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Method of establishing reference intervals of thyroid profile parameters

Metoda wyznaczania przedziałów referencyjnych parametrów profilu tarczycowego

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Słowa kluczowe

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None

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Summary

Introduction. In recent years, attention has been paid to the fact that the reference intervals applied in laboratories do not meet endocrinologists' expectations. This problem is of great importance with regard to thyroid profile parameters. According to some endocrinologists, the currently used reference ranges for these parameters have been defined for too wide groups.

Aim. The purpose of this study is the attempt to determine internal reference intervals of the basic thyroid profile parameters: TSH, fT4 and fT3 taking into account the specificity patients' population.

Material and methods. The analysis covers test results of TSH, fT4 and fT3 levels in serum collected by the Central Clinical Laboratory of the Laboratory Medicine Center of the University Clinical Center in Gdansk (UCML UCK). Only tests results collected in the period from 8 October 2010 to 26 October 2011 were taken into account. Moreover, only results of patients aged over 16 years with no additional population limits were considered relevant.

Results. The initial amount of data reached 67 430 registered test observations. After the application of all exclusion criteria, there were 16 480 test observations for 9726 patients in total to be considered in the analysis. The group under research included 6619 (68.0%) female patients and 3107 (32.0%) male patients. There were 2134 (32.2%) women under the age of 40 and 1008 (22.4%) men under the age of 40. TSH level in serum was estimated in 8252 individuals. Within this group, there were 5547 women (32.83% under the age of 40 and 67.17% aged over 40 years) and 2705 men (23.51% under the age of 40 and 76.49% aged over 40 years). FT4 level estimation was performed in 4916 patients: in 3565 women (35.57% under the age of 40 and 64.43% aged over 40 years) and 1351 men (21.76% under the age of 40 and 78.24% aged over 40 years). FT3 level was estimated in 3312 individuals: 2337 women (27.86% under the age of 40 and 72.14% aged over 40 years) and 975 men (20% under the age of 40 and 80% aged over 40 years).

Conclusions. Upper and poured value of referential scope for TSH for the full reference (without distinguishing to the sex and the age) took out appropriately 3.78 and 0.43 μIU/ml. Range referential suggested by the producer of reagent in sets used in the Central clinical laboratory setting of the profile of the thyroid gland are hesitating from 0.35 to 4.94 μIU/ml. The TSH range of reference in serum is lower than final values suggested by the producer of the set of reagents.

Based on test results of the entire reference group, amounted to the bottom and upper scope of free ratable values of the faction of the thyroid gland 11.13 and 15.87 pmol/L (fT4), as well as 2.92 and 5.41 pmol/L (fT3). Referential scopes suggested by the producer took out from 9.01 to 19.05 pmol/L (fT4) and from 2.63 to 5.70 pmol/L (fT3) in serum.

Streszczenie

Wstęp. W ostatnich latach coraz częściej zwraca się uwagę na to, że stosowane w laboratoriach przedziały referencyjne nie spełniają oczekiwań lekarzy endokrynologów. Pro-

blem ten ma duże znaczenie w odniesieniu do parametrów profilu tarczycowego. Zdaniem niektórych specjalistów z zakresu endokrynologii, wykorzystywane obecnie przedziały referencyjne dla tych parametrów zostały określone dla zbyt szerokich grup.

Cel pracy. Celem niniejszego badania była próba wyznaczenia wewnętrznych przedziałów referencyjnych, uwzględniających specyfikę populacji pacjentów i podstawowych parametrów profilu tarczycowego: TSH, fT4 i fT3.

Materiał i metody. Analizą objęto wyniki oznaczeń stężeń TSH, fT4 i fT3 w surowicy wykonanych w Centralnym Laboratorium Klinicznym Uniwersyteckiego Centrum Medycyny Laboratoryjnej Uniwersyteckiego Centrum Klinicznego (UCML UCK) w Gdańsku, w przedziale czasu od 8 października 2010 roku do 26 października 2011 roku. Analizowano wyniki badań osób w wieku powyżej 16 lat, bez dodatkowych ograniczeń populacyjnych.

