

Establishment of reference values of markers of non-thyroidal illness syndrome on a state-of-the-art fully automated ABEI-based chemiluminescence system

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ABSTRACT

Objective: To determine the reference values found in a reference population, free of any thyroidal as well as non-thyroidal illnesses.

Methods and Materials: Initially, 266 adult volunteers were included in the study from Mohi Uddin Islamic Medical College, and Mohi Uddin Teaching Hospital Mirpur Azad Jammu and Kashmir during 31st August 2022 to 30th August 2023., Pakistan after taking written informed consent. One hundred and twenty-four volunteers were selected (females 63; males 61) after exclusion of the volunteers with one or more of the exclusion criteria. Blood specimens were collected and analyzed for rT3, fT3, fT4 and TSH using Maglumi X8 (Snibe, China), an ABEI (N-(aminobutyl)-N-(ethyl)-isoluminol) based analyzer at Pathology Laboratory MOTH.

Results: Reference values of rT3, fT3, fT4 and TSH were found to be 13.3 – 19.8 ng/dL (0.2 – 0.30 nmol/L); 2.65 - 4.15 pg/mL (4.08 - 6.39 pmol/L); 10.6 - 16.2 pg/mL (13.64– 20.84 pmol/L) and 0.43 - 3.98 μ IU/mL (0.43 – 3.98 mIU/L), respectively.

Conclusion: Reference values determined in our own population should be used when diagnosing and monitoring NTIS and other thyroid disorders

Keywords: Free thyroxine (fT4), Free triiodothyronine (fT3), Non-Thyroidal Illness Syndrome, Reverse triiodothyronine (rT3), Thyroid stimulating hormone (TSH).

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INTRODUCTION

Non-Thyroidal Illness Syndrome (NTIS), previously called Euthyroid Sick Syndrome is characterized initially by increased reverse T3 (rT3), decreased free triiodothyronine (fT3), and later by decreased free thyroxine (fT4) and decreased thyroid-stimulating hormone (TSH) in a patient who is not a known case of thyroid-pituitary-hypothalamic axis disease [1]. Another important criteria for the diagnosis of NTIS is that all these hormones should come within the reference values after recovery from the particular illness which caused NTIS to ensue [2]. Frequency of NTIS has been reported to be 40%-70% in patients admitted in various critical care and high dependency

units of the hospitals but these patients have to be suspected and tested for the diagnosis of NTIS [1]. Insight into molecular mechanisms leading to NTIS is based on an extensive system of intra-cellular regulation of thyroid hormones involving three iodothyronine deiodinases enzymes serially called type 1 deiodinase (DIO1), type 2 deiodinase (DIO2) and type 3 deiodinase DIO3 [3]. Main biochemical role of DIO1 and DIO2 is to attack outer ring iodine of T4 to convert it to T3, the active form, while DIO3 attacks inner ring iodine of T4 to produce rT3 (inactivation pathway), and also attacks T3 to produce diiodothyronine (T2). by removal of iodine atoms at the inner ring (inactivation pathway) (Figure-I) [4].

It has been found that if the patient remains fasting during the acute illness, the decrease in fT3 and increase in rT3 may be a patient-friendly hormone change as it slows down the body metabolic rate⁵. If the patient is started with oral feeding early in the acute illness, these so-called beneficial changes may not take place [5]. In later stage of the prolonged critical illness, these changes may become harmful or at least non-beneficial, so chronology is the major

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determinant of NTIS [5]. Many acute and chronic diseases have been associated with NTIS including infections and inflammations of lungs, bowel and Central Nervous System CNS [6]. Similarly, NTIS has been found in post-operative stages of coronary artery bypass surgery, liver and kidney transplantation, fracture of hip joint surgery; cardiac conditions like acute coronary syndrome and congestive cardiac failure; many cancers; end stage renal disease and acute complications of diabetes; and finally, COVID-19 infection [7]. In Human Immunodeficiency Virus (HIV) infection the changes may be in opposite direction i.e. decrease in the reverse T3 and normal or increase fT3 and fT3/ fT [8]. NTIS has recently been found to be negatively correlated with recovery in children admitted in ICU with shock [9].

