INNOVATIVE TOOLS AND METHODS

Minimally invasive esophageal sponge cytology sampling is feasible in a Tanzanian community setting

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Abstract
Esophageal sponge cytology is an endoscopy alternative well accepted by patients with extensive data for accuracy in the context of adenocarcinoma. Few studies have assessed its feasibility in asymptomatic community members, and fewer still in East Africa, where esophageal squamous cell carcinoma (ESCC) rates are high. We aimed to assess the feasibility of a capsule-based diagnosis of esophageal squamous dysplasia (ESD), an ESCC precursor, which may benefit epidemiological and early detection research. We collected Cytosponge collections in 102 asymptomatic adults from Kilimanjaro, Tanzania. Uptake, acceptability and safety were assessed. Participants scored acceptability immediately following the procedure and 7 days later on a scale of 0 (least) to 10 (most acceptable). Slides from paraffin-embedded cell clots were read by two pathologists for ESD and other pathologies. All participants (52 men, 50 women, aged 30-77) swallowed the device at first attempt, 100 (98%) of which gave slides of adequate cellularity. Acceptability scores were 10 (53%), 9 (24%), 8 (21%), 7 (2%) and 6 (1%), with no differences by age, sex or time of asking. Cytological findings were esophageal inflammation (4%), atypical squamous cells of uncertain significance (1%), low-grade dysplasia (1%), gastritis (22%) and suspected intestinal metaplasia (6%). Setting-specific logistical and ethical considerations of study implementation are discussed. We demonstrate the safety, acceptability and feasibility of Cytosponge sampling in this setting, paving the way for innovative etiology and early-detection research. Targeted sampling strategies and biomarker development will underpin the success of such initiatives. The study protocol is registered on ClinicalTrials.gov (NCT04090554).

Abbreviations:
ASCUS, atypical squamous cells of unknown significance; EAC, esophageal adenocarcinoma; EC, esophageal cancer; ESD, esophageal squamous dysplasia; ESCC, esophageal squamous cell carcinoma; LSIL, low-grade squamous intraepithelial lesion; IARC, International Agency for Research on Cancer; NIMR, National Institute of Medical Ethics
1 | INTRODUCTION

Esophageal cancer (EC) was the seventh commonest cancer worldwide with the sixth highest number of cancer deaths in 2018. Esophageal squamous cell carcinoma (ESCC) accounted for ~84% of this estimated burden, exhibiting high incidence in East Africa along an apparent “corridor” spanning from Ethiopia in the north to the Eastern Cape of South Africa. In Tanzania, there were an estimated 2500 new EC cases and deaths in 2018. Despite documentation of this African ESCC corridor as early as the 1960s, its etiology is not fully understood. Poor prognosis of ESCC in East Africa necessitates etiological and early detection research.

Esophageal squamous dysplasia (ESD) is an established precursor to ESCC, with effective endoscopic therapies capable of definitive treatment at a preclinical stage and making ESD an early detection target. Additionally, ESD etiology partially overlaps with ESCC etiology, and studies of ESD determinants may inform ESCC etiology. Such studies are possible in areas or population subgroups where ESCC incidence rates and thus ESD population prevalence is high. In one of the few studies to investigate the prevalence of ESD in an East African community, an endoscopy survey of asymptomatic over 20 year olds in Bomet, Kenya, found that 15% had ESD (any grade), indicating that similarly high prevalence may occur in other East African settings.

ESD is diagnosed by endoscopy with Lugol’s iodine and morphological confirmation of biopsied lesions. Endoscopy is invasive and costly, which has led to the search for affordable less-invasive alternatives. A similar need for nonendoscopic diagnosis applies to Barrett’s esophagus, a precursor to esophageal adenocarcinoma (EAC). The Cytosponge is one alternative developed for Barrett’s by the Fitzgerald Lab at the UK MRC Cancer Unit. This “pill-on-a-string” is swallowed and dissolves to release a sponge, which, when retrieved, harvests cells from the esophagus. The cells are processed into a paraffin block to create a pseudobiopsy to ease pathological analysis. Biomarkers have been developed for Barrett’s esophagus, including an immunohistochemical biomarker (TFF3) and atypia, p53 and copy number assays for risk stratification. This approach is also needed for ESD, though data on the diagnostic accuracy using the Cytosponge for ESD are limited. A study among an asymptomatic Iranian population found 61% sensitivity for any grade (n = 18) and 100% sensitivity for high grade (n = 2) ESD.

In the United Kingdom, the device has been shown to be safe and acceptable to adult patients, with only three adverse events (two sponge detachments and a minor bleed) out of 4422 procedures, a 92% swallow success and higher acceptability than endoscopy without sedation. No data exist on the feasibility of using the Cytosponge within the Tanzanian healthcare and laboratory infrastructure, or its acceptability and safety among asymptomatic adults locally.

