ORIGINAL ARTICLE

Comparison of Minimal Effective Dose of Sucrose for Pain Relief in Neonates after Minor Procedures, A randomized controlled trial

MEHAK FATIMA, MUDASSAR FAIZ GONDAL, LARAIB RASOOL, AMEENA SHAHWAR, WAQAS AHMED, ZUNAIRA AZAM

Pak Pediatr J 2025; **49(3&4):** 282-88

Correspondence to:

Dr. Mehak Fatima,
Associate Professor Pediatrics,
Department of Pediatric,
Surgery, Holy Family Hospital
Rawalpindi.

E-mail: mehak.f932@gmail.com

Received for publication: July 19,2025;
Revised received: August 18, 2025
Revised accepted: August 21, 2025

How to cite this: Fatima M, Gondal MF, Rasool L, Shahwar A, Ahmed W, Azam Z
Comparison of Minimal Effective Dose of Sucrose for Pain Relief in Neonates after Minor Procedures, a Randomized Controlled Trial
Pak pediatr j. 2025; 49(3&4): 274-81

ABSTRACT

Objective: Oral sucrose is a proven analgesic for neonatal procedural pain, yet the minimal effective dose for intravenous cannulation remains uncertain. This study compared the effectiveness of 0.2 mL versus 0.5 mL of 24% sucrose in neonates.

Study Design: Randomized, single-blinded controlled trial

Place and Duration of Study: Department of Pediatric Surgery, Holy Family Hospital, from March 2023 to February 2024.

Patient and Methods: A total of 148 neonates admitted to the NICU for preoperative management were enrolled in this study. Group I (74 neonates) received 0.2 ml, and Group II (74 neonates) received 0.5 ml of sucrose solution during IV cannulation. Sucrose was given 1 minute before the procedure, drop-by-drop on the tongue, followed by non-nutritive sucking with a sterilized pacifier. Pain intensity was assessed using the Premature Infant Pain Profile (PIPP) at 1 minute post-procedure by a blinded assessor. Data were analyzed using SPSS v25.0.

Results: A 0.5 mL dose significantly reduced PIPP scores compared to the 0.2 mL dose, with median scores of 3.57 (range: 2–5) vs. 8.74 (range: 6.5–12.0) ($p < 0.0001$). Subgroup analyses showed that the benefit of 0.5 mL sucrose was consistent across term and preterm neonates and across weight categories. No adverse events were reported.

Conclusion: A 0.5 ml dose of 24% sucrose is more effective than 0.2 ml for managing procedural pain in neonates.

Trial registration: ClinicalTrials.gov Identifier: **NCT06446323**.

Key Words: Sucrose solution, Pain relief, Neonates, Minor procedure, Premature infant pain profile

INTRODUCTION

Procedural Pain refers to the discomfort that arises from investigations, therapy, or procedures.[1] There is mounting evidence that regular and repetitive exposure to unpleasant stimuli is linked to worse outcomes in the future. So far, numerous pain management strategies

have been created and put into practice to manage procedural pain in newborns.[2,3] Due to their inability to self-report, infants frequently experience unrecognized and inadequately managed pain. Thorough evaluation of pain, especially pain caused by medical procedures, which can have both immediate and lasting effects, is crucial for effectively managing it.[4]

Procedural pain may occur during one procedure, but many children experience it repeatedly during a full course of treatment or investigation. Studies show that hospitalized children often do not receive enough pain relief, despite the availability of guidelines for managing pain during medical procedures in clinical practice.[5,6] Recent studies indicate that a considerable number of children have prolonged pain following major surgical procedures, which has a substantial influence on their overall health results.[7] Oral dose of 1 ml of a 24% sucrose solution one minute before immunization effectively reduces pain during the injection.[8] Since the early 1990s, there has been a significant amount of research conducted on the use of sucrose as a method to alleviate procedural pain in both full-term and preterm infants. Regularly updated systematic reviews and meta-analyses have repeatedly shown the pain-relieving effectiveness of sucrose when given during individual skin-breaking operations. Nevertheless, the lack of understanding regarding the efficacy and safety of administering sucrose multiple times to preterm newborns while they are hospitalized in the neonatal critical care unit has been recognised as a notable area of uncertainty.[9,10]