A word about the terminology. The term 'normal range' is commonly used by the clinicians and many laboratory persons, but scientifically it is not a correct term as word 'normal' is a statistical term for parametric type of data, while word 'range' implies a single figure i.e. difference between two values in a distribution [9]. Textbooks of Clinical Chemistry and International Federation of Clinical Chemistry (IFCC) have recommended the use of term 'reference values' [10,11]. If reference values are determined from a previously collected large data set it is called *posteriori method*, if specimen collection is carried out prospectively in a reference population after careful exclusion of the disease for which the test is used, it is called *priori method* [12]. The term *partitioning* is used for the procedure of determining different reference values after dividing the reference population on the basis of sex or age [13]. Confounders like controllable factors are neutralized by standardization of the procedure for preparation of reference individuals and specimen collection [13]. Boot-strap method, which is a simple non-parametric method is most commonly used statistical method for the establishment of reference values [14]. A major advantage of using parametric method, however, is requirement of a smaller number of subjects but it involves complexed statistical procedure [15]. All these methods have their own inherent advantages and limitations [16]. The present was planned to establish reference values of various thyroid parameters like rT3, fT3, fT4 and TSH. These reference values will be of immense help for use in patients suspected of NTIS. Previously, these reference values are not available for any Pakistani population.

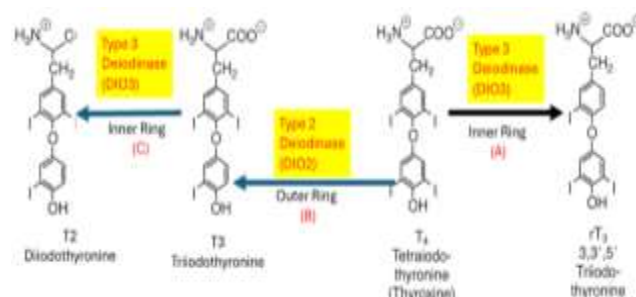


Figure-1: Action of type 3 deiodinase to produce reverse T3 (rT3) from thyroxine T (4) (A) and diiodothyronine (T2) from diiodothyronine T3 (B) and type 2 deiodinase for the production of T3 and T4 (C).

MATERIAL AND METHODS

A priori design was used for establishment of reference values [12]. The study was carried out at Mohi Uddin Islamic Medical College, Mirpur AJ&K, Pakistan (MIMC) and Mohi Uddin Teaching Hospital Mirpur AJ&K, Pakistan (MOTH) during 31st August 2022 to 30th August 2023. Proper permission from Ethical Review Board (ERB) of MIMC vide reference letter number MIMC-0582 dated 2nd August 2022. Two hundred and sixty-six volunteers (266) were evaluated for enrolment the study, mostly medical students and staff of MIMC and MOTH after taking their informed written consent. Exclusion criteria were age < 20 years and > 50 years, mild diseases, participants on any medications, pregnant participants, chronic illness thyroid disorders, menstrual irregularities and infertility. Textbooks, IFCC and Clinical and Laboratory Standards Institute (CLSI) USA have recommended using minimum sample size of 120 individuals for establishing reference values without partitioning [10,11,17]. So, we ensured that the number of our finally selected subjects should not drop below 120 in the present study. Pre-analytical factors were addressed carefully before blood specimens were collected and analyzed including:

a) Subject preparation: volunteers were briefed about the venipuncture to allay their anxiety;
b) Specimen collection: non-fasting condition, sitting relaxed for about 10 minutes prior to the venipuncture, no double prick and use of fine needle to reduce pain. Specimens were immediately transported to the laboratory, centrifugated immediately for serum separation. Specimen were stored in a -70°C freezer until analyzed. After exclusion of the volunteers meeting the exclusion criteria, blood specimens of 124 volunteers were analyzed for four hormones related to NTIS i.e. rT3, fT3, fT4 and TSH. Chemiluminescence-based autoanalyzer Maglumi-X8 by SNIBE (Shenzhen, China) was used for laboratory-based analyses. This is a continuous random access

chemiluminescence immunoassay (CLIA) automated system, uses nano-magnetic microbeads separation high throughput (180 tests/h) technology, the luminescence substrate being N-(aminobutyl)-N-(ethyl)-isoluminol (ABEI) [18]. Snibe (Shenzhen China) provided reagents and consumables for this project. Performance Characteristics of four hormone tests were found acceptable as given in Table-I including assay precision, linearity and linear regression as compared to the reference method. All laboratory results were blinded by allotting unique numbers to the volunteer's blood specimens. The lab results of outliers, however, were unblinded for repeating and referral to the related physicians for management of the disease.

Performance Characteristics of The Assay Parameters [18]: Following performance characteristics of the assay parameters were given by the manufacturers:

A) Precision: Defined by Intra-assay replication studies. Manufacturer's claim of Coefficient of Variation (CVs) were verified by performing in-house replication study of 20 replicates for each analyte and then applying F-Test of variance.

