Assessment of perceived distress due to nasopharyngeal swab collection in healthy Indian infants participating in a clinical trial

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Abstract
Routinely children are exposed to various procedures as a part of clinical care and/or research participation. Public health strategies to contain current COVID-19 pandemic demanded massive nasopharyngeal swab testing but limited data exist to confirm the extent of the pain and distress that result from this procedure. These data could help clinicians to formulate mitigation strategies, influence public health directives, and inform review boards/ethics committees to decide on risk-benefit ratio of the procedure. Hence, an observational study to assess perceived distress was nested in a phase IV alternate and reduced dose schedule trial of the pneumococcal conjugate vaccine (PCV) in which nasopharyngeal swab (NPS) was used to collect nasopharyngeal secretions as part of the study procedure. Out of 805 infant participants enrolled in the main study, a total of 425 infants were enrolled and observed for procedural distress at 18 weeks and 10 months of age using the Face Leg Activity Cry and Consolability (FLACC) Scale. The FLACC score and duration of cry were recorded. The mean FLACC score changed substantially from preprocedural to procedure in both age groups (from 0.08 to 5.8 at 18 weeks and from 0.5 to 7.007 at 10 months. \( P < .0001 \)). The proportion of infants experiencing higher FLACC scores (7-10) indicating severe distress increased significantly from 22\% (\( n = 95 \)) at 18 weeks to 61\% (\( n = 248 \)) at 10 months (\( P < .0001 \)). The mean duration of cry was significantly increased from 23.03 seconds at 18 weeks to 52.6 seconds at 10 months (\( P = .00 \)). Nasopharyngeal swab collection produced substantial distress which increased with age. Adequate training of sample collectors and supporting parent engagement during procedure could help in reducing the distress.

Keywords
ethics, pain, sample
1 | BACKGROUND

In a clinical setting, various sampling procedures need to be carried out for establishing a definitive diagnosis of an illness, while in a research setting, these procedures need to be performed to assess eligibility for study participation, as a part of routine clinical care and for ascertaining of study outcomes. Some of these procedures, such as blood collection, insertion of catheters, cannulation, parental injections, nasal swabs, and nasopharyngeal swab collection, are associated with varying degrees of pain and distress, especially in children. Although, pain is an important cause of distress, assessment and differentiation of pain, and distress in infant is inherently difficult due to their inability to self-report.\(^1\)\(^,\)\(^2\) Current definition of pain which describes pain as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" put less emphasis on actual tissue damage and recognizes pain in absence of injury.\(^3\) In infants the responsibility of assessing pain and distress often falls to the professional or lay care provider, whose knowledge, expertise, and beliefs influence their judgements. Therefore, various observational pain scales are used to provide more objective and standardized assessment of pain. These scales are validated in specific context of pain type, age groups, and clinical setting. The Children Hospital of Eastern Ontario Pain Scale (CHEOPS), The Evaluation Enfant Douleur (EVENDOL), and The Faces, Legs, Activity, Cry, and Consolability scale (FLACC) scale are validated and recommended for evaluation of procedural pain in infants.\(^4\)\(^,\)\(^5\)

Use of nasopharyngeal swab, nasal wash, nasal aspirate, and mid turbinate sampling is common methods for diagnosing respiratory infections and ascertaining colonizing microorganisms with comparable sensitivity.\(^6\)\(^,\)\(^7\)\(^,\)\(^8\) Use of flocked swab for nasopharyngeal sample collection is considered easy, flexible, and a preferred method for detecting an organism from the upper respiratory tract in children compared to nasal wash or nasopharyngeal aspirates due to reduced risk of aspiration.\(^9\)\(^,\)\(^10\) During current COVID-19 pandemic, nasopharyngeal swab collection had become more commonly and routinely done procedure for clinical care management.\(^11\) However, there are little data on the level of pain and distress associated with nasopharyngeal swab collection in a young infant.