Wyniki. Wejściowa baza danych liczyła 67 430 zarejestrowanych obserwacji pomiarowych. Po zastosowaniu wszystkich kryteriów wykluczenia do analiz zakwalifikowano łącznie 16 480 obserwacji pomiarowych dla 9726 pacjentów. Grupa badanych obejmowała 6619 (68,0%) kobiet i 3107 (32,0%) mężczyzn. Poniżej 40. r.ż. było 2134 (32,2%) kobiet i 1008 (22,4%) mężczyzn. U 8252 osób oznaczono stężenie TSH w surowicy. Wśród nich 5547 osób stanowiły kobiety (32,83% poniżej i 67,17% powyżej 40. r.ż.), a 2705 mężczyźni (23,51% poniżej i 76,49% powyżej 40. r.ż.). Oznaczenie stężenia fT4 wykonano u 4916 osób: 3565 kobiet (35,57% poniżej i 64,43% powyżej 40. r.ż.) i 1351 mężczyzn (21,76% poniżej i 78,24% powyżej 40. r.ż.). Oznaczenie stężenia fT3 wykonano u 3312 osób: 2337 kobiet (27,86% poniżej i 72,14% powyżej 40. r.ż.) i 975 mężczyzn (20% poniżej i 80% powyżej 40. r.ż.).

Wnioski. Górna i dolna wartość zakresu referencyjnego dla TSH dla pełnego odniesienia (bez rozróżnienia na płeć i wiek) wyniosła odpowiednio 3,78 i 0,43 μIU/ml. Zakres referencyjny sugerowany przez producenta odczynnika w zestawach używane w Centralnym Laboratorium Klinicznym profilu tarczycy wahają się od 0,35 do 4,94 μIU/ml. Zakres odniesienia TSH w surowicy jest niższy niż wartości końcowe sugerowane przez producenta zestawu odczynników.

Na podstawie wyników testu całej grupy odniesienia, dolny i górny zakres wartości referencyjnych swobodnych frakcji tarczycy wyniosły 11,13 i 15,87 pmol/L (fT4), a także 2,92 i 5,41 pmol/L (fT3). Referencyjne zakresy sugerowane przez producenta wyniosły od 9,01 do 19,05 pmol/L (fT4) i od 2,63 do 5,70 pmol/L (fT3) w surowicy.

INTRODUCTION

In recent years, attention has been paid to the fact that the reference intervals applied in laboratories do not meet endocrinologists' expectations. This problem is of great importance with regard to thyroid profile parameters. According to some endocrinologists, the currently used reference ranges for these parameters have been defined for too wide groups.

For example, the reference groups involved in the process of determining reference ranges included patients with subclinical hypothyroidism (1-4). This subclinical dysfunction is characterized by an elevated level of thyroid stimulating hormone (TSH) in serum as well as by free thyroxine (fT4) and free triiodothyronine (fT3) levels close to reference range ends. These factors disturb the process of determining reference ranges. Results of population-based studies have confirmed that subclinical hypothyroidism has been observed in 10% of the population (5). Early diagnosed subclinical hypothyroidism becomes more important because this dysfunction is one of the risk factors of the development of hypothyroidism, cardiovascular disorders, lipid disorders, miscarriage, depression, and other pathological conditions (6). Therefore, it has been more and more frequently emphasized that the upper TSH reference range end should be lowered. In a majority of laboratories, the reference range end currently oscillates between 4.0-5.0 μIU/mL (1-4, 7).

For many years, the need to define credible reference systems has been the reason of attempts to define reference ranges of laboratory test results in hospital population. According to the guidelines of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), clinical laboratories ought to verify the reference ranges that are used by these laboratories (8). Such procedure aims at the identification of population-specific deviations of laboratory test results. However, due to the considerably time-consuming and expensive nature of such activities, the establishment of internal reference intervals according to the IFCC guidelines exceeds the capacity of most laboratories. Alternatives are: the application of reference ranges described in literature, using reference ranges recommended by manufacturers of laboratory reagent kits, and "borrowing" reference range values from other laboratories. Nevertheless, such procedure involves a risk of disturbing factors such as the characteristics of the particular target population, equipment factors and methodological factors. The above mentioned conditions justify the need of searching new, more cost-efficient and accessible methods of establishing internal reference ranges.