B) Analytical Sensitivity: Was evaluated by measuring Limits of Blank (LOB) and Limits of Detection (LOD) separately for each of the four hormones. LOB and LOD indicate the ability of the test to measure the smallest quantity of the analyte (hormone).

C) Analytical Measurement Range (AMR): Indicates the whole range of concentrations of the analyte (hormone) that can be measured by the test.

D) Linearity: It corresponds to the correlation between reference test and the test under evaluation. It is depicted by 'r²' and acceptable 'r²' value is >0.097.

E) Linear Regression: is the quantitative relationship between the reference test and the test under

evaluation of performance. For each analyte (hormone) following equation is used: 'y = ax + b'

Quality Control (QC): Internal QC provided in the reagent kits was run with each batch of analysis while external Quality Assessment (EQA) was carried out by participating in NHS-EQAS® (Novative Healthcare Solutions-External Quality assessment Scheme). (<https://nhs-neqas.com/external-quality-controls/>), which is an ISO 17043 accredited External Quality Assessment Scheme (except for rT3).

Data was entered in Microsoft Excel® database for analyses. Non-parametric approach was used for calculation of reference values because of following reasons:

A) This is the recommended method by IFCC [11] and adopted by CLSI of USA [16];

B) The nature of the underlying distribution of the data does not matter.

C) No statistical expertise is required; the values obtained from reference individuals were simply put in rank order by concentration (rank 1 is the lowest, rank 2 is the next lowest, etc.).

D) The central 95% became the reference interval.

E) The 90% confidence limits of the endpoints of the interval was similarly just taken from the data points themselves.

F) One hundred and twenty observations provide enough data to determine both the central 95% of the distribution and the 90% confidence limits on both endpoints.

That is, with 120 observations, rank 3 is the 2.5th percentile; rank 118 is the 97.5th percentile; ranks 1 and 7 define the 90% confidence interval of the 2.5th percentile; and ranks 114 and 120 define the 90% confidence interval of the 97.5th percentile (10). G) Since no statistical difference between the two groups i.e. males and females was found, so separate reference intervals for different partitions were not needed and data was not collected for each partition.

Table-I: Performance Characteristics of NTIS Related Hormones (18)

Name of Hormone	Precision (Intra-assay) CV%	Analytical Sensitivity (LOB & LOD)	Analytical Specificity (Cross reactivity)	AMR	Linearity	Method Comparison (Linear Regression) (y=ax+b)
Reverse T3 ²¹	4.88%	5 & 10 ng/dL	None	5 – 100 ng/dL	r=0.997	y=0.985x + 0.007
Free T3 ²²	4.63%	0.2 & 0.4 pg/mL	T4: <0.01% T3: <0.01 %	0.2 – 50 pg/mL	r=0.9506	y= 0.970x + 0.02338
Free T4 ²³	4.24%	1.0 & 1.5 pg/mL	T4: <0.01% T3: <0.01 %	1.0 – 120 pg/mL	r=0.997	y= 1.019x + 0.442
TSH ²⁴	2.53%	0.001 & 0.006 mIU/L	None	0.001 – 100 mIU/L	r=0.9596	y=0.953x + 0.327

CV: Coefficient of Variation; LOB: Limit of Blank; LOD: Limit of Detection; AMR: Analytical Measurement range. 'r' is Pearson correlation coefficient;

*Linear Regression Equation (y=ax+b) 'y' is the index test, 'a' is slope, 'b' is y intercept and 'x' is the reference test value

RESULTS

One hundred and forty-two volunteers were excluded from the study due to falling in one of the exclusion criteria mentioned above. Figure-II provides insight into the reasons of exclusion of volunteers with exact number of volunteers in each category of exclusion. Figure 3 gives distribution of volunteers into different gender and age groups. Females (n=41) and Males (n=27) of 20-24 years of age were the most dominant group (Figure-III).

Post-analyses exclusion was done for outliers based on Dixon's Method (12) (0-3 per analyte) (Table-II). Three results of TSH were too high to be included in the data and were declared outliers. One participant had high TSH and low fT4 (primary hypothyroidism),

while in the other two, only TSH was raised (possibly sub-clinical hypothyroidism). All three were referred to the concerned physicians for repeating the tests and management of the disease. Details of the non-parametric procedure used for the determination of lower and upper reference values along with 90% Confidence Limits of all four hormones are given in Table-II. Table 3 indicates the reference values of 4 hormones in traditional and SI units along with the conversion factors for general utility. In Table-III we have also compared the reference values determined using Maglumi X8, reference values reported by the manufactures [18] and reference values established using Abbott Architect (US) [17].

