

# A REVIEW OF ULTRASOUND-GUIDED PNEUMATIC ENEMA IN THE REDUCTION OF INTUSSUSCEPTION IN CHILDREN: INSIGHTS FROM A TEACHING HOSPITAL IN NIGERIA

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## ABSTRACT

**Background:** Pneumatic reduction, as a non-operative mode of treating intussusception, is traditionally done under fluoroscopy guidance. However, fluoroscopy guidance is fraught with exposure to ionizing radiation and fluoroscopy machines may not be routinely available especially in low-and-middle income countries. The aim of this study was to evaluate the efficacy, safety and predictive factors of successful reduction of intussusception using the ultrasound-guided pneumatic technique.

**Materials & Methods:** This was a prospective cohort study of children, 3 to 36 months of age, who were confirmed to have intussusception by abdominal ultrasonography and had pneumatic (air) reduction under ultrasound guidance between December 2018 to December 2020. Patients with bowel perforation, peritonitis, non-responsive shock or recurrent intussusception were excluded. Success rate, time to reduction, complication (recurrence and perforation) rate, and predictive factors for successful reduction were evaluated.

**Result:** Thirty (36.1%) out of 83 patients who presented with intussusception during this period had ultrasound-guided pneumatic reduction. The mean age was  $6.1 \pm 1.6$  (range: 4 to 9) months. Male to female ratio was 2.3:1. About one-third of the patients presented within  $\leq 48$  hours of onset of symptoms. 21 patients (70%) had a successful reduction. The mean time to reduction was  $10.51 \pm 4.19$  (range: 3 to 16) minutes. One patient had recurrence giving a recurrence rate of 3.3%. There was no case of perforation. Patients who had successful reduction significantly had palpable abdominal mass ( $p = 0.0139$ ), serum sodium  $> 135 \text{ mmol/l}$  ( $p = 0.0419$ ) and serum potassium  $> 3.5 \text{ mmol/l}$  ( $p = 0.0318$ ). Only serum potassium  $> 3.5 \text{ mmol/l}$  (Odd ratio (OR), 6.9; 95% confidence interval (CI), 1.2 - 40.3;  $p = 0.03$ ) was significantly associated with successful reduction on multivariable analysis.

**Conclusion:** Ultrasound-guided pneumatic reduction of ileocolic intussusception is feasible, effective and safe. It may be used in the treatment of children who present with ileocolic intussusception. Serum potassium  $> 3.5 \text{ mmol/l}$  may suggest the likelihood of successful reduction.

**Keywords:** Intussusception; Pneumatic reduction; Ultrasound-guided; Children

## INTRODUCTION

Intussusception is the invagination of a segment of bowel (intussusceptum) into an adjacent segment of the bowel (intussusciens).<sup>1</sup> In ileocolic intussusception, which is common in children 3 – 36 months of age, the terminal ileum is the intussusceptum while the colon is the intussusciens. Intussusception is the commonest cause of intestinal obstruction in infancy and early childhood and as such, it is a pediatric surgical emergency.<sup>1-3</sup> Treatment may be operative or non-operative; with the non-operative mode of treatment, when not contra-indicated, being currently preferred.<sup>4</sup> Non-operative reduction of intussusception may be effected with either hydrostatic or pneumatic reduction.

Interestingly, it is established that pneumatic reduction is faster when compared with hydrostatic reduction.<sup>3</sup> Fluoroscopy has been traditionally used to guide pneumatic reduction of intussusception, however, ultrasound is portable, cheaper, readily available and has no ionizing radiation. Ultrasound is able to visualize the entire peritoneal cavity compared with fluoroscopy which visualizes only the intraluminal content.<sup>5,6</sup> Ultrasound-guided pneumatic reduction is not commonly reported. A recent systematic review called for more papers on efficacy and safety of ultrasound-guided pneumatic reduction.<sup>5</sup> Moreover, most reports on ultrasound-guided pneumatic reduction were done

on patients who presented early (within 24 – 48 hours).<sup>3,7,8</sup> This may not be of much benefit to clinicians in the low-and-middle income countries (LMICs) where late presentation (mostly after 48 hours) is still rife.<sup>9,10</sup> Papers reporting time to reduction are scant. The only report from Nigeria on experience with ultrasound-guided pneumatic reduction of intussusception in children was retrospective.<sup>1</sup>

The purpose of this study was to evaluate the efficacy and safety; and to identify the factors associated with successful reduction of intussusception using the ultrasound-guided pneumatic reduction technique. We hypothesized that ultrasound-guided pneumatic reduction of intussusception has a high success rate, and short time to reduction and low complication rates; and we sought to identify patient factors that may be related to successful reduction using this technique. Identifying such factors will guide counselling and discussion of outcome of care with the parents of children who would benefit from ultrasound-guided pneumatic reduction of intussusception.

