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ORIGINAL ARTICLE

First Referrals for Testing on Thyroid Disorders and the Influence of Age and Gender on Positive Test Results

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SUMMARY

Background: **Thyroid diseases can occur at different ages, and the type of disorder is somewhat age and gender related. The most reliable method of diagnosis is the measurement of free hormones in the blood. The aim of this study was to determine the number of positive findings in relation to the number of referrals and to consider the ways and methods for possible rationalization of referrals.**
Methods: **This study was carried out using a retrospective data analysis of the register of the emergency biochemical laboratory of the General County Hospital Vinkovci, Eastern Croatia. Over a six-month period in 2017, data for patients who were referred for the first time to the laboratory with the suspicion of thyroid disease was analysed. The number of referrals was compared to the number of positive test results. The positive test results were specified by the type of thyroid disorder and classified by age and gender.**
Results: **The study included 1,128 patients from the area of Eastern Croatia, including 306 (27.13%) males and 822 (72.87%) females. There were 234 (20.74%) positive findings of the total number of requests and most of them, 182 (78%), were for women. With respect to age, the highest number of positive findings (21.30%) was found in the group 41 - 60 years of age. Latent hypothyroidism was the most prevalent disease and patients with this diagnosis were mostly in the age group of over 50 years (59.15%).**
Conclusions: **There was a disproportionately larger number of referrals in comparison to the number of positive findings. Better education of medical experts and monitoring of the findings in the laboratory through the data registers are the strategies to lower the number of referrals and to improve the medical doctors` capabilities to discern between different thyroid disorders.**
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INTRODUCTION

The prevalence of newly diagnosed thyroid dysfunction has decreased in the last several years in the developed world, 1 - 3% of the population has hypothyroidism in iodine-replete areas, with a higher prevalence in elderly

people [1,2]. According to previous studies, the physiology of thyroid hormone regulation has been reviewed extensively. It is also known that regulation of thyroid hormone secretion is conducted from within the central nervous system, hypothalamus, and hypophysis, which allow modulation of the system from the periphery, via feedback from nutrient intake or via the autonomic nervous system [3,4]. Most of the secreted hormone is thyroxine (T4), while triiodothyronine (T3) is mostly generated in peripheral tissues by the process of deiodination from T4 under the action of the enzyme 5`-deiodinase [5].

Thyroid disease occurs independent of age and gender, but the type of disorder may be related to age and gender. The incidence and prevalence of both, hyperthyroidism (increased thyroid gland activity and hormones synthesis) and hypothyroidism (decreased thyroid gland activity and hormones synthesis) were found to increase with age, being more prevalent in women than in men. In younger people, it is more often that thyroid disease is caused by an immune mechanism, such as Hashimoto's thyroiditis or Basedow's disease. In elderly people, the common cause of thyroid disease is deregulation of the neuroendocrine link from the pituitary gland to the peripheral tissues [5]. The most common thyroid disorder is subclinical hypothyroidism, where the central neuroendocrine axis is not damaged, but there is resistance of peripheral tissues to hormone action. Measuring the level of free hormones is the most reliable diagnosis method since the concentrations of FT3 and FT4 are independent of the carrier protein concentration. Thyroid-stimulating hormone (TSH) concentration is the most sensitive marker of thyroid function, and still presents an initial test for suspected thyroid disease.

A negative feedback mechanism that exist between the pituitary hormone thyrotropin (TSH) and T4, keep the secretion of thyroid hormones within a normal range. The production of TSH, in turn, is regulated by the hypothalamic thyrotropin releasing hormone (TRH).

