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Authors: Vijaya Vinotha Muthuchandrasekar, Balaji Chinnasami, Aseema Fathima Z., Subash Sundar, Aamina Hussain, Aravind Murugesan

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Longitudinal hormonal dynamics during minipuberty and exploratory associations with vitamin D status in healthy term male infants

Vijaya Vinotha Muthuchandrasekar ¹, Balaji Chinnasami ¹, Aseema Fathima Z. ¹, Subash Sundar ¹,
Aamina Hussain ², Aravind Murugesan ³

¹ Department of Pediatrics, SRM Medical College Hospital and Research Center, Kattankulathur, Chengalpattu District, Tamil Nadu, India

² Department of Community Medicine, SRM Medical College Hospital and Research Center, Kattankulathur, Chengalpattu District, Tamil Nadu, India

³ Department of Radiology, SRM Medical College Hospital and Research Centre, Kattankulathur, Chengalpattu District, Tamil Nadu, India

Corresponding author: Balaji Chinnasami, e-mail: balajisrmt@gmail.com

ORCID

VM: <https://orcid.org/0009-0004-1512-1531>

BC: <https://orcid.org/0000-0001-9184-0168>

AFZ: <https://orcid.org/0009-0006-8345-0307>

SS: <https://orcid.org/0000-0002-6255-7125>

AH: <https://orcid.org/0000-0003-1787-7741>

AM: <https://orcid.org/0009-0001-3778-377X>

ABSTRACT

Introduction and aim. Minipuberty, the transient postnatal reactivation of the hypothalamic-pituitary-gonadal axis, is a critical window for male reproductive maturation. The study prospectively characterized hormonal dynamics during minipuberty and explored associations between 25-hydroxyvitamin D [25(OH)D] and gonadal hormones, adrenal corticosterone, and genital growth parameters.

Material and methods. Sixty healthy term male babies were enrolled; 52 completed follow-up. Testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), corticosterone, and 25(OH)D were measured on day 3 and at 3 months by LC-MS/MS and chemiluminescence immunoassay. Penile length and testicular volume were recorded at both visits.

Results. Testosterone increased from 47.18 to 142.62 ng/dL, LH from 1.31 to 2.46 mIU/mL, and FSH from 1.22 to 2.10 mIU/L (all $p < 0.001$), while corticosterone decreased from 211.19 to 86.25 ng/dL ($p < 0.001$). Several nominal associations were observed before multiple comparison correction; however, after

Bonferroni correction, only the inverse association between 25(OH)D and corticosterone at 3 months ($r=-0.576$, $p<0.001$) remained statistically significant. All other associations should be considered exploratory.

Conclusion. Only the inverse association between vitamin D and corticosterone remained statistically significant after correction, while other associations were exploratory and require confirmation in larger studies.

Keywords. corticosterone, minipuberty, neonates, testicular volume, testosterone, vitamin D

Introduction

The early postnatal months are marked by a well-recognized but transient endocrine phenomenon – minipuberty characterized by reactivation of the hypothalamic-pituitary-gonadal axis (HPG) axis before its subsequent quiescence until puberty.¹⁻³ In male infants, this window is driven by a sequential increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH), followed by a surge of testosterone that may approach early-pubertal concentrations between one and three months of age.¹⁻³ This androgen-rich period supports testicular descent, Leydig and Sertoli cell maturation, and phallic growth, and is thought to lay the groundwork for adult male reproductive function.^{1,3,4} Disruption of this window has been associated with cryptorchidism, micropenis, and impaired Sertoli cell proliferation, with potential long-term consequences for adult fertility and reproductive health. However, the factors that determine the magnitude and timing of this surge are still not well understood.

Beyond its established role in calcium homeostasis and skeletal health, vitamin D is increasingly recognized as a pleiotropic steroid hormone with direct reproductive relevance. Functional VDR and 1α -hydroxylase have been identified in human Leydig cells, Sertoli cells, and the hypothalamic-pituitary unit,^{5,6} suggesting tissue-level steroidogenic regulation. Experimental work indicates that 3β -HSD and 17β -HSD – enzymes central to androgen biosynthesis 3-HSD and 17-HSD can be upregulated by vitamin D, and intracellular calcium signaling in testicular cells is also a proposed target.^{6,7} In adult men, higher levels have been linked to better testosterone concentrations and reproductive outcomes in observational studies and clinical trials.^{8,9} Whether this vitamin D-steroidogenesis relationship extends to the neonatal period, particularly during the hormonally active minipuberty window, has not previously been examined in a prospective longitudinal study.