## MATERIALS AND METHODS

This is a prospective cohort study that was performed at the Paediatric Surgery Unit of the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu, Nigeria between December 2018 to December 2020 on children who presented to the children emergency room, during the period of the study, with intussusception confirmed by abdominal sonography. The study was approved by the UNTH Health Research and Ethics committee. Written informed consent was obtained from the parents or legal guardians. This study is compliant with Strengthening the Reporting of Observational studies in Epidemiology (STROBE)<sup>11</sup> and Strengthening the Reporting of Cohort Studies in Surgery (STROCSS)<sup>12</sup> guidelines.

Children, 3 to 36 months of age, who were clinically diagnosed to have intussusception which was confirmed by abdominal ultrasonography were included in the study. Patients who had bowel perforation, peritonitis, non-responsive shock or recurrent intussusception at presentation were excluded. Time to presentation was not used as a criterion for eligibility.

Screening for eligibility was done clinically by way of history taking, physical examination and intussusception confirmed by abdominal ultrasonography. The patients were resuscitated with intravenous fluids; size 10 nasogastric tube for gastric decompression and 8Fr 2-way urethral catheter to monitor urine output were passed. Intravenous antibiotics - Ceftriaxone 50mg/

kilogram body weight/day in two divided doses and Metronidazole 7.5mg/kilogram body weight/dose 8 hourly – were commenced. Serum electrolytes, urea, and creatinine; and Full Blood count were sought for (at presentation). Electrolyte imbalance was corrected when present and haemogram optimized to a minimum of 10g/dl. The patients proceeded to the radiological suite for pneumatic (air) reduction under ultrasound guidance when they were fully resuscitated. Preparation was made for immediate surgery if there was failed reduction and/or bowel perforation occurred.

## Procedure for Ultrasound-guided Pneumatic (air) reduction of Intussusception

Ultrasound-guided Pneumatic (air) reduction was performed with the patient in supine position in the radiological suite. Following application of the coupling gel on the surface of the abdomen, the Radiologist performed longitudinal and transverse scans using an ultrasound machine (GE Versana Essential S/W of Medical Systems (China) Co. LTD) with a high frequency (7.5MHz) linear transducer to locate the intussusception mass.

A size 22Fr 2-way Foley catheter, the part of the aneroid sphygmomanometer containing the hand bulb, release valve and aneroid gauge, a short tube (cut from a urine bag – with the bag cut off), Sterile water, and 5ml syringes constitute the materials needed for ultrasound-guided Pneumatic (air) reduction. Sedatives were not used during the procedure. The Foley catheter was inserted into the rectum and balloon inflated with 30mls of sterile water. An adhesive tape was firmly applied - extending from one greater trochanter to the other - to ensure a good anal seal. The release valve of the aneroid sphygmomanometer was connected to the drainage channel of the Foley catheter via the short tube (cut from the urine bag).

The hand bulb was then squeezed to release air into the bowel while monitoring the mass with ultrasound. The pneumatic pressure was carefully monitored using the aneroid gauge and not allowed to exceed 120mmHg at any time. The pulse rate and oxygen saturation of the patient were monitored during the procedure. Complete reduction was confirmed by the disappearance of the intussusception mass. Not more than three attempts at reduction were made. Each attempt was continued as far as the mass was progressively reducing as monitored with ultrasound. However, each attempt was discontinued if after 3 minutes the mass was no longer reducing as monitored with ultrasound<sup>2</sup>. Each attempt was discontinued by disconnecting the short tube from the Foley catheter; hence, reducing the pressure before another attempt

was started again. The time to reduction was also monitored with a stopwatch. At the end of the procedure, the adhesive tape was removed, the balloon was deflated, the catheter removed and patient monitored.