Primary hypothyroidism is the most common, while secondary and tertiary hypothyroidism are less common [6]. The main marker of subclinical hypothyroidism is slightly elevated values of TSH with proper concentration of T3 and T4 hormones. Hyperthyroidism is characterized by increased production of thyroid hormones T3 and T4. Non-thyroid disease or euthyroid syndrome usually occurs in patients who suffer from other illnesses and have no primary thyroid disorder. Proper concentration of FT3 and FT4 hormone values do not exclude thyroid dysfunction, while the normal FT3 and FT4 concentrations are most common in subclinical disease within the reference range [5]. In primary hypothyroidism, laboratory findings include low T4 and FT4 hormone concentrations with elevated TSH. In contrast to primary, secondary hypothyroidism laboratory findings usually include low concentrations of T4 and FT4, with normal or decreased TSH. In observing the effects of therapy in secondary hypothyroidism, TSH is not an appropriate marker as FT4, which should be in the middle of the reference range. In relation to hypothyroidism, hyperthyroidism is easier to diagnose. The concentration of T3 and T4 is increased, and TSH is suppressed. In the initial phase of Graves' disease, T3 is greater than T4. Despite better awareness, there is still a large proportion of the European population which unknowingly has laboratory evidence of thyroid dysfunction [7]. Overt thyroid diseases, both hyperthyroidism and hypothyroidism, are common public health concerns and associated with the diverse clinical manifestations. Severe thyroid disease, either due to increased or decreased thyroid gland function, may be life-threatening if not treated but is fortunately scarce.

The aim of this study is to determine the proportion of patients with positive findings among those referred to laboratory for the first time for testing on thyroid hormones and to determine the age- and gender-related distributions of particular patterns of thyroid diseases. The results are expected to inform PC providers regarding target groups for referrals for testing of thyroid hormones. The final aim is rationalization of referrals and the decrease of laboratory costs.

MATERIALS AND METHODS

Patients

This study was carried out by a retrospective data analysis of the register of the emergency biochemical laboratory of the General County Hospital Vinkovci, Eastern Croatia, an administrative centre (35,000 inhabitants) of a county in Eastern Croatia. Laboratory findings of thyroid hormones testing were considered for patients who had been referred to the Biochemical Laboratory for the first time with the suspicion of thyroid gland dysfunction. Information was not available for symptoms or comorbidity patterns, for which patients had been referred. Participants did not undergo additional studies for the purposes of this study, and according to the GDPR, personal data were protected on all levels. As we have used only data from the register with no personal data, informed consent was not required.

The data used were for the number of requests and the number of positive findings per months, for the first part (January - June) of the year 2017. Positive findings were classified as the thyroid dysfunction entities: hyperthyroidism, hypothyroidism, subclinical hypothyroidism, non-thyroidal illness syndrome, and other, non-specified thyroid dysfunctions. The distributions per age and gender were analyzed.

Analysis

Thyroid hormones were determined using the direct chemiluminescence method, that is widely accepted as a new standard method. ADVIA Centaur FT3 ReadyPack primary reagent and ADVIA Centaur FT4 ReadyPack primary reagent were used for determining the free T3 and free T4 level. ADVIA Centaur TSH-3 ReadyPack primary reagent was used to determine TSH.

Table 1. Number of requests in percentage related to the month.

Number of requests (%)							p *
n	January	February	March	April	May	June	0.073
1,128	190 (16.84)	195 (17.29)	232 (20.56)	159 (14.10)	195 (17.29)	157 (13.92)	

* χ^2 test.

Table 2. Number of requests related to age and gender.

Number of requests (%)						p *
Age group	0 - 20	21 - 40	41 - 60	60+	Total	0.087
Male	38 (23.17)	81 (25.15)	110 (27.29)	77 (33.47)	306 (27.13)	
Female	126 (76.83)	241 (74.85)	293 (72.71)	153 (66.53)	822 (72.87)	
Total	164 (100)	322 (100)	403 (100)	230 (100)	1,128 (100)	

* χ^2 test.

Table 3. Number of positive findings related to gender.

	Number of requests	Number of positive findings	p *
Male	306	52	< 0.001
Female	822	182	
Total	1,128	234	

* χ^2 test.

Table 4. Number of positive requests related to gender and specific thyroid disease.

Number of requests (%)							p *
	Latent hypothyroidism	Non-thyroid disease	Hypothyroidism	Hyperthyroidism	Other	Total	0.842
Male	14 (27)	3 (6)	8 (15)	10 (19)	17 (33)	52	
Female	57 (31.3)	16 (8.8)	26 (14.2)	26 (14.2)	57 (31.3)	182	
Total	71 (30)	19 (8)	34 (15)	36 (15)	74 (32)	234	

* χ^2 test.

Table 5. Comparison of different diseases by age group.

Number of requests (%)			p *
	Subclinical hypothyroidism	Non-thyroid disease	< 0.001
0 - 50	29 (40.85)	4 (21.05)	
50+	42 (59.15)	15 (78.95)	
Total	71 (100.00)	19 (100.00)	

* χ^2 test.