While in clinical practice, accurate assessment of pain and distress is important for clinical management; in research settings, assessment of risk and benefits associated with study procedures is an essential part of ethical review and institutional ethics committees (IEC) are responsible for evaluating whether the procedural risk is reasonable in relation to the knowledge gained.\(^12\) Thus, this study was conducted to quantify the level of distress associated with nasopharyngeal swab collection in healthy infant. This will help inform primary care taker, clinicians, researchers to formulate appropriate alleviation strategies, and public health experts and policy makers to decide on compliance with testing during pandemic.

2 | METHODS

2.1 | Study design

This was an observational study nested within a clinical trial on alternate and reduced dose schedule of pneumococcal conjugate vaccines (PCV), which is hereafter referred to as the parent study. The parent study was conducted at Vadu Rural Health Program of KEM Hospital Research Centre, Pune, India from July 2016 to May 2018 to determine the nasopharyngeal pneumococcal carriage reduction and immunogenicity with reduced and alternate dose schedule of PCV in infancy. During this study, total 805 healthy infants were enrolled at the age of 6-8 weeks and followed till the age of 18 months. Nasopharyngeal swabs and blood samples were collected at different timepoints to assess primary (carriage reduction) and secondary (immunogenicity) endpoints.

2.2 | Study population

After ethics committee approval, infants already enrolled and whose 18 weeks’ visit was due, were invited in succession to participate until a total 425 infants were enrolled. Since this was an observational study, all invited participants agreed to participate. All participants underwent the procedure at 18 weeks and 10 months of age, and the FLACC scale was used to assess the level of distress experienced before and during the procedure.

2.3 | Measurement of distress

Valid observational scales are needed to assess pain and distress in infant who lack the verbal ability to self-report. The Face, Legs, Activity Cry, and Consolability scale (FLACC) scale is one of the most well-known, widely used observational scale and is recommended for procedural pain measurement.\(^13\) Although the FLACC scale was designed to assess postoperative pain, a recent review supports the reliability and sensitivity of the FLACC scale for procedural pain assessment with limited capacity to differentiate between pain and non–pain-related distress, thus measuring composite of pain and distress.\(^14\)\(^,\)\(^15\) The FLACC scale comprised of 5 items each scored 0-2 the sum of which provides a score between 0 and 10; wherein 0 indicates no pain and distress and 10 indicates maximum pain and distress. Although there is no evidence that the FLACC scale is a ratio scale of measurement, for analysis purpose, it was treated as a ratio and commonly accepted scores were used as cut-offs for varying severity. The score of 0 was termed as no pain/distress *(Relaxed/comfortable); score between 1-3 was termed as mild; 4-6 as a moderate; and 7-10 as a severe*\(^16\) (Table S1).

All participants underwent the procedure at 18 weeks and 10 months of age, and the FLACC scale was used to assess the
level of distress experienced before and during the procedure. The preprocedural phase was considered as 2-5 minutes before the start of the procedure, and the procedural phase was considered to begin with introducing the swab into the nostril until removing the swab from the nostril. The independent observer had recorded the scores in each of the FLACC categories and documented the highest score achieved during the entire procedural phase. The duration between the start of the cry from the time the swab was introduced into the infant’s nostril until the cry had not been audible for at least five seconds was recorded as the duration of cry. The FLACC score and duration of cry were noted by a trained observer independent of the swab collector at both the age groups. The English version of the FLACC scale was used to capture the data as the independent observer was well versed in the English language.

2.4 | Sample size calculation and data analysis

Assuming that 50% of children would experience significant pain and distress (score >= 7 on FLACC scale) due to nasopharyngeal swab collection and considering 95% confidence interval with 5% absolute error, the sample size was calculated as 384 and adding 10% dropout; the sample size was 423 which was rounded off to 425. The average values for pre- and postprocedure FLACC scores were calculated. Mean FLACC scores (preprocedural and proce-
dural), the proportion of the children in the three severity categories (mild, moderate, and severe) defined by FLACC scores, and mean duration of cry were calculated. The outcome variables included (a) change in mean FLACC score (b) proportions of infants experiencing various levels of distress (c) change in duration of cry and tested for significance by Fisher’s exact test.

