Research Protocol

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Dentistry Section

Determination of the Levels of Estradiol (E2) in Urine and the Optimum Time Frame for Nasal Cartilage Moulding in Infants with Cleft Lip and Palate: A Research Protocol for a Prospective Observational Study

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ABSTRACT

Introduction: Cleft lip and palate are among the most common congenital craniofacial anomalies, which result from incomplete fusion of the facial processes when the fetus develops. After performing non surgical methods like nasoalveolar moulding, which is crucial to improve the nasal symmetry, the length of the columella and aesthetics of the face, before the surgical procedures are performed. In infants, the ability of the nasal cartilage is very much influenced by maternal hormones like Estradiol (E2), which is usually elevated in newborns for a short period of postnatal growth due to the placental transfer. Thus, understanding urinary estradiol levels will help us establish an effective time frame for initiating the presurgical procedures.

Need of the study: The effectiveness of PNAM depends on the malleability of nasal cartilage in early infancy, which is influenced by maternal hormones such as estradiol. However, the optimal biologically driven time frame for initiating PNAM has not been clearly established. Assessing urinary estradiol levels in infants may help identify the most favourable period for initiating PNAM to improve treatment outcomes in cleft lip and palate patients.

Aim: To assess urinary estradiol levels in infants with cleft lip and palate to identify the optimal time frame for initiating Presurgical Nasoalveolar Moulding (PNAM) based on hormonally influenced nasal cartilage malleability.

Materials and Methods: This will be a prospective observational study conducted in the Department of Orthodontics and Dentofacial Orthopaedics, in collaboration with the research house, Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra, India. The study will include convenience sampling, a total sample of 25 infants per group (cleft and non cleft) will be included, based on Lamorte power calculations and feasibility with infants registered under the Smile Train visiting the Departmental OPD. The study will be carried out over 12 months from January 2026 to December 2026. The primary inclusion criteria of age ≤3 months at initiation of PNAM, absence of systemic illness, and no prior orthopaedic intervention. Demographic parameters including age, gender, birth weight, and cleft type, were recorded. After obtaining parental consent, urine samples shall be collected from infants in labelled sterile vials and stored at -80°C until analysis. Estradiol levels were estimated using a standard ELISA technique. Samples were collected at four time points: V1 (0-15 days), V2 (30 days), V3 (60 days), and V4 (90 days) from birth. Intragroup comparisons were performed across visits, and intergroup comparisons were made between infants with cleft lip and palate, non-cleft infants, and standard textbook reference values to determine the optimum estradiol level and time frame for nasal cartilage moulding. Data were statistically analysed using repeated-measures Analysis of Variance (ANOVA), with $p<0.05$ considered statistically significant.

Keywords: Breast milk, Cartilage, Formula, Hyaluronic acid, Maternal oestrogen

INTRODUCTION

Cleft lip and palate are the 2nd most common congenital anomaly after club foot. Non syndromic clefts comprise a range of disorders, the cause of which remains unknown. The incidence of cleft lip and palate has been reported as 1.09 per 1,000 live births [1].

Cleft deformity is associated with significant abnormality in nasal cartilage morphology and asymmetry of alar cartilage, nasal base and columella. The lateral crura of the cartilage on the affected side are flat and concave, whereas on the normal side, they are stretched, leading to greater flatness. While the bilateral clefts present additional challenges like short, absent columella and the excessively wide prolabium [2].

Dr. B Grayson's PNAM appliance, with the scientific evidence of Matsuo's work of moulding the cartilage, works in the direction of moulding the disturbed nasal alar cartilage by stretching the nasal mucosal lining, and achieving non surgical columellar elongation and nasal symmetry pre-surgically, combining it with moulding of the alveolar processes in the cleft. [2].

This process of active moulding and repositioning utilises the inherent plasticity of cartilage in newborns. The temporary mouldability observed during the neonatal period is thought to result from elevated levels of Hyaluronic Acid (HA), a key component of the proteoglycan-rich intercellular matrix, which remains in circulation for several weeks after birth [2].

Oestrogen receptor may play an important role in the biosynthesis of HA. Many studies regarding the effect of Maternal Oestrogen (ME) on HA synthesis have been conducted. This suggests that an increase in oestrogen levels increases the levels of HA [3].

Oestrogen speeds up cartilage maturation, reduces linear bone growth, promotes endochondral ossification, enhances bone mineralisation, and causes dehydration of the cartilage matrix accompanied by an apparent increase in collagen [4].

The standard values of Estradiol (E2) in an adult female varies according to the menstrual phases i.e., in the follicular phases it is 5-20 µg/day, ovulatory phase: 15-35 µg/day and in luteal it is about 10-25 µg/day but in pregnancy markedly elevated values can be seen which may exceed upto 1000 µg/day in the third trimester. Thus, the E2 levels are physiologically high in newborns due to transplacental transfer of maternal oestrogen and later decline rapidly over the first few weeks of life [5].