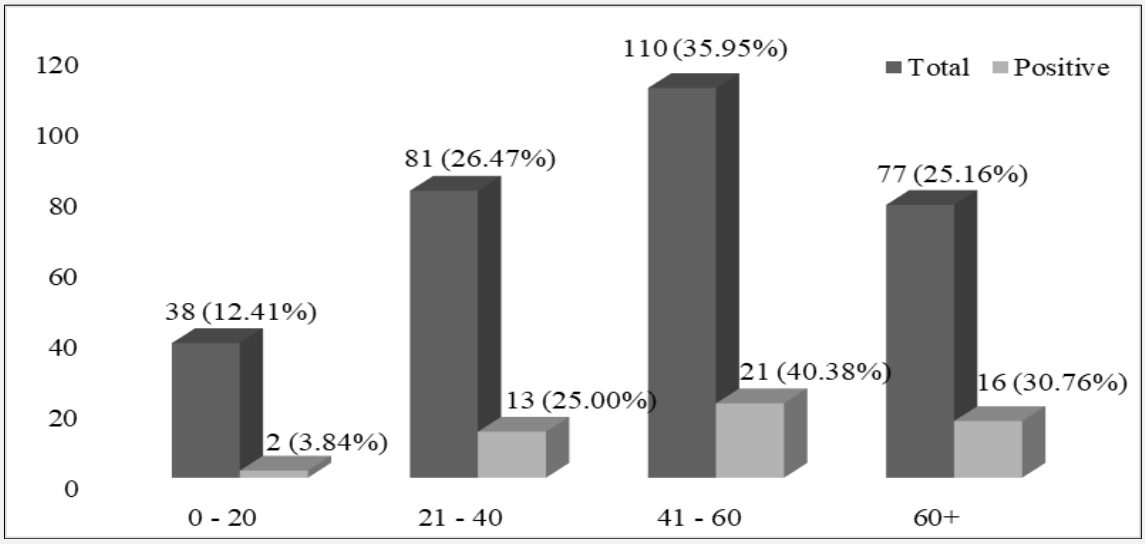

Figure 1. The ratio of positive and negative findings of male patients related to age.

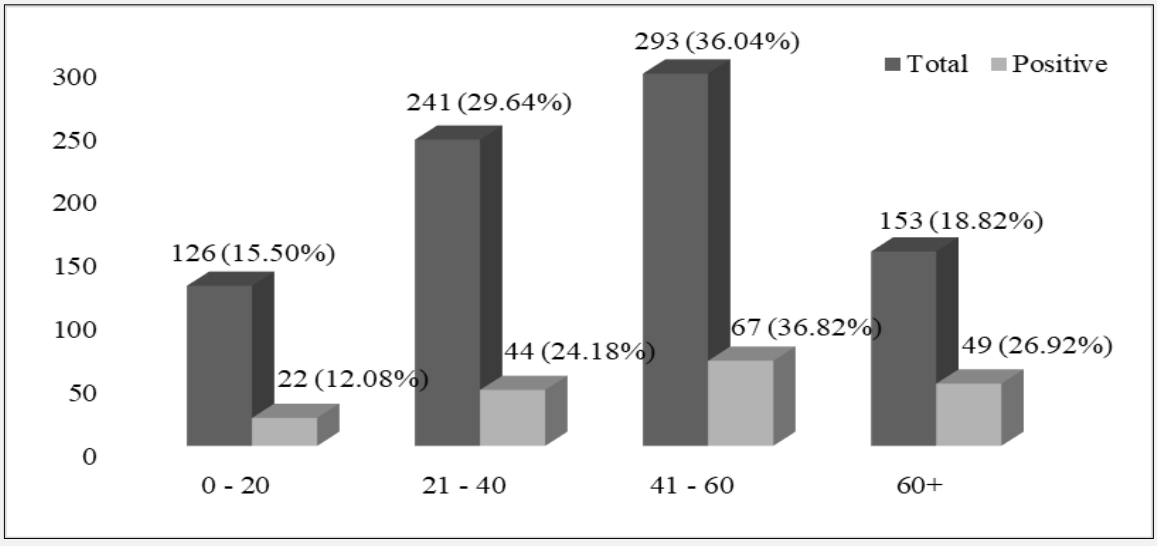

Figure 2. The ratio of positive and negative findings of female patients related to age.