AIM

The purpose of this study is the attempt to determine internal reference intervals of the basic thyroid profile parameters: TSH, fT4 and fT3 taking into account the specificity patients' population.

The structure of the study involves the model of retrospective analysis of thyroid profile parameter test

results collected in the database of the Central Clinical Laboratory of the University Clinical Center in Gdansk.

MATERIAL AND METHODS

The analysis covers test results of TSH, fT4 and fT3 levels in serum collected by the Central Clinical Laboratory of the Laboratory Medicine Center of the University Clinical Center in Gdansk (UCML UCK). Only tests results collected in the period from 8 October 2010 to 26 October 2011 were taken into account. Moreover, only results of patients aged over 16 years with no additional population limits were considered relevant.

A series of exclusion criteria was applied to filter the information from the database. The first exclusion criterion was a missing PESEL (i.e. the unique 11-digit personal identification number of Polish citizens). PESEL enables clear identification of patients and provides date of birth and gender required for the purpose of the analysis.

Another exclusion criterion was that the pre-analytical phase of the diagnostic process (involving i.e. preparation of individuals before collecting the sample, sample collection, transportation and storage of the biological material) was performed exclusively in the University Clinical Center (UCK) in order to ensure compliance of procedures.

Test results of patients of UCK departments and clinics with specializations posing risk of falsifying the established reference ranges (endocrinology and maternity clinics and medical practices, as well as intensive care units) were also rejected. The remaining exclusion criteria were as follows: patients participated in the multi-center public health research (9), level values were beyond the valid Central Clinical Laboratory (10), the same parameter was tested twice or more frequently within the analyzed period, and, simultaneously, there was a positive test result for thyroglobulin, thyroid peroxidase and thyrotropin hormone receptor antibodies.

Ethics

The study protocol has been approved by the Local Bioethics Committee of the Medical University of Gdansk. Due to the character of the analyzed data (results of routine laboratory tests collected due to various reasons) and the aggregate, retrospective character of the analysis, individual patient's consent was not required.

Laboratory estimation methods

In the Central Clinical Laboratory, the CMIA method (Abbott Laboratories Poland) was applied for quantitative determination of TSH, fT4 and fT3 in serum. The reference ranges recommended by the reagent kit producer for the specific parameters, obtained by means of AxSYM Ultrasensitive hTSH II and AxSYM Free T4 estimation results in healthy individuals are the following: 0.35-4.94 μIU/mL for TSH (99% confidence interval, a group of 549 patients), 9.01-19.05 pmol/L for fT4 (99% confidence interval, a group of 411individuals) and 2.63-5.7 pmol/L for fT3 (95% confidence interval, a group of 436 patients) (10).

Statistical analysis

Distribution normality of the results of the analyzed parameters was tested by means of the Cramer-von Mises normality test. Descriptive statistics such as mean and median were calculated for the groups under research. A non-parametric method was applied to establish the reference ranges of all the analyzed parameters. The Q 2.5 and Q 97.5 quantile values were adopted as the upper and the low end respectively. The data was calculated by means of Statistica 10.0 and the R 2.15.0 statistical computing package.

RESULTS

The initial amount of data reached 67 430 registered test observations. After the application of all exclusion criteria, there were 16 480 test observations for 9726 patients in total to be considered in the analysis. The group under research included 6619 (68.0%) female patients and 3107 (32.0%) male patients. There were 2134 (32.2%) women under the age of 40 and 1008 (22.4%) men under the age of 40. TSH level in serum was estimated in 8252 individuals. Within this group, there were 5547 women (32.83% under the age of 40 and 67.17% aged over 40 years) and 2705 men (23.51% under the age of 40 and 76.49% aged over 40 years). FT4 level estimation was performed in 4916 patients: in 3565 women (35.57% under the age of 40 and 64.43% aged over 40 years) and 1351 men (21.76% under the age of 40 and 78.24% aged over 40 years). FT3 level was estimated in 3312 individuals: 2337 women (27.86% under the age of 40 and 72.14% aged over 40 years) and 975 men (20% under the age of 40 and 80% aged over 40 years).