Patients who had a complete reduction of intussusception were taken back to the ward and monitored for symptoms of intussusception which include abdominal pain, vomiting, and passage of blood and mucus. The nasogastric tube was removed when the drainage was minimal, no longer bilious and there was no abdominal distension. Oral feeds were commenced when patients were able to pass feces and/or flatus. The intravenous fluids were stopped when oral intake was fully established; urethral catheter was then removed. Complete reduction as noted during ultrasound-guided pneumatic reduction complemented by disappearance of clinical features of intussusception was regarded as successful reduction.

Patients were then discharged when recurrence was ruled out by the absence of symptoms of intussusception. Abdominal ultrasonography was not routinely done to confirm recurrence before the patients were discharged from the hospital. The parents were advised of the possibility of recurrence of intussusception and the need to present early. Patients who did not have successful reduction proceeded to have surgery. Delayed repeat enema was not done.

The patients were followed up via face-to-face and telephone clinics. They were seen on the first, fourth and twenty-fourth week following discharge from the hospital and thereafter by phone calls. Each patient was followed up for a minimum of 24 months. The patients were clinically assessed for recurrent intussusception during each visit.

For the purpose of this study, successful reduction was defined as complete reduction during ultrasound-guided pneumatic reduction with resolution of symptoms of intussusception. The time to reduction was the time from insufflation of air to when complete reduction occurred by ultrasound assessment. Failed reduction was defined as presence of the intussusception mass after 3 attempts (or less than or equal to 3 attempts if perforation occurred) at reduction have been made. Recurrence was defined as return of symptoms of intussusception 12 hours or more after a successful reduction<sup>13</sup>. Patients who had return of symptoms of intussusception before 12 hours after a successful reduction were regarded to have had an incomplete reduction ab initio.

Successful reduction and recurrence were assessed by a Senior registrar in Paediatric Surgery who was not involved in the enema reduction.

The following variables were obtained for each patient: age, sex, weight, duration of symptoms prior to presentation, phone number of parents, date of admission, clinical features, outcome of non-operative reduction, details of operative treatment for failed reduction, and duration of follow up.

### Statistical analysis

Data management and analysis was done using Statistical Package for Social Sciences (SPSS) of IBM SPSS statistics for windows, version 20 (SPSS Inc., Chicago, Illinois). Numerical data was assessed for normality using Shapiro-Wilks test. Numerical variables were expressed as mean  $\pm$  standard deviation while categorical variables were expressed as percentages and presented in tables. Categorical data were compared using Fisher's exact test. Logistic regression analysis was applied to determine predictive factors for successful reduction of intussusception. All statistical tests were two-tailed. P-value  $<0.05$  was considered statistically significant.

## RESULTS

A total of 30 patients were recruited within the study period; completed the study and their data were analyzed.

The mean age of the patients at presentation was  $6.1 \pm 1.6$  (range: 4 to 9) months. 21 (70%) were males while 9 (30%) were females giving a male to female ratio of 2.3:1. The mean body weight at presentation was  $6.9 \pm 0.9$  (range: 5 to 8.7) kilograms. About one-third of the patients presented within  $\leq 48$  hours of onset of symptoms - the mean duration of symptoms prior to presentation was  $80.6 \pm 39.4$  (range: 24 to 168) hours (Table 1).

**Table 1:** Baseline characteristics of the patients

Variable	Frequency (N=30), n (%)
Sex	
Male	21 (70.0)
Female	9 (30.0)
Age (months)	
$\leq 6$	18 (60.0)
7 – 12	12 (40.0)
Body weight (kilogram)	
$\leq 7$	13 (43.3)
$> 7$	17 (56.7)
Duration of symptoms (hours)	
$\leq 48$	11 (36.7)
$> 48$	19 (63.3)