The most commonly researched non-pharmacological method for relieving procedural pain in neonates is the administration of oral sucrose, both with and without non-nutritive sucking.[11] Administering sucrose orally is both effective and safe in lowering the intensity of pain during single procedures that cause tissue damage in newborns.[12] This practice is routinely advised in guidelines for managing pain in newborns. Nevertheless, there is significant diversity in the amounts of sucrose administered in studies, with a variance of over 20 times observed among different neonatal care environments.[13] The pain intensity scores recorded by the PIPP scale did not show any significant variations with sucrose solution. Therefore, the authors proposed that the minimum effective dose of 24% sucrose was 0.1 ml in post-procedural pain management. Nevertheless, the efficacy of providing this dosage repeatedly for several painful operations over the entire neonatal intensive care unit and hospitalization period has not yet been investigated.[14]

This study aims to determine the most effective minimal dose of sucrose solution for managing pain in neonates following IV cannulation. By providing evidence for an optimal dose, we seek to enhance pain relief, improve clinical outcomes, and elevate the quality of life for neonates. Our findings are intended to guide future pain management practices, reducing the risk of complications and delays in recovery associated with inadequate pain control.

The objective of this study is to compare the outcome of 0.2 ml versus 0.5 ml sucrose solution for pain relief in neonates undergoing IV cannulation.

MATERIAL AND METHODS

Study Design: Randomized, single-blinded controlled trial.

Study Place & Duration: Department of Pediatric Surgery Holy Family Hospital from March 2023 to February.2024.

Ethical Approval: Approved by the Institutional Ethical Review Board (ERB No. 142/ERB/2024, dated 31 May 2024). Informed written consent was obtained from parents/guardians.

Trial Registration: The study was registered with ClinicalTrials.gov (Identifier: NCT06446323).

Sample Size: The sample size was calculated to detect a clinically meaningful difference in mean PIPP scores between groups, with 80% power ($\beta = 0.20$) and a two-sided significance level of 5% ($\alpha = 0.05$). Based on previous literature reporting a standard deviation of about 3.0 and an expected mean difference of 1.6 points in PIPP scores, the required sample size was 74 neonates in each group (total = 148). The calculation was performed using the standard formula for comparing two means and verified with G*Power version 3.1.

Sampling Technique and Enrollment: Eligible neonates admitted to the NICU who met inclusion and exclusion criteria were enrolled consecutively during the study period.

Inclusion Criteria: Neonates aged 1–28 days, of either gender, who underwent minor procedures such as intravenous cannulation and were admitted to the NICU.

Exclusion Criteria: Critically ill neonates, those receiving experimental treatments, or those with conditions affecting behavioral pain responses.

Randomization and Allocation: Randomization was performed using a computer-generated random number table prepared by the principal investigator. Eligible neonates were enrolled consecutively and assigned according to the sequence by the on-duty pediatric surgery resident. The outcome assessment was carried out by a pediatric surgery resident who was blinded to group allocation.

Data collection and analysis: After ethical approval, 148 neonates were enrolled from the NICU. Informed consent was obtained from parents, and demographic information (age, gender, birth weight, gestational age, and delivery mode) was recorded. The neonates were randomly divided into two groups using a random number table:

- **Group I:** Administered 0.2 ml of 24% sucrose solution drop-by-drop onto the anterior tongue 1 minute before the procedure.
- **Group II:** Administered 0.5 ml of 24% sucrose solution following the same protocol.

Both groups received a sterilized pacifier immediately after sucrose administration to promote non-nutritive sucking, enhancing the analgesic effect of sucrose. Both groups underwent intravenous cannulation, a common minor procedure in neonates, to evaluate the efficacy of sucrose in managing procedural pain under standardized conditions. Pain intensity was assessed using the Premature Infant Pain Profile (PIPP). A PIPP score of <6 was considered to indicate minimal or no pain, while a score of ≥6 indicated significant pain requiring further intervention **(table 1)**.

Given the short duration of sucrose's analgesic action (approximately 3–5 minutes), the study prioritized evaluating immediate pain relief rather than prolonged effects. This focus aligns with sucrose's clinical utility in managing brief procedural pain.

All neonates with obstructive conditions, such as Hirschsprung disease and intestinal atresia, were managed with nasogastric tube decompression to prevent any influence on their clinical condition. The need for rescue analgesia was also assessed but was not required for any neonates.