The simplicity and inexpensiveness of the Cytosponge make it an attractive tool for resource-limited settings with high ESCC incidence.

What’s new?

Endoscopy is often the standard method for diagnosing esophageal squamous cell carcinoma (ESCC). However, in resource-limited regions, lower-cost alternatives are urgently needed, especially for early-detection screening programs. Esophageal sponge cytology is one such alternative, but would it be useful for large-scale screening? In this preliminary study, the authors tested this “pill-on-a-string” approach in a region of Tanzania with high ESCC incidence. They found that the method is acceptable, safe, and feasible for screening asymptomatic community members in a high-risk region. These results will aid in the design of novel aetiology, biomarker, and early-detection research studies.

Recognizing this potential, we aimed to evaluate the feasibility of Cytosponge collections in the community setting of Kilimanjaro by assessing: (a) willingness to participate and device acceptability, including the ability to swallow the device; (b) device safety; (c) sample processing quality in the context of laboratory infrastructure; and (d) the sample prevalence of Cytosponge morphologically detected ESD and esophageal pathologies, with post hoc gastric findings also reported. We also summarize key logistical, ethical and clinical considerations in the setup of this de novo field study.

2 | MATERIALS AND METHODS

2.1 | Study design

We conducted a feasibility study of administering the Cytosponge in a target of 100 age- and sex-stratified asymptomatic community participants and volunteers in Tanzania, and assessed device acceptability immediately after sponge administration and 7 days later. The study was conducted at the Kilimanjaro Clinical Research Institute (KCRI) Majengo Unit, a clinical research unit in an urban suburb of Moshi, Kilimanjaro. This is within the catchment area of a completed ESCC case-control study, part of the Esophageal Squamous Cell Carcinoma African PrEvention research (ESCCAPE; http://escape.iarc.fr) project. Majengo was selected owing to the need for emergency access to endoscopy in this pilot phase, for example, to remove the sponge endoscopically in the event of a detachment or to treat variceal bleeding given the high incidence of liver disease in this population. The site was equipped with a backup generator to power refrigerators during frequent power cuts. Private rooms for Cytosponge procedures and interviews, and participant waiting areas were designated, and a full-time study nurse (AD) and research assistant (TN) were employed.
2.2 | Participant recruitment

Cytosponge appointments were conducted from September to December 2019. Inclusion criteria were adult (≥30 years) residents of Kilimanjaro District, approached ad hoc, informed of the study and invited to participate. Other participants volunteered for the study having heard about it. As this was the first use of the Cytosponge in Africa, the study was based on a small community sample but did not employ population-based random sampling. The target age and sex distribution were 1:1 male:female ratio within each age band, where 20% were 30 to 39 years; 20% 40 to 49 years; 30% 50 to 59 years and 30% were 60 years and older. The following exclusion criteria were employed: food/drink within previous 4 hours; pregnancy; dysphagia; self-reported history of oropharyngeal/esophageal/gastric cancer; prior esophageal surgery; esophageal varices; stricture or requiring esophageal dilation; self-reported liver cirrhosis; history of hematemesis; recent use of anticoagulants; cardiac event within previous 6 months; unable to provide informed consent. Participants were reimbursed 20 000 TSh (~$8.70 USD).

2.3 | Questionnaire data

Participants underwent an interviewer-administered questionnaire before Cytosponge collection. Data were collected on age; sex; participation motive; ethnicity; HIV status and therapy; residential location; education level; religion; EC family history; EC history of friends/neighbors/colleagues; alcohol consumption (ever/never), consumption (ever/never) of strong alcohol (ie, >30% alcohol by volume [ABV]); smoked tobacco use (ever/never) and smokeless tobacco use (ever/never). Data were collected using REDCap tools hosted at the International Agency for Research on Cancer (IARC).

2.4 | Cytosponge procedure

One week of on-site training was delivered by a senior research nurse (IDB) in the Cytosponge (Europlaz Technologies, UK) standard operating procedure (SOP). After inspection for defects, the capsule was placed toward the back of the tongue while the string was held by the participant. The capsule was swallowed with water and 5 minutes was allowed for it to dissolve in the stomach. The string was used to retrieve the sponge, which was placed into a pot of SurePath preservative (Becton Dickinson). Samples were refrigerated at 4°C to 6°C and transported weekly to the KCMC pathology laboratory.

2.5 | Cytosponge acceptability and safety

After the procedure, participants were shown a visual analogue scale13 and asked to score the procedure from 0 to 10 (0 being “completely unacceptable” and 10 being “completely acceptable”). The closest Swahili translations were haikubaliki and inakubalika, respectively. The number of attempts to swallow the capsule was recorded. Adverse events were reported in accordance with Good Clinical Practice (GCP) guidelines for clinical trials and independently assessed for relatedness and severity. Participants were given emergency contact details of an endoscopy nurse. Telephone calls were made 7 days after appointments, during which acceptability scores were requested again, and details and duration of any discomfort.

