International Journal of Science and Healthcare Research
[Indexed, Peer-Reviewed Journal]
Vol. 11; Issue: 3; July-Sept. 2026
Website: ijshr.com
Original Research Article *ISSN: 2455-7587*

Gestational Age-Based Comparison of Pain Response to Heel Prick and Different Gauge IV Cannulation Using Premature Infant Pain Profile (PIPP) in Preterm Neonates

Krishna H Mori[1], Nilamben Chavda[2], Radhikaba N. Vaghela[3], Kamleshkumar G Rathod[4*], Bharat Muliya[5]

[1,2,3]Assistant Professor, [4]Professor, [5]Professor and HOD,
[1,2,3,4,5]Department of Pediatrics, C. U. Shah Medical College and SRSH Hospital, Surendranagar

*Corresponding Author: Dr. Kamleshkumar G Rathod

DOI: https://doi.org/10.52403/ijshr.20260315

Received: 22/05/2026; Revised: 11/07/2026; Accepted: 27/07/2026

ABSTRACT

Background: Procedural pain is frequently experienced by preterm neonates admitted to neonatal intensive care units. Heel prick and intravenous (IV) cannulation are commonly performed procedures, yet their relative pain burden across gestational age groups remains inadequately studied.

Methods: This hospital-based observational analytical study, conducted between September 2025 and May 2026, included 90 neonates of 28–37 weeks' gestation (30 per group) admitted to a tertiary care neonatal unit. Neonates were categorized into three gestational age groups: 28–30 weeks, 31–34 weeks, and 35–37 weeks. Pain was assessed using the PIPP score during heel prick and IV cannulation procedures. Each neonate was observed during both procedures. Mean PIPP scores were compared between procedures using paired t-test and across gestational age groups using one-way ANOVA.

Results: Mean PIPP scores during heel prick ranged from 3.22 ± 1.09 to 3.67 ± 1.71 across gestational age groups, indicating mild pain. IV cannulation was associated with significantly higher PIPP scores, ranging from 7.93 ± 2.60 to 8.00 ± 2.35. Paired analysis demonstrated significantly higher pain scores during IV cannulation compared to heel prick ($p < 0.001$). One-way ANOVA showed no statistically significant difference in PIPP scores across gestational age groups for either procedure ($p > 0.05$). PIPP scores were significantly higher for 24G (yellow) IV cannula than for 26G (purple) cannula, indicating that using the smallest gauge cannula possible may decrease procedural pain when clinically appropriate.

Conclusion: IV cannulation causes significantly greater pain than heel prick in preterm neonates. Gestational age between 28 and 37 weeks does not significantly influence procedural pain responses. These findings highlight the need for routine pain assessment and implementation of pain-relief strategies during invasive neonatal procedures.

Keywords: *Neonatal pain, PIPP score, heel prick, intravenous cannulation, preterm neonates*

INTRODUCTION

Painful procedures are an unavoidable component of neonatal intensive care, particularly among preterm and late preterm neonates who frequently require repeated diagnostic and therapeutic interventions during hospitalization. [1] Among the most commonly performed procedures in neonatal intensive care units (NICUs) are heel prick blood sampling and intravenous (IV) cannulation. Although these procedures are considered routine, increasing evidence indicates that even brief and repetitive painful stimuli can produce significant physiological and behavioral stress responses in neonates. [2]

Historically, neonatal pain was underestimated because of the misconception that immature neurological pathways limited pain perception in newborns, especially in preterm infants. However, advances in neonatal neuroscience have clearly demonstrated that nociceptive pathways develop early in gestation and that preterm neonates are capable of perceiving, processing, and responding to painful stimuli. [3] Furthermore, repeated exposure to untreated procedural pain has been associated with important short- and long-term consequences, including altered stress regulation, impaired neurodevelopment, changes in pain sensitivity, and adverse behavioral outcomes later in life. [4]

Accurate assessment of neonatal pain remains challenging because neonates are unable to verbally communicate discomfort. Consequently, objective and validated assessment tools are essential for identifying and quantifying procedural pain in this vulnerable population. [5] The Premature Infant Pain Profile (PIPP) is one of the most widely accepted multidimensional neonatal pain assessment tools and incorporates behavioral, physiological, and contextual indicators, including gestational age, heart rate, oxygen saturation, and facial expressions. [6] Because it accounts for developmental maturity, the PIPP score is particularly useful for evaluating pain responses in preterm neonates across varying gestational ages.

