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Research Article

Establishment Of Reference Range For Free Thyroxine Hormone (FT4) In Neonates: A Cross Sectional Study

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Abstract:
Introduction: Reference intervals play a crucial role in clinical laboratory test interpretation and patient care. Accurate reference intervals for free thyroxine (FT4) in neonates are crucial for diagnosing and managing thyroid-related conditions but are currently lacking due to neonatal-specific physiological variations. This study aims to establish precise FT4 reference ranges to improve neonatal thyroid function assessment and clinical care.
Aim: To establish a reference range for free thyroxine hormone (FT4) in neonates
Materials and Methods: This cross-sectional study was conducted at the Obstetrics and Gynecology (OBG) and Neonatal Intensive Care Unit (NICU) of Father Muller Medical College and Hospital, Mangalore from January 2019 to December 2019. A total of 240 healthy term neonates (37-42 weeks of gestation) with no congenital anomalies, infections, or significant perinatal complications were included in the study. Serum free thyroxine (FT4) levels were measured using the Roche Cobas e601 electrochemiluminescence method. Blood samples were collected from neonates within the first week of life. Data analysis was performed following Clinical and Laboratory Standards Institute (CLSI) guidelines. Medians, 2.5th, and 95th percentiles for FT4 were calculated to determine the reference range. Demographic parameters such as gestational age, birth weight, and sex of the neonates were recorded.
Results: The established reference range for FT4 in neonates was found to be 1.4-4.1 ng/dl. There was no significant difference between genders (p- value= 0.987), with male neonates having a range of 1.4-4.2 ng/dl and female neonates having a range of 1.4-3.68 ng/dl.
Conclusion: This study highlights the importance of establishing accurate reference intervals for clinical decision-making. The reference range for FT4 in neonates differs from that in adults, emphasizing the need for age-specific reference interval. Further studies with larger sample sizes are warranted to validate and refine gender-specific reference intervals for FT4 in neonates.

Keywords: Reference range, Free thyroxin hormone (FT4), Neonates, CLSI guidelines.

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INTRODUCTION

Reference intervals (RIs) are crucial in clinical laboratory testing, aiding in the interpretation of test results and guiding patient care decisions. These intervals, specific to various factors such as age, gender, and ethnicity, are essential for accurate diagnosis and treatment. Nearly 80% of physicians' medical decisions are based on information provided by laboratory reports. Typically, this information is provided in the form of a reference interval or medical decision limit. A reference interval as defined by Ceriotti "is an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others" [1]. No reference interval is completely "wrong" or "right". The majority of RIs in use today refer to the central 95% of reference population of subjects. By definition, 5% of all results from healthy people will fall outside of the reported range and, as such, will be flagged as being "abnormal" [1].

Reference intervals are indispensable in evaluating laboratory result [2]. Every laboratory has to establish its own reference range. There are many problems associated with the calculation of reference interval, therefore instead of performing a new reference interval study, laboratories and manufacturers refer to studies done many decades ago or, when both the methods and the population were very different [1].

Thyroid hormones play a pivotal role in neonatal development, influencing brain development, growth, and metabolism. Free thyroxine hormone (FT_4), the unbound form of thyroxine, reflects thyroid function and is vital in diagnosing thyroid disorders in neonates. FT_4, the unbound form of the thyroid hormone thyroxine (T_4), is representative of thyroid status. Therefore, serum FT_4 levels reflect the health of the thyroid gland and can assist in thyroid disease diagnosis. Measurement of FT_4 is essential for screening congenital hypothyroidism (CH) and preventing intellectual disability through early diagnosis and treatment in newborn screening programs [3].

Currently there is no reference range established for FT_4 in neonates in Indian population. Considering the importance of reference interval in clinical decision making, a study of establishing reference interval for FT_4 in neonates is conducted.

Establishing precise reference intervals for free thyroxine (FT4) in neonates is crucial for accurate thyroid function assessment and diagnosis, yet such data is lacking for the Indian population. This study aims to fill this gap by determining FT4 reference ranges for healthy Indian neonates.

MATERIALS AND METHODS: This retrospective observational hospital cross sectional study was done at Father Muller medical college, Mangalore. Data collection occurred from January 2019 to December 2019, and data analysis was performed from January 2020 to March 2020. The study included 240 healthy neonates as per CLSI guidelines [4]. After obtaining institutional ethical clearance(FMMCIEC/CCM/51/2018), FT_4 values of healthy neonates analyzed in clinical biochemistry laboratory are collected from laboratory information system (LIS) for a period of one year.

Serum FT_4 results of healthy babies less than one month old, babies weighing more than 2.5kg.**,** babies having normal head circumference and babies born within the normal gestational age were included in the study.

More than one-month old babies, newborns with conditions or concomitant medications likely to affect thyroid function, chromosomal abnormality**,** born with inappropriate body weight, height and head circumferences for gestational age, newborns who had congenital anomaly, intra uterine growth retardation, thyroid disease themselves or their mothers, and pituitary disease and abnormal TSH values were excluded from the study. FT_4 assay was done in neonate's serum sample based on electrochemiluminescence (competitive principle) in Roche Cobas e601[5]. This assay was unaffected by icterus, hemolysis, lipemia, and biotin and auto antibodies of thyroid hormones can interfere with this assay.