**Table 2:** Clinical features and laboratory findings of the patients

Variable	Frequency (N=30), n (%)
Symptoms	
Vomiting	
Present	24 (80.0)
Absent	6 (20.0)
Abdominal pain	
Present	27 (90.0)
Absent	3 (10.0)
Abdominal distension	
Present	13 (43.3)
Absent	17 (56.7)
Rectal bleeding	
Present	23 (76.7)
Absent	7 (23.3)
Signs	
Maximum temperature (°C)	
≤ 38.5	10 (33.3)
> 38.5	20 (66.7)
Palpable abdominal mass	
Present	23 (76.7)
Absent	7 (23.3)
Palpable rectal mass	
Present	8 (26.7)
Absent	22 (73.3)
Location of abdominal mass	
Right lower	1 (3.3)
Right upper	12 (40.0)
Left upper	1 (3.3)
Left lower	16 (53.4)
Laboratory	
White blood cell count (/mm <sup>3</sup> )	
≤11.0 x 10 <sup>9</sup> /l	4 (13.3)
>11.0 x 10 <sup>9</sup> /l	26 (86.7)
Serum sodium (mmol/l)	
≤ 135	11 (36.7)
> 135	19 (63.3)
Serum potassium (mmol/l)	
≤ 3.5	8 (26.7)
> 3.5	22 (73.3)

The most common symptoms the patients presented with were abdominal pain (n=27, 90%), vomiting (n=24, 80%) and rectal bleeding (n=23, 76.7%). Most of the patients had their maximum body temperature measured during hospital admission to be >38.5°C (n=20, 66.7%); 23 patients (76.7%) had a palpable abdominal mass, mostly (n=16, 53.4%) felt in the left lower quadrant of the abdomen. Most (n=22, 73.3%) of the patients had their white blood cell count elevated above 11.0 x 10<sup>9</sup>/l (Table 2). The mean duration of follow-up was 41.1±5.6 (range: 24 to 47) months.

Twenty-one patients had successful ultrasound-guided pneumatic reduction giving a success rate of 70%. The

mean time to reduction in the patients that had successful reduction was 10.51 ±4.19 (range: 3 to 16) minutes. One patient had recurrence 36 hours after successful reduction giving a recurrence rate of 3.3%. He had a successful repeat ultrasound-guided pneumatic (air) reduction. There was no case of perforation during ultrasound-guided pneumatic reduction in any of the patients.

Concerning the patients who had failed reduction, most (n=8, 26.7%) had bowel resection and anastomosis while one patient (3.3%) had manual reduction. They all had edematous ileocolic intussusception.

**Table 3:** Predictive factors for successful ultrasound-guided pneumatic reduction

Variable	Successful reduction (N=21), n(%)	Failed reduction (N=9), n(%)	P-value
Sex			
Male (21)	14 (66.7)	7 (33.3)	0.6814
Female (9)	7 (77.8)	2 (22.2)	
Age (months)			
≤ 6 (18)	12 (66.7)	6 (33.3)	0.7036
7 – 12 (12)	9 (75.0)	3 (25.0)	
Body weight (kilogram)			
≤ 7 (13)	8 (61.5)	5 (38.5)	0.4434
> 7 (17)	13 (76.5)	4 (23.5)	
Duration of symptoms (hours)			
≤ 48 (11)	9 (81.8)	2 (18.2)	0.2481
> 48 (19)	12 (63.2)	7 (36.8)	
Vomiting			
Present (24)	18 (75.0)	6 (25.0)	0.3287
Absent (6)	3 (50.0)	3 (50.0)	
Abdominal pain			
Present (27)	20 (74.0)	7 (26.0)	0.2069
Absent (3)	1 (33.3)	2 (66.7)	
Abdominal distension			
Present (13)	10 (76.9)	3 (23.1)	0.6908
Absent (17)	11 (64.7)	6 (35.3)	
Rectal bleeding			
Present (23)	15 (65.2)	8 (34.8)	0.3830
Absent (7)	6 (85.7)	1 (14.3)	
Maximum temperature (°C)			
≤ 38.5 (10)	8 (80.0)	2 (20.0)	0.6749
> 38.5 (20)	13 (65.0)	7 (35.0)	
Palpable abdominal mass			
Present (23)	19 (82.6)	4 (17.4)	0.0139
Absent (7)	2 (28.6)	5 (71.4)	
Palpable rectal mass			
Present (8)	7 (87.5)	1 (12.5)	0.3742
Absent (22)	14 (63.6)	8 (36.4)	
Location of abdominal mass			
Right upper (12)	6 (50.0)	6 (50.0)	0.1139
Left lower (16)	13 (81.3)	3 (18.7)	
White blood cell count (/mm <sup>3</sup> )			
≤11.0 x 10 <sup>9</sup> /l (4)	2 (50.0)	2 (50.0)	0.5632
>11.0 x 10 <sup>9</sup> /l (26)	19 (73.1)	7 (26.9)	
Serum sodium (mmol/l)			
≤ 135 (11)	5 (45.5)	6 (54.5)	0.0419
> 135 (19)	16 (84.2)	3 (15.8)	
Serum potassium (mmol/l)			
≤ 3.5 (8)	3 (33.3)	5 (66.7)	0.0318
> 3.5 (22)	18 (85.7)	4 (14.3)	

