus). C-D: Adenocarcinoma, Site: Stomach C: H&E-stained section composed of signet-ring cells D: Pap-stained smear showing signet-ring cells (40× objective). E-F: Adenocarcinoma, Site: Stomach, E: H&E-stained section composed of neoplastic cells with vesicular nuclei, F: Cluster of tumour cells with feathering and gland opening (40× objective, Site: Stomach). Scale bar =100µm

DISCUSSION

In the present study, 94.3% of cases were diagnosed concordantly between histology and imprint cytology. The measure of agreement between the two methods was estimated as almost perfect by Cohen's *Kappa* (0.857, *P*<.001). The results corroborate the views of previous researchers who claim that imprint cytology is an invaluable adjunct to histology[12] which remains the gold standard for the detection of gastrointestinal malignancy.[12] In sub-Saharan Africa, the use of imprint cytology in the diagnosis of GIT lesions is not well documented but there are some academic works which are publicly available online (digital repositories) which highlight the use of touch imprint cytology in the diagnosis of malignancy in the oral cavity,[23] head and neck lesions[24] or prostatic gland[25] in Kenya.

In Egypt, the use of touch imprint cytology was evaluated in ovarian tumors.[26] The performance of imprint cytology in the above studies is in agreement with this study which reported the sensitivity of 89.7% and specificity of 96.1%. For example, the sensitivity of cytology in the diagnosis of squamous cell carcinoma in the oral cavity was 81.5% while the specificity was 100%.[23] In the study about head and neck lesions,[24] the performance was as follow: the sensitivity =94.9%, specificity = 88.5% and the overall accuracy =92.1%. While histology remains the method of choice with regard to cancer diagnosis, these findings support the notion that cytology can be an alternative to histology in low income settings countries.[27] Elsewhere, the study of Dhakhwa *et al* showed a higher sensitivity and specificity of 91.6% and 100% respectively.[12] The difference with our study (sensitivity =89.7%, specificity =96.1%) might be due to some cases that were reported negative for malignancy on cytology and later reported as positive on histology. However, in the same study, Dhakhwa *et al* showed that cytology may diagnose malignancy in cases which were initially negative on histology, this was proven by a repeat of the biopsy.[12] In such cases, "there must be unequivocally malignant cells in the touch smear,"[12] the author said.

Small clusters of malignant cells may also be missed when a conclusive tissue pattern is lacking on histology.[12] These and other similar few cases contributed to the slight differences in performance compared to the above-mentioned study. In cases with positive cytology and negative histology, it was however recommended that a repeat biopsy be done or correlate with clinical and endoscopic findings to confirm the diagnosis.

CONCLUSION

Histology gives a definite diagnosis and has a high success rate but is dependent upon processing techniques and long-time involved in these techniques. And for any lesion, surgeons want to have the definitive diagnosis which helps in planning for surgery and patient counselling. Thus, touch imprint can help to assess the quality of the biopsy taken as it can provide results intraoperatively without waiting for histopathology results. This may even enable early planning of further course of action by the clinician and help the patient by avoiding repeated procedures that may be required in case of inadequate biopsies.[15] Also, in cases where surgeons might need to provisionally report a case as positive or negative for malignancy in a short period of time with minimum additional effort, touch imprint may help.[33] The touch preparations can have impact on the initial cost but also will have reduced other additional costs resulting from repeated procedures for inadequate biopsy. It will also reduce travel or transport costs to the patients due to repeated procedures.

STUDY LIMITATIONS

Although imprint cytology has shown a good performance for detecting malignancy, benign lesions, it was unable to diagnose some benign lesions such as polyp, atrophy, oedema, and foveolar hyperplasia which were diagnosed by histology. This is due to the nature of these lesions; it has been shown that lesions such as hyperplastic polyps cannot be recognized using cytology[31]. The colonoscopy was not being fully performed at the time of sample collection; hence only one sample was obtained. The fact that all histological reports were not available for comparison with all cytological results at the time of data analysis is another limitation that we think might have influenced the findings in one way or another. Some tissue biopsies were reported from private laboratories and therefore could not be traced for review.

RECOMMENDATIONS

More studies are required to explore the potential of imprint cytology in diagnosis of various lesions in GIT as well as in other tissues, especially using a large number of participants. The role of imprint cytology in tumour typing is not also well known, further researches are recommended. Moreover, increasing the number of clinical cytologists through well designed training can have a crucial role in alleviating the case load to pathologists.

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