Statistical analysis:

It was done using SPSS version 23. Normality of the data was determined using Kolmogorov–Smirnov test. Data was summarized using descriptive statistics (mean ± standard deviation) for normally distributed data and median for skewed data. To obtain reference interval, 2.5th and 97.5th percentile was used. The comparison of reference interval between the genders was made using Mann–Whitney U-test. A p-value less than 0.05 was considered statistically significant.

Results:

Reference intervals are age, ethnicity and method dependent. Total 240 neonates were enrolled in the study, among that 137(57%) were males and 103(43%) were females. The mean gestational age of the neonates was 39 weeks, with a range spanning from 37 to 42

weeks. The mean birth weight was recorded at 3.1 kg, with individual weights ranging from 2.5 kg to 4.0 kg. Table 1 shows the summary of the results of FT_4. Reference interval of male babies is between 1.4-4.2ng/dl and reference interval of FT_4 in female neonates lies between 1.4-3.68ng/dl. There was no statistical significance in reference interval among male and female, so single reference interval is used that is between 1.4-4.1ng/dl.

Table 1: Reference interval of FT_4 based on gender

Parameters	Study Participants (n= 240)		Male (n=137)		Female (n= 103)		p-value (Male vs Female
	Median	Reference Interval (2.5^{th} - 97.5^{th} percentile)	Median	Reference Interval (2.5^{th} - 97.5^{th} percentile)	Median	Reference interval (2.5^{th} - 97.5^{th} percentile)	0.987
FT_4 (ng/dl)	2.39	1.4-4.1	2.29	1.4-4.2	2.5	1.4-3.68	

DISCUSSION

Reference intervals are essential for clinical laboratory test interpretation and patient care. A test result by itself is of little value unless it is reported with the appropriate information for its interpretation. Typically, this information is provided in the form of a reference interval or medical decision limit. Hence every laboratory has to establish its own reference intervals as they play an important role in clinical decision making.

The establishment of accurate reference intervals for free thyroxine (FT4) is essential for the early diagnosis and management of thyroid disorders, particularly congenital hypothyroidism, in neonates. This study aimed to determine the FT4 reference interval for healthy neonates in the Indian population, filling a significant gap in the existing literature.

The current study conducted on 240 neonates revealed that the mean gestational age was 39 weeks, with birth weights ranging from 2.5 kg to 4.0 kg. The male neonates accounted for 57% of the study, while females made up 43%. The reference interval for FT4 in male babies was found to be between 1.4-4.2 ng/dl, and for female neonates, it ranged from 1.4-3.68 ng/dl. Despite the lack of statistical significance between male and female reference intervals, a single reference interval of 1.4-4.1 ng/dl was adopted for both genders [5] are in accordance with the findings published by the Canadian CALIPER study [6]. A study was conducted in babies of American population aging from 1 to 12 month says that FT_4 reference intervals were very similar for males and females of all ages and ranged between 1.3–2.8 ng/dL [7]. In contrast, the study conducted in Nairobi population [8,9] and in Iran population demonstrated age and sex differences within the neonatal period [10].

Establishing accurate reference intervals is crucial as they are influenced by various factors such as age, gender, and ethnicity. Studies emphasize the importance of defining local reference intervals based on the specific population to ensure precision in clinical assessments [11,12, 13]. Furthermore, the reference intervals for thyroid hormones like TSH and FT4 are known to be affected by age, gender, iodine nutrition, and ethnicity, highlighting the need for tailored reference values [14, 15].

Variations in reference intervals can stem from differences in population definitions, including factors like ethnicity, diet, and genetics. Therefore, it is essential to consider these aspects when establishing reference intervals to ensure their accuracy and relevance to the target population [16, 17].

By establishing specific reference intervals, healthcare providers can more accurately assess thyroid function in neonates and identify any potential abnormalities that may require further evaluation or intervention.

Accurate reference intervals are crucial for clinical decision-making, especially in neonatal care. This study fills a gap in the literature by establishing an FT4 reference range for neonates in the Indian population. Further studies with larger sample sizes are needed to validate and refine gender-specific reference intervals.

CONCLUSION:

The study underscores the importance of establishing accurate reference intervals for FT4 in neonates, aiding in the diagnosis and management of thyroid disorders in this population. The established reference range of 1.4-4.1 ng/dl for FT4 in neonates in the Indian population serves as a valuable tool for clinicians in interpreting thyroid function tests and guiding patient care. By providing a standardized reference interval, healthcare providers can effectively monitor and evaluate thyroid hormone levels in neonates, facilitating early detection and management of thyroid-related disorders.

the discussion on reference intervals for FT4 in neonates highlights the significance of considering age, gender, and ethnicity when establishing reference values to ensure accurate clinical assessments and interpretations. Tailoring reference intervals to specific demographic groups is essential for providing precise and reliable diagnostic information in neonatal care.

Conflict of Interest: None

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Author's contributions:

Archana Augustine: Conceptualization, Methodology, Data curation, Writing- Original draft preparation.

Arun Kumar, Sandesh K S: Visualization, Investigation, Supervision.

Shruthi Rai P: Writing the draft and reviewing.

Shivarajashankara Y M: Reviewing and Editing.

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