The levels of the ME and HA are seen to be highest in the initial days of the birth and start declining as the ME levels decrease, from 93 micrograms/L (49-153) at 1-3 months of age to 20 micrograms/L (9-40) at 2-3 years and 16 micrograms/L (6-32) at 4-18 years [5]. It is therefore suggested that the nasal cartilage moulding should be initiated in the early days [2].

Amongst the factors that influence the levels of oestrogens and maintains its titre/level are the mother's milk and fortified milk. Breast milk and certain infant formulas may contain substances with oestrogen- like activity, potentially extending the effects of oestrogen in the body; however, these effects typically diminish between six and 12 months of age [6].

Normal sucking and suckling are considered to be the most difficult tasks for the cleft infant because of the presence of the cleft palate [7]. Many infants are deprived of the mother's milk as abnormal sucking and suckling activity leads to reduced milk production. Also, the patients visiting the Institute are from low socioeconomic status who cannot afford fortified milk as a top feed for their infants. And mostly prefer feeding the baby with cow's or buffalo's milk. It is thought that this can further reduce the titer of the HA early as compared to that of normal infants. Thereby, reducing the scope of moulding the nasal cartilage for an elongated time.

The PNAM treatment should begin as early as possible after birth [2]. In the perinatal period, elevated maternal oestrogen leads to an increase in HA levels. HA contributes to reduced elasticity of cartilage, ligaments, and connective tissues by breaking down the intracellular matrix. Neonatal cartilage exhibits the greatest plasticity immediately after birth, gradually decreasing as the infant grows. This heightened plasticity is likely due to elevated levels of HA associated with maternal oestrogen transferred to the infant. Around six weeks of age, the cartilage begins to lose its flexibility. As a result, PNAM is most effective when initiated within the first three to four months of life [2].

Estradiol tends to enhance the elasticity and malleability of the nasal cartilage by increasing water content and HA inside the matrix. But the duration and variation of the elevated estradiol levels in the neonates remain ill-defined. The following study is thereby planned to evaluate the titer/levels of oestrogen/estradiol in samples collected from infants with cleft lip and palate so as to determine the optimum time frame for nasal cartilage moulding.

There are very few studies in the literature where the levels of HA and ME are measured in cleft infants to treat them under any treatment protocol.

A cleft patient has to undergo treatment right from birth to adolescence. If the skeletal severity is too significant, then skeletal/jaw surgeries are an additive treatment required. The multidisciplinary modality includes treatment for jaw and teeth correction, speech and hearing training, surgical facial reconstructions, etc., PNAM therapy is an additive burden on the parents, which is initiated since birth.

The PNAM technique is beneficial to the infants regarding cartilage moulding, which should be initiated early. If the levels of E2 are determined before initiation of any therapy for the infant, the reported advantages can be availed. Also, it will be easy to determine whether the PNAM therapy is beneficial or an additional emotional and financial burden for the parents in the initial days of the treatment for their cleft child.

Correlative, non invasive study to give a scientific validation for hormonal levels and cartilage malleability.

Urine samples have been widely utilised in epidemiologic studies to assess endogenous hormone levels. These samples have proven stable both with and without added ascorbic acid, during short-term storage at 4°C for up to 48 hours, as well as during long-term storage at -80°C for up to a year, and have remained reliable through multiple freeze-thaw cycles. Therefore, the study aimed to determine the levels of estradiol in urine and the optimum time frame for nasal cartilage moulding in infants with cleft lip and palate. And the objectives were-

1. To measure the levels of Estradiol (E2) in infants with and without cleft lip and palate;
2. To compare the levels of Estradiol (E2) in infants with and without cleft lip and palate with standard established values;
3. To determine the optimum time frame to mould the nasal cartilage → this will be assessed by statistically getting the mean superior outcome and the predictive performance, ANOVA.

REVIEW OF LITERATURE

Studied the effects of oestrogen on cartilage [4]. They induced osteoarthritis in rabbits and then treated them by injecting the oestradiol component. It was found that oestrogen accelerated cartilage maturation, stimulates endochondral ossification, diminish linear bone growth.

Uzuka M et al., in 1980, studied the relation between HA and oestrogen [3]. It was observed that administration of oestradiol in the mouse skin resulted in an increase in the levels of HA. The glycosaminoglycan present in mouse skin contains two components, i.e., HA and dermatan sulphate. Administration of oestradiol increased the levels of only HA and not dermatan sulphate. They also stated that the level of hyaluronic acid is dependent on the age of the subject.

Performed pre-operative non surgical correction of cleft lip nasal deformity [2]. Active moulding of the nasal cartilage is due to the plasticity of the cartilage in infants. This temporary plasticity is due to the high levels of HA (component of intercellular proteoglycan matrix), which is found circulating in the infants for several weeks after birth.