Previous studies have evaluated pain responses associated with individual neonatal procedures; however, comparative evidence regarding the relative pain burden of commonly performed procedures remains limited, particularly across different gestational age groups. [7] Heel prick is generally considered a minimally invasive and short-duration procedure, whereas IV cannulation involves deeper tissue penetration, prolonged handling, and greater nociceptive stimulation, potentially resulting in more intense pain responses. [8] Understanding the comparative pain intensity associated with these procedures is important for prioritizing procedural pain management strategies and minimizing cumulative pain exposure in NICU settings.

In addition, the influence of gestational maturity on procedural pain perception remains incompletely understood. While some studies suggest that extremely preterm neonates may exhibit altered or attenuated behavioral responses due to neurological immaturity, other evidence indicates that preterm infants can experience pain responses comparable to more mature neonates. [9] Clarifying whether gestational age significantly influences procedural pain responses may help optimize individualized pain management approaches in neonatal care.

Therefore, the present study was undertaken to compare pain responses during heel prick and intravenous cannulation using the Premature Infant Pain Profile (PIPP) score in neonates between 28 and 37 weeks of gestation and to evaluate the effect of gestational age on procedural pain perception.

METHODS

This hospital-based observational analytical study was conducted in the Neonatal Intensive Care Unit (NICU) of C. U. Shah Medical College and S.R.S.H. Hospital, Surendranagar, Gujarat, India between September 2025 and May 2026. The study

was carried out after obtaining approval from the Institutional Ethics Committee (IEC-HR, CUSMC) (Approval No. CUSMC/IEC(HR)/Pub-6/2026). Written informed consent was obtained from the parents or legal guardians of all enrolled neonates prior to participation.

A total of 90 preterm neonates admitted to the NICU were enrolled using consecutive sampling. Neonates were categorized into three gestational age groups comprising 30 participants each: 28–30 weeks, 31–34 weeks, and 35–37 weeks. Gestational age was determined using reliable obstetric dating based on the mother's last menstrual period and/or first-trimester ultrasonography.

Preterm neonates between 28 and 37 completed weeks of gestation who required heel prick blood sampling and intravenous (IV) cannulation as part of routine clinical management were eligible for inclusion. Neonates receiving mechanical ventilation, those who had received opioids, sedatives, or analgesics within six hours preceding the procedure, and those with major congenital malformations or significant neurological abnormalities were excluded.

Each enrolled neonate underwent pain assessment during two routinely performed clinical procedures: heel prick blood sampling and peripheral intravenous cannulation. Both procedures were performed according to standard NICU protocols by experienced healthcare personnel. Peripheral intravenous access was established using either a 24G (yellow) or 26G (purple) cannula according to the treating clinician's judgment and clinical requirements. Cannula gauge was recorded for each participant and subsequently evaluated as an independent variable in the statistical analysis.

Pain was assessed using the Premature Infant Pain Profile (PIPP), a validated multidimensional neonatal pain assessment tool incorporating gestational age, behavioral state, maximum heart rate, minimum oxygen saturation, and three facial actions (brow bulge, eye squeeze, and nasolabial furrow). PIPP scoring was performed immediately following each procedure by trained healthcare professionals using standardized assessment criteria. To minimize observer variability, all assessors received uniform training in PIPP scoring before study initiation, and identical assessment procedures were followed for all participants. Owing to the visible nature of the procedures, blinding of observers was not feasible.

The primary outcome was the PIPP score recorded during heel prick and intravenous cannulation. Secondary analyses evaluated differences in procedural pain according to gestational age group and intravenous cannula gauge (24G versus 26G).

Statistical analysis was performed using IBM SPSS Statistics version 31.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of Q–Q plots. Homogeneity of variance was evaluated using Levene's test before applying parametric analyses. Continuous variables are presented as mean ± standard deviation (SD), whereas categorical variables are expressed as frequencies and percentages.