Table-II: Non-Parametric Determination of Reference Values of NTIS Related Hormones [10] (Number of Blood Specimen Analyzed = 124).

Name of Hormone	Outliers	Rank No of Percentile		Original Value Corresponding to the Rank Number		90% Confidence Limits (90%CL)		Summary	
	Excluded from Data (By Dixon Method) ²⁵	Lower: 0.025	Upper: 0.975	Lower Limit (2.5 percentile)	Upper Limit (97.5 percentile)	Lower Reference Limits Rank #	Upper Reference Limits Rank# (n+1) -7 & (n+1)-1	Lower Limit (90% CL)	Upper Limit (90% CL)
Reverse T3	0	0.025 x (124+1) = 3.125 i.e. #3	0.0975 x (124+1) = 121.875 i.e. #122	13.3 ng /dL	19.8 ng /dL	#1 and #7 Values: 12.1 & 14.7 ng/dL	#118 & #124 Value: 18.5 & 20.9 ng/dL	13.3 (12.1 and 14.7) ng/dL	19.8 (18.5 and 20.9) ng/dL
Free T3	0	0.025 x (124+1) = 3.125 i.e. #3	0.0975 x (124+1) = 121.875 i.e. #122	2.65 pg/mL	4.15 pg/mL	#1 and #7 Values: 0.98 & 2.65 pg/mL	#118 & #124 Value: 3.98 & 4.48 pg/mL	2.65 (2.48 and 2.7) pg/mL	4.15 (3.98 and 4.48) pg/mL
Free T4	1	0.025 x (123+1) = 3.1 i.e. #3	0.0975 x (123+1) = 120.9 i.e. #121	10.6 pg/mL	16.2 pg/mL	#1 and #7 Values: 10.1 & 11.3 pg/mL	##117 & #123 Value: 15.7 & 16.5 pg/mL	10.6 (10.1 and 11.3) pg/mL	16.2 (15.7 & 16.5) pg/mL
TSH	3	0.025 x (121+1) = 3.05 i.e. #3	0.0975 x (121+1) = 118.95 i.e. #119	0.43 mIU/L	3.98 mIU/L	#1 and #7 Values: 0.31 & 0.76 mIU/L	#115 & #121 Value: 3.82 & 4.12 mIU/L	0.43 (0.31 and 0.76) mIU/L	3.98 (3.82 & 4.12) mIU/L

Table-III: Reference values NTIS related hormones determined by different systems in traditional and SI units.

Name of Hormone	Reference Values Reported in Present Study On Maglumi X8 (SNIBE) Traditional Units (SI Units)	Reference Values Reported by Manufacturer On Maglumi X8 (SNIBE) Traditional Units (SI Units) (18)	Reference Values Reported on Abbot Architect Traditional Units (SI Units) (17)	Conversion Factor (Traditional to SI Units)
Reverse T3	13.3 – 19.8	3.1 – 9.5	10 – 28	ng/dL to nmol/L
ng/dL (nmol/L)	(0.2 – 0.30)	(0.48 – 1.46)	(0.15 – 0.43)-	X 0.0154
Free T3	2.65 - 4.15	2.00 – 4.20	2.10 – 4.40	pg/mL to pmol/L
pg/mL (pmol/L)	(4.08 - 6.39)	(1.86 – 6.43)	(3.23 – 6.77)	X 1.54
Free T4	10.6 - 16.2	8.9-17.2	8 – 27	pg/mL to pmol/L
pg/mL (pmol/L)	(13.6– 20.8)	(11.4 - 22.1)	(10.3 – 34.7)	X 1.287
TSH	0.43 - 3.98	0.3-4.5	0.4 – 4.2	μIU/mL to mIU/L
μIU/mL (mIU/L)	(0.43 – 3.98)	(0.3-4.5)	(0.4 – 4.2)	X 1

