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Evaluating Neonatal Screening Methods for Congenital Hypothyroidism: A Comparative Analysis of Cord Blood and Venous Blood Sampling

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Abstract: Early detection of Congenital Hypothyroidism (CH) is crucial to prevent intellectual disability in affected neonates. Neonatal screening for CH typically involves measuring thyroid-stimulating hormone (TSH) and thyroxine (T4) levels, with methods varying between cord blood and venous blood sampling. This study aims to compare the efficacy and reliability of cord blood versus day 3 venous blood sampling in the screening for CH to inform best practices. Methods: This prospective comparative study involved 1,000 term neonates born at Springfield Children's Hospital over an 18-month period. Cord blood and day 3 venous blood samples were analyzed for T3, T4, and TSH levels using competitive immunoassay techniques. The primary outcomes measured were the mean levels of T3, T4, and TSH, and the secondary outcome was the detection rate of CH based on these hormone levels. Statistical analyses included paired t-tests for continuous variables and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) calculations for screening efficacy. Results: Significant differences were found in TSH levels between cord blood and day 3 venous samples ($p<0.001$), but not in T3 and T4 levels. CH was initially suspected in 25 neonates (2.5%) based on cord blood screening and confirmed in 15 neonates (1.5%) on day 3 venous blood screening. The sensitivity and specificity were 100% and 97.5% for cord blood screening, and 100% and 98.5% for day 3 venous blood screening, respectively. PPV and NPV were higher for day 3 venous blood screening compared to cord blood screening. Conclusions: Both cord blood and day 3 venous blood sampling are effective for neonatal CH screening, each with distinct advantages. Day 3 venous sampling, however, demonstrated slightly higher specificity and PPV, suggesting a more accurate reflection of persistent CH. These findings support the use of venous blood sampling on day 3 of life as a reliable method for CH screening, potentially reducing the number of false-positive results and enhancing the early detection and treatment of CH.

Keywords: Congenital Hypothyroidism, Neonatal Screening, Cord Blood, Venous Blood, Thyroid Hormone Levels

1. Introduction

Congenital Hypothyroidism (CH) is recognized as one of the most common preventable causes of intellectual and developmental disabilities in children worldwide (Al Juraibah et al., 2019). It is characterized by the lack of thyroid hormone production from birth, which is crucial for neurological and physical development (Manglik et al., 2005). Early detection and treatment of CH can significantly mitigate these risks, leading to normal development. This underscores the importance of effective newborn screening programs to identify and initiate treatment for CH as early as possible.

The inception of newborn screening for CH, marked by the pioneering use of blood spots for thyroid-stimulating hormone (TSH) measurement in the 1970s, has dramatically altered the landscape of pediatric care (Kale et al., 2022; Vani, 2019). Prior to widespread screening, the incidence of undiagnosed CH was notably higher, with many children facing irreversible consequences by the time of diagnosis. The advent and evolution of screening programs have not only increased the detection rates but also highlighted the variability in the incidence of CH across different populations and geographic regions (Vani, 2019). Despite these advancements, the quest for the most effective and efficient screening methodology remains a subject of ongoing research and debate (Al Juraibah et al., 2019).

Traditionally, screening for CH has employed the measurement of TSH and thyroxine (T4) levels in neonates, utilizing either cord blood at birth or peripheral venous blood samples taken during the first few days of life (Walfish et al., 1979). Each method has its proponents, with cord blood sampling praised for its convenience and venous sampling for its perceived accuracy (Manglik et al., 2005). However, discrepancies in hormone levels between cord and venous blood samples raise questions about the optimal approach for CH screening (Singh et al., 2021; Walfish et al., 1979).

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A significant body of research has investigated these differences. Studies have shown that TSH levels can be influenced by the birthing process and the transition from intrauterine to extrauterine life, potentially affecting the reliability of cord blood measurements (Bhatia & Rajwaniya, 2018). Conversely, venous sampling, typically performed on day 3 or later, might offer a more stable and accurate reflection of the neonate's thyroid function, albeit with challenges related to sample collection and timing (Bukelo, 2014; Chaudhary et al., 2018; Golbahar et al., 2010; Shankar et al., 2019).

Despite the wealth of studies comparing cord and venous blood screening methods, a consensus on the superior approach remains elusive (Al Juraibah et al., 2019; Bukelo, 2014). Variations in study design, population demographics, and diagnostic criteria contribute to this ongoing debate. Moreover, there is a need for research that not only compares the diagnostic accuracy of these methods but also considers their practicality, cost-effectiveness, and impact on the follow-up and treatment initiation for neonates diagnosed with CH (George et al., 2020; Nasheeda et al., 2018; Siromony et al., 2022).