Neonatal vitamin D insufficiency is a widespread problem in India, with prevalence estimates ranging from 50% to over 90% in some series.¹⁰⁻¹² Contributing factors include prolonged indoor confinement of newborns, limited dietary sources, reduced skin synthesis related to skin pigmentation, and frequently depleted maternal stores at delivery. If this deficiency coincides with the minipuberty window when androgen-driven genital development and early reproductive programming are underway – the long-term consequences may extend well beyond infancy.

To date, a single published study by KInç et al.¹³ studied a mixed-sex Turkish cohort at a single cross-sectional time point (30–45 days). No prospective dual time point study has previously been conducted that captured the entire HPG axis alongside objective genital growth measures in Indian male neonates.

Aim

The aim of this study was to prospectively characterize hormonal dynamics during minipuberty and to explore associations between serum 25(OH)D levels and testosterone, LH, FSH, corticosterone, penile length, and mean testicular volume on day 3 and at three months of age in healthy term male infants.

Material and methods

Study design and setting

This was a prospective observational study conducted at the SRM Medical College Hospital and Research Institute, Chennai, Tamil Nadu, between June 2024 and December 2025. Institutional Ethics Committee approval was obtained (SRMIEC-ST0724-8011), and written informed consent was obtained from each infant's parent or guardian before enrollment.

Study population

We enrolled term singleton male infants born at 37–42 weeks gestation. The growth classification appropriate for gestational age (AGA; birth weight 10th–90th centile) or small for gestational age (SGA; birth weight below the 10th centile) was assigned using the INTERGROWTH-21st newborn size standards.¹⁴ Infants were excluded if born to mothers with diabetes mellitus, hypertension, or pre-eclampsia; if they had major congenital malformations, genital anomalies (hypospadias, cryptorchidism, micropenis, ambiguous genitalia), chromosomal or genetic disorders, endocrine pathology, or critical illness; or if they were born of multiple pregnancy. Maternal conditions, including diabetes mellitus, hypertensive disorders, and hypothyroidism, were recorded; however, due to the exclusion criteria, only maternal hypothyroidism was present in the final cohort and was included as ‘maternal complications’ in the regression model.

Procedures and timeline

Clinical assessments occurred at two pre-specified visits: day 3 of life and 3 months of age (day 90±5). On day 3 of life, anthropometric measurements were recorded according to the INTERGROWTH-21st protocol.¹⁴ At 3 months, weight, length, and head circumference were measured according to WHO Child Growth Standards.¹⁵ All values were recorded as the mean of three consecutive readings taken on the same day.

The stretched penile length (SPL) was assessed using a rigid ruler placed on the compressed suprapubic fat pad, measuring from the pubic symphysis to the tip of the glans; three consecutive readings were averaged.

The testicular volume was determined by high resolution transscrotal ultrasound on a Philips Affiniti 70 system (12–4 MHz transducer) using the ellipsoid formula (length×width×height×0.523). A single experienced pediatric radiologist performed all sonographic examinations, remaining blind to hormonal data throughout.

At each visit, a 3 ml venous blood sample was centrifuged at 3,000 rpm for 10 minutes; the resulting serum was stored at -70 ° C until batch analysis. Testosterone and corticosterone levels were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS); gonadotropins (LH, FSH) by chemiluminescence immunoassay, all processed at a nationally accredited reference laboratory maintaining validated pediatric reference intervals. Oral vitamin D3 (400 IU/day) was started from the day of hospital discharge according to the IAP guidelines.¹⁶