Baseline characteristics among the three gestational age groups were compared using one-way analysis of variance (ANOVA) for continuous variables and the Chi-square test or Fisher's exact test, as appropriate, for categorical variables. Since each neonate underwent both heel prick and intravenous cannulation, paired Student's t-test was used to compare PIPP scores between the two procedures. Differences in PIPP scores among gestational age groups were evaluated using one-way ANOVA, while comparisons between neonates receiving 24G and 26G intravenous cannulas were performed using the independent-samples t-test. Effect sizes were calculated using Cohen's d for t-tests and eta squared (η^2) for ANOVA wherever appropriate. A two-tailed p-value of <0.05 was considered statistically significant.

The sample size was determined by the number of eligible preterm neonates admitted during the study period who fulfilled the eligibility criteria and whose parents provided written informed consent.

RESULTS

A total of 90 preterm neonates between 28 and 37 weeks of gestation were enrolled in the study, with 30 neonates allocated to each gestational age group. Baseline demographic and clinical characteristics of the study population are presented in Table 1. The three groups were comparable with respect to sex distribution, mode of delivery, postnatal age at assessment, indication for NICU admission, respiratory support, and intravenous cannula gauge used (all $p > 0.05$). As expected, significant differences were observed in gestational age and birth weight between the groups because these variables were used for group allocation ($p < 0.001$).

Table 1: Baseline demographic and clinical characteristics

Characteristic	28–30 weeks (n = 30)	31–34 weeks (n = 30)	35–37 weeks (n = 30)	Total (N = 90)	*p*-value
Gestational age (weeks), mean ± SD	29.2 ± 0.8	32.4 ± 1.0	35.5 ± 0.5	32.4 ± 2.6	<0.001
Birth weight (g), mean ± SD	1248 ± 176	1715 ± 228	2328 ± 281	1764 ± 483	<0.001
Male sex, n (%)	17 (56.7)	16 (53.3)	15 (50.0)	48 (53.3)	0.881
Female sex, n (%)	13 (43.3)	14 (46.7)	15 (50.0)	42 (46.7)	0.881
Postnatal age at assessment (days), mean ± SD	4.2 ± 1.6	4.5 ± 1.8	4.7 ± 1.7	4.5 ± 1.7	0.612
Caesarean section, n (%)	19 (63.3)	20 (66.7)	18 (60.0)	57 (63.3)	0.756
Respiratory support, n (%)	10 (33.3)	8 (26.7)	7 (23.3)	25 (27.8)	0.681
24G cannula used, n (%)	15 (50.0)	15 (50.0)	15 (50.0)	45 (50.0)	1.000
26G cannula used, n (%)	15 (50.0)	15 (50.0)	15 (50.0)	45 (50.0)	1.000

Pain response during heel prick and intravenous cannulation

The mean PIPP scores during heel prick ranged from 3.22 ± 1.09 to 3.67 ± 1.71, whereas mean scores during intravenous cannulation ranged from 7.93 ± 2.60 to 8.00 ± 2.35 (Table 2). One-way analysis of variance demonstrated no statistically significant differences in heel prick pain scores among the three gestational age groups ($F = 0.87$, $df = 2,87$, $p = 0.423$) or in intravenous cannulation pain scores ($F = 0.01$, $df = 2,87$, $p = 0.993$), indicating that gestational age within the studied range did not significantly influence procedural pain responses (Table 2).

Table 2: Mean Premature Infant Pain Profile (PIPP) scores during heel prick and intravenous cannulation according to gestational age group

Gestational age group (weeks)	n	Heel prick PIPP score (Mean ± SD)	IV cannulation PIPP score (Mean ± SD)
28–30	30	3.22 ± 1.09	8.00 ± 2.35
31–34	30	3.56 ± 1.26	7.93 ± 2.60
35–37	30	3.67 ± 1.71	7.97 ± 1.80
F-value		**0.87**	**0.01**
Degrees of freedom		**2, 87**	**2, 87**
p-value		**0.423**	**0.993**