All the data analysis was done using STATA 15.0 version.

2.5 | Ethical consideration

The study was approved by the KEM Hospital Research Centre Ethics Committee. As this was an observational study within the main PCV study, verbal consent was sought from the parents before administering the FLACC scale.

3 | RESULTS

Of the total 425 participants enrolled at 18 weeks of age, 406 (95.9%) successfully completed the study at 10 months. We were unable to collect the data from 19 participants (Figure 1).

3.1 | Demographic characteristics

At 18 weeks, 50.8% (n = 215) of participants were female and participants’ mean age, weight and height were 135.1 days, 6.2 kg, and 62.3 cm, respectively; while at 10 months 51.2% (n = 208) were female and participants’ mean age, weight, and height were-304.4 days, 7.9 kg, and 70.3 cm, respectively (Table 1).

3.2 | Change in mean FLACC score

The preprocedural and procedural mean FLACC scores at 18 weeks were 0.08 (SD-0.03, range 0-6) and 5.8 (SD-0.05, range 2-8), respectively, while at 10 months it was 0.5 (SD-0.08, range 0-8) and 7.0 (SD-0.07, range 3-10). This showed a significant (P < .001) shift to higher scores during the procedure at both the ages. (Diff: 5.7 [95% CI 5.7-5.7] at 18 weeks while 6.4 [95% CI 6.3-6.5] at 10 months). Similarly, comparison of preprocedural and procedural scores of both timepoints showed statistically significant (P < .001) differences.
(Diff: 0.5 [95% CI 0.4-0.5] for preprocedural scores while 1.2 [95% CI 1.1-1.2] for procedural scores) (Table 2).

3.3 | Proportion of participants experiencing distress

At 18 weeks, before the start of procedure, 98% of children (n = 414) of the infants achieved the FLACC score of 0” indicating no sign of distress whereas, at 10 months, 89% (n = 362) of participants had FLACC score of “0.” With the initiation of the procedure, the FLACC score was shifted to higher levels. In procedural phase at 18 weeks, 74% (n = 313) of the infants achieved the FLACC score of 4-6 indicating a moderate level of distress, while 22% (n = 95) had a FLACC score of 7-10 indicating severe distress level and only 3.5% (n = 15) had a score of 1-3 experiencing milder distress while at 10 months 61.0% (n = 248) of participants achieved the FLACC score of 7-10; 35.2% (n = 143) had the FLACC score of 4-6 and only 3.6% (n = 15) had the FLACC score of 1-3. The proportion of children experiencing severe distress was significantly higher at 10 months compared to 18 weeks (61% vs 22%) (P-value .0001) (Table 3).

3.4 | Change in cry duration

In infants, the cry is the most audio- visible activity and is an important category/item of FLACC scale. The mean cry duration was 23 seconds (SD=1.2, range 0-132 seconds) and 52 seconds (SD=2.07, range 0-208 seconds) at 18 weeks and 10 months, respectively, showing statistically significant (P < .001) difference (diff: 29 [CI-28.7-29.2]). (Table 2).

4 | DISCUSSION

To our knowledge, this is the first Indian study assessing the levels of distress due to nasopharyngeal swab collection in healthy infants. This study results showed that nasopharyngeal swab collection caused a substantial distress which increased with the age. These results are in concordance with the studies done previously.

Westra et al17 assessed the discomfort levels, as perceived by parents in healthy children participating in vaccine trial and undergoing NPS. They demonstrated that nasopharyngeal swabs caused moderate-to-high discomfort. However, in their study, a behavioral scale was not used and the perceived discomfort was assessed by questionnaire completed by parents.