Statistical analysis

Statistical analyses were carried out in SPSS version 27.0 (IBM, Armonk, NY, USA). Continuous variables are reported as mean±SD or median (Q1–Q3) depending on normality, assessed using the Shapiro-Wilk test. Changes from day 3 to the third month were tested using the Wilcoxon signed rank test. Comparisons between groups (AGA versus SGA) used the Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Spearman's rank order correlation assessed relationships between vitamin D and each hormonal and genital growth outcome. A post hoc power analysis was performed based on the observed correlation between vitamin D levels and mean testicular volume. With an effect size (r) of 0.30, an alpha level of 0.05, and a total sample size of 52 participants, the statistical power was approximately 74%, indicating a moderate power for detecting moderate associations in this cohort. To account for multiple comparisons, Bonferroni correction was applied within each analytical set. For correlation analyzes between vitamin D and hormonal parameters (16 comparisons), the adjusted significance threshold was set at $p < 0.003$. For the correlations between vitamin D and genital parameters (6 comparisons), the corrected significance level was $p < 0.008$. Significance was established at $p < 0.05$ (two-tailed). Correlation analyses and group-based comparisons address different analytical questions and may not yield concordant results. Consequently, statistically significant correlations do not necessarily translate into significant differences between groups defined by threshold-based categorization.

Results

Participant characteristics

Of the 60 infants enrolled, 52 completed both study visits and were included in the final analysis; 8 were lost to follow-up. Of the 52 infants, 27 (51.9%) delivered by lower-segment caesarean section (LSCS), 24 (46.2%) by normal vaginal delivery and 1 (1.9%) by vacuum-assisted delivery. Maternal hypothyroidism

was documented in 14 mothers (26.9%). The mean birth weight was 2.73 ± 0.34 kg and the mean birth length was 47.83 ± 1.75 cm. Demographic and anthropometric data for both visits are summarized in Table 1. The mean vitamin D at birth was 25.26 ± 18.66 ng/mL, suggesting relatively low vitamin D levels in this cohort. After three months of standard supplementation (400 IU/day), the mean of the group increased to 49.43 ± 23.50 ng/mL, though with considerable variability between individuals.

Table 1. Baseline and follow-up demographic and anthropometric characteristics (n=52)*

Variable	Value (n (%) or mean \pm SD)
Growth status: AGA	28 (53.8%)
Growth status: SGA	24 (46.2%)
Mode of delivery: LSCS	27 (51.9%)
Mode of delivery: normal vaginal delivery	24 (46.2%)
Mode of delivery: vacuum-assisted	1 (1.9%)
Maternal hypothyroidism	14 (26.9%)
Birth weight (kg)	2.73 ± 0.34
Birth length (cm)	47.83 ± 1.75
Head circumference at birth (cm)	33.66 ± 1.24
Weight at 3 months (kg)	5.50 ± 0.78
Length at 3 months (cm)	59.14 ± 3.31
Head circumference at 3 months (cm)	39.51 ± 2.22

* AGA – appropriate for gestational age, SGA – small for gestational age, LSCS – lower segment caesarean section

Minipuberty hormonal surge: longitudinal changes

Wilcoxon signed rank testing confirmed significant hormonal shifts during the two visits (Table 2). Testosterone increased from a birth median of 47.18 (18.97–95.69) ng/dL to 142.62 (92.62–274.34) ng/dL at three months ($Z=-5.828$, $p<0.001$) – a nearly three-fold increase. LH increased from 1.31 (1.21–1.88) to 2.46 (1.96–3.04) mIU/mL ($Z=-5.231$, $p<0.001$) and FSH from 1.22 (0.89–1.72) to 2.10 (1.61–2.55) mIU/L ($Z=-5.009$, $p<0.001$), confirming physiological activation of the HPG axis during minipuberty. In contrast,

corticosterone decreased from 211.19 (124.88-377.34 ng / dL) at birth to 86.25 (47.39–141.16) ng/dL at 3 months ($Z=-5.282$, $p<0.001$), consistent with the anticipated regression of the fetal adrenal cortex during early postnatal life.

Table 2. Longitudinal changes in hormonal levels from birth to 3 months (Wilcoxon signed-rank test, $n=52$)

Parameter	Birth median (Q1–Q3)	3-month median (Q1–Q3)	Z	p
Testosterone (ng/dL)	47.18 (18.97–95.69)	142.62 (92.62–274.34)	-5.828	<0.001
LH (mIU/mL)	1.31 (1.21–1.88)	2.46 (1.96–3.04)	-5.231	<0.001
FSH (mIU/L)	1.22 (0.89–1.72)	2.10 (1.61–2.55)	-5.009	<0.001
Corticosterone (ng/dL)	211.19 (124.88–377.34)	86.25 (47.39–141.16)	-5.282	<0.001

Spearman correlation between vitamin D levels and hormonal parameters

Vitamin D levels showed differential hormonal associations between the two time points (Table 3). At birth, neonatal vitamin D levels were positively correlated with testosterone ($r=0.335$, $p=0.015$) and negatively with corticosterone ($r=-0.275$, $p=0.048$); no significant relationships were observed for LH or FSH at birth. At 3 months, a strong inverse correlation was observed between vitamin D levels and corticosterone ($r=-0.576$, $p<0.001$). No significant vitamin D associations with LH or testosterone were detected at the three-month visit.