On univariable analysis, patients who had successful reduction were significantly associated with palpable abdominal mass ( $p=0.0139$ ), serum sodium  $>135\text{mmol/l}$  ( $p = 0.0419$ ) and serum potassium  $>3.5\text{mmol/l}$  ( $p = 0.0318$ ) (Table 3).

On multivariable analysis, only serum potassium  $>3.5\text{mmol/l}$  (Odd ratio (OR), 6.9; 95% confidence interval (CI), 1.2 - 40.3;  $p=0.03$ ) was significantly associated with successful reduction (Table 4).

**Table 4:** Multivariate logistic regression for prediction of possible successful reduction

Variable	OR (95% CI)	P-value
Presence of palpable abdominal mass	2.032 (0.219 – 18.895)	0.533
Serum Sodium $> 135 \text{ mmol/l}$	0.995 (0.815 – 1.215)	0.963
Serum potassium $> 3.5 \text{ mmol/l}$	6.969 (1.205 – 40.301)	0.030

OR: Odds ratio; 95% CI: 95% Confidence interval

## DISCUSSION

Ileocolic intussusception is initially treated by non-operative methods except when operative treatment is indicated.<sup>2</sup> Hydrostatic enema reduction is commonly done in most Low-and-Middle Income Countries due to the widespread availability of ultrasound; fluoroscopy machines are not readily available.<sup>14</sup> However, with the advent of pneumatic reduction under ultrasound guidance, pneumatic reduction is bound to gain widespread acceptance in the LMICs. The goal of this study was to evaluate the efficacy, safety and predictors of successful ultrasound-guided pneumatic reduction.

We found that ultrasound-guided pneumatic reduction has an acceptably high success rate, short time to reduction and low recurrence and perforation rates. Presence of palpable abdominal mass, Serum sodium >135 mmol/l and serum potassium >3.5mmol/l were found in successful reduction. It was done using easily accessible materials, hence, justifying its utility and adaptability to the LMICs. We were able to recruit suitable patients without recourse to their time to presentation. This will help enhance the applicability of the results of this study in settings like ours where most of these patients present late.

Ultrasound-guided pneumatic reduction combines the benefits of use of ultrasound and pneumatic enema in reduction of intussusception. Ultrasound has no ionizing radiation and this is of great benefit to children who are ten times more radiosensitive when compared to adults.<sup>15</sup> The use of ultrasound in guiding pneumatic reduction is in keeping with the As Low As Reasonably Achieved (ALARA)<sup>16</sup> principle and Image gently campaign.<sup>17</sup>

The success rate in this study is consistent with the success rate in similar studies.<sup>3,6,7</sup> This is in keeping with the finding that the intracolonic pressures achieved during pneumatic reduction is high.<sup>5</sup> It is believed that the reverberations of air in the inflated colon significantly reduces visualization and this has discouraged some radiologists and pediatric surgeons in the practice of ultrasound-guided pneumatic reduction.<sup>18</sup> However, the intussusception mass is easily seen as the air is only proximal to it.<sup>19</sup> Moreover, the oedematous ileocaecal valve and residual ileoileal intussusception are easily recognized during ultrasound-guided pneumatic reduction than during fluoroscopy-guided pneumatic reduction.<sup>6</sup> This was demonstrated by Mensah *et al.*<sup>20</sup> who had to take their patients back to the ultrasound room, following fluoroscopy-guided pneumatic reduction, to confirm complete reduction and rule out any residual intussusception.

Delayed repeat enema was not practiced in this study as it is not a part of the unit protocol in line with patient safety. However, delayed repeat enema has been suggested to improve the success rate of pneumatic reduction.<sup>3,15,19</sup> Dahab *et al.*<sup>15</sup>, established a success rate of 66% in their initial trial, which is less than the success rate in this study. Their final success rate was 78% after a second trial. Although, Singh *et al.*<sup>6</sup>, did not do delayed repeat enema in their study; they still achieved a success rate of 88%. The practice of delayed repeat enema is likely to be utilized in our unit in the future. Moreover, the role of the use of sedatives in pneumatic enema reduction has not been established.<sup>4,5,18</sup> Sedatives were not used during pneumatic reduction in this study.

