THE JOURNAL OF
CLINICAL PSYCHIATRY
THE OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF CLINICAL PSYCHOPHARMACOLOGY

Supplementary Material

Article Title: Increased Prevalence of Subclinical Hypothyroidism and Thyroid Autoimmunity in Depressed Adolescents: Results From a Clinical Cross-Sectional Study in Comparison to the General Pediatric Population

Author(s): Raphael Hirtz, MD, PhD; Manuel Föcker, MD; Lars Libuda, PhD; Jochen Antel, PhD; Dana Öztürk; Cordula Kiewert, MD; Martin Munteanu, MD; Triinu Peters, PhD; Dagmar Führer, MD; Denise Zwanziger, PhD; Michael Thamm; Johannes Hebebrand, MD; and Corinna Grasemann, MD

DOI Number: 10.4088/JCP.20m13511

List of Supplementary Material for the article

Disclaimer

This Supplementary Material has been provided by the author(s) as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

Supplementary Material

Increased Prevalence of Subclinical Hypothyroidism and Thyroid Autoimmunity in Depressed Adolescents: Results from a Clinical Cross-Sectional Study in Comparison to the General Pediatric Population

Hirtz R, MD PhD, Föcker M, MD, Libuda L, PhD, Antel J, PhD, Öztürk D, Kiewert C, MD, Munteanu M, MD, Peters T, PhD, Zwanziger D, PhD, Führer D, MD, Thamm M, Hebebrand J, MD, Grasemann C, MD

Overview

Methods

Questionnaires - KiGGS

The Strength and Difficulties Questionnaire (SDQ) screens for mental health symptoms as well as positive attitudes in children and adolescents assessing 5 dimensions (emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems, and prosocial behavior) by 25 items. Each item is scored on a 3-point Likert scale (0-2) with higher scores indicating more problems. By summing the subscores from each dimension, a total score can be calculated and used to classify results as normal, borderline, or abnormal [1].

Health-related quality of life (HRQoL) was measured by the KINDL-R, which consists of 24 items assessing 6 dimensions of HRQoL (physical well-being, emotional well-being, self-esteem, family, friends, everyday functioning) scored on a five-point Likert scale (1-5). A total score can be calculated and transformed to values between 0 to 100. Higher scores indicate a better quality of life [2]. While in children younger than 11 years we used the scores on the parent-proxy form of self-administered questionnaires, in children older than 11 years we referred to the scores of the self-report form of the questionnaires [3].

Criteria for Reference Sample – KiGGS

Unimpaired mental health was defined by a score on the KINDL-R above the 10. percentile, a score on the SDQ indicating no significant mental health problem (i.e., no classification of the SDQ total score as 'abnormal'), no attendance of a psychiatrist or psychologist within the last 12 months, and no intake of psychotropic drugs

Anthropometric Measures

A physical examination was performed upon admission, including assessment of body length and body weight.

Height was determined in upright posture using a wall-mounted stadiometer. Height was recorded with a precision of 0.1 cm. Bodyweight was measured wearing underwear by an electronic scale displaying weight with a precision of 0.1 kg. BMI was determined by the ratio of weight in kg and the height in meters squared (kg/m^2) [4].

Laboratory Studies – Blood sampling

Blood samples from depressed patients were obtained from an antecubital vein in monovettes® (Sarstedt, Germany) in the early morning after an overnight fast. Aliquots were transferred at 4° C within an hour after blood sampling to the laboratory of the University Hospital Essen for analyses.

Blood samples from participants of the KiGGS survey were collected after a median fasting period of 2 hours using a vacutainer system. Whole blood was stored at 4°C and serum at -40°C before the transfer of samples to the central laboratory of the RKI within 3 days after sample collection [5].

Laboratory Studies – z-Transformation

The z-transformation of laboratory parameters was performed employing RefCurv (Version 0.4.4, https://refcurv.com [6]) relying on the information of the distribution of TSH and fT4 concentrations from age- and sex-specific percentile charts, which have been published for participants of the KiGGS survey [5] or were provided by Siemens based on an individual data usage agreement in addition to information from the package insert.