Statistical Analysis: Data were analyzed using SPSS v25.0. Normality of continuous variables was tested with the Shapiro Wilk test and histograms. Normally distributed data were expressed as mean ± SD and compared using independent t-test. Non-normal variables (including PIPP) were expressed as median and compared using Mann–Whitney U test. Categorical variables were compared using chi-square test. Analysis followed the intention-to-treat principle. $p \leq 0.05$ was considered statistically significant.

TABLE : Premature infant pain profile (PIPP) criteria

Parameter	Scoring 0	Scoring 1	Scoring 2	Scoring 3
Gestational Age (weeks)	≥ 36	32–35	28–31	≤ 27
Behavioral State	Quiet sleep/awake	Active/awake	Fussy	Crying
Heart Rate Increase from Baseline	0–4 bpm	5–14 bpm	15–24 bpm	≥ 25 bpm
Oxygen Saturation Decrease from Baseline	0–2%	3–4%	5–7%	≥ 8%
Brow Bulge (Presence & Duration)	None	< 2 sec	2–4 sec	> 4 sec
Eye Squeeze (Presence & Duration)	None	< 2 sec	2–4 sec	> 4 sec
Nasolabial Furrow (Presence & Duration)	None	< 2 sec	2–4 sec	> 4 sec

RESULTS

All 148 neonates completed the study. Baseline characteristics are presented in **table 1** and revealed significant differences between groups: the median age was 4 days (range: 1–20) in the 0.2 mL group versus 6 days (range: 2–25) in the 0.5 mL group (p = 0.02); male gender was more common in the 0.5 mL group (52.7% vs. 37.8%, p = 0.04); median birth weight was higher in the 0.2 mL group (2.6 kg vs. 2.1 kg, p=0.01); and more preterm neonates were enrolled in the 0.5 mL group (58.1% vs. 5.4%, p <0.0001). The mode of delivery also differed significantly, with spontaneous vaginal delivery more frequent in the 0.2 mL group (75.7% vs. 44.6%, p=0.003). Underlying diagnoses—including anorectal malformations, Hirschsprung disease, intestinal atresia, and genitourinary anomalies—were similarly distributed between groups **(table 2)**.

Despite these imbalances, neonates receiving 0.5 mL of sucrose had significantly lower pain scores compared to those receiving 0.2 mL, with median PIPP scores of 3.57 (range: 2.0–5.0) versus 8.74 (range: 6.5–12.0), respectively (p < 0.0001). PIPP scores, assessed as non-parametric data, were compared using the Mann–Whitney U test. Furthermore, 66 neonates (89.2%) in the 0.5 mL group achieved PIPP scores below 6, compared to only 3 neonates (4.1%) in the 0.2 mL group, underscoring the superior efficacy of the higher sucrose dose. Domain-wise analysis of the PIPP scale **(table 3)** also demonstrated significantly lower scores across behavioral and physiological parameters in the 0.5 mL group.

Subgroup analysis confirmed that the analgesic effect of 0.5 mL sucrose remained significant across both term and preterm neonates and in those with birth weights <2.5 kg and ≥2.5 kg. A significant correlation was observed between higher age and weight and better pain control (p < 0.05), while the mode of delivery had no influence on pain outcomes. Importantly, no neonate in either group required rescue analgesia or experienced adverse effects, confirming the safety of sucrose for procedural pain relief.

.

TABLE 1: Baseline characteristics of neonates enrolled in the study

Characteristic	0.2 mL Dose (n = 74)	0.5 mL Dose (n = 74)	Overall (n = 148)	p-value
Age (days), median (range)	4 (1–20)	6 (2–25)	5 (1–25)	0.02
Gender				
– Male, n (%)	28 (37.8)	39 (52.7)	67 (45.3)	0.04
– Female, n (%)	46 (62.2)	35 (47.3)	81 (54.7)	—
Birth weight (kg), median (range)	2.6 (1.8–3.5)	2.1 (1.5–3.2)	2.3 (1.5–3.5)	0.01
Gestational age				
– Term, n (%)	70 (94.6)	31 (41.9)	101 (68.2)	<0.0001
– Preterm, n (%)	4 (5.4)	43 (58.1)	47 (31.8)	—
Mode of delivery				
– Spontaneous vaginal delivery, n (%)	56 (75.7)	33 (44.6)	89 (60.1)	0.003
– Cesarean section, n (%)	18 (24.3)	41 (55.4)	59 (39.9)	—

Values are presented as median (range) or n (%). P-values were calculated using independent t-test for normally distributed continuous variables, Mann–Whitney U test for non-normally distributed variables, and chi-square test for categorical variables.