Statistical analysis

The results of this study were presented in tables and graphics as ratios and absolute numbers. The χ^2 test was used to examine the difference between categorical variables. All p-values are two-sided. The significance of differences determined by statistical testing was expressed at the level $p < 0.05$.

The statistical software package Statistica for Windows 2010 (version 10.0, StatSoft Inc., Tulsa, OK, USA) was used for data analysis.

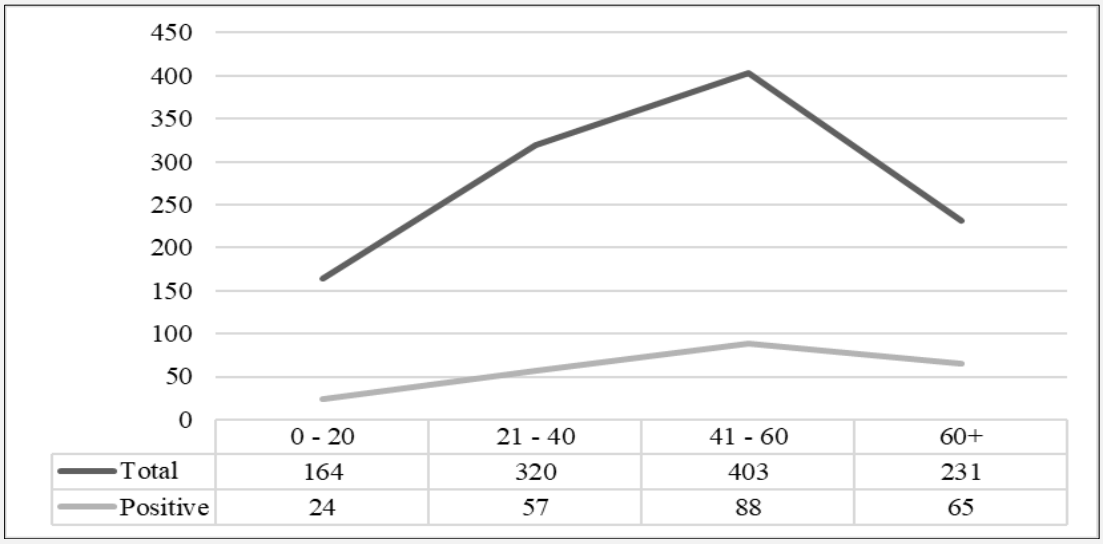

Figure 3. The ratio of requests and positive findings related to age and gender.

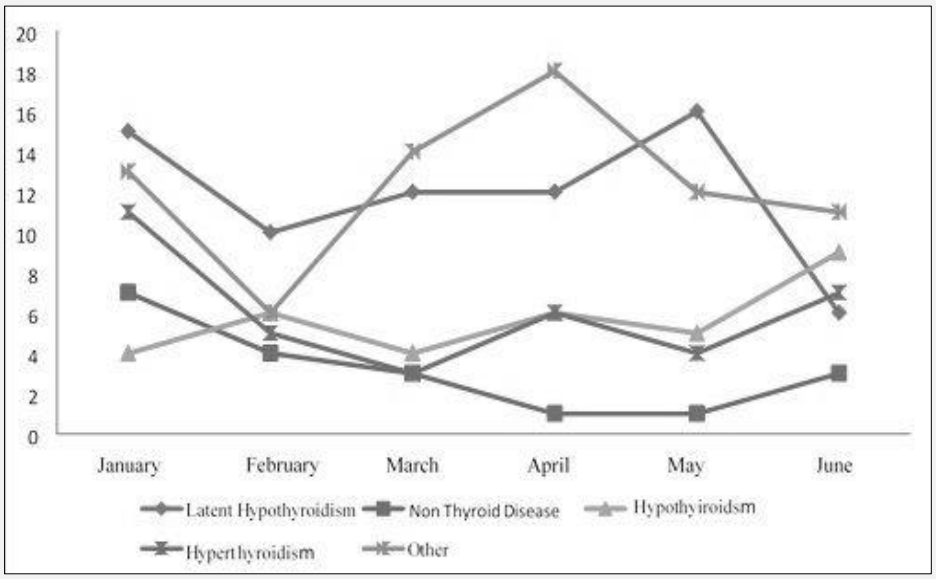

Figure 4. Number of positive findings per month in relation to the type of disease.

