



# Evaluating the Analgesic Efficacy of Oral Glucose in Mitigating Acute Vaccination-Induced Pain in Term Neonates: A Randomized Controlled Clinical Trial

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## ABSTRACT

**Background:** Neonates frequently encounter painful stimuli during routine clinical procedures. Among these, vaccination stands out as a prevalent source of distress, necessitating the adoption of safe, accessible, and efficacious analgesic interventions. This study aimed to investigate the analgesic efficacy of oral glucose administration in attenuating pain associated with Hepatitis B vaccination among term neonates.

**Methods:** This randomized controlled trial was conducted at Shahid Mustafa Hospital, Ilam, Iran, enrolling 90 term neonates scheduled for Hepatitis B vaccination. Participants were randomly allocated into three distinct cohorts: intervention, placebo, and control. Two minutes prior to the immunization, the intervention group received 2 mL of 25% glucose solution, while the placebo group received an equivalent volume of sterile water. The control group received no intervention. Pain severity was evaluated using the Neonatal Infant Pain Scale (NIPS) within a 1–2-minute timeframe following the procedure. To ensure objectivity, all vaccinations were administered by an expert nurse blinded to the group assignments. Statistical analysis was performed using SPSS version 16, utilizing ANOVA and LSD post-hoc tests to compare groups.

**Results:** Baseline characteristics, including gestational age, anthropometric measurements (weight, height, head circumference), sex, and mode of delivery, were comparable across all groups with no statistically significant differences. While the glucose-treated group exhibited lower pain scores compared to the sterile water group, this difference did not reach statistical significance ( $p=0.63$ ). However, a significant reduction in pain intensity was observed in both the intervention and sterile water groups when compared to the control group ( $p<0.001$ ).

**Conclusion:** The findings indicate that glucose administration is associated with decreased pain responses in neonates undergoing minor medical procedures. Oral sweet solutions represent a practical, safe, and effective non-pharmacological strategy for pain management during routine immunization in newborn infants.

The authors declare no conflicts of interest.

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## Introduction

There is ample evidence indicating that both term and preterm babies feel pain in response to annoying stimuli [1-2]. In response to pain, newborns activate acute physiological, behavioral, metabolic, and hormonal responses. One of the devastating long-term consequences of pain includes delayed and abnormal neurological and behavioral development since the event coincides with the critical moment of neural development [3].

Painful and stressful stimuli increase catecholamine levels, heart rate, blood pressure, and intracranial pressure. Neonates' responses to pain include a reduction in blood oxygen saturation and heart rate, acidosis, an increase in blood glucose level, and respiratory rate dysregulation. Furthermore, the hormones released by pain may aggravate tissue damage and delay wound healing, thereby increasing the risk of infection, the length of hospitalization, and the likelihood of mortality [4-5]. There is ample evidence suggesting that pain control during infancy can beneficially improve physiological, behavioral, and hormonal outcomes [3]. Therefore, pain control is regarded as an important and integral component of health care during infancy. In this regard, professional societies emphasize the importance of pain as the fifth vital sign and highlight the need to boost healthcare staff's awareness of pain control strategies [6]. For nearly half a century, intramuscular injections have been an indispensable part of drug administration [7]. Neonates commonly face painful procedures such as heel blood sampling, venous blood collection, and vaccine administration. Accordingly, hepatitis B virus (HBV) vaccination is considered a routine part of neonatal care, which generally bears pain for newborns [8]. Historically, pain relief in neonates has involved both pharmacological and non-pharmacological interventions. Nevertheless, the recent clinical focus has increasingly favored non-pharmacological methods, primarily due to safety considerations and the risk of adverse reactions inherent in drug-based therapies during minor procedural pain [9]. Among these strategies are repositioning, swaddling, limiting movements, hugging, shaking, playing music, reducing environmental stimuli, skin-to-skin contact, and non-nutritive sucking [10].

One of the methods that has been traditionally used and has recently been subjected to extensive research is the consumption of sweeteners. A 2024 network meta-analysis by Efendi et al. demonstrated that sweet solutions, including glucose, significantly reduce pain in preterm infants, with 25–30% glucose showing comparable efficacy to sucrose [11].