The same procedure was applied to z-transform BMI data from participants of the present study as well as the KiGGS survey according to reference data for German children and adolescents [4].

Statistical Analysis – Confounder: Vitamin D Level

TPO+ has been related to vitamin D levels, even though the evidence is limited [7,8]. To investigate a significant effect of vitamin D deficiency on thyroid autoimmunity, we compared TPO-Ab titer, TSH, and fT4 concentrations between depressed patients with (< 12 ng/dl) and without vitamin D deficiency (> 12 ng/dl) by two-sample t-tests. Also, we tested for bivariate correlations between vitamin D status and thyroid functioning and thyroid

autoimmunity. As the H_0 (*no* difference) was the favored outcome, no correction for multiple comparisons was applied.

Statistical Analysis – Miscellaneous

Likelihood ratio confidence intervals (CI, 95%), that have been shown to provide superior results in low prevalence conditions [9], were estimates for the prevalence of TPO+ as well as subclinical and overt thyroid dysfunction in both samples.

The Kolmogorov-Smirnov test assessed the normality of the dependent variable (BDI-II scores) and residuals in multiple regression. The non-normally distributed BDI-II score was rank-transformed according to Templeton [10], preserving its mean and standard deviation. The (modified) Breusch-Pagan test evaluated homoscedasticity, and the Durbin-Watson test excluded the autocorrelation of residuals. Outlier detection relied on Cook's distance.

Statistical Analysis - Demographics

Depressed patients with TPO+ or thyroid dysfunction were compared to depressed patients without thyroid dysfunction (no subclinical or overt thyroid dysfunction, no thyroid autoimmunity, no pre-existing, physician-diagnosed thyroid disease, no levothyroxine prescription) concerning age and BMI by two-sample t-tests. χ2-tests of independence and Fisher's exact test in case of cell counts < 5 were employed for comparisons with regard to the variables outlined in Supplementary Table 1. All analyses were corrected for multiple comparisons, as outlined above.

Results

Vitamin D Status

There was no significant difference in z-standardized TSH ($t(353) = 0.72$, $p = .472$; s. Table 3) or fT4 levels ($t(339) = 0.36$, $p = .717$) as well as TPO-Ab titers ($t(358) = -1.61$, $p = .108$) between vitamin D deficient and sufficient depressed adolescents in the present study. Moreover, vitamin D status did neither correlate with z-standardized TSH ($r(353 = .01$, $p = .847$) and fT4 levels ($r(339) = .04$, $p = .435$) nor TPO-Ab titers ($r(358) = -.09$, $p = .079$).

Demographics

Depressed adolescents affected by TPO+ significantly differed from depressed adolescents without thyroid dysfunction regarding the frequency of pre-existing, physician-diagnosed thyroid disease ($p_{Fisher's\ exact\ test\ (FET)} = 1.6 \times 10^{-5}$; see Table 3 for summary statistics and Supplementary Table 1 for detailed statistical results) as well as the frequency of levothyroxine prescription ($p_{FET} = 1.6 \times 10^{-5}$). In depressed adolescents with SCHYPO, only the frequency of thyroid autoimmunity ($p_{FET} = 5 \times 10^{-6}$) was significantly higher than in depressed adolescents with unremarkable thyroid function.

Sensitivity Analyses

When only subjects with TPO-Ab levels two-fold above the cut-off were considered, the prevalence of TPO+ in adolescent depression remained increased in comparison to the general adolescent population without evidence of impaired mental health (clinical sample: 3.9%, 95%-CI [2.2–6.2]; KiGGS survey: 2.1%, 95%-CI [1.6–2.7]; OR 1.88, 95%-CI [1.03-3.45], p=.037, Table 4).