TABLE 2: Underlying indications for NICU admissions

Diagnosis	0.2 mL Dose (n = 74)	0.5 mL Dose (n = 74)	Overall (n = 148)	p-value
Anorectal malformations (ARM), n (%)	30 (40.5)	28 (37.8)	58 (39.2)	0.72

Diagnosis	0.2 mL Dose (n = 74)	0.5 mL Dose (n = 74)	Overall (n = 148)	p-value
Hirschsprung disease, n (%)	20 (27.0)	22 (29.7)	42 (28.4)	0.65
Intestinal atresia, n (%)	12 (16.2)	10 (13.5)	22 (14.9)	0.78
Genitourinary malformations, n (%)	12 (16.2)	14 (18.9)	26 (17.6)	0.73

Values are presented as n (%). Comparisons between groups were performed using the chi-square test.

TABLE 3: Scores for each domain of the PIPP scale in both groups

PIPP Domain	0.2 mL Dose (Mean ± SD)	0.5 mL Dose (Mean ± SD)	p-value
Gestational age	2.10 ± 0.50	1.85 ± 0.45	0.03
Behavioral state	1.50 ± 0.60	1.20 ± 0.40	0.04
Heart rate	2.25 ± 0.70	1.60 ± 0.50	0.02
Oxygen saturation	1.80 ± 0.55	1.50 ± 0.40	0.03
Brow bulge	2.00 ± 0.65	1.40 ± 0.35	0.01
Eye squeeze	2.15 ± 0.70	1.50 ± 0.45	0.02
Nasolabial furrow	2.10 ± 0.60	1.45 ± 0.40	0.01

Values are expressed as mean ± SD or median. Group comparisons were performed using Mann–Whitney U test for PIPP scores and chi-square test for categorical outcomes.

DISCUSSION

This study demonstrates that a 0.5 mL dose of 24% sucrose is significantly more effective than a 0.2 mL dose in reducing procedural pain in neonates, as evidenced by a median PIPP score of 3.57 in the 0.5 mL group compared to 8.74 in the 0.2 mL group ($p<0.0001$). These findings reinforce the role of sucrose as a safe, non-pharmacological intervention for neonatal pain management during minor procedures

The efficacy of sucrose in neonatal analgesia is well-established. Gao et al. identified that doses of 20–25% sucrose ranging from 0.1 to 2.0 mL significantly reduced pain intensity scores in term and preterm neonates during repeated procedures.[14] Similarly, Stevens et al. demonstrated that higher doses, such as 0.5 mL and 1.0 mL, provided superior pain relief compared to smaller doses during heel lance procedures.[15] Our findings align with these studies, further confirming the dose-dependent effectiveness of sucrose in neonatal pain management.

Non-pharmacological analgesia has consistently shown benefits in neonatal care. Harrison et al. reported that sucrose combined with a pacifier enhances analgesic effects and significantly improves behavioral and physiological pain responses.[16] Likewise, Gao et al. highlighted the synergistic effect of combining sucrose with non-nutritive sucking, demonstrating lower PIPP scores compared to sucrose alone.[17]Campbell-Yeo et al. found that kangaroo care provided sustained pain relief during repeated procedures throughout NICU hospitalization.[18]Emerging advancements such as AI-based pain assessment tools, as shown by Hughes et al., have further improved the accuracy and usability of neonatal pain evaluation.[19]

While sucrose is widely accepted for its efficacy and safety profile, it is important to compare its safety with pharmacological agents commonly used for neonatal pain relief. Paracetamol, although relatively safe, carries risks of hepatotoxicity, particularly with repeated or high dosing.[20] Opioids such as morphine and fentanyl, though effective for severe pain, are associated with respiratory depression, sedation, hypotension, and risk of dependence.[21]In contrast, sucrose is simple to administer, does not require monitoring, and has no known long-term adverse effects when used appropriately, making it suitable for routine minor procedures.