2.6 | Sample processing and cytopathology examination

One week training in sample processing and cytopathology review was delivered by a biomedical scientist (MB) and histopathologist (MOD). Cytosponge samples were processed into formalin-fixed paraffin embedded (FFPE) blocks by adapting an SOP.18 Agarose gel (Sigma-Aldrich) was substituted for plasma-thrombin and stirred into the centrifuged cell pellets to form a clot. Blocks were transported to IARC where slides were prepared by cutting two serial sections from each block and staining with hematoxylin and eosin (H&E). Slides were scanned at 40x magnification and uploaded to the SlidePath Digital Image Hub, where they were read independently by two pathologists (MOD and BA-A). A consensus on nonconcordant reviews was reached by joint review remotely.

2.7 | Statistical analysis

Comparisons of categorical variables between age groups (dichotomized using the median age of 51 years as a cut point) and sex were performed using the chi-squared test, or Fisher’s exact test if cells contained <10 observations. Statistical analysis was performed using R version 3.6.1.

3 | RESULTS

3.1 | Participant recruitment and characteristics

Figure 1 summarizes participant recruitment. In total, 144 people were engaged, 31 (21.5%) of whom were ineligible. Of the 31, exclusions were made for one or more of pregnancy (n = 9, 29%), cardiac events or related medication (n = 8, 25.8%), dysphagia (n = 7, 22.6%), history of haematemesis (n = 5, 16.1%), peptic ulcer (n = 5, 16.1%), possible history of oropharyngeal cancer (n = 5, 16.1%), liver cirrhosis (n = 2, 6.5%) and prior esophageal surgery (n = 1, 3.2%). Of 113 eligible individuals, 11 (9.7%) refused participation, giving 102 consenting participants. Of the 11 (9.7%) refusals, with a suggestion of more in men (8 refusals; 13%) than women (3 refusals; 6%), most (>80%) refused at the initial invitation or did not turn up to the appointment. Only one person (9.1%) refused after seeing the device.

Participants characteristics are presented in Table 1. Near equal numbers of men and women were recruited, aged between 30 and 77 years old and a mean age of 50.6 ± 11.6. Most participants (75/102; 74%) had at least primary education. Chagga was the most
frequently reported ethnicity (60%), higher among women (37/50; 74%) than men (24/52; 46%), partly owing to a higher number of male Muslim participants (15/52; 28.8% vs 8/50; 16%) who reported “other” ethnicity. Christianity was the predominant religion (79/102; 78%). Alcohol consumption (ever) was reported by 31 of 52 (60%) males and 28 of 50 (56%) females; and tobacco usage by 40% of males and 2% of females. The self-reported HIV prevalence was 6.9% (n = 7 known positives). A high reporting of family history of EC was found among women (12/50; 24%) compared to men (3/52; 3.8%).

### 3.2 | Cytosponge acceptability

Acceptability outcomes are presented in Table 2. Among consenting participants, 82% gave checking their health status as their motivation for participating. All participants swallowed the device at first attempt. Immediate postprocedure acceptability scores are plotted in Figure 2. Scores ranged between 6 and 10, with the majority (52.9%) of participants scoring 10, followed by 9 (23.5%) and 8 (20.6), giving a mean of 9.3. Follow-up contact 7-day postprocedure was successful for 91 (89%) participants (Table 2). On average, there was no difference between the immediate acceptability score and that at follow-up (mean difference: +0.08, 95% CI: −0.20, +0.36); however, 20% (18/91) of participants reduced their score by 1 or 2. Discomfort was reported by 21 (22%) of those followed-up, 18 (86%) of whom reported a sore throat—an anticipated side effect. Symptoms of discomfort persisted for more than 2 days in 10 (47.6%) of those who experienced them, but were not assessed beyond 7 days.

### 3.3 | Cytosponge safety

Out of 103 opened devices, one was defective upon inspection (knotted suture) and not used. Out of 102 procedures, one adverse event occurred. Shortly after sponge withdrawal, a 49-year-old female vomited visible amounts of blood in water. The participant underwent a precautionary endoscopy the following day. No active bleeds were observed in the esophagus, but a gastric ulcer was identified as the likely cause of the bleed. None of the study participants contacted the study nurse due to new or concerning symptoms during the 7 day follow-up period. A small number of mild immediate side effects were reported, including six participants who vomited or regurgitated water and one episode of coughing.

### 3.4 | Sample processing quality

Cytology slides were prepared for 102 participants, with an additional slide from a separate FFPE block for three participants due to poor cell clots in the initial block. These were prepared by reprocessing cells remaining in the leftover sample. Of these 105 slides, four were excluded due to low cellularity. Two of those excluded were from participants with a second FFPE block, giving one readable slide for 100 of 102 participants.