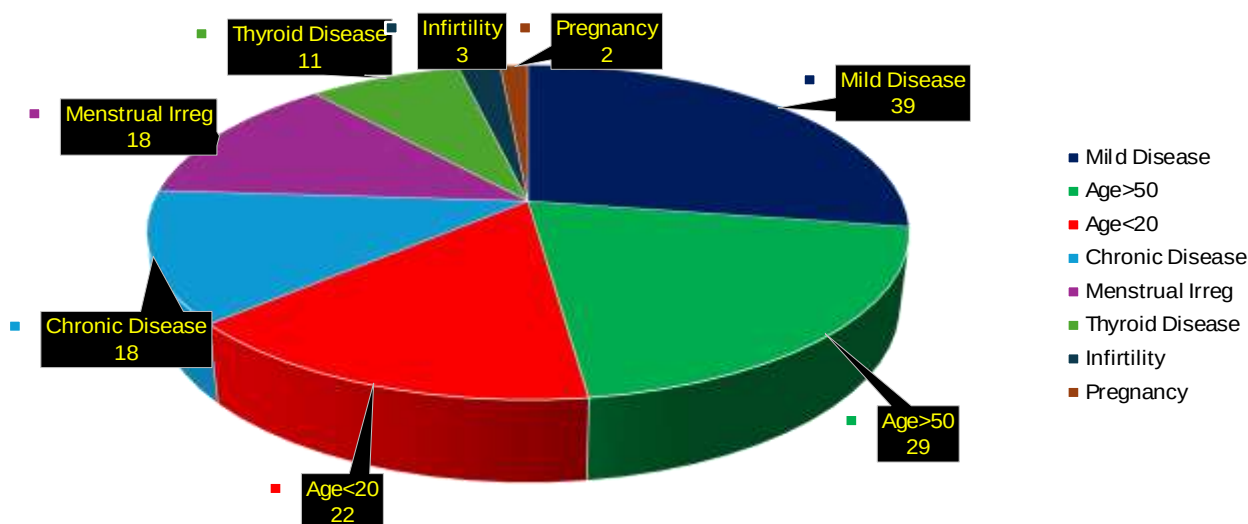


Figure-II: Distribution of volunteers excluded from study (n = 142)

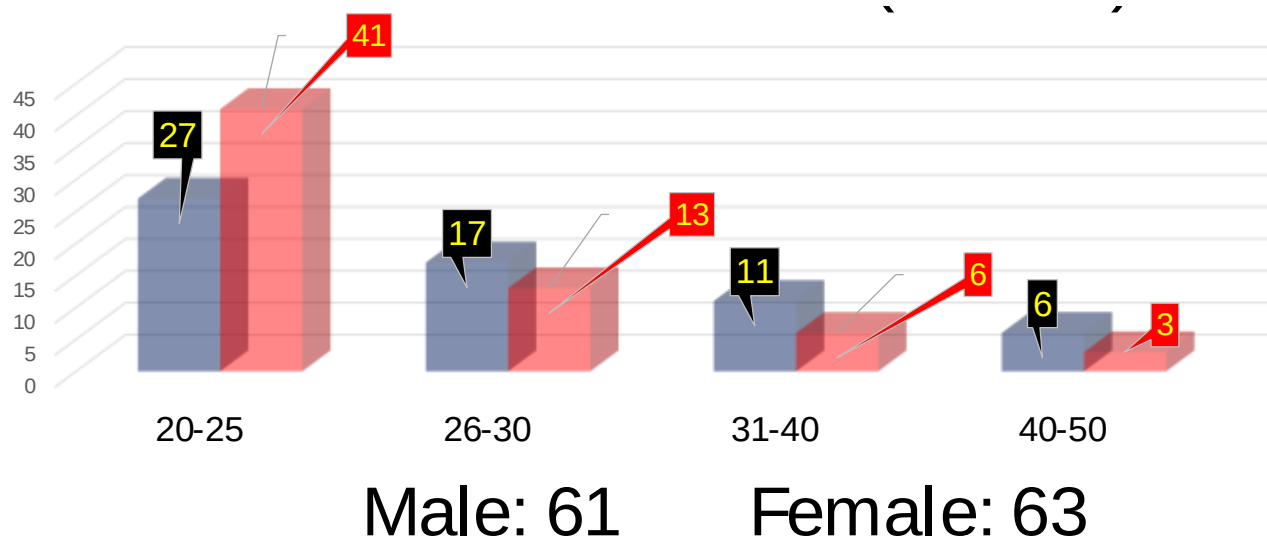


Figure-III: Age and gender distribution of selected volunteers (n= 124).

