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Serum trace elements show association with thyroperoxidase autoantibodies in Thyroid Imaging Reporting and Data System (TI-RADS) 4 nodules

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Thyroid nodule (TN) has been becoming a great concern worldwide due to its high incidence. Although some studies have reported associations between trace elements exposure and the risk of TNs, the linkage was not inconclusive. The present study aimed to identify the association of selected serum trace elements (Ca, Mg, V, Fe, Co, Cu, Zn, Se, Mn and Mo) with TNs among general adults. A cross-sectional study was conducted in January 2021 in Chengdu, China. 1282 subjects completed the questionnaire and gave at least one human biological material after an overnight fast, venous blood, and urine, including 377 TN participants defined through ultrasound. Various trace elements in serum specimens were determined by inductively coupled plasma mass spectrometry. Thyroid functions were tested by chemiluminescent microparticle immunoassay (CMIA). The associations between trace elements levels and the risk of TNs were examined by restricted cubic splines (RCS) regression and bayesian kernel machine regression (BKMR) models. TNs were more common in females ($P<0.001$) and in the elderly ($P<0.001$) and that they were also frequently associated with fertility, marital status, annual household income, drinking, anxiety, vitamin supplement, tea consumption, hypertension and hyperlipidemia. After adjusting for confounders by a propensity score matching model, the association between trace elements concentrations and TNs risk was found to be statistically insignificant in the RCS (P for nonlinear > 0.05) and BKMR models. FT3 or T4 (total or free) increased significantly with increasing total trace elements mixture levels. In TI-RADS-4 TN subjects, TPO-Ab level increased significantly with increasing total trace elements mixture levels in the high-dose range. Ca, Zn, Mo at their 75th percentile showed positive individual effects on TPO-Ab, which was examined to be interactive. The detection of trace elements for TNs in general adults may be of no significance, but once individuals classified as TI-RADS-4 TNs are detected with abnormal TPO-Ab, Ca, Zn and Mo level are recommended to measure. The substantive association on it still needs to be continuously explored in the future.

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Thyroid nodule (TN) is a common discrete lesion in the thyroid gland, acounting for 20% to 67% in randomly selected populations[1]. They are radiologically distinct from the surrounding thyroid parenchyma and are frequently identified in patients with no symptoms who are undergoing routine physical examination or incidentally during a radiologic procedure such as ultrasonography (US) imaging[2]. Approximately 5% of TNs undergo progressive enlargement and 10% are at risk of malignancy, warranting intervention[3]. All TNs are considered to require ultrasound assessment for the need of further diagnosis of possible thyroid cancer through fine needle aspiration biopsy (FNA). Similarly, inadequate selection of TN for assessment can result in missed diagnoses of clinically relevant thyroid cancer[4]. Risk factors that increase the probability of nodules include advancing age, female sex, iodine deficiency, metabolic disorder, history of radiation, and childhood irradiation[5–7].

Trace elements in the external environment, such as iodine (I), calcium (Ca), magnesium (Mg), vanadium (V), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), manganese (Mn) and molybdenum (Mo), have been linked to the occurrence and development of TN, although underlying mechanism remains unclear[8–10]. Thyroid disease effects of elemental mixtures that are not sufficient considered for nonlinear correlations and elemental interactions may be biased, resulting in contradictory findings, such as negative associations of Zn levels with TN in a European metropolis and positive associations in Guangdong, China[11,12]. The importance has been identified that the single, interactive, and overall effects of trace elements require multiple methods for comprehensive interpretation[13]. Notably, changes in serum trace element concentrations can significantly affect thyroid function levels[14,15], but it is not well known whether this association also exists in individuals with TNs. Moreover, studies have focused on investigating the relationship between trace element levels and TNs in pregnant women and thyroid disease patients, fewer research have been conducted on the general population[9,16].

Given the sparsity and inconsistent results of studies linking trace elements and TNs, in the present study we performed a cross-sectional study to identify the association of selected serum trace elements (Ca, Mg, V, Fe, Co, Cu, Zn, Se, Mn and Mo) with TNs among general adults, using restricted cubic splines (RCS) regression and bayesian kernel machine regression (BKMR) models adjusted by urine iodine as a covariate.

Methods

Study population

In order to examine the relationships of human exposure with thyroid health in the adults, we conducted a cross-sectional study in January 2021 in Chengdu, China. The adults who visited Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital for physical examination were recruited. The volunteers with history of malignant tumor, thyroidectomy, or severe liver and kidney dysfunction as well as potential occupational exposure were excluded, and the pregnant and lactating women were also excluded. Finally, 1282 subjects completed the questionnaire, underwent thyroid ultrasonographic examination, and gave at least one human biological material after an overnight fast, venous blood, and urine, were sampled by the nurses. All volunteers were told the purpose of this program and signed informed consent. The protocol was approved by the Ethics Committee of China National Center for Food Safety Risk Assessment (No. 2019008) and Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital (No. 2020467). Moreover, the collection of human biological samples in our study has been approved by Ministry of Science and Technology (MOST) of China in 2020. All methods were performed in strict adherence to the Ethical Review Measures for Biomedical Research Involving Human Subjects issued by the National Health Commission of China (2016), the Helsinki Declaration of the World Medical Association (WMA), and the International Ethical Guidelines for Biomedical Research Involving Human Subjects issued by the Council for International Organizations of Medical Sciences (CIOMS).