### 3.5 | Cytological findings

The prevalence of cytological findings among 100 participants with a readable slide is shown in Table 3. No significant findings were made in the esophageal squamous cells of 91 (91%) participants. Significant inflammation was found in the squamous cells of four (4%) participants: acute esophagitis (n = 2); eosinophilic esophagitis (n = 1) and ulcer slough (n = 1). Atypical squamous cells of uncertain significance (ASCUS) were found for one (1%) participant: a 49-year-old female. Low-grade squamous intraepithelial lesion (LSIL)—low-grade ESD—was found for one (1%) participant who was a 69-year-old female. The presence of columnar cells was observed on the slides of 90% of
participants, an indication that the Cytosponge had deployed in the stomach and sampled the full length of the esophagus. No significant findings were made in the gastric columnar cells of 75 (75%) participants. Significant gastric inflammation was found for 22 (22%) participants: chronic (n = 9; 9%) and active chronic (n = 13; 13%) gastritis. Intestinal metaplasia (IM) was found in six (6%) participants’ cells, three of them not accompanied by gastritis. Barrett’s esophagus could not be ruled out despite a low expected prevalence of this disease locally.

### TABLE 1  Characteristics of the study participants overall and by sex

|                           | Males   | Females | Total  |
|---------------------------|---------|---------|--------|
| All participants, n (row %)| 52 (51) | 50 (49) | 102 (100) |
| Age group (years)         |         |         |        |
| 30 to 39                  | 11 (21.2)| 10 (20) | 21 (20.6) |
| 40 to 49                  | 11 (21.2)| 10 (20) | 21 (20.6) |
| 50 to 59                  | 14 (26.9)| 15 (30) | 29 (28.4) |
| 60 to 75                  | 14 (26.9)| 15 (30) | 29 (28.4) |
| 75+                       | 2 (3.8) | 0 (0)   | 2 (2)   |
| Range                     | 30–77   | 30–71   | 30–77   |
| Mean ± SD                 | 50.5 ± 12.3 | 50.6 ± 10.9 | 50.6 ± 11.6 |
| Ethnic group              |         |         |        |
| Chagga                    | 24 (46.2)| 37 (74) | 61 (59.8) |
| Pare                      | 3 (5.8) | 1 (2)   | 4 (3.9)  |
| Maasai                    | 2 (3.8) | 0 (0)   | 2 (2)   |
| Other                     | 23 (44.2)| 12 (24) | 35 (34.3) |
| Education                 |         |         |        |
| Primary or below          | 34 (61.5)| 41 (82) | 75 (73.5) |
| Started or completed secondary | 14 (26.9)| 9 (10)  | 23 (22.5) |
| University/tertiary       | 4 (7.7) | 0 (0)   | 4 (3.9) |
| Religion                  |         |         |        |
| Christian Catholic        | 24 (46.2)| 19 (38) | 43 (42.2) |
| Christian Protestant/Anglican | 12 (23.1)| 20 (40) | 32 (31.4) |
| Christian Evangelist      | 1 (1.9) | 3 (6)   | 4 (3.9)  |
| Muslim                    | 15 (28.8)| 8 (16)  | 23 (22.5) |
| Family history of ECa     |         |         |        |
| Yes                       | 3 (3.8) | 12 (24) | 15 (14.7) |
| No                        | 49 (94.2)| 38 (76) | 87 (85.3) |
| Know anyone with ECb      |         |         |        |
| Yes                       | 11 (21.2)| 17 (34) | 28 (27.5) |
| No                        | 41 (78.8)| 33 (66) | 74 (72.5) |
| Ever alcohol consumption  |         |         |        |
| Yes                       | 31 (59.6)| 28 (56) | 59 (57.8) |
| No                        | 21 (40.4)| 22 (44) | 43 (42.2) |
| Ever strong alcohol consumptionc | 10 (32.3)| 2 (7.1) | 12 (20.3) |
| No                        | 21 (67.7)| 26 (92.9)| 47 (79.7) |
| Ever tobacco smoking      |         |         |        |
| Yes                       | 18 (34.6)| 0 (0)   | 18 (17.6) |
| No                        | 34 (65.4)| 50 (100)| 84 (82.4) |
| Ever smokeless tobacco    |         |         |        |
| Yes                       | 5 (9.6) | 1 (2)   | 6 (5.9)  |
| No                        | 47 (90.4)| 49 (98) | 96 (94.1) |
| HIV statusd               |         |         |        |
| HIV-positive              | 1 (1.9) | 6 (12)  | 7 (6.9)  |
| HIV-negative              | 39 (75) | 36 (72) | 75 (73.5) |
| Unknown                   | 12 (23.1)| 8 (16)  | 20 (19.6) |

| aSelf-reported family history of esophageal cancer. |
| bSelf-reported history of esophageal cancer in acquaintances such as friends, neighbors and colleagues; |
| cDefined as >30% ABV. |
| dSelf-reported HIV status. |