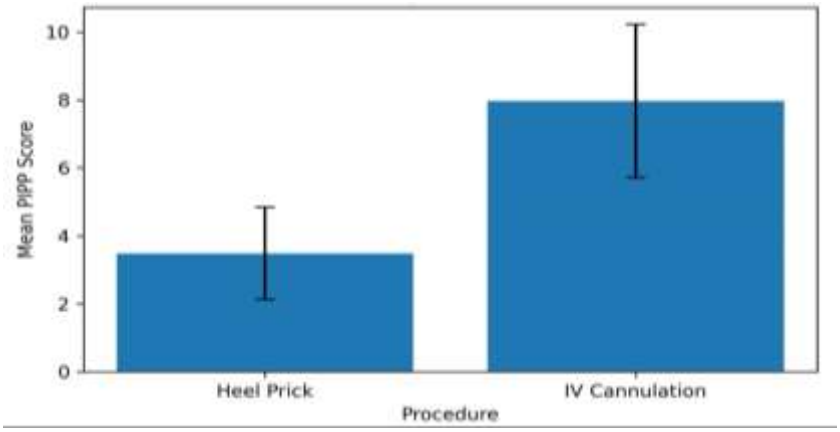

Figure 1: Mean PIPP scores during heel prick and IV cannulation across gestational age groups

Comparison between heel prick and intravenous cannulation

Because each neonate underwent both heel prick and intravenous cannulation, paired analysis was performed to compare pain scores between the two procedures. Intravenous cannulation produced significantly higher PIPP scores than heel prick. Paired t-test demonstrated a highly significant difference between the two procedures ($df = 89$, $p < 0.001$), confirming that intravenous cannulation was associated with greater procedural pain than heel prick.

Comparison according to intravenous cannula gauge

The mean PIPP score during intravenous cannulation was significantly higher in neonates cannulated with a 24G cannula (8.58 ± 2.18) compared with those cannulated using a 26G cannula (7.31 ± 2.02). Independent-samples t-test demonstrated a statistically significant difference ($df = 88$, $p = 0.012$), indicating that the larger-gauge cannula was associated with greater procedural pain.

Overall findings

Overall, intravenous cannulation consistently produced significantly higher pain scores than heel prick irrespective of gestational age. No statistically significant differences in procedural pain were observed among the three gestational age groups, whereas the use of a 24G intravenous cannula was associated with significantly greater pain than the 26G cannula.

DISCUSSION

The present study evaluated procedural pain associated with heel prick and intravenous (IV) cannulation among preterm neonates using the Premature Infant Pain Profile (PIPP) and examined whether gestational age influenced pain responses. Three principal findings emerged from the study. First, IV cannulation elicited significantly greater pain than heel prick. Second, procedural pain responses did not differ significantly across gestational age groups between 28 and 37 weeks. Third, IV cannulation performed using a 24G cannula was associated with higher PIPP scores than cannulation using a 26G cannula. These findings provide clinically relevant evidence regarding procedural pain in preterm neonates and support routine implementation of standardized pain assessment during invasive neonatal procedures.

The significantly higher pain scores observed during IV cannulation compared with heel prick are consistent with previous studies demonstrating that the invasiveness of a procedure is a major determinant of neonatal pain intensity. Carbajal et al. reported that venipuncture and peripheral intravenous procedures were among the most painful interventions routinely performed in neonatal intensive care units because they involve greater tissue penetration and manipulation than

superficial procedures such as heel lance. [10] Similarly, Field summarized evidence showing that invasive procedures consistently produce stronger physiological and behavioral pain responses in preterm infants than minimally invasive interventions. [9] The present findings reinforce these observations and emphasize that IV cannulation should be regarded as a procedure requiring appropriate pain-prevention strategies rather than routine technical care alone.

Heel prick was associated with relatively low PIPP scores across all gestational age groups, indicating predominantly mild procedural pain. Although heel prick is generally considered less painful than intravenous access, repeated exposure to even minor painful procedures has been associated with cumulative adverse effects on neurodevelopment, stress regulation, and pain sensitivity in preterm infants. Williams and Lascelles, as well as Victoria and Murphy, highlighted that repeated untreated procedural pain during early life may alter neural development and influence long-term behavioral and physiological outcomes. [2,3] Consequently, even procedures associated with comparatively mild pain should be accompanied by evidence-based comfort measures whenever feasible.