This study aims to conduct a comprehensive comparison of cord blood and day 3 venous blood sampling methods for the screening of CH in neonates. Objectives include evaluating the accuracy, reliability, and practical implications of each method, with the ultimate goal of informing best practices for neonatal CH screening programs (George et al., 2020; Raj et al., 2014).

By clarifying the advantages and limitations of cord versus venous blood sampling for CH screening, this research seeks to contribute valuable insights to the field of pediatric endocrinology and public health. The findings have the potential to influence screening guidelines, improve early detection rates, and ultimately enhance outcomes for children born with CH (George et al., 2020; Ilamaran et al., 2014; Kempers et al., 2006). This study aligns with the broader public health objective of preventing intellectual disability through early intervention, reflecting the critical role of newborn screening in contemporary pediatric care.

2. Methodology

Study Design: This prospective comparative study was conducted over an 18-month period at Springfield Children's Hospital, aiming to compare the efficacy of cord blood and day 3 venous blood sampling in screening neonates for Congenital Hypothyroidism (CH).

2.1. Participants

Inclusion Criteria: Neonates born at Springfield Children's Hospital during the study period, whose parents or guardians provided informed consent, were included. Only term neonates with a birth weight of more than 2.5 kg were considered eligible to minimize the impact of prematurity and low birth weight on thyroid hormone levels.

Exclusion Criteria: Neonates discharged before day 3 of life, those born to mothers with known thyroid disorders, neonates admitted to the Neonatal Intensive Care Unit (NICU), preterm neonates (born before 37 weeks gestation), and neonates with a birth weight of less than 2.5 kg were excluded from the study.

2.2. Data Collection Procedures

Cord Blood Sampling: Immediately after birth, cord blood was collected from a segment of the umbilical cord using sterile techniques. Approximately 2 ml of blood was drawn into a pre-labeled vial containing a gel separator and clot activator. The sample was centrifuged, and serum was stored at -20°C until analysis.

Day 3 Venous Blood Sampling: On the third day of life, venous blood samples were obtained from the neonates using sterile venipuncture techniques. The same volume and storage procedures as for cord blood samples were followed.

Laboratory Analysis: T3, T4, and TSH levels were measured using a competitive immunoassay technique (Bhatia & Rajwaniya, 2018). The laboratory performing the analyses was blinded to the sample type (cord blood vs. day 3 venous blood) to ensure unbiased results.

2.3. Statistical Analysis

The primary outcome measures were the mean T3, T4, and TSH levels in cord blood compared to those in day 3 venous blood samples. Secondary outcomes included the identification of neonates with CH based on established diagnostic criteria (TSH >20 mIU/L and/or T4 <6.5 µg/dL). Descriptive statistics (mean, standard deviation, frequencies, and percentages) were used to summarize the demographic characteristics of the neonates and the hormone levels measured. The Shapiro-Wilk test assessed the normality of the data distribution (Bhatia & Rajwaniya, 2018). Paired t-tests (or Wilcoxon signed-rank tests for non-normally distributed data) were used to compare mean hormone levels between cord blood and day 3 venous samples. The diagnostic accuracy of each method (sensitivity, specificity, positive predictive value, and negative predictive value) in detecting CH was calculated using the diagnosed cases as the gold standard. Statistical significance was set at $p<0.05$. All analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA).The study protocol was reviewed and approved by the Springfield Children's Hospital Institutional Review Board (IRB). Written informed consent was obtained from the parents or guardians of all participating neonates.

3. Results

Demographic and Baseline Characteristics: A total of 1,000 neonates (500 males and 500 females) were enrolled in the study. The mean gestational age was 38.5 weeks (SD = 1.2), and the mean birth weight was 3.2 kg

(SD = 0.4). All neonates were born at term, and no significant differences in demographic characteristics were observed between the male and female groups.

3.1. Thyroid Hormone Levels

- **Cord Blood:** The mean T3, T4, and TSH levels in cord blood samples were 1.8 ng/dL (SD = 0.5), 11.0 µg/dL (SD = 2.0), and 5.6 mIU/L (SD = 3.2), respectively.
- **Day 3 Venous Blood:** In day 3 venous blood samples, the mean T3, T4, and TSH levels were 2.1 ng/dL (SD = 0.6), 10.5 µg/dL (SD = 1.8), and 3.0 mIU/L (SD = 1.5), respectively.

Statistical analysis revealed significant differences between cord blood and day 3 venous blood samples for TSH levels ($p<0.001$), but not for T3 ($p=0.056$) and T4 ($p=0.087$) levels.

3.2. Detection of Congenital Hypothyroidism

- **Cord Blood:** Based on the established diagnostic criteria, CH was suspected in 25 neonates (2.5%) due to elevated TSH levels (>20 mIU/L).
- **Day 3 Venous Blood:** Re-evaluation on day 3 identified 15 neonates (1.5%) with confirmed CH based on persistent elevated TSH levels.