RESULTS

The study was conducted on 1,128 patients over a period of six months (from January to June of 2016). Of the total number of participants, 306 were male (27.13%) and 822 were female (72.87%). Most requests were submitted in March (20.56%) and the least in June (13.92%), but there is no significant difference observed in months and number of request (χ^2; p = 0.073) (Table 1).

Most requests were submitted for women for ages 41 - 60. Obtained results did not show a significant statistical difference in the number of requests and gender ($p = 0.087$) (Table 2).

There were 234 positive findings (20.74%) of the total number of requests. Most of them were women ($n = 182$). Obtained results showed a significant statistical difference in the number positive findings and female gender ($p < 0.001$) (Table 3).

Furthermore, given the number of positive findings, patients were classified according to gender and specific thyroid disease. No statistically significant difference was found ($p = 0.842$) (Table 4).

In male gender, most of the requests and positive findings were for ages 41 - 60. For age 20 or less, there were only 3.84% positive findings (Figure 1).

There are 18.25% positive findings in women of age 21 - 40. Most positive findings were described in women of age 60+ (32.02%). Obtained results did not show a significant statistical difference in the number of positive findings and female gender (Figure 2).

Concerning age, most positive requests were for ages 41 - 60 in both genders. According to them, most of the positive findings were described in the same age. Obtained results showed a significant statistical difference in the number of positive findings and age of 41 - 60 (χ^2; $p = 0.035$) (Figure 3).

Figure 4 shows the number of positive findings sorted by disease type by month. The least number of patients suffer from non-thyroid disease. No statistically significant difference was found χ^2; $p = 0.287$.

Elderly people often suffer from non-thyroid disease more than others (Table 5).

DISCUSSION

There is a lack of strong evidence which promotes screening for thyroid dysfunctions in asymptomatic adults, and the current data suggest the need for more comprehensive guidelines for the recommendations in laboratory testing of thyroid function that can help identify patients who may be predisposed to developing thyroid dysfunction. Pathophysiology of thyroid disorder presents a big challenge for family medicine practice, who are the first in identifying those patients. Many people who are seriously ill have abnormal thyroid test results but no other evidence of thyroid dysfunction. Patients with comorbidity or previous autoimmune disease require more complex clinical management. As it has been previously said, TSH presents a sensitive marker of the thyroid status, but sometimes, when an abnormal serum TSH value is obtained, the usual next step is to repeat the measurement of TSH and also measure a serum free T4. The study was conducted for five groups of disease: subclinical hypothyroidism, non-thyroid disease, hypothyroidism, hyperthyroidism, and other diseases related to thyroid dysfunction. It is important to emphasize, that all data is collected from biochemical emergency laboratory, with no previous knowledge of patients medical history. According to the group of diseases associated with thyroid dysfunction, this implies findings which present a challenge for the family medicine doctor in properly treatening, for example findings that are close to the limit of the benchmarks. The study was conducted on 1,128 patients, 822 were female. Most requests were submitted in March and the least in June. These results confirm previous studies, that various forms of thyroid dysfunction are more common in women [8,9]. According to the number of requests, most of them came from patients aged 41 - 60, and the lowest number of requests was for patients age 20 or less. Most of the positive findings were for ages 41 - 60. Based on given results, most of the positive findings were associated with latent hypothyroidism, significantly more in women, but results also suggest that subclinical hypothyroidism was found more often in males compared to other thyroid disorders. Hyperthyroidism presents serious cardio-metabolic issues, that indicate proper treatment of these conditions, universally suggested by international guidelines. Hyperthyroidism, defined by decreased levels of TSH and the presence of elevated free T3 and free T4, is common in the general population and progressively increases with age. This fact is in correlation to a study by Delitala, who investigated the prevalence of hyperthyroidism and proved that 15.40% of participants, aged 75 and more, suffer from hyperthyroidism [10]. Overall, focusing at the months included in the study, the most positive findings were in January. These findings are expected, because exposure to cold weather can affect the thyroid gland. An association of thyroid disorder and cold exposure was confirmed in research by Mustafa S. et al., who investigated the effects of chronic cold exposure on thyroid gland function in rabbits using radionuclide tracer and thyroid hormones secretion concentration. They concluded that the decrease in rabbit body weights may be related to the increase in metabolism due to the increase of thyroid hormones [11]. Therefore, there is a lack of studies focused on the type of thyroid disorder and exposure to cold or warm. In future research more attention should be focused on the correlation between weather conditions and the occurrence of thyroid disease (colder or warmer weather, as an indicator of increased metabolism requirements which could act as an additional helping factor for the onset of thyroid dysfunction). For better interpretation of the results, one more division was made, exclusively for two diseases. Latent hypothyroidism was the most prevalent disease (41.50%) for ages over 50 and least prevalent above the age of 65. One of the ideas before starting this reseach was that the incidence of non-thyroid disease in the elderly is higher, which was proven correct. A cross section was made for only two age groups: 0 - 50 and 50+, and results showed that in those patients in age 0 - 50, there are only 21.05% who suffered from suspected non-thyroid disease. On the other hand, there are 78.95% of patients over the age of 50, who had a find-