All TNs were defined to be present when sized > or equal to 2 mm in diameter and classified according to the Thyroid Imaging Reporting and Data System (TI-RADS) released by the American College of Radiology[17]. To ensure the accuracy of the diagnosis, two registered physicians who both had a professional certificate for ultrasonography (awarded by the China National Health and Family Planning Commission) conducted thyroid ultrasonographic examination using B-mode US imaging (MX7, Mindray Shenzhen, P.R. China) with a 13 MHz linear array probe. Finally, 377 TNs were diagnosed.

A total of 905 controls were recruited participants who were confirmed by ultrasound to have no nodules and collected using the same recruitment criteria as those used for participants with TNs. To optimize inter-group comparability, we performed a propensity score matching (PSM) between groups. Studies designed PSM to remove the effects of confounding in multiple environmental and public-healthy analyses[18,19]. As a post-randomization method, PSM can summarize all of the relevant confounding in a single composite score to initiate unbiased and balanced comparisons[20].

Measurement of elements

A single, venous blood samples after an overnight fast (approximately 5 mL), and urine samples after an overnight fast (approximately 10 mL) were collected for in the study hospital. Venous blood samples were drawn from each participant after an overnight fast (8–10 h) by the professional workers using non-anticoagulant vacuum sampling vessel. Subsequently, the blood samples were centrifuged at 3500 rpm for 10 min at 4 °C and the upper serum were transferred into several 500 μl centrifuge tubes for frozen storage at − 80 °C until analysis.

The serum samples were used to measure trace elements concentrations by using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher Scientific, USA) following the reference method for the determination of trace elements in blood[21]. For quality-control purposes, we measured the blood control samples (CLINCHEK Level-1, Level-3, Recipe, Munich, Germany) to ensure that the measured values were within the target ranges[22,23]. A comprehensive analysis of the Certified Reference Materials (CRMs) for blood, detailed in SM Table S1.

Urine specimens were used to measure urinary iodine concentration using a DIONEX ICS-5000 + ion chromatography (IC, Thermo Scientific, USA). A Standard Reference Material (SRM), Mercury, Perchlorate, and Iodide in Frozen Human Urine (SRM 3668, National Institute of Standards and Technology, USA) was applied for the validation of detection of urinary iodine. Urine iodine was standardized using urine creatinine levels measured by the HITACHI 7180 chemical analyzer[24].

Measurement of thyroid functions

Chemiluminescent microparticle immunoassay on the ARCHITECT i2000SR (ABBOTT DIAGNOSTICS, Abbott Park, IL, USA) was employed to quantify the thyroid functions in serum within 24h after venous sampling[25]. Thyroid parameters in blood samples were analysed by the standard laboratory tests, including the analysis of serum TSH, tri-iodothyronine (T3), tetra-iodothyronine (T4), free T3 (FT3) and free T4 (FT4), as well as autoantibodies (ab) to TSH receptor (TR-Ab) and thyroglobulin (TG-Ab), thyroperoxidase (TPO-Ab)[26,27].

Covariates

Height and weight were measured at the time of recruitment. Body mass index (BMI) was calculated by as the ratio of the weight in kilograms to the square of height in meters (kg/m^2). Data on socio-demographic characteristics, lifestyle information, dietary habits and underlying diseases were collected using a standardized questionnaire by well-trained workers. Socio-demographic characteristics included age, gender, ethnicity, education level, marital status, fertility, occupation, household income and family history of thyroid cancer. Lifestyle information included smoking, drinking, physical activity, sleep quality, anxiety status, irritability status and vitamin supplement. The types of dietary habits included tea, fish, shellfish, shrimp and crabs, seaweed, smoked meat and cruciferous vegetables.

Statistical analysis

R software (version 3.6.1) under RStudio (version 1.2.5001) was utilized for data analyses. Data were presented as mean [standard deviation (SD)] or frequency [corresponding proportion (%)], for continuous and categorical variables respectively. PSM was performed between groups using the R Package "Matching" (version 4.9-2) as follow: ①Standardized mean differences (SMD) were determined to compare baseline characteristics between groups. And the SMD greater than or equal to 0.1 was considered as a confounding factor[28]. ②Logistic regression model was used to calculate the propensity scores. ③K-nearest neighbor algorithm was used to make a 1:2 match without replacement using the caliper of width equal to 0.2 of the standard deviation of the legit of the propensity scores[29]. ④SMD was used again to determine the balance after PSM.