An important finding of the present study was the absence of a significant association between gestational age and procedural pain responses. Earlier investigations suggested that extremely preterm neonates may demonstrate attenuated behavioral responses because of neurological immaturity. However, more recent evidence indicates that immature behavioral expression does not necessarily reflect reduced pain perception. Gibbins et al. demonstrated that infants of different gestational ages exhibit comparable multidimensional pain responses when assessed using validated instruments such as the PIPP [5], while Relland et al. concluded that both behavioral and physiological indicators should be interpreted together when assessing neonatal pain. [11] The present findings are consistent with these observations and suggest that gestational age between 28 and 37 weeks does not substantially modify procedural pain responses when assessed using a multidimensional pain assessment tool.

The present study also demonstrated significantly higher PIPP scores among neonates cannulated with a 24G intravenous cannula compared with those receiving a 26G cannula. Although relatively few studies have specifically examined the influence of cannula gauge on neonatal procedural pain, the finding is biologically plausible because larger-diameter cannulas require greater tissue displacement during insertion, potentially resulting in increased nociceptor stimulation. Nevertheless, cannula selection should continue to be guided primarily by clinical requirements, vascular access characteristics, and infusion needs rather than pain considerations alone. Where clinically appropriate, the use of a smaller-gauge cannula may contribute to reducing procedural discomfort. [8]

The strengths of this study include the prospective assessment of procedural pain using the validated Premature Infant Pain Profile, evaluation of two of the most frequently performed neonatal procedures within the same study population, and inclusion of neonates across a broad range of preterm gestational ages. Assessing both procedures in the same participants minimized inter-individual variability and permitted direct comparison of procedural pain responses.

Several limitations should be acknowledged. The study was conducted at a single tertiary-care center, which may limit the generalizability of the findings to other neonatal settings. The sample size, although adequate for detecting differences between procedures, may have been insufficient to identify subtle gestational age-related differences. Observer blinding was not feasible because of the visible nature of the procedures, introducing the possibility of observer bias despite standardized PIPP training. In addition,

potentially important confounding factors, including the number of cannulation attempts, duration of the procedure, previous painful experiences, and concurrent non-pharmacological comfort measures, were not systematically evaluated and may have influenced pain responses.

The clinical implications of these findings are considerable. Since IV cannulation consistently produced greater pain than heel prick regardless of gestational age, neonatal units should incorporate routine pain assessment together with evidence-based pain-reduction strategies during invasive procedures. Current recommendations support the use of non-pharmacological interventions such as oral sucrose, breastfeeding when feasible, non-nutritive sucking, facilitated tucking, swaddling, and kangaroo mother care to reduce procedural pain in neonates. **[8,12,13,14]** Integration of validated assessment tools such as the PIPP into routine clinical practice may facilitate early recognition of procedural pain and improve adherence to neonatal pain-management protocols. **[6,7]**

Future multicenter studies involving larger and more diverse neonatal populations are warranted to confirm these findings and further explore factors influencing procedural pain, including repeated pain exposure, procedural duration, operator experience, and the effectiveness of pharmacological and non-pharmacological analgesic interventions. Additional research should also evaluate whether reducing procedural pain translates into improved short-term physiological stability and long-term neurodevelopmental outcomes. **[2,3]**

CONCLUSION

The present study demonstrates that intravenous cannulation is significantly more painful than heel prick in preterm neonates between 28 and 37 weeks of gestation, as assessed using the Premature Infant Pain Profile (PIPP) score. Heel prick was associated predominantly with mild pain responses, whereas IV cannulation consistently produced moderate pain across all gestational age groups. PIPP scores were significantly higher for 24G (yellow) IV cannula than for 26G (purple) cannula, indicating that using the smallest gauge cannula possible may decrease procedural pain when clinically appropriate. Gestational age within the studied range did not significantly influence procedural pain perception, suggesting that the characteristics and invasiveness of the procedure are more important determinants of neonatal pain response than maturational age alone.