Table 3. Spearman correlation between vitamin D levels and hormonal parameters at birth and three months ($n=52$)*

Hormone	Vitamin D at birth r (p)	Vitamin D at 3 months r (p)
Testosterone (birth)	0.335 (0.015)	0.163 (0.250)
LH (birth)	0.248 (0.089)	0.207 (0.158)
FSH (birth)	-0.067 (0.650)	0.036 (0.807)

Hormone	Vitamin D at birth r (p)	Vitamin D at 3 months r (p)
Corticosterone (birth)	-0.275 (0.048)	-0.210 (0.136)
Testosterone (3 months)	0.238 (0.089)	0.096 (0.499)
LH (3 months)	0.068 (0.630)	0.044 (0.759)
FSH (3 months)	-0.323 (0.020)	-0.146 (0.301)
Corticosterone (3 months)	-0.154 (0.276)	-0.576 (<0.001)

* values are Spearman r with two-tailed p values

Correlations between vitamin D and genital growth parameters

Vitamin D levels showed a consistent positive relationship with mean testicular volume at both visits (Table 4). Vitamin D levels were moderately correlated with testicular volume at birth ($r=0.304$, 95% CI: 0.034–0.533, $p=0.028$) and a comparable association with testicular volume at 3 months ($r=0.290$, 95% CI: 0.019–0.522, $p=0.037$). Vitamin D levels at three months were similarly associated with concurrent testicular volume ($r = 0.304$, 95% CI 0.036-0.534, $p = 0.027$). Penile length did not show correlation with vitamin D levels in either assessment.

Table 4. Spearman correlation between vitamin D levels and genital parameters (n=52)*

Outcome	Exposure	n	r	95% CI	p
Mean testicular volume (birth)	Vitamin D at birth	52	0.304	0.034 to 0.533	0.028
Mean testicular volume (3 months)	Vitamin D at birth	52	0.290	0.019 to 0.522	0.037
Mean testicular volume (3 months)	Vitamin D at 3 months	52	0.306	0.036 to 0.534	0.027
Penile length (birth)	Vitamin D at birth	52	-0.084	-0.348 to 0.199	0.554
Penile length (3 months)	Vitamin D at birth	52	0.211	-0.064 to 0.452	0.133

Outcome	Exposure	n	r	95% CI	p
Penile length (3 months)	Vitamin D at 3 months	52	0.136	-0.143 to 0.393	0.335

* 95% CI calculated using Fisher's z transformation

Multiple comparison correction

Several correlations between vitamin D levels and hormonal parameters demonstrated statistical significance using the conventional threshold of $p < 0.05$. Moderate associations were observed between vitamin D at birth and testosterone at birth, between vitamin D and corticosterone at birth, and a negative correlation between vitamin D and FSH at three months. However, after Bonferroni correction for multiple comparisons (adjusted threshold $p < 0.003$ for 16 hormonal comparisons), only the strong inverse correlation between vitamin D levels and corticosterone at three months ($r = < 0.576$) retained statistical significance. The remaining associations did not survive correction and should therefore be interpreted with caution.

Table 5. Multivariate linear regression analysis for the determinants of mean testicular volume at three months (n=52)*

Predictor variable	B (unstandardized)	SE	Standardized β	95% CI for B	p
Vitamin D at birth (ng/mL)	0.003	0.001	0.411	0.001–0.005	0.008
Growth status (SGA vs AGA)	-0.078	0.050	-0.335	-0.179– 0.023	0.127
Birth weight (kg)	-0.017	0.088	-0.050	-0.195– 0.161	0.845
Birth length (cm)	0.003	0.010	0.045	-0.017– 0.023	0.760
Head circumference (cm)	0.001	0.015	0.009	-0.029– 0.031	0.957
Maternal complications	-0.017	0.036	-0.066	-0.089– 0.055	0.641