The time to reduction is also in consonance with the finding by Singh *et al.*<sup>6</sup> This is unsurprising because of the high intracolonic pressure seen in pneumatic reduction. Some studies have also noted that time to reduction becomes shorter with increasing effective intracolonic pressures.<sup>21,22</sup> Although, time to reduction is not commonly reported in most papers on experience with ultrasound-guided pneumatic reduction, it is way of quantifying how fast this technique is compared to fluoroscopy-guided pneumatic reduction.

Interestingly, the complication (perforation and recurrence) rates noted in this study are low and this is similar to what was obtainable in other studies.<sup>1,3,5,21</sup> Bowel perforation was not noted in this study. This may be because the pressure was gradually increased and maintained between 80 to 120mmHg. Careful patient selection also plays a role.<sup>21</sup> However, when perforation occurs during the procedure, there is usually a loss of inflating pressure and the intraperitoneal air appears noticeably as an echogenic line with a posterior ring-down artifact.<sup>6</sup> Recurrence, when it occurs, is treated with the same procedure without fear of undue exposure to ionizing radiation.

Agreeably, serum potassium >3.5 mmol/l was significantly associated with successful reduction. It is possible that with continuous loss of potassium from the mucus in the colon, (which forms a part of the red currant jelly stool) the serum potassium progressively gets depleted with time. However, a recent study which modified the Chang Mai University Intussusception scoring system replaced the method of reduction with hyponatremia.<sup>23</sup> Hyponatremia (serum sodium <136 mmol/l) and hypokalemia (<3.4mmol/l) were regarded as an unfavorable factor for a successful pneumatic reduction. A Palpable abdominal mass was also found to be significantly associated with successful ultrasound-guided pneumatic reduction in another study<sup>1</sup>. Other studies noted that

successful reduction was inversely associated with body weight, duration of symptoms, distal location of the mass, haemogram, and body temperature.<sup>24,25</sup> This variance may be accounted for by differences in population characteristics.

This study may be limited by the small sample size and the fact that the pneumatic reduction under ultrasound guidance was done by different members of the unit with varying expertise. However, all the team members were sufficiently trained prior to the commencement of the study. Also, radiographic features of intussusception were not considered during the assessment of predictive factors for successful ultrasound-guided pneumatic reduction in this study. Convincingly, this study will be of great help and assistance to radiologists and surgeons (especially in the LMICs) who are involved in the care of children with intussusception.

### Limitations of the study

With only 30 patients undergoing ultrasound-guided pneumatic reduction, the sample size may be too limited to generalize the results of this study broadly. A larger sample size could strengthen the reliability of findings. Also, the study was conducted in a single tertiary hospital, limiting the external validity of the findings, as outcomes may differ in other healthcare settings with varying levels of expertise and resources. However, with a 70% success rate, the study demonstrates that ultrasound-guided pneumatic reduction is effective and safe. This finding supports this approach as a viable alternative to fluoroscopy-guided methods, especially in low-resource settings.

### CONCLUSION

Ultrasound-guided pneumatic reduction of ileocolic intussusception is feasible, effective and safe. It may be used in the treatment of children who present with ileocolic intussusception. Serum potassium >3.5mmol/l may suggest the likelihood of successful reduction. This finding and the usefulness of sedation during Ultrasound-guided pneumatic reduction are areas requiring further exploration.

### Declarations

#### Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the UNTH Health Research and Ethics committee with number: NHREC/05/01/2008B-00002458-IRB00002323.

### Consent to participate

Written informed consent was obtained from parents/legal guardians.

### Consent to publish

Parents/legal guardians signed informed consent regarding publishing their data and photographs.

### Funding

None

### Conflict of interest

None of the authors have any conflict of interest

### Author's contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by ISC. The first draft of the manuscript was written by ISC and critically reviewed by SOE, UOE, EPN, and ICO. All authors commented on previous versions of manuscript. All authors read and approved the final manuscript.

### Data availability

The datasets generated during/and/or analyzed during the current study are available from the corresponding author on reasonable request.

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