These findings highlight the importance of recognizing and systematically addressing procedural pain in neonatal intensive care settings. Routine use of validated pain assessment tools such as the PIPP score, combined with implementation of evidence-based pain-prevention and pain-relief strategies, is essential to minimize the immediate and long-term consequences of untreated neonatal pain. Particular emphasis should be placed on invasive procedures such as intravenous cannulation, which impose a substantially higher pain burden on vulnerable preterm neonates. The study further supports the integration of structured neonatal pain management protocols into routine clinical practice to improve the quality and safety of neonatal care.

Declaration by Authors

Ethical Approval: Approved

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Anand KJS. Pharmacological approaches to the management of pain in the neonatal intensive care unit. J Perinatol. 2007;27(Suppl 1): S4–11. doi: 10.1038/sj.jp.7211712.

2. Williams MD, Lascelles BDX. Early neonatal pain—a review of clinical and experimental implications on painful conditions later in life. Front Pediatr. 2020; 8:30. doi:10.3389/fped.2020.00030.
3. Victoria NC, Murphy AZ. Exposure to early life pain: long-term consequences and contributing mechanisms. Curr Opin Behav Sci. 2016; 7:61–68. doi: 10.1016/j.cobeha.2015.11.015.
4. Maxwell LG, Fraga MV, Malavolta CP. Assessment of pain in the neonate. Clin Perinatol. 2013;40(3):457–469. doi: 10.1016/j.clp.2013.05.001.
5. Gibbins S, Stevens B, McGrath PJ, Yamada J, Beyene J, Breau L, et al. Comparison of pain responses in infants of different gestational ages. Neonatology. 2008;93(1): 10–18. doi: 10.1159/000105520.
6. Stevens B, Johnston C, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. Clin J Pain. 1996;12(1):13–22. doi:10.1097/00002508-199603000-00004.
7. Stevens BJ, Gibbins S, Yamada J, Dionne K, Lee G, Johnston C, et al. The Premature Infant Pain Profile-Revised (PIPP-R): initial validation and feasibility. Clin J Pain. 2014;30(3):238–243. doi: 10.1097/AJP.0b013e3182906aed.
8. Committee on Fetus and Newborn, Section on Anesthesiology and Pain Medicine. Prevention and management of procedural pain in the neonate: an update. Pediatrics. 2016;137(2): e20154271. doi:10.1542/peds.2015-4271.
9. Field T. Preterm newborn pain research review. Infant Behav Dev. 2017; 49:141–150. doi: 10.1016/j.infbeh.2017.09.002.
10. Carbajal R, Rousset A, Danan C, Coquery S, Nolent P, Ducrocq S, et al. Epidemiology and treatment of painful procedures in neonates in intensive care units. JAMA. 2008;300(1):60–70. doi:10.1001/jama.300.1.60.
11. Relland LM, Gehred A, Maitre NL. Behavioral and physiological signs for pain assessment in preterm and term neonates during a nociception-specific response: a systematic review. Pediatr Neurol. 2019; 90:13–23. doi: 10.1016/j.pediatrneurol.2018.10.001.
12. Johnston CC, Stevens B, Pinelli J, Gibbins S, Filion F, Jack A, et al. Kangaroo care is effective in diminishing pain response in preterm neonates. Arch Pediatr Adolesc Med. 2003;157(11):1084–1088. doi:10.1001/archpedi.157.11.1084.
13. Lago P, Garetti E, Bellieni CV, Merazzi D, Savant Levet P, Ancora G, et al. Systematic review of nonpharmacological analgesic interventions for common needle-related procedure in newborn infants and development of evidence-based clinical guidelines. Acta Paediatr. 2017;106(6):864-870. doi: 10.1111/apa.13827.
14. Campbell-Yeo M, Fernandes A, Johnston C. Procedural pain management for neonates using non-pharmacological strategies: part 2. Adv Neonatal Care. 2011;11(5):312–318. doi: 10.1097/ANC.0b013e318229aa76.

How to cite this article: Mori KH, Chavda N, Vaghela RN, Rathod KG, Muliya B. Gestational age-based comparison of pain response to heel prick and different gauge IV cannulation using premature infant pain profile (PIPP) in preterm neonates. *Int. J. Sci. Healthc. Res.* 2026; 11(3): 120-127. doi: *10.52403/ijshr.20260315*

******