Before PSM, descriptive analyses of the study population characteristics were performed according to study outcomes (TN, non-TN). Wilcoxon rank sum test, Mann–Whitney U tests and the χ^2 tests were used to analyze differences according to the data distributions. Concentrations of serum trace elements were presented by density plot matrix, and results shown in SM Figure S1. Because of the severely skewed distribution of trace element concentrations and thyroid function indexes, natural logarithm and Box-cox transformation were applied to the regression models, while the crude model was not adjusted through covariates except for standardized urine iodine. The covariates significantly associated with TN were set into the PSM model all at once, and data filtering results of the matching process were listed in SM Table S2.

After PSM, we applied restricted cubic splines (RCS) and bayesian kernel machine regression (BKMR) to assess the associations of trace elements with TNs and thyroid function indexes. The dose–response relationships between trace elements and risk of TN was visualized and examined by RCS, as RCS had been widely described as a valid strategy to realize the correlation analysis between continuous exposure and binary outcomes[30,31]. These curves were composed of the odds ratios (ORs) in the logistic regression equation, which 95% CIs were also presented. Odd ratio for the reference value was considered as 1, adjusted by standardized urine iodine. Knots in the splines were set at 5th, 25th, 50th, 75th and 95th percentile, respectively[32]. BKMR, a nonparametric highdimensional exposure–response function using kernel machine regression, was used to estimate the effects of individual trace elements, the overall mixture effect and interactions between trace elements[33]. All BKMR models were adjusted for standardized urine iodine with 10,000 iterations using the Markov Chain Monte Carlo sampler. To evaluate the joint effect of trace element, the value of the exposure–response function when all trace elements were at a particular quantile was compared to all of them were at their median value. In addition, for indicators that have shown statistical significance in joint effects, individual effects of each trace element were estimated by subtracting the mean outcome value when the single element was at the 25th percentile with the mean value of the outcome when that element was at its 75th percentile, where all of the remaining elements were fixed to a particular quantile (25th, 50th and 75th percentile). To evaluate specific interaction parameters, the single element risks (associated with a change from its 25th to 75th percentile) when the remaining elements were fixed at their 25th percentile was compared to when they were fixed at their 75th percentile.

All tests were 2-sided with an α level of 0.05. RCS and BKMR were implemented with the R packages "rms" (version 6.5-0) and "bkmr" (version 0.2.2), respectively.

Results

Characteristics of participants

Before PSM, the socio-demographic characteristics of all 1282 participants (377 TNs and 905 non-TNs) included in this study are presented in Table 1 and Fig. 1. There were 115 sets of men and 262 sets of women. Mean BMI in the control group was 37.48 ± 8.09 kg/m^2 and 42.91 ± 9.38 kg/m^2 in the TNs group. There were significant

Variable	TN (–) (n = 905)	TN (+) (n = 377)	*p*-value[a]
Age (years, Mean ± SD)	37.48 ± 8.09	42.91 ± 9.38	< 0.001
BMI (kg/m^2, Mean ± SD)	22.48 ± 3.06	22.65 ± 3.02	0.349
Female, n (%)	528 (58.34)	262 (69.50)	< 0.001
Ethnicity, n (%)			0.094
Han	870 (96.13)	363 (96.29)	
Tibetan	7 (0.77)	0 (0.00)	
Yi	6 (0.66)	2 (0.53)	
Hui	6 (0.66)	0 (0.00)	
Others	16 (1.77)	12 (3.18)	
Fertility, n (%)			< 0.001
Non	206 (23.09)	54 (14.32)	
One child	514 (56.80)	263 (69.76)	
Two child	163 (18.01)	53 (14.06)	
Three child	19 (2.10)	7 (1.86)	
Marital status, n (%)			< 0.001
Unmarried	153 (16.92)	29 (7.69)	
Married	725 (80.09)	331 (87.80)	
Divorce	27 (2.99)	17 (4.51)	
Annual household income (Ten-thousand CNY, %)			0.006
< 10	8 (0.88)	7 (1.86)	
10 ~ 20	585 (64.64)	211 (55.97)	
20 ~ 30	192 (21.22)	85 (22.55)	
> 30	120 (13.26)	74 (19.63)	
Occupation, n (%)			0.252
Doctor	348 (38.45)	134 (35.54)	
Nurse	213 (23.54)	108 (28.65)	
Technician	82 (9.06)	29 (7.69)	
Worker	64 (7.07)	20 (5.31)	
Executive	38 (4.20)	10 (2.65)	
Researcher	17 (1.88)	8 (2.12)	
Others	143 (15.80)	68 (18.04)	
Education level, n (%)			0.952
Primary	14 (1.55)	5 (1.33)	
Middle	37 (4.09)	16 (4.24)	
High	603 (66.63)	259 (68.70)	
College	186 (20.55)	71 (18.83)	
Master	65 (7.18)	26 (6.90)	
Family history of thyroid cancer, n (%)	16 (1.77)	9 (2.39)	0.465
Underlying diseases			
Hypertension, n (%)	37 (4.09)	26 (6.90)	0.034
Diabetes, n (%)	12 (1.33)	11 (2.92)	0.050
Hyperlipidemia, n (%)	62 (6.85)	46 (12.20)	0.002
Hyperuricemia, n (%)	29 (3.20)	15 (3.98)	0.488
Drinking, n (%)			0.011
Never	565 (62.43)	268 (71.09)	
Drinking	275 (30.39)	90 (23.87)	
Abstained	65 (7.18)	19 (5.04)	
Smoking, n (%)			0.354
Never	754 (83.31)	319 (84.62)	
Smoking	106 (11.71)	35 (9.28)	
Abstained	45 (4.97)	23 (6.10)	
Physical activity, n (%)			0.112
Never	273 (30.17)	91 (21.14)	
Low-Intensity[b]	360 (39.78)	165 (43.77)	
Middle-Intensity[c]	177 (19.56)	85 (22.55)	
High-Intensity[d]	95 (10.50)	36 (9.55)	
Continued			

