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Establishment of Reference Range for FT4 in Neonates

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Research Article

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ABSTRACT

Background: Reference Intervals (RIs) are essential for clinical laboratory test interpretation and patient care. Reference intervals are indispensable in evaluating laboratory result. Every laboratory has to establish its own reference range. Methods for estimating a reference range is expensive difficult to perform, often inaccurate and non-reproducible. Despite the importance of RIs globally there are very few publications on RIs for neonates TFT, is primarily due to technical issues.

FT4, the unbound form of the thyroid hormone thyroxin (T4), is representative of thyroid status. Therefore, serum FT4 levels reflect the health of the thyroid gland and can assist in thyroid disease diagnosis. During fetal development, thyroid hormones are important in brain cell migration as well as differentiation of neurons, oligodendrocytes, astrocytes, and microglia Therefore, adequate levels of maternal T4 are important for appropriate fetal neurodevelopment. Neonatal screening can help in the timely diagnosis of newborn thyroid diseases such as congenital hypothyroidism and thus prevent developmental or growth problems.

Objective: To establish reference range for FT4 in neonates.

Methods: Samples are collected from OBG and NICU wards and test for serum FT4 is done by using electrochemiluminescence method in Roche Cobbas 601. Data obtained is analyzed based on CLSI guidelines for determining reference range for FT4. Medians, 2.5th, 5th, 95t, 97.5th percentile for free T4 is calculated. Range is calculated using median and 2.5th and 97.5th percentile values.

Result: This study established a reference range for FT4 in neonates based on CLSI guidelines and it is 1.4-4.1 ng/dl.

Conclusion: Clinical biochemistry results are always accompanied by reference range. Various factors such as age, sex, geography, method of estimation etc. will affect the reference range. The reference range for FT4 measure by electrochemiluminasence method in adults varies from

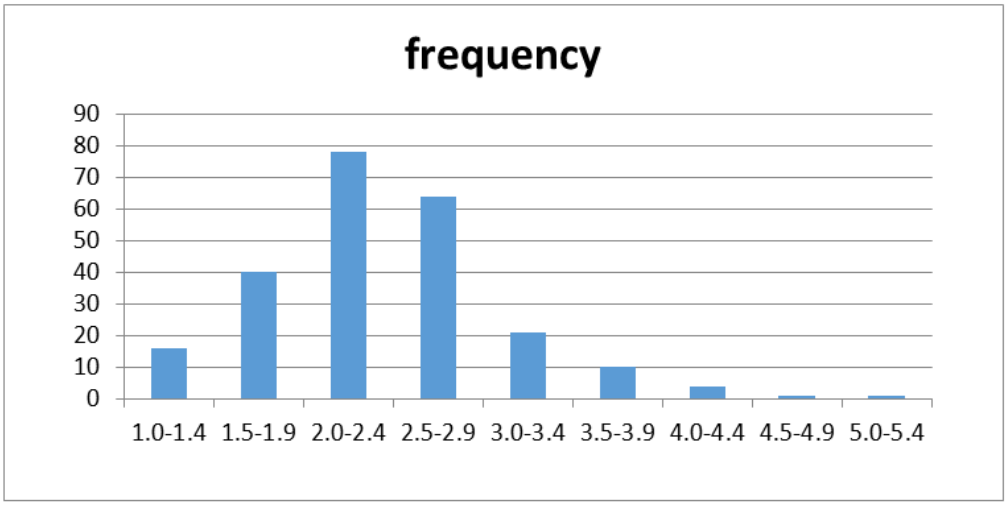
that measured in neonates. In adults the reference range is 0.93-1.7 ng/dl and in neonates it is 1.4-4.1 ng/dl.

INTRODUCTION

Reference intervals are essential for clinical laboratory test interpretation and patient care. 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 (RI) or medical decision limit. An RI 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 RI 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". 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 RI, therefore instead of performing a new reference interval study, laboratories and manufacturers refer to studies done many decades ago, when both the methods and the population were very different [3].

Methods for estimating a reference range is expensive difficult to perform, often inaccurate and non-reproducible. Recruiting a valid group of reference subjects and obtaining informed consent in today's environment is costly, time-intensive, and virtually an impossible task for most laboratories. The challenge is further magnified in establishing RIs for different age groups; uncommon sample types timed collections, challenge tests, and serial measurements (Figure 1).

Figure1. Frequency distribution of sample group.



Thyroid hormone values are population and method dependent. Age and sex specific reference intervals are important pre-requisite for interpreting thyroid hormone measurement in children, only few studies have reported age-specific pediatric reference value for TSH, FT3, FT4 so far. No sex –difference were found for TSH and FT4 between aged matched serum samples [4].