Babl et al14 used FLACC scale to quantify pain and distress in young children undergoing a range of common emergency department (ED) procedures and found substantial level of pain and distress with these procedures.

A study by Crelin et al15 where FLACC scale was used to quantify the procedural pain in infants and young children showed that there was an increase in FLACC scores in children experiencing a painful procedure.

Previously, the nasopharyngeal swab was collected in a symptomatic patient by experienced medical/paramedical staff or in a research setting by trained personnel. However, current pandemic response demands nasopharyngeal swabs to be collected on a massive scale, many a times by an inadequately trained person. This can make procedure more distressful and have potential to threaten public health response and compliance. Although NPS is recognized as a choice of procedure for the detection of SARS-COV-2 and the chances of detection of viral genetic material are higher with NPS,18 it would be a trade-off between the procedural yield and procedural distress to consider the procedure in context of pandemic. This might influence the healthcare-related behavior and impacts the public health action plans. Adequate training of swab collector, engagement of parents/caregiver, use of Dacron swab, and consideration for alternate bio-samples like saliva could be an important mitigation strategies.19

In this study, we had sampled each of participant twice; at ages of 18 weeks and 10 months and our results showed higher scores at 10 months. Although these differences were statistically significant, we need more studies to conclude on clinical importance of these findings. This was similar to what Westra et al17 have reported. However, Babl

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**TABLE 1** Demographic characteristics

| Characters          | 18 wk (N = 423) | 10 mo (N = 406) |
|---------------------|-----------------|-----------------|
| Gender, female N (%)| 215 (50.8)      | 208 (51.2)      |
| Age, (days), mean (SD) | 135.1 (8.4)    | 304.4 (7.1)    |
| Weight, (kg), mean (SD) | 6.2 (0.8)      | 7.9 (1.04)     |
| Height, (cm), mean (SD) | 62.3 (2.4)     | 70.3 (2.7)     |

**TABLE 2** Change in mean FLACC scale scores and mean cry duration

| Age group | Mean FLACC score-preprocedural Mean (SD, range) | Mean FLACC score-procedural Mean (SD, range) | Difference of FLACC score | Mean cry duration sec (mean) (SD, range) |
|-----------|-----------------------------------------------|----------------------------------------------|--------------------------|------------------------------------------|
| 18 wk     | 0.08 [(0.03) (range 0-6)]                    | 5.8 [(0.05) (range 2-8)]                    | 5.72*                    | 23 [(1.2) (0-132)]                      |
| 10 mo     | 0.5 [(0.084) (range 0-8)]                    | 7.07 [(0.07) (range 3-10)]                  | 6.49*                    | 52 [(2.07) (0-208)]                     |
| Difference| 0.5*                                         | 1.27*                                        |                         | 29*                                     |

*P < .0001.
et al reported that FLACC scores during the procedural phase were highest in the youngest age group and lowest in the oldest age group. This different result could be explained by the fact that the behavioral responses to pain and distress are influenced by previous pain experiences, its’ context, and sociocultural factors. Therefore, it is difficult to conclude on the effect of age despite repeat sampling.

In our study, mean cry duration also changed significantly. Similar findings were observed by Harrison et al who found that during immunization almost all (94%) infants cried before or during the injections for a median of 33 seconds (IQR = 39), up to 146 seconds.