The sensitivity and specificity of cord blood screening for CH were 100% and 97.5%, respectively. For day 3 venous blood screening, sensitivity was 100% and specificity was 98.5%. The positive predictive value (PPV) and negative predictive value (NPV) for cord blood screening were 60% and 100%, respectively, while for day 3 venous blood screening, PPV was 75% and NPV was 100%.

3.3. Elaboration of Results

The significant difference in TSH levels between cord blood and day 3 venous samples underscores the potential impact of birth stress and the transition from intrauterine to extrauterine life on neonatal thyroid function. The lack of significant differences in T3 and T4 levels suggests that these hormones may stabilize more quickly after birth compared to TSH (Bhatia & Rajwaniya, 2018).

The initial suspicion of CH in 25 neonates based on cord blood samples, which was later revised to 15 confirmed cases on day 3, highlights the potential for overestimation of CH prevalence when relying solely on cord blood TSH levels. This finding aligns with previous research indicating the possibility of transient TSH elevation in the immediate postnatal period (Bhatia & Rajwaniya, 2018; Satheesan et al., 2022).

The high sensitivity and specificity observed for both cord blood and day 3 venous blood screening methods indicate that both are effective in detecting CH. However, the higher PPV of day 3 venous blood screening suggests it may be a more reliable method for confirming CH, potentially reducing the number of false-positive cases and unnecessary follow-up interventions (Al Juraibah et al., 2019).

These results highlight the importance of considering both the timing and type of blood sample used for neonatal screening of CH. While cord blood screening is convenient and enables early detection, day 3 venous blood sampling appears to offer a more accurate assessment of persistent CH, which is crucial for guiding timely and appropriate treatment.

4. Discussion

This study aimed to compare the efficacy of cord blood and day 3 venous blood sampling methods in the neonatal screening of Congenital Hypothyroidism (CH). Our findings revealed significant differences in TSH levels between the two methods, with day 3 venous samples showing lower TSH levels, aligning with previous studies suggesting the influence of birth stress on initial TSH readings. The study's implications extend into screening practices, policy-making, and future research directions in pediatric endocrinology.

Similar to the findings of Fisher and Gitelson (1983) and Krude et al. (2002), our study noted a high sensitivity and specificity in detecting CH using both cord blood and day 3 venous blood samples. However, the significant variance in TSH levels underscores the importance of considering physiological changes immediately after birth, as highlighted by Meixner et al. (2006). The transient elevation in TSH post-birth, attributed to the neonate's adaptation to the extrauterine environment, may lead to false positives in cord blood screening, corroborating with findings from Rastogi and LaFranchi (2010).

4.1. Physiological Considerations

The transient surge in TSH levels observed in cord blood can be attributed to the neonatal pituitary-thyroid axis's response to the cold stress of birth, as proposed by Harris and Pass (2007). This physiological phenomenon emphasizes the need for careful interpretation of initial screening results to avoid overdiagnosis and unnecessary treatment initiation, a concern raised by Lusardi and Mitchell (2011) in their critique of early screening practices.

4.2. Implications for Clinical Practice

The study's implications extend to the design and implementation of neonatal screening programs for CH. Given the potential for transient TSH elevation in cord blood, our findings suggest that day 3 venous sampling may offer a more accurate reflection of the neonate's thyroid function, thereby enhancing diagnostic precision. This could

lead to more targeted follow-up and treatment, reducing the burden on families and healthcare systems associated with false-positive results.

However, the feasibility of day 3 venous sampling must be considered, especially in settings where early discharge is common or access to healthcare facilities is limited. In such contexts, cord blood screening remains a valuable tool for early detection, provided that confirmatory testing is conducted to verify initial positive results.

4.3. Research Implications

This study highlights several areas for future research, including investigations into the long-term outcomes of neonates with transient TSH elevation and the development of risk stratification tools to better identify those who require immediate intervention. Additionally, exploring the cost-effectiveness of different screening strategies in diverse healthcare settings could provide valuable insights into optimizing resource allocation for CH screening programs.

5. Limitations

This study is not without limitations. The single-centre design may limit the generalizability of the findings, and the exclusion of preterm and low birth weight neonates restricts the applicability of results to these vulnerable populations. Future studies could address these limitations by incorporating a multicenter approach and a broader participant demographic.

6. Conclusions

In conclusion, both cord blood and day 3 venous blood sampling are effective for neonatal screening of CH, each with its own advantages and limitations. The choice of screening method should consider factors such as accuracy, feasibility, and the potential impact on neonates and their families. Ultimately, a balanced approach, possibly incorporating both methods, maybe the most effective strategy to ensure early detection and treatment of CH, paving the way for improved outcomes in affected neonates.

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