Comparing the prevalence of thyroid dysfunction and autoimmunity between participants of the KiGGS survey and the subsample of patients with a BDI-II diagnosed depression ascertained by either the K-SADS-PL or clinical assessment, we found the same pattern of

findings with very similar prevalence figures as in patients with a diagnosis of a MDD solely based on the BDI-II (s. Supplementary Table 2).

Multiple Regression – SCHYPO and TPO+

In SCHYPO, BDI-II scores were neither associated with thyroid hormone levels considering bivariate correlations (z-TSH: $r(29) = -.21$, $p = .257$; z-fT4: $r(29) = .25$, $p = .184$, TPO-Ab: $r(29) = -.04$, $p = .818$) nor multiple regression (s. Table 3) or the variance in BDI scores accounted for by thyroid parameters ($R^2_{thyroid}$ $F(3, 27) = 1.01$, $p = .405$). This also applied to depressed adolescents with TPO+ (z-TSH: $r(19) = -.10$, $p = .657$; z-fT4: $r(19) = .08$, $p = .718$, TPO-Ab: $r(19) = -.16$, $p = .503$, $R^2_{thyroid}$ $F(3, 17) = 0.26$, $p = .851$; Supplementary Table 3).

Discussion

Prevalence of Thyroid Dysfunction and Autoimmunity in the General Pediatric Population

The prevalence of TPO+ in children and adolescents of the KiGGS survey without evidence of impaired mental health was determined at 3.1%, which is well in line with results from 2 previous studies (2.9% - 3.4%[11,12]) but below estimates from another 3 studies (4.6 – 8.2%; [13,14,15]), even when considering the upper limit of the confidence interval for TPO+. Without comparing each previous study with the present study in detail, comparability between studies is limited. The studies conducted by Taubner et al. [13] (~4.6%, age range 12-20 years), Kaloumenou et al. [14] (8.2%, Tanner stage ≥ II) and Zois et al. [15] (8.2%, 12-18 years) but also the study conducted by Kabelitz et al. [12] were sampled from single cities in either Germany (Leipzig, Berlin) or Greece (Athens, Konitsa). As shown by Loviselli et al. [11], there is considerable heterogeneity in the prevalence of TPO+ even between communities from the same region with comparable iodine supply (0% - 7.3%, on average 2.9%). Thus, results from these studies might have been regionally confounded in contrast to the present and representative sample of German children and adolescents from the KiGGS survey.

The prevalence of SCHYPO of 2.1% in the general pediatric population of the KiGGS study agrees with 2 previous studies and an estimated prevalence of SCHYPO of 1.7% to 2.9% [16,17]. Both these estimates originate from representative studies highlighting the need to exclude regional confounding as likely present in studies investigating the prevalence of TPO+ and discussed above.

Prevalence in SCHYPO

Recently, Luft et al. [18] reported a prevalence of SCHYPO of 6.1%. Unfortunately, the time of day when blood was sampled is not mentioned. In the present study, blood was drawn in the early morning when TSH levels peak[19], which may explain a higher prevalence of SCHYPO in comparison to the study by Luft et al. [18]. Moreover, in the present study, the evaluation of thyroid functioning relied on age- and sex-specific reference ranges but not a fixed cut-off, which may also explain different prevalence figures between studies.

Despite these considerations regarding methodological aspects of the evaluation of thyroid functioning, the diagnosis of depression in the present study and study by Luft et al. [18] relied on different criteria. Luft et al. [18] report that diagnoses were established according to DSM-IV and DSM-5 criteria, but this information is not detailed. In the present study, we found a prevalence of SCHYPO of 9.1% when depression was diagnosed according to the BDI-II and 8.2% when diagnosed according to the K-SADS-PL or clinical assessment. Despite only a small difference in these figures and widely overlapping confidence intervals, the lower bound of the confidence interval for SCHYPO in adolescent depression according to the K-SADS-PL or clinical assessment (5.7%) includes the prevalence of SCHYPO reported by Luft et al. [18]. Thus,

when depression is diagnosed according to the DSM-IV criteria as operationalized by BDI-II, the number of depressed adolescents with SCHYPO may slightly be overestimated when considering the sample by Luft et al. [18] as reference.