3.6 | Participant follow-up

Participants with ASCUS or LSIL were invited to undergo chromoendoscopy with confirmatory biopsy and morphological evaluation. Both declined follow-up. Participants with significant inflammation in esophageal squamous cells are being invited to a consultation with a local clinician to ascertain information on symptoms. Participants with either gastritis or IM are being invited to be tested for *Helicobacter pylori* infection.
| Reason for agreeing                | Males | Females | P value for sex difference | ≤51 years old | >51 years old | P value for age difference | Total  |
|-----------------------------------|-------|---------|-----------------------------|---------------|---------------|-----------------------------|--------|
| Knows/knew someone with EC       | 6 (11.5) | 7 (14) | 0.59                        | 5 (9.6)       | 8 (16)        | 0.76                        | 13 (12.7) |
| To help research                  | 1 (1.9)  | 3 (6) |                           | 2 (3.8)       | 2 (4)         | 4 (3.9)                     |        |
| To check their health             | 44 (84.6) | 40 (80) |                           | 44 (84.6)     | 40 (80)       | 84 (82.4)                   |        |
| General interest                  | 1 (1.9)  | 0 (0) |                           | 1 (1.9)       | 0 (0)         | 1 (1)                       |        |
| Attempts to swallow               | 1 (1.9)  | 0 (0) |                           | 1 (1.9)       | 0 (0)         | 1 (1)                       |        |
| >1 (max 3 allowed)                | 0 (0)    | 0 (0) |                           | 0 (0)         | 0 (0)         | 0 (0)                       |        |
| Acceptability score (0–10)        |        |        |                            |               |               |                             |        |
| <6                                | 0 (0)    | 0 (0) | 0.63                        | 0 (0)         | 0 (0)         | 0.79                        | 0 (0)  |
| 6                                 | 1 (1.9)  | 0 (0) |                            | 1 (1.9)       | 0 (0)         | 1 (1)                       | 1 (1)  |
| 7                                 | 0 (0)    | 2 (4) |                            | 1 (1.9)       | 1 (2)         | 2 (2)                       | 2 (2)  |
| 8                                 | 12 (23.1)| 9 (18)|                            | 10 (19.2)     | 11 (22)       | 21 (20.6)                   |        |
| 9                                 | 13 (25)  | 11 (22)|                           | 13 (25)       | 11 (22)       | 24 (23.5)                   |        |
| 10                                | 26 (50)  | 28 (56)|                           | 27 (51.9)     | 27 (54)       | 54 (52.9)                   |        |
| Mean ± SD                         | 9.2 ± 0.94 | 9.3 ± 0.91 |                           | 9.2 ± 0.96    | 9.3 ± 0.88    | 9.3 ± 0.92                   |        |
| Follow-up contact made?           |        |        |                            |               |               |                             |        |
| Yes                               | 47 (90.4) | 44 (88)| 0.76                        | 51 (98.1)     | 40 (80)       | <0.01                       | 91 (89.2) |
| No                                | 5 (9.6)  | 6 (12)|                            | 1 (1.9)       | 10 (20)       | 11 (10.8)                   |        |
| The following rows only apply to those with whom follow-up contact was made (n = 91 in total) |        |        |                            |               |               |                             |        |
| Acceptability score at >7 days postprocedure |        |        |                            |               |               |                             |        |
| <6                                | 0 (0)    | 0 (0) | 0.68                        | 0 (0)         | 0 (0)         | 0.08                        | 0 (0)  |
| 6                                 | 1 (2.1)  | 1 (2.3)|                            | 2 (3.9)       | 0 (0)         | 2.2                         | 4 (4.4) |
| 7                                 | 3 (6.4)  | 1 (2.3)|                            | 2 (3.9)       | 2 (5)         | 4 (4.4)                     |        |
| 8                                 | 6 (12.8) | 6 (13.6)|                           | 9 (17.6)      | 3 (7.5)       | 12 (13.2)                   |        |
| 9                                 | 9 (19.1) | 9 (20.5)|                           | 11 (21.6)     | 7 (17.5)      | 18 (19.8)                   |        |
| 10                                | 28 (59.6)| 27 (61.4)|                           | 27 (52.9)     | 28 (70)       | 55 (60.4)                   |        |
| Mean ± SD                         | 9.3 ± 1.1 | 9.4 ± 0.97 |                           | 9.2 ± 1.1     | 9.5 ± 0.85    | 9.3 ± 1                     |        |
| Change in score after 7 days      |        |        |                            |               |               |                             |        |
| −4                                | 0 (0)    | 1 (2.3)| 0.83                        | 1 (2)         | 0 (0)         | 0.2                         | 1 (1.1) |
| −3                                | 2 (4.3)  | 1 (2.3)|                            | 2 (3.9)       | 1 (2.4)       | 3.3                         | 3 (3.3) |
| −2                                | 5 (10.6) | 4 (9.1) |                            | 6 (11.8)      | 3 (7.5)       | 9 (9.9)                     |        |
| −1                                | 6 (12.8) | 3 (6.8) |                            | 6 (11.8)      | 3 (7.5)       | 9 (9.9)                     |        |
| 0                                 | 14 (29.8)| 20 (45.5)|                           | 18 (35.3)     | 16 (40)       | 34 (37.4)                   |        |
| +1                                | 14 (29.8)| 10 (22.7)|                           | 13 (25.5)     | 11 (27.5)     | 24 (26.4)                   |        |