DISCUSSION

This is the first study in Pakistan in which reference values of rT3 has been determined along with other markers of NTIS and the novelty of present study is measuring of rT3 on an immunoassay system! Carrying out a test on an immunoassay autoanalyzer is like making the test 'public' because of the ease of automation. The instrument we used for analysis of these hormones is the state-of-the-art random access fully automated CLIA-based system (Maglumi X8). CLIA is the ultimate development of commonly used Enzyme-Linked Immunosorbent Assay (ELISA), that required immense time and manpower resources with many potential sources of errors [19]. The data related to the reference values of rT3 established on a CLIA system is extremely scanty. The reference values of all four hormones have been compared with reference

values determined on another chemiluminescence autoanalyzer i.e. Architect (Abbott) [17]. Values of TSH of two systems are closest followed by ft3, rT3 and ft4 (Table-III). Similarly, reference values reported by the manufacturers (Snibe-China) (18) are comparable to those reported in the present study (Table-III) except for the discrepancy in the upper values of rT3, probably because of the wide age range used by the manufacturer [18].

rT3 is a unique thyroid hormone which is secreted in an extra-thyroidal site and is used for the exclusion of thyroid diseases [20]. There has been a close contest in the NTIS literature between rT3 and ft3 as diagnostic markers for NTIS, with ft3 mostly winning the contest of diagnostic marker of NTIS. The probable reasons are easy availability and more consistent decrease of ft3 in NTIS, while rT3 and rT3 / ft3 is

increased in most of the cases but may be normal or even decreased in some situations (2,6). With the advent of better analytical method and equipment, however, (e.g. CLIA and Maglumi by Snibe-China), rT3 is gaining popularity. Moreover, in the presence of central hypothyroidism (pituitary or hypothalamic origin), rT3 will be quite helpful to the physicians. Although role of rT3 in NTIS is questionable, Forestier et al (2009) have shown that increase in rT3 is the most plausible mortality predictor out of the all NTIS related hormones in elderly living independently with a mortality prediction of up to 98.5% [21]. Mortality predicted by decreased fT3, fT4 and TSH has also been reported [21].

It is important to note that NTIS has two faces; acute illness and chronic illness, one is good and the other may be bad, depending on the context [5]. More interestingly, 'fasting' in the first week of acute illness generates the sequence of event leading to NTIS i.e. initially increased rT3 and decreased fT3 and subsequently decreased fT4 and decreased TSH. Beneficial effects NTIS as a result of macronutrient deficiency in early acute illness has been proved and some actual clinical benefits of this 'early fasting' has also been reported e.g. improvement in conditions like ICU-acquired weakness, kidney injury and growth hormone secretagogues [5]. It has been implicated that this situation is developed to decrease the metabolic rate of the body and conserve energy [5]. Coming to the chronic phase of NTIS that occurs in prolonged critically ill patients who stay on intensive medical care for weeks or months. In these patients the most prominent feature is decreased TSH in spite of low fT3 and low fT4, flouting the strong negative feedback of thyroid hormone -TSH axis i.e. a two-fold change in T4 may cause a 100-fold change in TSH [22]. Researchers have found answers to this discrepancy in one step higher control center i.e. hypothalamus; a reduction in gene expression of Thyroid Releasing Hormone (TRH) was found in specific areas of hypothalamus. Some researchers have reported raised TSH in NTIS, too, probably due to failure in the physiological down-regulation of the hypothalamus-pituitary-thyroid axis considered as a protective survival response in NTIS [23].

An important question is what are the factors which cause the typical hormonal changes seen in NTIS? From time to time various cytokines have been implicated e.g. interleukin 6 (IL-6) and other related cytokines are elevated in NTIS patients preceding the decrease in serum fT3 and fT4. More recently, an increase in type 2 deiodinase (DIO2) has been found

to be mediated by a cytokine named nuclear factor κ B (NF- κ B) leading to the local increase in the expression of T3 in hypothalamus and suppression of Thyroid Releasing Hormone (TRH) and ensuing of central hypothyroidism. The treatment of NTIS is shrouded with controversy; a near consensus is that increased rT3 and decreased fT3 of early NTIS should not be treated with thyroid replacement as it is a protective response of the body; whereas decreased fT4 and decreased TSH of late illness is a sort of central hypothyroidism and may be treated with T3 [24]. A preprint of this article has been published earlier [25]

CONCLUSION

We have reported reference values of four hormones for use in clinical practice for diagnosing and monitoring of non-thyroidal illness, a very common condition present in critically ill patient. These values are Reverse T3: 13.3 – 19.8 ng/L (0.2 – 0.30 nmol/L), Free T3: 2.65 - 4.15 pg/dL (4.08 - 6.39 pmol/L), Free T4: 10.6 - 16.2 pg/dL (13.6– 20.8 pmol/L)) and TSH: 0.43 - 3.98 μ IU/mL (0.43 – 3.98 mIU/L).