Thyroid Dysfunction other than SCHYPO

In contrast to SCHYPO, there was no evidence of an increased risk of either overt hypothyroidism or subclinical and overt hyperthyroidism. In contrast, Leo et al. [20] reported a prevalence of subclinical hyperthyroidism of 6.7% in 134 psychiatric adolescents inpatients, which is well above the finding of the present study and a recent study by Luft et al. [18]. The sample size of the present study as well as the study by Luft et al. [18], however, were (much) larger. Moreover, recent findings relied on latter generations of TSH assays with higher precision in the lower measurement range and on more reliable pediatric reference ranges for TSH [21] than in the study by Leo et al. [20]. Also, the study by Leo et al. [20] was not confined to patients with depression but included patients with diverse psychiatric diagnoses. Thus, we conclude that there is likely no increased prevalence of subclinical hyperthyroidism in adolescent depression.

Zader et al. [22] recently reported a 3.4-fold increased risk of depression in children and adolescents (primarily) affected by overt autoimmune hyperthyroidism, also referred to as Grave's disease, with a crude incidence of 1:3.000 to 1:10.000 [23,24]. Considering these prevalence figures, a patient with overt hyperthyroidism due to Grave's disease in a sample of depressed adolescents is unlikely. Indeed, the only patient to evidence a laboratory pattern of overt hyperthyroidism in the present study was a patient on inadequate levothyroxine replacement therapy. Summarizing, considering the prevalence of Grave's disease in children and adolescents, the finding by Zader et al. [22] is well in line with results from the present as well as previous studies regarding the prevalence of overt hyperthyroidism in adolescent depression.

subgroup 1	subgroup 2	test	variable	p-value	test statistics	df
no thyroid affection	TPO-Ab positive	t-test	age	.361	-0.92	308
		t-test	z-BMI	.419	-0.81	308
		FET	sex	.117		
		t-test	BDI-II	.367	-0.90	308
		$\chi 2$	BDI-II severity	.095	4.67	2
		FET	L-Thyroxin	**.00002**		
		FET	thyroid disease	**.00002**		
		$\chi 2$	psychotropic medication	.782	0.11	1
		FET	oral contraceptive	.402		
		FET	smoking	.090		
no thyroid affection	subclinical hypothyroidism	t-test	age	.420	0.81	318
		t-test	z-BMI	.190	-1.31	318
		$\chi 2$	sex	.504	0.45	1
		t-test	BDI-II	.287	1.07	318
		FET	L-Thyroxin	.097		
		$\chi 2$	BDI-II severity	.397	1.85	2
		FET	thyroid disease	.097		
		$\chi 2$	psychotropic medication	.854	0.03	1
		FET	oral contraceptive	.728		
		$\chi 2$	smoking	.463	0.54	1
		FET	TPO positive	**.0000005**		

Supplementary Table 1. Statistical results of the comparison of anthropometric and demographic variables as well as covariates among depressed adolescents. FET = Fisher's exact test. SPSS does not provide a test statistic. $\chi 2$ = $\chi 2$ test of independence. t-test = two-sample t-test. Bold typed p-values indicate significant differences between the indicated groups.

thyroid disorder	prevalence (%)	test statistic	p-value	OR
thyroid autoimmunity (TPO > 35 IU/ml)	6.3 [3.9 - 9.6]	$\chi^2(1, N = 2{,}613) = 7.73$	.005	2.09 [1.23 - 3.56]
thyroid autoimmunity (TPO > 70 IU/ml)	4.2 [2.3 - 7.0]	$\chi^2(1, N = 2{,}613) = 5.00$	.025	2.05 [1.08 - 3.91]
subclinical hypothyroidism	8.2 [5.3 - 11.9]	$\chi^2(1, N = 2{,}597) = 33.69$	6.4×10^{-9}	4.16 [2.47-7.00]
severe subclinical hypothyroidism	0.4 [0.0 - 1.6]	FET	.290	4.16 [0.38 - 45.97]
overt hypothyroidism	0.0	FET	1.000	*
subclinical hyperthyroidism	0.4 [0.0 - 1.6]	FET	.067	0.19 [0.03 - 1.39]
overt hyperthyroidism	0.4 [0.0 - 1.6]	FET	1.000	1.09 [0.14 - 8.72]