Mode of delivery	-0.029	0.031	-0.124	-0.092	0.368
				0.034	

* Multivariate linear regression adjusted for growth status, maternal complications, mode of delivery, and baseline anthropometric parameters, model fit: $R^2=0.227$, adjusted $R^2 = 0.105$, $F = 1.851$, $p = 0.111$, B unstandardized regression coefficient, SE standard error, β – standardized beta coefficient, CI confidence interval

In the multivariate linear regression analysis, vitamin D level on day 3 showed an association with mean testicular volume at 3 months ($B=0.003$, $\beta=0.411$, 95% CI: 0.001–0.005, $p=0.008$), after adjustment for growth status (AGA/SGA), maternal complications, mode of delivery, birth weight, birth length, and head circumference (Table 5); however, these findings should be interpreted cautiously given the general model and are considered exploratory. This indicates that each 1 ng/mL increase in birth vitamin D level was associated with a 0.003 unit increase in testicular volume at three months, independent of these covariates. None of the remaining variables showed a statistically significant association with testicular volume (all $p>0.05$). The general fit of the model was modest ($R^2=0.227$, adjusted $R^2=0.115$, $F=1.851$, $p=0.101$). However, it must be explicitly acknowledged that the overall regression model did not reach statistical significance ($F=1.850$, $p=0.101$), indicating that the model as a whole does not significantly predict testicular volume. Therefore, the individual coefficient of vitamin D should therefore be interpreted with caution and cannot be used to support independent predictive conclusions; these regression findings are presented for exploratory purposes.

Discussion

This study prospectively characterized hormonal dynamics during minipuberty in healthy term male infants, demonstrating the expected increase in testosterone, LH, and FSH, along with a decline in corticosterone. In addition, exploratory analyses were performed to examine potential associations between vitamin D status and endocrine and growth parameters. The longitudinal hormonal trajectories observed in this cohort – including testosterone surge, gonadotropin increase, and corticosterone decline – are consistent with well-established minipuberty physiology described in the prior literature,¹⁻³ supporting that these changes represent expected postnatal endocrine patterns.

A positive association between vitamin D and neonatal testosterone was observed; however, this was not remain significant after correction for multiple comparisons and should therefore be interpreted as exploratory. While biologically plausible given the presence of vitamin D receptors in Leydig cells,^{5,6} any mechanistic interpretation remains speculative and hypothesis-generating in the absence of robust statistical support.

An important statistical caveat must be acknowledged. After applying the Bonferroni correction for multiple comparisons, most of the correlations reported in this study, including those between vitamin D and testosterone and between vitamin D and testicular volume, were no longer statistically significant at the corrected threshold ($p < 0.003$ for hormonal parameters; $p < 0.008$ for genital parameters). Only the inverse correlation between vitamin D and corticosterone at three months ($r = -0.576$, $p < 0.001$) survived correction. Consequently, all associations reported in this study should be interpreted as exploratory and hypothesis-generating rather than confirmatory, and must be replicated in larger, adequately powered, independent cohorts before any clinical inferences are drawn.

It should also be noted that all statistical analyses in this study were predefined in the study protocol, carried out using standard validated methods, and no post hoc data-driven model selection was performed; the analytical approach was determined independently of the results.

Attenuation of this association at three months may reflect several possibilities, including a potential threshold effect or the dominant influence of gonadotropin-driven stimulation during peak minipuberty. However, these interpretations are speculative and cannot be confirmed within the constraints of the present data.

The absence of a significant association between vitamin D levels and gonadotropins (LH, FSH) at either visit is noteworthy and was similarly reported by KInç et al.,¹³ who found no significant correlation between vitamin D status and gonadotropins in boys at 30 to 45 days of life. Taken together, these data raise the possibility that, to the extent vitamin D is associated with minipuberty hormones, the association may be at a peripheral rather than central level at the gonadal rather than the hypothalamic-pituitary level. This remains speculative in the absence of experimental or interventional evidence.

Associations between vitamin D levels and testicular volume were observed at both timepoints; however, these were not statistically significant after correction for multiple comparisons and should therefore be interpreted as exploratory. While the direction of association was consistent, the moderate effect sizes and loss of significance after correction limit the strength of inference. Any biological interpretation remains tentative and should be considered hypothesis-generating given the lack of statistical significance after correction.