(Continues)
| Discomfort experienced? | Yes | No | P value for sex difference | ≤51 years old | >51 years old | P value for age difference | Total |
|-------------------------|-----|----|----------------------------|--------------|--------------|--------------------------|-------|
|                         |     |    |                            | Males | Females | ≤51 years old | >51 years old |                           |       |
|                         |     |    |                            | 5 (10.6) | 5 (11.4) | 4 (7.8) | 6 (15) | 10 (11) |
|                         |     |    |                            | 1 (2.1) | 0 (0) | 1 (2) | 0 (0) | 1 (1.1) |
| Mean (95% CI)           | 0.11 | 0.05 |                            | −0.3, 0.51 | −0.35, 0.44 | −0.48, 0.32 | −0.11, 0.66 | 0.08 |

| Discomfort experienced? | Yes | No | P value for sex difference | ≤51 years old | >51 years old | P value for age difference | Total |
|-------------------------|-----|----|----------------------------|--------------|--------------|--------------------------|-------|
|                         |     |    |                            | Males | Females | ≤51 years old | >51 years old |                           |       |
|                         |     |    |                            | 8 (17) | 13 (29.5) | 13 (25.5) | 8 (20) | 0.62 | 21 (22.2) |
|                         |     |    |                            | 39 (83) | 31 (70.5) | 38 (74.5) | 32 (80) | 70 (77.8) |
| If experienced, reported symptoms of discomfort | Sore/irritated throat |                           | 7 (87.5) | 11 (84.6) | NA | 11 (84.6) | 7 (87.5) | NA | 18 (85.7) |
|                         | Other* |     |                            | 1 (12.5) | 2 (15.4) | 2 (15.4) | 1 (12.5) | 3 (14.3) |

| Duration of discomfort (assessed at day 7) | <1 hour | 1-6 hours | 6-24 hours | 1-2 days | >2 days | P value for sex difference | ≤51 years old | >51 years old | P value for age difference | Total |
|-------------------------------------------|---------|-----------|-----------|---------|---------|--------------------------|--------------|--------------|--------------------------|-------|
|                                          | Males   | Females  | ≤51 years old | >51 years old |                           |       |
|                                          |         |          | Males | Females | ≤51 years old | >51 years old |                           |       |
|                                          | 0 (0)   | 1 (7.7)  | 0 (0) | 1 (12.5) | NA | 1 (12.5) | NA | 1 (4.8) |
|                                          | 1 (12.5) | 2 (15.4) | 3 (23.1) | 0 (0) | 3 (14.3) |       |       |
|                                          | 3 (37.5) | 3 (23.1) | 4 (30.8) | 2 (25) | 6 (28.6) |       |       |
|                                          | 0 (0)   | 1 (7.7)  | 1 (7.7) | 0 (0) | 1 (4.8) |       |       |
|                                          | 4 (50)  | 6 (46.2) | 5 (38.5) | 5 (42.5) | 10 (47.6) |       |       |

Note: P values refer to comparisons between sexes and between age groups using Fisher’s exact test for categorical variables and Student’s t-test for continuous variables.

*Included unspecified discomfort (n = 1), excess saliva in the mouth (n = 1) and possibly unrelated back pain (n = 1).
We successfully conducted a Cytosponge feasibility study in Tanzania—the first use of any such device in Tanzania, and the first use of the Cytosponge in Africa. The device was swallowed and a sample was collected from all 102 participants, who reported extremely high acceptability, and 100 of which were prepared into slides of adequate cellularity. One adverse advent occurred—bleeding which may have resulted from a gastric ulcer. The following cytological findings were made: significant esophageal inflammation (4%), ASCUS (1%), LSIL (1%), gastritis (22%) and intestinal metaplasia (6%), three of which were not accompanied by gastritis. These findings require follow-up but indicate the potential utility of the approach.

The study benefited from strong international collaboration and intensive on-site training. This ensured a complete swallow with entry of the device into the stomach in 90% participants and allowed sample collection and processing procedures to be minimally adapted with no infrastructural modifications, yielding a high compliance of sample quality for examination.