Supplementary Table 2. Comparison of depressed adolescents with a BDI-II score above 13 and a confirmed diagnosis according to the K-SADS-PL or clinical assessment with the KiGGS survey participants. Prevalence in percent, in brackets 95% confidence interval. FET = Fisher`s exact test, SPSS does not provide a test statistic. OR = odds ratio, FDR = false discovery rate. * 0% prevalence in depressed adolescents, therefore no OR. Note: Altogether 16 patients had missing information on TSH, fT4 or both.

Depressed adolescents with subclinical hypothyroidism (N=31)

Model	variables	β	t-value	p	R^2 model
1	#			n.s.	
	#			n.s.	
	#			n.s.	
	#			n.s.	
2	z-TSH	-0.19	-0.95	.349	.101
	z-fT4	0.31	1.53	.138	
	TPO-Ab	0.14	0.64	.526	

Depressed adolescents with TPO-Ab positivity (N=21)

Model	variables	β	t-value	p	R^2 model
1	#			n.s.	
	#			n.s.	
	#			n.s.	
	#			n.s.	
2	z-TSH	-0.12	-0.35	.731	.044
	z-fT4	-0.09	-2.60	.798	
	TPO-Ab	-0.20	0.77	.454	

Supplementary Table 3. Results of the multiple regression analysis from depressed adolescents with subclinical hypothyroidism and TPO-Ab positivity. Model 1 includes covariates entered as a first block of regressors and chosen by stepwise regression. Model 2 includes the covariates identified by the previous step of analysis as well as the thyroid parameters. β = standardized regression coefficient, z-standardized variables are labeled with the prefix 'z' (e.g., z-TSH). n.s. = not significant.