ing that indicates a non-thyroid disease. It is important to emphasize that non-thyroid disease was the least represented of all diseases, and usually it is associated with elderly age. The percentage of women with latent hypothyroidism was 31%. Compared to the study of Wilson GR et al., where 4 - 8.5% of the study population had latent hypothyroidism, while this percentage increased to as much as 20% in women over the age of 60 [12]. If we concentrate on the cost of the study and the number of requests, there were only 20.74% of positive findings, which is about a fifth of the total number of referrals. In conclusion, there were a large number of requests in correlation to a small number of positive findings. In order to reduce additional costs, we need to improve the education of clinicians, improve national guidelines of required referrals for thyroid disease, available medical history, and send short notes with request paper. A similar idea was described in a research paper by Premawardhana LD. They concluded that measurements of thyroid hormones and TSH are expensive and of limited clinical value. Also, the contribution of abnormal tests is low, tests are inconsistent and often difficult to interpret [13]. To sum up, women at all ages are at risk and the risk of thyroid disease increases with age. It is clear, that latent hypothyroidism is the most prevalent disease, with an extremely large number. TSH is the cheapest marker test for women with suspected latent hypothyroidism. In order to prevent this, this marker could be done in the elderly with atrial fibrillation, in women with osteoporosis and reduced bone density, in elderly with CVD and with cognitive impairment. Thyroid hormones play important roles in metabolism, and the cardiovascular system is also very sensitive to thyroid function. However, not only overt thyroid dysfunction, but also mild thyroid dysfunction shows increased risk of coronary heart disease and mortality, which should not be overlooked at all [9,14,15,]. Increasing TSH in overt and mild hypothyroidism is also found to be associated with unfavorable lipid concentrations (decreased HDL levels and hypercholesterolemia) [16,17]. Meng et al. investigated an association between thyroid function and metabolic syndrome and found that serum TSH and FT3 levels were positively associated with MS. Their research suggests the importance of monitoring TSH and FT3 in the population for MS risk assessment, especially in the elderly [18]. Vaidya et al. in their research, prospectively illustrated that testing only women with high-risk pregnancies, as the consensus guidelines recommend, would fail to identify about one-third of women with hypothyroidism [19,20]. On the other hand, the current position of the US government concluded that there was insufficient evidence to support screening for thyroid dysfunction in nonpregnant, asymptomatic adults [21].

Limitations of our study deserve additional comments. First, this was a retrospective observational study, focused on data from an emergency biochemistry laboratory, with no patient medical history, so the cause and effect relationship cannot be discerned. Further prospective studies should be carried out to answer the causality question. Second, we did not obtain data on thyroid antibodies, which might influence thyroid hormone. So, we could not comment on the putative impact of thyroid autoimmunity. Third, as it was conducted only in the County Hospital Vinkovci, for more accurate data, it would be best to extend this research to other Centers in other counties and compare the results. Fourth, the research period of six months cannot provide concrete research results, as it does not cover at least a whole year.

CONCLUSION

In general, our results also suggest a disproportionately large number of referrals in comparison to the number of positive findings. Latent hypothyroidism is the most prevalent disease and most often includes the age group over 50 years for all thyroid diseases. The current evidence is insufficient to assess the balance between the benefits and harms of screening for thyroid dysfunctions in asymptomatic adults. The current data on the prevalence of thyroid disease is limited across the various age groups, and this implies the need for more precise and comprehensive recommendations for laboratory testing. Furthermore, there is a pressing need to easily identify and cheap measurable biomarkers of thyroid hormone status.