FT4, the unbound form of the thyroid hormone thyroxin (T4), is representative of thyroid status. Therefore, serum FT4 levels reflect the health of the thyroid gland and can assist in thyroid disease diagnosis. During fetal development, thyroid hormones are important in brain cell migration as well as differentiation of neuronsoligodendrocytes, astrocytes, and microglia Therefore, adequate levels of maternal T4 are important for appropriate fetal neurodevelopment. Neonatal screening can help in the timely diagnosis of newborn thyroid diseases such as congenital hypothyroidism and thus prevent developmental or growth problems [5].

Congenital hypothyroidism (CH) is one of the most important causes of preventable mental retardation. Iodine deficiency and excess are the primary factors that affect thyroid-stimulating hormone (TSH) levels and increase the recall rates for CH screening. Newborns with TSH levels above 10 mIU/mL and FT4 levels within the normal range are diagnosed as cases of transient hyperthyrotropinemia and are treated with low-dose Na-L-T4.

Currently there is no reference range established for FT4 in neonates in Indian population. Considering the importance of RI in clinical decision making, a study of establishing reference interval for FT4 in neonates is conducted. This study is to establish reference range for free thyroxin hormone in neonates by using nonparametric method given by CLSI guideline (C28-A2).

MATERIALS AND METHODS

This observational descriptive record based study was done at Father Muller medical college for a period of one year (2019). The study included 240 healthy neonates as per CLSI guidelines. After obtaining institutional ethical clearance, FT4 values of healthy neonates analyzed in clinical biochemistry laboratory are collected from Laboratory Information System (LIS) for a period of one year. Reference range for FT4 in neonates is calculated based on CLSI guidelines [6].

Serum FT4 results of healthy babies less than one month old, babies weighing more than 2.5 kg, 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.

The following test is performed in neonate's serum sample:

Method of analysis

Precision of instrument was checked regularly before the analysis and reagent were calibrated to the instrument before the analysis of sample. FT4 assay was done in neonate's serum sample based on electrochemiluminasence (competitive principle). This assay was unaffected by icterus, hemolysis, lipemia, and biotin and auto antibodies of thyroid hormones can interfere with this assay.

The data collected from clinical biochemistry laboratory is analyzed based on the CLSI guidelines for establishing reference range for FT4. The median, 2.5th percentile, 97.5th percentile was calculated and range was established (Table 1).

Table 1. Table showing class interval and frequency of sample group.

Class Intervals (CI)	Frequency
1.0-1.4	16
1.5-1.9	40
2.0-2.4	78
2.5-2.9	64
3.0-3.4	21
3.5-3.9	10
4.0-4.4	4
4.4-4.9	1
5.0-5.4	1

Reference range is established by following nonparametric method given by CLSI guidelines. According to that:

1. First, the n reference values are sorted in ascending order of magnitude.

2. Median is found out by plotting frequency distribution graph

3. The percentiles are calculated by the formula 0.025(n+1) and 0.975(n+1).

4. The lower limit of RI is equal to 2.5th percentile and the upper limit is equal to 97.5th percentile with confidence interval of 90%.

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. RI of male babies is between 1.4-4.2 ng/dl and RI of FT4 in female neonates lies between 1.4-3.68 ng/dl. There was no statistical significance in reference interval among male and female, so single reference interval is used. So single reference interval was being used that is between 1.4-4.1 ng/dl (Tables 2 and 3).

Table 2. Showing class intervals and frequency of male babies.

Class Intervals	Frequency
1.0-1.4	10
1.5-1.9	23
2.0-2.4	49
2.5-2.9	29
3.0-3.4	11
3.5-3.9	6
4.0-4.4	3
4.5-4.9	1
5.0-5.4	1

Table 3. Showing class intervals and frequency of female babies.

Class Intervals	Frequency
1.0-1.4	6
1.5-1.9	17
2.0-2.4	26
2.5-2.9	36
3.0-3.4	10
3.5-3.9	5
4.0-4.4	1
4.5-5.0	0

Reference intervals are essential for clinical laboratory test interpretation and patient care. Methods for estimating them are expensive, difficult to perform, often inaccurate and non-reproducible. Nearly 80% of physician's medical decisions are based on information provided by laboratory reports (Figures 2 and 3).

Figure 2. Frequency distribution of male babies.