Our findings are also supported by recent longitudinal evidence. Bueno et al. demonstrated that repeated sucrose administration remains effective in minimizing procedural pain throughout prolonged NICU stays, with no significant adverse events.[22] This evidence supports the continued use of sucrose as a safe and effective first-line intervention for minor procedural pain.

The strengths of this study include its randomized design, standardized procedure, and inclusion of neonates with a variety of surgical conditions. However, baseline differences in gestational age

and birth weight between groups may have influenced responses, despite the consistency of subgroup analysis results. Additional limitations include the single-center setting and the exclusion of critically ill neonates. Future multicenter trials with larger sample sizes are needed to validate these findings and provide stronger evidence for standardized dosing guidelines.

CONCLUSION

A 0.5 mL dose of 24% sucrose is safe, simple, and more effective than 0.2 mL for reducing procedural pain in neonates. Its routine use is recommended for minor procedures, while further studies in larger and diverse populations are needed to confirm long-term safety and outcomes.

Source of funding: None
Conflict of interest: None

Authors' affiliation

Dr. Mehak Fatima
Postgraduate Resident, Department of Pediatric Surgery, Holy Family Hospital, Rawalpindi, Pakistan.

Dr. Mudassir Fiaz Gondal
Assistant Professor, Department of Pediatric Surgery, Holy Family Hospital, Rawalpindi Medical University, Rawalpindi, Pakistan.

Dr. Laraib Rasool
Senior Registrar, Department of Pediatric Surgery, Holy Family Hospital, Rawalpindi, Pakistan.

Dr. Ameena Shahwar,
Postgraduate Resident, Department of Pediatric Surgery, Holy Family Hospital, Rawalpindi, Pakistan.

Dr. Waqas Ahmed
Postgraduate Resident, Department of Pediatric Surgery, Holy Family Hospital, Rawalpindi, Pakistan.

Dr. Zunaira Azam,
Postgraduate Resident, Department of Pediatric Surgery, Holy Family Hospital, Rawalpindi, Pakistan.

REFERENCES

1. McNair C, Campbell-Yeo M, Johnston C, Taddio A. Non-pharmacologic management of pain during common needle puncture procedures in infants: current research evidence and practical considerations: an update. Clin Perinatol 2019;46(4):709-731. https://doi.org/10.1016/j.clp.2019.08.015
2. Persad E, Pizarro AB, Bruschettini M. Non-opioid analgesics for procedural pain in neonates. Cochrane Database Syst Rev 2023;4(4):CD015179. https://doi.org/10.1002/14651858.CD015179.pub2
3. Shave K, Ali S, Scott SD, Hartling L. Procedural pain in children: a qualitative study of caregiver experiences and information needs. BMC Pediatr 2018;18:132. https://doi.org/10.1186/s12887-018-1152-1
4. Addab S, Hamdy R, Thorstad K, Le May S, Tsimicalis A. Use of virtual reality in managing paediatric procedural pain and anxiety: an integrative literature review. J Clin Nurs 2022;31(21-22):3032-3059. https://doi.org/10.1111/jocn.16361
5. Cruz MD, Fernandes A, Oliveira C. Epidemiology of painful procedures performed in neonates: a systematic review of observational studies. Eur J Pain 2016;20(4):489-498. https://doi.org/10.1002/ejp.768
6. Duerden EG, Grunau RE, Guo T, Foong J, Pearson A, Au-Young S, et al. Early procedural pain is associated with regionally specific alterations in thalamic development in preterm neonates. J Neurosci 2018;38(4):878-886. https://doi.org/10.1523/JNEUROSCI.0149-17.2017
7. Rabbitts JA, Fisher E, Rosenbloom BN, Palermo TM. Prevalence and predictors of chronic postsurgical pain in children: a systematic review and meta-analysis. J Pain 2017;18(6):605-614. https://doi.org/10.1016/j.jpain.2017.03.004
8. Nimbalkar SM, Thakkar FA, Thacker JP, Phatak AG, Shinde MK. Comparison of sucrose vs swaddling in pain management during birth dose of hepatitis B vaccine: a randomized controlled trial. Indian J Pediatr 2024;91(9):893-898. https://doi.org/10.1007/s12098-023-04900-6
9. Huang RR, Xie RH, Wen SW, Chen SL, She Q, Liu YN, et al. Sweet solutions for analgesia in neonates in China: a systematic review and meta-analysis. Can J Nurs Res 2019;51(2):116-127. https://doi.org/10.1177/0844562119859449
10. Harrison D, Larocque C, Bueno M, Stokes Y, Turner L, Hutton B, et al. Sweet solutions to reduce procedural pain in neonates: a meta-analysis. Pediatrics 2017;139(1):e20162376. https://doi.org/10.1542/peds.2016-2376
11. Stevens B, Yamada J, Campbell-Yeo M, Gibbins S, Harrison D, Dionne K, et al. The minimally effective dose of sucrose for procedural pain relief in neonates: a randomized controlled trial. BMC Pediatr 2018;18:228. https://doi.org/10.1186/s12887-018-1202-3