In 2012, De Jong M et al., conducted serial measurements of gonadotropin and estradiol levels in infant urine samples collected at one week, one month, three months, and six months of age [8]. This study offered a detailed assessment of the postnatal activation of the Hypothalamic-Pituitary-Gonadal (HPG) axis in infants. The findings indicated that estradiol levels are initially high in cord blood serum but decline rapidly after birth, reaching pre-pubertal levels by approximately four months of age. Maslarinec G et al., in 2015, suggested the use of urine as a source to be evaluated for the level of E1 and E2 [9].

Cağlar MK investigated the link between low oestrogen and labial adhesions, but findings are often interpreted to show that while hypoestrogenism creates a vulnerable state, it is likely not the sole cause. There was no significant difference in serum estradiol levels between girls with and without adhesions [10].

MATERIALS AND METHODS

This will be a prospective observational study and will be carried out over 12 months from January 2026 to December 2026. The study protocol was reviewed and approved by the IEC of Datta Meghe Institute of Higher Education and Research (IEC-DMIMS(DU)/IEC/Dec-2019/8587). Convenience sampling of cleft lip ± palate patients presenting to the Smile Train Centre Department of Orthodontics and Dentofacial Orthopaedics, the study will be carried out over a period of 12 months.

The study at DMIMS will recruit 25 infants with CLP and 25 non cleft controls using convenience sampling. Following parental consent, demographic data and baseline nasal and alveolar measurements, including columella length, nostril dimensions, alar base width, and alveolar cleft gap, will be recorded. Urine samples will be collected at four time points: 0-15 days, 30, 60, and 90 days, and stored at -80°C. Estradiol levels will be quantified using Enzyme-linked Immunosorbent Assay (ELISA) [11]. PNAM therapy will be initiated early, utilising custom appliances with nasal stents to mould the alar cartilage and elongate the columella, with weekly adjustments [11,12].

The standard reference values quoted in the textbook of Medical Physiology by John Edward Hall will be taken for comparison with infants with and without cleft lip and palate, for the normal range of oestrogen present in infants from birth to 4 months [13]. The standard values of ME and HA were found to be 93 micrograms/l (range: 49-153) at 1-3 months of age, decreasing to 20 micrograms/l (range: 9-40) by 2-3 years, and further dropping to 16 micrograms/l (range: 6-32) between 4 and 18 years of age.

Inclusion criteria: Infants up to three months of age and with a complete cleft lip and/or palate (unilateral or bilateral) for whom pre-surgical nasal moulding is planned will be included in the study.

Exclusion criteria: Infants more than three months old and with syndromic clefts, partial/incomplete clefts, major congenital anomalies, or infants with medical contraindications to nasal moulding will be excluded from the study.

Sample size calculation: Convenience sampling of eligible infants presenting during the 1-year recruitment window. Based on the incidence of the cleft population, which is reported to be around 1 in 800-1000 live births, corresponding to approximately 27,000–33,000 affected neonates annually. The suggested sample size = 22 (depending on the patient visit number to the Smile Train Unit). Sample size taken for the following study n=25 in each group.

One kit includes 96 wells, out of which seven will be used to determine the standard deviation. Estradiol levels in infants show well-documented physiological variability, and age-specific SD values have been reported in standard medical physiology and paediatric endocrinology literature.

The standard formula used for sample size calculation was:

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \times \alpha^2}{d^2}$$

Where:

- n = required sample size per group
- $Z_{\alpha/2}$ = standard normal deviate for 95% confidence level (1.96)
- Z_{β} = standard normal deviate for 80% power (0.84)
- σ = estimated standard deviation
- d = minimum clinically meaningful difference

Values substituted:

- $Z_{\alpha/2}$ = 1.96

Zβ = 0.84

- σ = standard deviation was derived from the expected range of 15-20 pg/ml for the age
- d= clinically relevant difference in estradiol levels across early infancy.

$$n = \frac{(1.96 + 0.84)^2 \times \alpha^2}{d^2}$$

Substituting these values yielded a minimum sample size of 22 subjects per group. To account for potential dropouts and improve study robustness, the final sample size was rounded up to 25 infants per group.

Outcome measures:

- Primary outcome: Correlation between E2 level and nasal moulding outcome, which will be measured as columellar length (mm) and a Nasal Symmetry Index (NSI) [2], to obtain a time window for successful moulding.
- Secondary outcome: Magnitude of changes occurring, feasibility (feasibility to the practical applicability of using urinary estradiol estimation to guide PNAM in infants, and not to treatment success).