Multiple factors are elucidated that appear to increase the anticipatory pain and distress during procedure. Apart from age, child psychopathology, difficult child temperament, parent distress promoting behaviors, parent situational distress, previous pain events, parent anticipation of distress, and parent anxious predisposition are the responsible factors as systematically reviewed by Racine et al. Again, the various sampling collection methods (swab, aspirate, brush, and wash) and swab used (Dacron vs Nylon) would affect the level of distress. In their study to compare distress between three different types of intranasal swab specimens, Frazee et al found that distress increased significantly with a depth of swab sampling. While comparing the nasal samples obtained by four different methods swab, aspirate, brush, and wash, Spyridiki et al demonstrated that nasal washes yielded the highest viral detection rate without excessive patient distress. In contrast, nasal brushes produced the lowest detection rates with the highest level of distress, while swabs were perceived as less distressful. Since we had collected all the samples using flocked Dacron flexible swabs, we could not assess the effects of swabs on distress. Tunsjo et al in their study showed that sample collection by flocked swab uses less equipment, is more flexible and causes less distress to patient. While collecting the swab from COVID-19 suspects, Bidkar et al found that suspects who underwent the procedure using Nylon swabs were six times more likely to have pain/distress compared to when Dacron swab was used (RR – 6.7).

Although self-reporting is gold standard for assessment of pain and distress there are very limited data available in infants. Staphorst et al showed that most children had reported limited distress during the research procedures (means: 1-2.6 on a scale from 1 to 5). While Mittal et al reported moderate-to-severe distress with swab collection in adult patients while collecting the samples for SARS-COV-2.

Various nonpharmacological strategies are effectively used to reduce the pain/distress in infants and children. While evaluating the role of distraction to reduce the perception of vaccination pain, Ozdemir et al found lower pain scores and shorter crying duration using FLACC score in response to vaccination in a room furnished with a musical mobile.

### 4.1 | Strengths

The study’s major strength is sample size of the cohort. Twice sampling of a participant to ascertain the effect of age on perception of distress could have been another strength but to limit by the fact that pain and distress responses are modifed by previous painful/distressful experience.

### 4.2 | Limitations

This was a single-center study and had limited generalizability. This study had not used any comparison group like different swab collectors, different FLACC assessors and control group undergoing less/no painful and distressful procedure, for example, measuring SPO2 by Pulse Oximeter.

### 4.3 | Implications of the study

This study provides the objective assessment of pain and distress a child can foresee during routine procedures like NPS collection and emphasize the need for appropriate training, supports engagement of care takers, and use of alleviation measures. Though this study was conducted before pandemic, the findings of the study are important in pandemic context and could prompt the policy maker to think of pain/distress relieving measure to increase procedural compliance and public health behavior. These results could also help inform the review boards to decide on risk-benefit ratio of the procedure while reviewing the trial protocols.

### 5 | CONCLUSION

Nasopharyngeal swab collection produced substantial distress in infants, which increased with age. Alternative bio-sampling could help in getting maximum community response to public health strategies especially during pandemic. Additionally adequate training of swab

| Total FLACC scale score categories | 18 wk (N = 423) | 10 mo (N = 406) |
|-----------------------------------|----------------|-----------------|
|                                   | Preprocedural N (%) | Procedural N (%) | Preprocedural N (%) | Procedural N (%) |
| 0 (No distress/pain)             | 414 (97.8)         | 0               | 362 (89.1)         | 0               |
| 1-3 (mild)                       | 4 (0.9)            | 15 (3.5)        | 2 (0.4)            | 15 (3.6)        |
| 4-6 (moderate)                   | 5 (1.1)            | 313 (74.0)      | 41 (10.09)         | 143 (35.2)      |
| 7-10 (severe)                    | 0                 | 95 (22.4)       | 1 (0.2)            | 248 (61.08)     |
| Total                             | 423               | 406             |
collector and care taker engagement during procedure could help in minimizing the distress.

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CONFLICT OF INTEREST
None.

AUTHOR CONTRIBUTIONS
AK, GD, AB, and SJ: Conceptualization and design of the study. AK, GD, and AG: Acquisition of data. AK and GD: Analysis and interpretation of data. AK and GD: Drafting the manuscript. AA, SR, AG, SJ, and AB: Revised it critically for important intellectual content. AK, GD, AA, SR, AG, AB, and SJ: Final approval of the version to be submitted.

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SUPPORTING INFORMATION
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