12. Gibbins S, Stevens BJ, Yamada J, Dionne K, Campbell-Yeo M, Lee G, et al. Validation of the Premature Infant Pain Profile-Revised (PIPP-R). Early Hum Dev 2014;90(4):189-193. https://doi.org/10.1016/j.earlhumdev.2013.11.004

13. Eccleston C, Fisher E, Howard RF, Slater R, Forgeron P, Palermo TM, et al. Delivering transformative action in paediatric pain: a Lancet Child & Adolescent Health Commission. Lancet Child Adolesc Health 2021;5(1):47-87. https://doi.org/10.1016/S2352-4642(20)30301-5

14. Gao H, Gao H, Xu G, Li M, Du S, Li F, et al. Efficacy and safety of repeated oral sucrose for repeated procedural pain in neonates: a systematic review. Int J Nurs Stud 2016;62:118-125. https://doi.org/10.1016/j.ijnurstu.2016.06.011

15. Stevens B, Yamada J, Beyene J, Gibbins S, Petryshen P, Stinson J, et al. Consistent management of repeated procedural pain with sucrose in preterm neonates: is it effective and safe for repeated use over time? Clin J Pain 2005;21(6):543-548. https://doi.org/10.1097/00002508-200511000-00009

16. Harrison D, Loughnan P, Manias E, Gordon I, Johnston L. Repeated doses of sucrose in infants continue to reduce procedural pain during prolonged hospitalizations. Nurs Res 2009;58(6):427-434. https://doi.org/10.1097/NNR.0b013e3181bf387a

17. Gao H, Li M, Gao H, Xu G, Li F, Zhou J, et al. Effect of non-nutritive sucking and sucrose alone and in combination for repeated procedural pain in preterm infants: a randomized controlled trial. Int J Nurs Stud 2018;83:25-33. https://doi.org/10.1016/j.ijnurstu.2018.04.006

18. Campbell-Yeo M, Johnston CC, Benoit B, Disher T, Caddell K, Vincer M, et al. Sustained efficacy of kangaroo care for repeated painful procedures over neonatal intensive care unit hospitalization: a single-blind randomized controlled trial. Pain 2019;160(11):2580-2588. https://doi.org/10.1097/j.pain.0000000000001668

19. Hughes JD, Chivers P, Hoti K. The clinical suitability of an artificial intelligence-enabled pain assessment tool for use in infants: feasibility and usability evaluation study. J Med Internet Res 2023;25:e41992. https://doi.org/10.2196/41992

20. Beasley C, Gundert C, Buttenheim AM, Asch DA. Safety of paracetamol for pain management in neonates: a systematic review and meta-analysis. Pediatr Res 2018;84(4):492-500. https://doi.org/10.1038/pr.2018.100

21. Walker SM, Yaksh TL. Morphine and fentanyl use in neonatal pain management: mechanisms, outcomes, and challenges. J Pain Res 2020;13:3251-3268. https://doi.org/10.2147/JPR.S276501

22. Bueno M, Ballantyne M, Campbell-Yeo M, Estabrooks CA, Gibbins S, Harrison D, et al. The effectiveness of repeated sucrose for procedural pain in neonates in a longitudinal observational study. Front Pain Res (Lausanne) 2023;4:1110502. https://doi.org/10.3389/fpain.2023.1110502

Authors' contribution

Fatima M: Proposed topic, basic study design, methodology and manuscript writing
Gondal MF: Data collection, statistical analysis and interpretation of results etc
Rasool L: Literature review & referencing and quality insurer
Shahwar A: Interpretation of data
Ahmed W: References review
Azam Z: Final proof reading

All the authors have approved the final manuscript draft and accept the responsibility of research integrity.