A 2025 systematic review and meta-analysis by Medeiros et al. confirmed that 25% glucose effectively

lowers pain scores during invasive procedures in premature neonates [12].

The analgesic mechanism is thought to involve activation of sweet taste receptors and release of endogenous opioids; recent genetic evidence by Farinella et al. (2023) suggests that variations in the SLC2A1 gene influence individual analgesic response to glucose [13]. However, there are contradictory results between studies, and also there is a lack of adequate studies on the analgesic effects of glucose in comparison with placebos.

## Objectives

Regarding that pain-free experiences are a basic need and right of all humans, particularly newborns, this study was conducted to compare the pain-reducing effects of a 25% glucose solution with a placebo in neonates receiving the HBV vaccine.

## Methods

In terms of study design, a sample size calculation was performed to ensure statistical robustness, establishing a requirement of  $n=30$  participants per arm based on a-priori power analysis and established literature. With respect to the intervention, neonates in the treatment and placebo cohorts were orally administered 2 mL of 25% glucose solution and an equivalent volume of distilled water, respectively, via syringe over a controlled 30-second interval. Following a two-minute latency period, Hepatitis B immunizations were performed by a single, designated clinician to maintain procedural uniformity and mitigate observer bias. Pain responses were simultaneously quantified utilizing the Neonatal Infant Pain Scale (NIPS), with continuous monitoring conducted during the 1–2-minute timeframe immediately following the injection.

## Study Design & Participants

This double-blinded randomized clinical trial was conducted on 90 eligible newborns in the Shahid Mostafa Khomeini Hospital of Ilam City. Then the neonates were randomly allocated to either of the three groups of 25% sucrose solution, distilled water, and no intervention. Entry criteria included term birth (either by natural delivery or through cesarean section), being healthy, a body weight above 2500 grams, and a first-minute Apgar score of  $> 7$ . Exclusion criteria were the presence of medical problems, congenital anomalies, being breastfed half an hour prior to the intervention, and the use of narcotic drugs by the mother.

## Sampling & Sample Size

Subject recruitment was initiated using a non-probability convenience sampling approach. To ensure methodological rigor, a target sample size of 90 neonates was computed via a-priori power analysis, incorporating

a 10% contingency buffer to account for anticipated participant attrition. Following enrollment, infants were systematically distributed across three distinct arms—25% sucrose solution, distilled water (placebo), or a non-intervention control—by employing a randomization protocol derived from a table of random numbers to effectively mitigate selection bias.

$$n = \left( \frac{Z_{1-\alpha/2}}{d} \right)^2 \times \delta^2; n = \left( \frac{1.96}{0.1} \right)^2 \times 0.2 = 76$$

### Data Collection Tools

Data acquisition was facilitated via a structured demographic inventory and the Neonatal Infant Pain Scale (NIPS). As a multidimensional behavioral assessment tool, the NIPS quantifies pain intensity by evaluating six clinically relevant parameters: facial mimicry, cry characteristics, respiratory rhythm, limb posture, and state of arousal. The instrument's scoring rubric ranges from a score of 0 (pain-free) to 7 (maximal pain severity). The psychometric properties of the scale were rigorously evaluated, confirming high content validity, and reliability was established with a Pearson correlation coefficient of ( $r=0.93$ ).

### The Main Study

Under the same conditions, neonates in the first (i.e., intervention) and second (i.e., placebo) groups were fed with 2 mL of 25% oral glucose solution and 2 mL of distilled water, respectively, through a syringe within 30 seconds. There was no intervention in the third group. Two minutes after the intervention, an HBV vaccine was injected by the same individual, and simultaneously, pain intensity was determined within 1-2 minutes using the NIPS scale. The sample size in this study was determined as  $n=30$  per group using a sample size calculation formula and according to previous similar studies.

### Data Analysis

Quantitative data were processed using SPSS software (version 26). To ensure internal validity, baseline equivalence between study cohorts was verified across key demographic and clinical variables, specifically Apgar scores, mode of delivery, sex, anthropometric indices (head circumference, weight, and height). Comparisons of mean pain intensity across the three study arms were performed using one-way analysis of variance (ANOVA). Post-hoc pairwise differences were subsequently isolated via the Least Significant Difference (LSD) test. Statistical significance was predefined at a threshold of  $p<0.05$ .