The outcome of nasal moulding will be assessed by evaluating changes in nasal morphology from baseline (V1) to the final visit (V4). The primary measures will include columella length and an NSI, calculated by comparing linear dimensions of the affected and non affected sides, with values closer to 100% indicating improved symmetry [2]. Secondary outcomes will include nostril height and width, alar base width, and reduction in alveolar cleft gap measured on standardised photographs and dental casts. A clinician-rated nasal aesthetic score and parental satisfaction rating will also be recorded. All measurements will be performed using standardised photographic protocols and digital callipers, with assessments carried out by two blinded examiners to ensure reliability.

Outcomes will be assessed through standardised measurements, clinician-rated aesthetics, and parental satisfaction, focusing on columella length and the NSI. The NSI expresses the degree of symmetry between the cleft and non cleft sides of the nose by comparing corresponding nasal dimensions like the nostril width and height, alar base width, and the columella deviation.

STATISTICAL ANALYSIS

Data will be entered and managed using Microsoft Excel 2021. Statistical analysis will be performed using IBM Statistical Package for Social Sciences (SPSS) Statistics version 26.0. A mixed-design repeated-measures ANOVA will be used to assess the effects of group (cleft/non cleft) and time (V1–V4) on urinary Estradiol (E2) levels and PNAM outcomes. Correlation analysis will be performed to evaluate the association between E2 levels and PNAM outcomes. A p-value <0.05 will be considered statistically significant.

The study timeline, including participant recruitment, urine sample collection, laboratory analysis, data management, statistical analysis, and manuscript preparation, is depicted in [Table/Fig-1].

Activity/Phase	1-2	3-4	5-6	7-8	9-10	11-12
Literature review and protocol finalisation						
Ethical approval and logistics						
Training and pilot calibration						
Procurement of kits and materials						
Participant recruitment						
Urine sample collection and E2 estimation						
Nasal moulding and follow-up						
Data entry and preliminary analysis						
Statistical analysis and interpretation						

Manuscript drafting and review						

[Table/Fig-1]: GNATT chart showing the proposed study timeline

REFERENCES

[1] Reddy SG, Reddy RR, Bronkhorst EM, Prasad R, Ettema AM, Sailer HF, et al. Incidence of cleft lip and palate in the state of Andhra Pradesh, South India Indian J Plast Surg. 2010;43(2):184-89.
[2] Cutting CB, Grayson BH, Brecht LE, Santiago P, Wood R, Kwon S. Presurgical columellar elongation and primary retrograde nasal reconstruction in one-stage bilateral cleft lip and nose repair. Plast Reconstr Surg. 1998;101(3):630-39.
[3] Uzuka M, Nakajima K, Ohta S, Mori Y. The mechanism of estrogen-induced increase in hyaluronic acid biosynthesis, with special reference to estrogen receptor in the mouse skin. Biochim Biophys Acta. 1980;627(2):199-206.
[4] Talwar RM, Svoboda K, Harper RP. Effects of estrogen on chondrocyte proliferation and collagen synthesis in skeletally mature articular cartilage. Journal of Oral and Maxillofacial Surgery. 2006;64(4):600-09.
[5] Trivedi P, Cheeseman P, Mowat AP. Serum hyaluronic acid in healthy infants and children and its value as a marker of progressive hepatobiliary disease starting in infancy. Clin Chim Acta. 1993;215(1):29-39.
[6] Effects of Infant Diets on Estrogen Activity and Development. Clinicaltrials.gov US National Library of Medicine, NIH.
[7] Kharbanda OP. Textbook of Orthodontics: Diagnosis and Management of Malocclusion and Dentofacial Deformities, Elsevier India, 2009.
[8] De Jong M, Rotteveel J, Heijboer AC, Cranendonk A, Twisk JW, Weissenbruch MM. Urine gonadotropine and Testosterone levels in male very low birth weight infants. Horm Res Paediatr. 2012;78(3):173-79.
[9] Maslarinec G, Beckford F, Morimoto Y, Frankee AA, Stanzyk FZ. Association of estrogen measurements in serum and urine of premenopausal women. Biomarkers in Medicine. 2015;9(5).
[10] Cağlar MK. Serum estradiol levels in infants with and without labial adhesions: The role of estrogen in the etiology and treatment. Pediatr Dermatol. 2007;24(4):373-75.
[11] Bradlow HL, Sepkovic DW, Klug T, Osborne MP. Application of an improved ELISA assay to the analysis of urinary estrogen metabolites. Steroids. 1998;63:406-13.
[12] Thompson AL, Whitten PL, Johnson ML, Lampl ML. Non-invasive methods for estradiol recovery from infant fecal samples. Front Physio. 2010;1:148. Doi: 10.3389/fphys.2010.00148.
[13] Hall JE. Guyton and Hall Textbook of Medical Physiology. 13th ed. Philadelphia (PA): Saunders Elsevier; 2016. 1148

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