References

1. Rothenberger A, Becker A, Erhart M, Wille N, Ravens-Sieberer U. Psychometric properties of the parent strengths and difficulties questionnaire in the general population of German children and adolescents: results of the BELLA study. *Eur Child Adolesc Psychiatry.* 2008;17 Suppl 1:99-105.
2. Ravens-Sieberer U, Erhart M, Wille N, Bullinger M. Health-related quality of life in children and adolescents in Germany: results of the BELLA study. *Eur Child Adolesc Psychiatry.* 2008;17 Suppl 1:148-156.
3. Ellert U, Ravens-Sieberer U, Erhart M, Kurth BM. Determinants of agreement between self-reported and parent-assessed quality of life for children in Germany-results of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS). *Health Qual Life Outcomes.* 2011;9:102.
4. Neuhauser H, Schienkiewitz A, Rosario AS, Dortschy R, Kurth B-M. Referenzperzentile für anthropometrische Maßzahlen und Blutdruck aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS). *Gesundheitsberichterstattung.* 2013.
5. Dortschy R, Rosario AS, Scheidt-Nave C, et al. Bevölkerungsbezogene Verteilungswerte ausgewählter Laborparameter aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS). *Gesundheitsberichterstattung.* 2009:135.
6. Winkler C, Linden K, Mayr A, et al. RefCurv: A Software for the Construction of Pediatric Reference Curves. *arXiv preprint arXiv:190109775.* 2019.
7. Kim D. The role of vitamin D in thyroid diseases. *Int J Mol Sci.* 2017;18(9):1949.
8. Nettore IC, Albano L, Ungaro P, Colao A, Macchia PE. Sunshine vitamin and thyroid. *Rev Endocr Metab Disord.* 2017;18(3):347-354.
9. Agresti A. On logit confidence intervals for the odds ratio with small samples. *Biometrics.* 1999;55(2):597-602.
10. Templeton GF. A two-step approach for transforming continuous variables to normal: implications and recommendations for IS research. *Commun Assoc Inf Syst.* 2011;28(1):4.
11. Loviselli A, Velluzzi F, Mossa P, et al. The Sardinian Autoimmunity Study: 3. Studies on circulating antithyroid antibodies in Sardinian schoolchildren: relationship to goiter prevalence and thyroid function. *Thyroid.* 2001;11(9):849-857.
12. Kabelitz M, Liesenkotter KP, Stach B, et al. The prevalence of anti-thyroid peroxidase antibodies and autoimmune thyroiditis in children and adolescents in an iodine replete area. *Eur J Endocrinol.* 2003;148(3):301-307.
13. Taubner K, Schubert G, Pulzer F, et al. Serum concentrations of anti-thyroid peroxidase and anti-thyroglobulin antibodies in children and adolescents without apparent thyroid disorders. *Clin Biochem.* 2014;47(1-2):3-7.
14. Kaloumenou I, Mastorakos G, Alevizaki M, et al. Thyroid autoimmunity in schoolchildren in an area with long-standing iodine sufficiency: correlation with gender, pubertal stage, and maternal thyroid autoimmunity. *Thyroid.* 2008;18(7):747-754.
15. Zois C, Stavrou I, Kalogera C, et al. High prevalence of autoimmune thyroiditis in schoolchildren after elimination of iodine deficiency in northwestern Greece. *Thyroid.* 2003;13(5):485-489.

16. Salerno M, Capalbo D, Cerbone M, De Luca F. Subclinical hypothyroidism in childhood - current knowledge and open issues. *Nat Rev Endocrinol.* 2016;12(12):734-746.
17. Hunter I, Greene SA, MacDonald TM, Morris AD. Prevalence and aetiology of hypothyroidism in the young. *Arch Dis Child.* 2000;83(3):207-210.
18. Luft MJ, Aldrich SL, Poweleit E, et al. Thyroid Function Screening in Children and Adolescents With Mood and Anxiety Disorders. *J Clin Psychiatry.* 2019;80(5).
19. Ehrenkranz J, Bach PR, Snow GL, et al. Circadian and circannual rhythms in thyroid hormones: determining the TSH and free T4 reference intervals based upon time of day, age, and sex. *Thyroid.* 2015;25(8):954-961.
20. Leo RJ, Batterman-Faunce JM, Pickhardt D, Cartagena M, Cohen G. Utility of thyroid function screening in adolescent psychiatric inpatients. *J Am Acad Child Adolesc Psychiatry.* 1997;36(1):103-111.
21. Bailey D, Colantonio D, Kyriakopoulou L, et al. Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from the CALIPER cohort. *Clin Chem.* 2013;59(9):1393-1405.
22. Zader SJ, Williams E, Buryk MA. Mental health conditions and hyperthyroidism. *Pediatrics.* 2019:e20182874.
23. Hanley P, Lord K, Bauer AJ. Thyroid disorders in children and adolescents: a review. *JAMA Pediatr.* 2016;170(10):1008-1019.
24. Lee HS, Hwang JS. The treatment of Graves' disease in children and adolescents. *Ann Pediatr Endocrinol Metab.* 2014;19(3):122.
25. Besier T, Goldbeck L, Keller F. Psychometric properties of the Beck depression inventory-II (BDI-II) among adolescent psychiatric patients. *Psychother Psychosom Med Psychol.* 2008;58(2):63-68.
26. Straub J, Plener PL, Koelch M, Keller F. Agreement between self-report and clinician's assessment in depressed adolescents, using the example of BDI-II and CDRS-R. *Z Kinder Jugendpsychiatr Psychother.* 2014;42(4):243-252.