### Ethics and Consent

This investigation was conducted in full accordance with the Declaration of Helsinki and received formal clearance from the Institutional Review Board (IRB) of Ilam University of Medical Sciences (Protocol ID:

IR.MEDILAM.REC.22/52/999/18). Prior to participation, written informed consent was secured from the legal guardians of all enrolled neonates. Guardians were provided with a comprehensive briefing regarding the research objectives, procedural phases, and their right to voluntary withdrawal at any stage without prejudice. To ensure participant privacy, all gathered datasets were anonymized and maintained under strict confidentiality protocols throughout the study.

## Results

A cohort of 90 neonates was evenly allocated across three parallel study arms: 25% oral glucose solution, distilled water (sham/placebo), and an unmanipulated control group. An assessment of baseline parameters—encompassing gestational maturity, anthropometric indices (birth weight, recumbent length, and cranial circumference), biological sex, and delivery modality—confirmed demographic homogeneity across the cohorts, with no statistically discernible variance ( $p>0.05$ ; Table 1).

Regarding the primary outcome, one-way analysis of variance demonstrated significant divergence in procedural pain responsiveness among the groups ( $F$ -test,  $p=0.002$ ). Mean pain scores ( $\pm$  " SD) followed an ascending gradient:  $3.00 \pm 1.66$  in the 25% glucose cohort,  $4.05 \pm 1.23$  in the distilled water cohort, and  $5.20 \pm 1.03$  in the control cohort. Subsequent post-hoc pairwise evaluations via the Least Significant Difference metric revealed marked, statistically robust pain attenuation in both the glucose and distilled water arms relative to the untreated controls ( $p=0.001$ ). However, the degree of pain alleviation did not reach statistical significance when contrasting the oral glucose and distilled water cohorts ( $p=0.63$ ; Table 2).

## Discussion

Assessment of pain in neonates is crucial and requires careful attention [14]. Our findings substantiate that the pre-procedural administration of 2 mL of 25% oral glucose solution two minutes prior to hepatitis B vaccination yields a robust antinociceptive effect relative to untreated controls, confining mean neonatal pain scores to the mild threshold ( $NIPS \leq 3$ ). Interestingly, the absence of a statistically discernible divergence in pain mitigation between the glucose and distilled water (placebo) cohorts implies that orosensory tactile stimulation, swallowing reflex activation, or the orogastric volumetric effect itself may independently confer a baseline degree of pain attenuation [2,13].

The analgesic effect of glucose in neonates has been extensively studied in recent years.

**Table 1- Comparison of newborns' demographic specifications between the study groups**

| Variables                  | Glucose |        |      | Distilled water |        |      | Control |        |      | P     |
|----------------------------|---------|--------|------|-----------------|--------|------|---------|--------|------|-------|
|                            | Mean    | SD     | %    | Mean            | SD     | %    | Mean    | SD     | %    |       |
| Gestational age (weeks)    | 38.6    | 0.8    | -    | 38.8            | 1.1    | -    | 39.1    | 0.7    | -    | 0.296 |
| Weight (g)                 | 3306.67 | 280.31 | -    | 3309.31         | 288.72 | -    | 3263.33 | 272.89 | -    | 0.532 |
| Height (cm)                | 50.10   | 1.26   | -    | 50.35           | 1.22   | -    | 50.27   | 1.28   | -    | 0.631 |
| Head circumference (cm)    | 34.47   | 1.81   | -    | 34.22           | 1.83   | -    | 33.73   | 1.78   | -    | 0.481 |
| Gender (girl)              | -       | -      | 46.7 | -               | -      | 48.8 | -       | -      | 50   | 0.469 |
| Type of delivery (natural) | -       | -      | 66.7 | -               | -      | 69.4 | -       | -      | 73.3 | 0.617 |