LIMITATION

Major limitation of the study is that no age specific reference values have been determined due to resource constrains.

RECOMMENDATION

In the present study volunteers belonged to all provinces and areas of Pakistan. We will recommend to use these reference values not only in Pakistan but also in surrounding countries and in overseas populations living in other countries. Moreover, facilities for estimation of all these four hormones on a state-of-the-art fully automated ABEI-based chemiluminescence system be made available in hospital laboratories, so that diagnosis and monitoring of NTIS is carried out effectively by the physicians.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Aamir Ijaz: Substantial contribution to study design, acquisition of data, manuscript writing, critical reviews, final approval, responsible for every aspect of the research

Furqan Shahid: Substantial contribution to acquisition of data, critical reviews, final approval, responsible for every aspect of the research

Shamaila Najeeb Paracha: Substantial contribution to analysis and interpretation of data, manuscript writing, critical reviews, final approval, responsible for every aspect of the research

Farzana Habib: Substantial contribution to study design, critical review, manuscript writing, final approval, responsible for every aspect of the research

Muhammad Bilal Shaukat, Sara Najeeb: Substantial contribution to acquisition of data, critical reviews, final approval, responsible for every aspect of the research

REFERENCES

- da Silveira CD, de Vasconcelos FP, Moura EB, da Silveira BT, Amorim FF, Shintaku LS, *et al.* Thyroid function, reverse triiodothyronine, and mortality in critically ill clinical patients. *Indian J Crit Care Med.* 2021; 25(10): 1161–6. DOI: <https://doi.org/10.5005/jp-journals-10071-24001>
- Chopra IJ. Nonthyroidal illness syndrome or euthyroid sick syndrome? *Endocr Pract.* 1996; 2(1): 45–52. DOI: <https://doi.org/10.4158/ep.2.1.45>
- Sabatino L, Vassalle C, Del Seppia C, Iervasi G. Deiodinases and the three types of thyroid hormone deiodination reactions. *Endocrinol Metab (Seoul).* 2021; 36(5): 952–64. DOI: <https://doi.org/10.3803/enm.2021.1198>
- Fliers E, Boelen A. An update on non-thyroidal illness syndrome. *J Endocrinol Invest.* 2021; 44(8): 1597–607. DOI: <https://doi.org/10.1007/s40618-020-01482-4>
- Van den Berghe G. Non-thyroidal illness in the ICU: A syndrome with different faces. *Thyroid.* 2014; 24(10): 1456–65. DOI: <https://doi.org/10.1089/thy.2014.0201>
- Ganesan K, Anastasopoulou C, Wadud K. Euthyroid sick syndrome. In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing; 2024 [updated 2022 Dec 8; cited 2024]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK482219/>
- Cauteruccio M, Vitiello R, Perisano C, Covino M, Sircana G, Piccirillo N, *et al.* Euthyroid sick syndrome in hip fractures: evaluation of postoperative anemia. *Injury.* 2020;51 Suppl 3: S9–12. DOI: <https://doi.org/10.1016/j.injury.2020.07.006>
- El-Nawawy A, Elwafa RAHA, Khalil Abouahmed A, Rasheed RA, Omar OM. Evaluation of non-thyroidal illness syndrome in shock patients admitted to pediatric intensive care unit in a developing country. *Eur J Pediatr.* 2024; 183(2): 769–78. DOI: <https://doi.org/10.1007/s00431-023-05338-w>
- Dodge, Yadolah (ed.), *The Oxford Dictionary of Statistical Terms* (Oxford, 2003; online Edn, Oxford Academic, 31 Oct. 2023). DOI: <https://doi.org/10.1093/oso/9780198509943.001.0001>
- Horowitz G, Jones GRD. Establishment and use of reference intervals. In: Rifai N, Horvath AR, Wittwer CT, editors. *Tietz textbook of clinical chemistry and molecular diagnostics*. 6th ed. Philadelphia (PA): Elsevier; 2018. p. 170–94. DOI: <https://doi.org/10.1016/B978-1-4160-6164-9.00005-6>