It was tested if the distributions of the TSH, fT4, fT3 and log(TSH) variables follow normality distribution. The hypothesis regarding normality of these distributions was rejected (Cramer-von Mises normality test; $p < 0.001$). Therefore, the non-parametric method was adopted to establish reference ranges. The TSH, fT4 and fT3 level reference ranges in question are presented in the tables 1-3 with regard to the whole group and to the specific age- and gender subgroups.

Tab. 1. Descriptive statistics of the concentration of thyrotropin hormone (μIU/ml) in the examined group with the whole and with taking into account of the sex and the age

Group	N		Mean	Median	2.5th centile	97.5th centile
Total	8	252	1.48	1.28	0.43	3.78
Gender						
female	5	547	1.50	1.30	0.43	3.83
male	2	705	1.43	1.23	0.43	3.51
Age (years)						
≤ 40	2	457	1.58	1.39	0.48	3.73
> 40	5	795	1.44	1.22	0.41	3.78

Tab. 2. Descriptive statistics of the concentration of the thyroxin (pmol/l) in examined for group with the whole and including the sex and the age

Group	N		Mean	Median	2.5th centile	97.5th centile
Total	4	916	14.73	14.66	11.13	18.57
Gender						
female	3	565	14.83	14.75	11.29	18.63
male	1	351	14.47	14.39	10.74	18.35
Age (years)						
≤ 40	1	562	14.50	14.42	11.21	18.41
> 40	3	354	14.84	14.78	11.13	18.62

Tab. 3. Descriptive statistics of the concentration triiodothyronine (pmol/l) in the examined group with the whole and including the sex and the age

Group	N		Mean	Median	2.5th centile	97.5th centile
Total	3	312	4.25	4.28	2.92	5.41
Gender						
female	2	337	4.26	4.28	2.95	5.40
male	975		4.23	4.27	2.87	5.43
Age (years)						
≤ 40	846		4.48	4.49	3.27	5.52
> 40	2	466	4.17	4.20	2.86	5.34

DISCUSSION

For the purpose of this research, we applied the model of retrospective analysis in order to define internal reference systems concerning the basic parameters of the thyroid profile for a central clinical laboratory of a large university center. In order for the analysis to be more homogenous, we used a series of exclusion criteria to select the members of the reference group. These criteria referred to both clinical and practical aspects.

Above all, we assumed that patients of some of the Central Clinical Laboratory clinics may be characterized by extreme values of the thyroid profile parameters. In the selection process of reference patients, we applied the health concept dedicated to establishing reference values. The rejection of patients of some clinics and departments has been the element of health definition formulated to establish internal laboratory reference ranges (as in the notion of relative health, suffering from one disease does not exclude being healthy in other aspects). Therefore, as indicated in the IFCC guidelines (8), we excluded test results of patients of endocrinology and maternity clinics and medical practices, as well as intensive care units. In the first case, the aim was to exclude cases of thyroid dysfunction and other endocrinological disorders that might influence the level of TSH, fT4 and fT3. Due to similar reasons, results of patients with identified antithyroid antibodies in the thyroid profile were excluded from the research. The presence of antithyroid antibodies in patient's serum may indicate the occurrence of autoimmunological thyroid disorders (11). According to the recommendations of the National Academy of Clinical Biochemistry (NACB), such individuals should be excluded from reference groups (12). While some patients were not involved in the research due to antithyroid antibodies, patients from intensive care units were excluded on different grounds. We assumed that basic disorders that had been the reasons of hospitalization might result in significant homeostasis impairment, and thyroid hormones are important homeostasis regulators (11). Next, the exclusion of maternity clinics' patients was justified by the NACB guidelines. According to them, in the case of pregnant women other TSH, fT4 and fT3 reference values should be considered depending on the trimester (12). In the case of most researchers, due to frequent fluctuation of TSH levels and thyroid hormones in pregnant women, such female patients are not involved in the research aimed at defining reference ranges (3, 13). Patients whose particular thyroid profile parameters were tested more than once within a short period were also excluded from the research. Among the potential reasons of such a repeat test are: differential diagnostics of thyroid – and other endocrinological disorders, monitoring of the results of endocrinological therapy, and the parameter level results beyond the official reference range (14). It is expected that a group of patients with a repeat thyroid profile test involves a significantly higher risk of thyreopathy. Estimations test results were also excluded when they were beyond the reference ranges suggested by the producer of the laboratory equipment applied in the research center (10). This decision is related with the fact that the current official reference ranges of the thyroid profile are questioned by endocrinologists. Many of these specialists claim that the current reference ranges were established incorrectly because the reference groups included patients with undiagnosed chronic lymphocytic thyroiditis (1-4).