**Table 2- The comparison of mean and SD of pain intensity in the neonates receiving 25% glucose, distilled water, and no intervention using the ANOVA test**

| Variables       | N  | Mean | SD   | 95% CI      |             | P     |
|-----------------|----|------|------|-------------|-------------|-------|
|                 |    |      |      | Lower bound | Upper bound |       |
| 25% Glucose     | 30 | 3    | 1.66 | 2.52        | 3.48        | 0.002 |
| Distilled water | 30 | 4.05 | 1.23 | 3.59        | 4.51        |       |
| Control         | 30 | 5.20 | 1.03 | 4.81        | 5.58        |       |
| Total           | 30 | 3.70 | 1.75 | 3.33        | 4.06        |       |

A 2024 network meta-analysis by Efendi et al., which included 42 randomized trials with 2,733 preterm infants, concluded that sweet solutions (sucrose and glucose) are effective in reducing procedural pain, with glucose ranking among the top interventions [10].

The exact neurophysiological cascade governing carbohydrate-induced antinociception remains incompletely characterized; nonetheless, prevailing hypotheses attribute this phenomenon to central endogenous opioid exocytosis triggered by the stimulation of orolingual sweet-taste gustatory receptors. Providing mechanistic depth to this variability, Farinella et al. (2023) unveiled genomic evidence associating an SLC2A1 single-nucleotide polymorphism (rs1105297) with divergent antinociceptive responsiveness to sweet solutions among neonates, suggesting that host genomic architecture dictates therapeutic sensitivity and offering a tenable biological rationale for divergent clinical findings across published cohorts [12].

A growing body of contemporary randomized clinical trials has corroborated the efficacy of enteral carbohydrate regimens in procedural pain mitigation. In a controlled cohort of term newborns, Petleshkova et al. (2022) observed that combining 25% glucose with non-nutritive sucking produced pronounced pain suppression during intramuscular vaccination protocols [2]. Concurrently, Mahmood et al. (2023) established that pre-procedural delivery of a 2 mL aliquot of 25% oral glucose markedly blunted venipuncture-induced behavioral reactivity compared to untreated controls, yielding a subdued mean NIPS score of 2.8 [13]. Expanding beyond the immediate neonatal window, an updated trial by Thyr et al. (2022) affirmed significant antinociceptive utility among infants aged 3–12 months receiving scheduled immunizations [15]. Comparable clinical benefits have been reported by Sajedi et al.

(2021) in mature neonates and Okan et al. (2022) across premature cohorts [16–17]. Longitudinal appraisal by Kassab et al. (2020) further demonstrated the enduring analgesic utility of 25% oral glucose for immunization-induced discomfort [18]. Complementing these data, subsequent experimental validation by Bembich et al. (2021) [19], highlighted that co-administering hypertonic glucose alongside non-nutritive sucking or maternal containment significantly minimizes acute distress. Synthesizing these cumulative findings, the 2023 Chinese evidence-based clinical practice guideline explicitly advocates the routine employment of sweet oral solutions—specifically 25% glucose—as an established first-line procedural comfort strategy in neonatal settings [20].

Conflicting clinical evidence regarding the efficacy of glucose in neonatal pain management underscores a critical absence of standardized dosing protocols.

Future studies must focus on establishing uniform guidelines to resolve these discrepancies and ensure optimal therapeutic outcomes [21]. Furthermore, high-level evidence syntheses—including updated Cochrane reviews by Stevens et al. (2024) [22] and Bueno et al. (2020) [23]—consistently articulate the need for robust, adequately powered trials directly benchmarking sweet solutions against inert volumetric placebos. In this regard, our observation of comparable pain relief between oral glucose and distilled water corroborates longitudinal datasets reported by Ahuja et al. (2020) [24], despite contrasting findings from other contemporaneous trials, such as Mahmood et al. (2023), which noted distinct statistical divergence between active sugar solutions and water [13].

## Conclusion

Overall, oral glucose solutions can be claimed to have significant analgesic effects in infants. Glucose is a simple carbohydrate that can be immediately absorbed through oral mucosa, explaining its fast-acting function. Therefore, regarding the cheapness, accessibility, and immediate analgesic effects of oral glucose, it is recommended to use this substance to alleviate pain in infants undergoing invasive procedures such as heel blood sampling, venipuncture, vaccination, umbilical vein catheterization, etc.

Since we found no study that could exactly match ours in terms of design, the concentration of oral glucose solution, and the selected painful procedure, it is recommended to further conduct similar studies in different age groups.

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