- Ichihara K, Boyd JC. IFCC Committee on Reference Intervals and Decision Limits. The IFCC recommendations for determining reference intervals: strengths and limitations. *J Lab Med.* 2009; 33(2): 45–51. DOI: <https://doi.org/10.1515/JLM.2009.016>
- Masca NG, Hensor EM, Cornelius VR, Buffa FM, Marriott HM, Eales JM, *et al.* RIPOSTE: A framework for improving the design and analysis of laboratory-based research. *Elife.* 2015; 4: e05519. DOI: <https://doi.org/10.7554/elife.05519>
- Bjerner J. Age-dependent biochemical quantities: an approach for calculating reference intervals. *Scand J Clin Lab Invest.* 2007;67(7):707–22. DOI: <https://doi.org/10.1080/00365510701342070>
- Linnert K. Non-parametric estimation of reference intervals by simple and bootstrap procedures. *Clin Chem.* 2000; 46(6): 867–9. Available from: DOI: <https://doi.org/10.1093/clinchem/46.6.867>
- Kim T, Choi H, Lee SM. Parametric and non-parametric estimation of reference intervals for routine laboratory tests: An analysis of health check-up data for 260 889 young men in the South Korean military. *BMJ Open.* 2022; 12(7): e062617. DOI: <https://doi.org/10.1136/bmjopen-2022-062617>
- Boyd JC. Defining laboratory reference values and decision limits: Populations, intervals, and interpretations. *Asian J Androl.* 2010; 12(1): 83–90. Available from: <https://pmc.ncbi.nlm.nih.gov/article/S1533-739683/pdf/aja20099a.pdf>
- Adeli K, Ceriotti F, Nieuwesteeg M. Reference information for the clinical laboratory. In: Rifai N, Horvath AR, Wittwer CT, editors. *Tietz textbook of clinical chemistry and molecular diagnostics*. 6th ed. Philadelphia (PA): Elsevier; 2018. p. 1745–89.
- Snibe Diagnostic. MAGLUMI Rev T3, fT3, fT4, TSH (CLIA) [package insert]. Document No. 006 RevT3-IFU-en-EU-V10.3 [cited 2024 Jun 10]. Available at: <https://hoachatxetnghiem.com.vn/en/downloads/snibe-maglumi-rev-t3-clia.pdf>
- Kricka LJ, Park JY. Principles of immunochemical techniques. In: Rifai N, Horvath AR, Wittwer CT, editors. *Tietz textbook of clinical chemistry and molecular diagnostics*. 6th ed. Philadelphia (PA): Elsevier; 2018. p. 361–3. DOI: <https://doi.org/10.1016/B978-1-4160-6164-9.00016-0>
- Luongo C, Dentice M, Salvatore D. Deiodinases and their intricate role in thyroid hormone homeostasis. *Nat Rev Endocrinol.* 2019; 15(8): 479–88. DOI: <https://doi.org/10.1038/s41574-019-0218-2>
- Forestier E, Vinzio S, Sapin R, Schlienger JL, Goichot B. Increased reverse triiodothyronine is associated with shorter survival in independently-living elderly: the Alsanut study. *Eur J Endocrinol.* 2009; 160(2): 207–14. DOI: <https://doi.org/10.1530/eje-08-0519>
- Anwar MS, Ansari U, Ahmed A, Bashir S. Thyroid disorders. In: Ijaz A, editor. *Chemical pathology for the beginners*. Lahore: Azeem Sons; 2018. p. 197–212. Available from: https://www.amazon.com/Chemical-Pathology-Beginners-Aamir-Ijaz/dp/3659575305/detailBullets_feature_div

23. Fliers E, Alkemade A, Wiersinga WM, Swaab DF. Hypothalamic thyroid hormone feedback in health and disease. *Prog Brain Res.* 2006; 153: 189–207. DOI: [https://doi.org/10.1016/S0079-123\(06\)53011-0](https://doi.org/10.1016/S0079-123(06)53011-0)
24. Boelen A, Platvoet-ter Schiphorst MC, Bakker O, Wiersinga WM. The role of cytokines in the lipopolysaccharide-induced sick euthyroid syndrome in mice. *J Endocrinol.* 1995; 146(3): 475–83. DOI: <https://doi.org/10.1677/joe.0.1460475>
25. Ijaz A, Anjum MU, Ahmed F, Paracha SN, Habib F, Shaukat MB, et al. Establishment of reference values of markers of non-thyroidal illness syndrome on a state-of-the-art fully automated ABEI-based chemiluminescence system. Preprint. medRxiv. 2024. DOI: <https://doi.org/10.1101/2024.06.26.24309539>