Few studies have employed capsule sponges in Africa. Prototypes date back to the 1960s, but devices comparable in design to the Cytosponge include a locally manufactured device used on >2000 inhabitants in South Africa. A low proportion of swallowing failures (<2%) and suboptimal cellularity (~3%) were reported. A later study evaluated the Oesotest as a clinical triage in Nairobi, Kenya. An unspecified number of patients with dysphagia were unable to swallow, but the device was successful in 60 endoscopy referrals and moderate to good agreement was found with endoscopy findings, that is, two histologically confirmed ESCC cases were also detected by the Oesotoest. Our 100% swallow success and high acceptability in Tanzanian are similarly encouraging. A notable feature of previous studies is sample preparation, whereby sponges were smeared onto slides and spray-fixed, which gives a lower cell yield than FFPE clots. We noted a variability of cellularity in our slides, but only ~2% were suboptimal for evaluation.

Compared to studies using the Cytosponge device, our median acceptability score of 10 (from 0 to 10) is higher than the 6 from a pooled analysis of 2672 UK procedures, and our mean (9.3) higher than 7.3 from 80 in the United States. Our swallow success rate was also 100% compared to 91% and 93%, but patients suffered from reflux disease and eosinophilic esophagitis in both studies, respectively. The single bleeding event in our study is high compared to 2 of 4422 in pooled UK data, but a gastric ulcer meant we
could not conclusively attribute bleeding to the procedure. Cytosponges were swallowed by all 300 of 301 asymptomatic individuals in Iran, with no complications.\textsuperscript{16} Our findings may be limited by choice of study site—a research unit where participation rates are boosted by awareness, engagement and trust. Such high acceptability may not be received in rural communities, or elsewhere in East Africa. Nevertheless, they provide a much-needed basis for ongoing research.

Although we primarily aimed to assess feasibility, findings from other East African ESCC hotspots suggested a higher prevalence of squamous atypia/dysplasia. An endoscopy survey of 305 asymptomatic adults in Kenya found dysplasia in 15%,\textsuperscript{8} albeit with a more sensitive screening modality. Despite less refined processing protocols, a South African sponge cytology study reported higher prevalence of atypia (4.8%-14%), dysplasia (1.4%-8%) and carcinoma (0%-2.8%) in 1000 asymptomatic individuals.\textsuperscript{20} Several factors likely contributed to

\textbf{FIGURE 3}  Situation-specific considerations of the Cytosponge administration in a new low-resource setting. Considerations 1 to 3 are presented top to bottom along the cascade of planning, recruitment and follow-up care, followed by overarching ethical considerations.
our lower prevalence: (a) without p53 staining, the sensitivity of the Cytosponge for low-grade ESD is only 39% to 80% based on Iranian findings\textsuperscript{16}, (b) our study was conducted in an urban suburb, whereas more ESCC cases originate from rural villages in this setting; and (c) healthy-participation effect—the sample was not a general population random sample and includes volunteers. Therefore, if ESD is to be used as an early detection target or surrogate epidemiological outcome in this setting, targeted sampling of high-risk populations and geographic locations will be needed, and a sensitive ESD biomarker. Proliferation markers and those of carcinogen exposures may also prove useful assays, as shown for PAHs.\textsuperscript{24} Conversely, the high prevalence of gastritis detected (22%) is consistent with expectations given the local prevalence of *H pylori* infection.

The greatest findings and considerations made in the present study derived from taking a procedure which to date has mostly been administered in people and health facilities in high resource settings, and administering it in a contrasting setting with different sociocultural contexts and medical infrastructure. Some of the questions, considerations and concerns that arose are provided in Figure 3 and may help to frame discussions in future studies. Suggested decisions are not provided as these would need to be made in context and by each setting’s team.

In conclusion, the feasibility of Cytosponge sampling was demonstrated in a Tanzanian setting where endoscopy services are resource limited. Further research is needed to define the test’s accuracy in this setting, which may lend support for risk stratification, targeted population sampling and downstream bioassay selection to realize the potential of this appropriate health technology for early detection and etiological investigation of ESCC.

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CONFLICT OF INTEREST

The Cytosponge device was designed by RCF and the MRC licensed the technology to Covidien GI Solutions, now part of Medtronic. Medtronic have had no influence on the design, conduct or analysis of this study. RCF and MOD are named inventors on patents pertaining to the Cytosponge and related assays. RCF and MoD are shareholders and consultants for Cyted Ltd. All other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are stored at the International Agency for Research on Cancer, where on-site access is welcomed via research collaboration.