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Declaration of Interest:
The authors declare that they have no conflict of interest.

References

1. Ingoe L, Phipps N, Armstrong G, Rajagopal A, Kamali F, Razvi S. Prevalence of treated hypothyroidism in the community: analysis from general practices in North-East England with implications for the United Kingdom. Clin Endocrinol (Oxf) 2017;87(6): 860-4 (PMID: 28782887).

2. Jeon MJ, Kim WG, Kwon H, et al. Excessive Iodine Intake and Thyrotropin Reference Interval: Data from the Korean National Health and Nutrition Examination Survey. Thyroid 2017;(7):967-72 (PMID: 28471294).

3. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev 2014;94(2):355-82 (PMID: 24692351).

4. Shahid MA, Ashraf MA, Sharma S. Physiology, Thyroid Hormone. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan 2021 May 12 (PMID: 29763182).

5. Cooper DS, Landenson PW. The Thyroid Gland. In: Gardner DG, Shoback D. Greenspan`s basic and clinical endocrinology. 10th ed. New York: McGraw-Hill; 2017. p. 163-226. https://accessmedicine.mhmedical.com/content.aspx?bookid=2178&sectionid=166248172

6. Chiovato L, Magri F, Carle A. Hypothyroidism in Context: Where We've Been and Where We're Going. Adv Ther 2019 Sep; 36(2):47-58 (PMID: 31485975).

7. Madariaga AG, Palacios SS, Guillen-Grima F, Galofre JC. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. J Clin Endocrinol Metab 2014;99(3):923-31 (PMID: 24423323).

8. Tognini S, Polini A, Pasqualetti G, et al. Age and gender substantially influence the relationship between thyroid status and the lipoprotein profile: results from a large cross-sectional study. Thyroid 2012;22:1096-103 (PMID: 23050788).

9. Jones DD, May KE, Geraci SA. Subclinical thyroid disease. Am J Med 2010;123:502-4 (PMID: 20569751).

10. Delitala AP. Subclinical Hyperthyroidism and the Cardiovascular Disease. Horm Metab Res 2017:49(10);723-31 (PMID: 289155 31).

11. Mustafa S, Elgazzar A. Influence of chronic exposure to cold environment on thyroid gland function in rabbits. Horm Metab Res 2014 Jul;46(8):546-9 (PMID: 24446160).

12. Wilson GR, Curry RW Jr. Subclinical Thyroid Disease. Am Fam Physician 2005;72(8):1517-24 (PMID: 16273818).

13. Premawardhana LD. Thyroid testing in acutely ill patients may be an expensive distraction. Biochem Med (Zagreb) 2017;27(2):300-7 (PMID: 28694722).

14. Cooper DS, Biondi B. Subclinical thyroid disease. Lancet 2012; 379:1142-54 (PMID: 22273398).

15. Rodondi N, den Elzen WP, Bauer DC, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA 2010;304:1365-74 (PMID: 20858880).

16. Walsh JP, Bremner AP, Bulsara MK, et al. Thyroid dysfunction and serum lipids: a community-based study. Clin Endocrinol (Oxf) 2005;63:670-5 (PMID: 16343102).

17. Waring AC, Rodondi N, Harrison S, et al. Thyroid function and prevalent and incident metabolic syndrome in older adults: the Health, Ageing and Body Composition Study. Clin Endocrinol (Oxf) 2012;76:911-8 (PMID: 22187968).

18. Meng Z, Liu M, Zhang Q, et al. Gender and Age Impacts on the Association Between Thyroid Function and Metabolic Syndrome in Chinese. Medicine (Baltimore) 2015;94(50):e2193 (PMID: 266 83929).

19. Vaidya B, Anthony S, Bilous M, et al. Detection of thyroid dysfunction in early pregnancy: Universal screening or targeted high-risk case finding? J Clin Endocrinol Metab 2007;92:203-7 (PMID: 17032713).

20. Surks MI, Ortiz E, Daniels GH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA 2004;291:228-38 (PMID: 14722150).

21. LeFevre ML, U.S. Preventive Services Task Force. Screening for thyroid dysfunction: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2015;162(9):641-50 (PMID: 25798805).