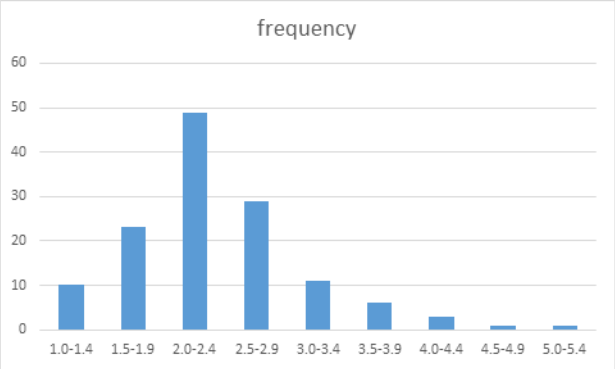
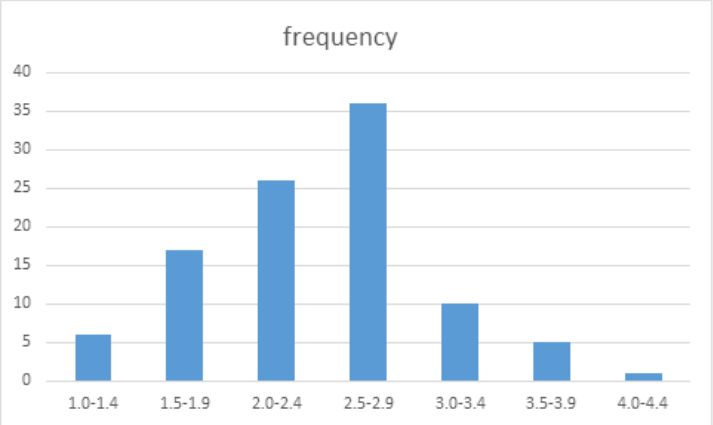


Figure 3. Frequency distribution of female babies.



DISCUSSION

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 RI or medical decision limit.

One of the most important elements of a laboratory test is the reference interval, the values that help clinician to interpret their patient's test results. Hence every laboratory has to establish its own reference intervals as they play an important role in clinical decision making.

FT4, the unbound form of the thyroid hormone thyroxine (T4), is representative of thyroid status. Therefore, serum FT4 levels reflect the health of the thyroid gland and can assist in thyroid disease diagnosis.

During fetal development, thyroid hormones are important in brain cell migration as well as differentiation of neurons, oligodendrocytes, astrocytes, and microglia. Therefore, adequate levels of maternal T4 are important for appropriate fetal neurodevelopment. Neonatal screening can help in the timely diagnosis of newborn thyroid diseases such as congenital hypothyroidism and thus prevent developmental or growth problems

There are no studies done on establishing reference range of FT4 in neonates in Indian population. Population based RI are a tool of interpretation of individual laboratory results,

This present study was conducted in healthy neonates of Indian population established a RI for FT4, and it is 1.4-4.1 ng/dl.

When this study is repeated by grouping the samples based on gender, it is found that the RI of males and females varies slightly from each other; however this requires further study as the sample size was not adequate according to the guidelines. This research can be extended further to establish RI for male and female babies separately.

A study was conducted in babies of American population aging from 1 to 12 month says that FT4 reference intervals were very similar for males and females of all ages and ranged between 1.3–2.8 ng/dL similar study was conducted in the children of same population tells that the RI of FT4 is between 0.33 ng/dl to 0.91 ng/dl.

These differences in RI are due to variations in many factors such as gender, ethnicity, population and method of estimation as RI of FT4 is population, gender and ethnicity specific. It varies from population to population based on the geographical distribution, life style and various other reasons. The level are also decreases as age increases, hence the RI of FT4 in neonates are more than that in adults.

Establishing RI for all biochemical parameters are important as they vary in different age group, gender and also based on various methods of estimation. There are no studies done on establishing RI for FT4 in Indian population. Hence this study helps the clinician in treating the babies with thyroid disorders.

CONCLUSION

Every laboratory has to establish its own RI as RIs are age, gender, population and method specific. These are the values which helps clinicians to interpret their patient's test results. Like many other biochemical parameters RI of FT4 is also age, gender, ethnicity and method dependent,. Hence the RI for FT4 hormone in neonates varies from that of adults. Therefore, in this study reference interval for FT4 in neonates in Indian population is established by following nonparametric method given by CLSI guidelines. According to this study RI for FT4 in neoanates is between 1.4-4.1 ng/dl. When the data are grouped based on gender it is found that the reference interval for male and female babies is slightly vary from each other. The RI of FT4 in males is between 1.4-4.2 ng/dl whereas in females it is between 1.4-3.68 ng/dl. However, further studies are required to establish RI for male and female babies separately as the sample size taken in our study is less than the number prescribed by the CLSI.

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