ETHICS STATEMENT

The study received ethical approval from the National Institute for Medical Research Tanzania (NIMR/HQ/R.8a/Vol.IX/1994); Tumaini University Kilimanjaro Christian Medical College (830) and IARC Ethics Committee (IEC 17-04). Clinical trial approval was obtained from the Tanzanian Food and Drug Authority (TFDAA0018/CTR/0012/08). All participants provided written informed consent. All authors had access to the study data and reviewed and approved the final manuscript.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Arnold M, Ferlay J, van Berge Henegouwen MI, Soerjomataram I. Global burden of oesophageal and gastric cancer by histology and subsite in 2018. Gut. 2020;69:1564-1571.
3. McCormack V, Menya D, Munishi M, et al. Informing etiologic research priorities for squamous cell esophageal cancer in Africa: a review of setting-specific exposures to known and putative risk factors. Int J Cancer. 2017;140:259-271.
4. McGlashan ND. Oesophageal cancer and alcoholic spirits in Central Africa. Gut. 1969;10:643-650.
5. Wang G, Abnet C, Shen Q, et al. Histological predictors of oesophageal squamous cell carcinoma: results from a 13 year prospective follow up study in a high risk population. Gut. 2005;54:187-192.
6. Inoue H, Minami H, Kaga M, Sato Y, Kudo SE. Endoscopic mucosal resection and endoscopic submucosal dissection for esophageal dysplasia and carcinoma. Gastrointest Endosc Clin. 2010;20:25-34.
7. Wei W, Abnet C, Lu N, et al. Risk factors for oesophageal squamous dysplasia in adult inhabitants of a high risk region of China. Gut. 2005;54:759-763.
8. Mwachiro MM, Burgert SL, Lando J, et al. Esophageal squamous dysplasia is common in asymptomatic Kenyans: a prospective, community-based, cross-sectional study. Am J Gastroenterol. 2016;111:500-507.
9. Dawsey SM, Fleischer DE, Wang GQ, et al. Mucosal iodine staining improves endoscopic visualization of squamous dysplasia and squamous cell carcinoma of the esophagus in Linxian, China. Cancer. 1998;83:220-231.
10. Codipilly DC, Qin Y, Dawsey SM, et al. Screening for esophageal squamous cell carcinoma: recent advances. Gastrointest Endosc. 2018;88:413-426.
11. Lao-Sirilex P, Boussioutas A, Kadri SR, et al. Non-endoscopic screening biomarkers for Barrett’s oesophagus: from microarray analysis to the clinic. Gut. 2009;58:1451-1459.
12. Ross-Innes CS, Debiram-Beecham I, O’Donovan M, et al. Evaluation of a minimally invasive cell sampling device coupled with assessment of trefoil factor 3 expression for diagnosing Barrett’s esophagus: a multi-center case–control study. PLoS Med. 2015;12:e1001780.
13. Kadri SR, Lao-Sirieix P, O'Donovan M, et al. Acceptability and accuracy of a non-endoscopic screening test for Barrett's oesophagus in primary care: cohort study. BMJ. 2010;341:c4372.
14. Fitzgerald RC, di Pietro M, O'Donovan M, et al. Cytosponge-trefoil factor 3 versus usual care to identify Barrett's oesophagus in a primary care setting: a multicentre, pragmatic, randomised controlled trial. The Lancet. 2020;396:333-344.
15. Ross-Innes CS, Chettouh H, Achilleos A, et al. Risk stratification of Barrett's oesophagus using a non-endoscopic sampling method coupled with a biomarker panel: a cohort study. Lancet Gastroenterol Hepatol. 2017;2:23-31.
16. Roshandel G, Merat S, Sotoudeh M, et al. Pilot study of cytological testing for oesophageal squamous cell dysplasia in a high-risk area in northern Iran. Br J Cancer. 2014;111:2235-2241.
17. Januszewicz W, Tan WK, Lehovsky K, et al. Safety and acceptability of esophageal Cytosponge cell collection device in a pooled analysis of data from individual patients. Clin Gastroenterol Hepatol. 2019;17:647-656 e1.
18. Paterson AL, Gehrung M, Fitzgerald RC, O'Donovan M. Role of TFF3 as an adjunct in the diagnosis of Barrett's esophagus using a minimally invasive esophageal sampling device—the Cytosponge™. Diagn Cytopathol. 2020;48:253-264.
19. Burrell RJ. Distribution maps of esophageal cancer among Bantu in the Transkei. J Natl Cancer Inst. 1969;43:877-889.
20. Jaskiewicz K, Venter FS, Marasas WF. Cytopathology of the esophagus in Transkei. J Natl Cancer Inst. 1987;79:961-967.
21. Lazarus C, Jaskiewicz K, Sumeruk R, et al. Brush cytology technique in the detection of oesophageal carcinoma in the asymptomatic, high risk subject; a pilot survey. Cytopathology. 1992:3:291-296.
22. Muriithi RW, Muchiri LW, Lule GN. Esophageal cytology sponge diagnostic test results in kenyatta national referral hospital, Kenya. Acta Cytol. 2014;58:483-488.
23. Katzka DA, Smyrk TC, Alexander JA, et al. Accuracy and safety of the cytospone for assessing histologic activity in eosinophilic esophagitis: a two-center study. Am J Gastroenterol. 2017:112:1538-1544.
24. Abedi-Ardekani B, Kamangar F, Hewitt SM, et al. Polycyclic aromatic hydrocarbon exposure in oesophageal tissue and risk of oesophageal squamous cell carcinoma in North-Eastern Iran. Gut. 2010;59:1178-1183.