The lack of a vitamin D–penile length association may have a plausible biological basis. Phallic enlargement during minipuberty depends mainly on local conversion of testosterone to dihydrotestosterone (DHT) via 5α -reductase in corporeal tissue, rather than on circulating testosterone per se.¹⁹ Because the associations of vitamin D in this dataset were with testosterone rather than DHT, the absence of a corresponding penile length effect is mechanistically coherent.

The inverse vitamin D–corticosterone relationship was the most notable finding of this study. This association was statistically significant after correction for multiple comparisons. The association at birth was modest ($r = 0.275$, $p = 0.048$) but became markedly stronger at three months ($r = -0.576$, $p < 0.001$),

coinciding with a near-60% decrease in corticosterone over the same interval, a trajectory consistent with regression of the fetal adrenal zone in postnatal life.²⁰ This association is biologically plausible: VDR is expressed in the adrenal cortex,^{21,22} and experimental data suggest that vitamin D may suppress CRH and ACTH at the hypothalamic-pituitary level⁶ while also modulating adrenal steroidogenic enzymes. Therefore, it is possible that higher vitamin D status may be associated with a more rapid postnatal decline in glucocorticoid production, a recognised component of neonatal metabolic transition, although residual confounding and the observational design preclude any causal interpretation. Future work with serial HPA axis markers and appropriate confounder adjustment will be necessary to further characterize this relationship.

Compared to Kılınç et al.,¹³ the strengths of this study lie in its prospective longitudinal design that spans two key timepoints across the minipuberty window, including the hormonal axes of gonadal and adrenal hormonal axes, and the use of objective ultrasound-based testicular volume. These design features provide a comprehensive characterization of early postnatal endocrine physiology in male infants and represent the primary novel contribution of this work.

Study limitations

The single-center design limits generalizability. The moderate sample size (n=52) reduces the statistical power for subgroup analyses. No maternal vitamin D data were available. Near-universal supplementation (400 IU/day after discharge) may have contributed to changes in vitamin D levels by three months, potentially reducing variability within the cohort and thus limiting our ability to examine the effects of sustained deficiency; therefore, all associations at three months should be interpreted in the context of supplemented, rather than naturally deficient, vitamin D status, and these findings cannot be extrapolated to prolonged deficiency. No serial corticosterone or HPA axis markers were obtained. The observational design precludes causal inference. Potential confounders including growth status (AGA/SGA), maternal hypothyroidism, mode of delivery, and baseline anthropometrics were not adjusted for in the primary correlation analyses – multivariable regression models addressing this are presented separately. Despite the application of the Bonferroni correction, the study remains at risk of type I error due to multiple comparisons and limited sample size. The study cannot determine whether vitamin D associations reflect a direct biological effect or are mediated by unmeasured factors that covariate with vitamin D status in this population.

Conclusion

In this prospective longitudinal study of healthy term Indian male newborns, the expected hormonal dynamics of minipuberty was confirmed, including a rise in testosterone, LH, and FSH and a decrease in corticosterone. The only association between vitamin D status and endocrine parameters that remained

statistically robust after correction for multiple comparisons was the inverse relationship between 25(OH)D and corticosterone at three months. All other associations, including those with testosterone and testicular volume, did not survive Bonferroni correction and should be considered exploratory, requiring confirmation in larger independent studies. These findings do not permit causal conclusions and should be interpreted within the limitations of an observational design and a supplemented cohort. Taken together, the results highlight the importance of longitudinal endocrine assessment during minipuberty, while underscoring the need for more research to clarify the role of vitamin D in this context.

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Declarations

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Authors' contributions

Conceptualization, B.C.; Methodology, B.C. and V.V.M.; Investigation, V.V.M. and A.F.Z.; Formal Analysis, B.C. and A.H.; Data Curation, V.V.M.; Writing – Original Draft Preparation, V.V.M.; Writing – Review & Editing, B.C., A.M. and S.S.; Visualization, A.M.; Supervision, B.C.; Resources, S.S. and A.H.

Conflicts of interest

The authors declare no competing interests.

Data availability

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

Ethics approval

The protocol was approved by the Institutional Ethics Committee of SRM Medical College Hospital and Research Institute (approval number: SRMIEC-ST0724-8011).

Use of AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language refinement and manuscript polishing. After using these tools, the authors reviewed and edited all content as needed and assume full responsibility for the content of the published article.

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