There were not only clinical conditions but also practical aspects considered in the process of defining the reference group. The aim of the analysis was to establish internal reference ranges for the Central Clinical Laboratory. Therefore, test results of patients redirected from other centers were excluded as well. This exclusion also concerns participants of multicenter clinical research (9). Such procedure enabled the definition of hospital population that became the basis for the establishment of reference ranges. Reference ranges based on the specific hospital population build the most adequate reference system for results of patients from this population. Moreover, owing to this procedure it was possible to avoid discrepancies in the pre-analytical phase. Establishing the reference ranges, we followed the IFCC guidelines (8). According to them, in cases of distributions that do not follow the normality pattern, the non-parametric method should be applied to establish reference ranges. As far as TSH is concerned, upper and low reference range ends were established for the complete reference group (with no gender and age distinction) and amounted to 3.78 and 0.43 μIU/mL, respectively. The reference range suggested by the producer of the reagent kits used in the Central Clinical Laboratory

to test thyroid profile parameters ranges from 0.35 to 4.94 μIU/mL (10). Based on the results of this study, the upper TSH reference range end in serum is lower than the end values suggested by the reagent kit producer. This may influence the frequency of diagnosis of subclinical hypothyroidism (15, 16). Based on the test results of the complete reference group, low and upper reference range ends of free fractions of thyroid hormones amounted to 11.13 and 15.87 pmol/L (fT4) as well as 2.92 and 5.41 pmol/L (fT3). The reference ranges suggested by the producer of the laboratory equipment range from 9.01 to 19.05 pmol/L (fT4) and from 2.63 to 5.70 pmol/L (fT3) in serum. No information questioning current fT4 and fT3 level reference ranges has been found in the available literature. The thyroid profile reference ranges resulting from this research take into account the specificity of patients' population. Owing to this fact, the reference ranges in question may facilitate differential diagnostics of thyroid disorders. However, despite the simplicity and cost-efficiency of the methodology of establishing reference ranges that was applied in this study, the potential limitations of this method should be considered as well. The most important limitation is the lack of possibility to relate laboratory test results to the real endocrinological condition of the patient. As a consequence, it is impossible to exclude potential patients with clinical pathologies of the thyroid gland or other disorders significantly impairing thyroid profile parameters from the research group. Certainly, although the risk of occurrence of this negative disturbing factor was minimized by strict exclusion criteria and large size of the research group, the new suggested reference range values need to be further verified under clinical conditions by means of the ROC analysis.

CONCLUSIONS

Upper and poured value of referential scope for TSH for the full reference (without distinguishing to the sex and the age) took out appropriately 3.78 and 0.43 μIU/ml. Range referential suggested by the producer of reagent in sets used in the Central clinical laboratory setting of the profile of the thyroid gland are hesitating from 0.35 to 4.94 μIU/ml. The TSH range of reference in serum is lower than final values suggested by the producer of the set of reagents.

Based on test results of the entire reference group, amounted to the bottom and upper scope of free ratable values of the faction of the thyroid gland 11.13 and 15.87 pmol/L (fT4), as well as 2.92 and 5.41 pmol/L (fT3). Referential scopes suggested by the producer took out from 9.01 to 19.05 pmol/L (fT4) and from 2.63 to 5.70 pmol/L (fT3) in serum.

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