Variable	TN (−) (n = 905)	TN (+) (n = 377)	*p*-value[a]
Sleep quality, n (%)			0.534
Excellent	202 (22.32)	94 (24.93)	
Common	486 (53.50)	189 (50.13)	
Poor	174 (19.23)	71 (18.83)	
Bad	43 (4.75)	23 (6.10)	
Anxiety, n (%)			0.026
Never	93 (10.28)	56 (14.85)	
Occasionally	706 (78.01)	269 (71.35)	
Frequently	106 (11.71)	52 (13.79)	
Irritability, n (%)			0.603
Never	114 (12.60)	40 (10.61)	
Occasionally	683 (75.47)	290 (76.92)	
Frequently	108 (11.93)	47 (12.47)	
Vitamin supplement, n (%)			0.017
Never	489 (54.03)	190 (50.40)	
Occasionally	352 (38.90)	142 (37.67)	
Frequently	64 (7.07)	45 (11.94)	
Tea consumption, n (%)			0.014
Never	32 (3.54)	11 (2.92)	
< Once per week	440 (48.62)	154 (40.85)	
1–2 times per week	224 (24.75)	125 (33.16)	
3–5 times per week	209 (23.09)	87 (23.08)	
Fish consumption, n (%)			0.134
Never	50 (5.52)	33 (8.75)	
< Once per week	813 (89.83)	325 (86.21)	
1–2 times per week	25 (2.76)	9 (2.39)	
3–5 times per week	17 (1.88)	10 (2.65)	
Shellfish consumption, n (%)			0.459
Never	63 (6.96)	32 (8.49)	
< Once per week	655 (72.38)	259 (68.70)	
1–2 times per week	31 (3.43)	11 (2.92)	
3–5 times per week	156 (17.24)	75 (19.89)	
Shrimp and crabs consumption, n (%)			0.477
Never	66 (7.29)	37 (9.81)	
< Once per week	726 (80.22)	293 (77.72)	
1–2 times per week	30 (3.31)	11 (2.92)	
3–5 times per week	83 (9.17)	36 (9.55)	
Seaweed consumption, n (%)			0.694
Never	66 (7.29)	30 (7.96)	
< Once per week	758 (83.76)	309 (81.96)	
1–2 times per week	39 (4.31)	15 (3.98)	
3–5 times per week	42 (4.64)	23 (6.10)	
Smoked meat consumption, n (%)			0.745
Never	64 (7.07)	27 (7.16)	
< Once per week	771 (85.19)	323 (85.68)	
1–2 times per week	33 (3.65)	16 (4.24)	
3–5 times per week	37 (4.09)	11 (2.92)	
Cruciferous vegetable consumption, n (%)			0.769
Never	8 (0.88)	3 (0.80)	
< Once per week	511 (56.46)	214 (56.76)	
1–2 times per week	379 (41.88)	159 (42.18)	
3–5 times per week	7 (0.77)	1 (0.27)	

Table 1. Baseline characteristics. TN, Thyroid nodule; BMI, Body mass index; CNY, Chinese Yuan; SD, Standard deviation. [a]Student's t-test and Analysis of Variance for continuous variables; Chi-square test for categorical variables. [b]Breathing smoothly and feeling comfortable, such as walking, fishing, darts, Tai Chi. [c]Slightly shortness of breath, such as brisk walking, dancing, play badminton. [d]Shortness of breath, sweating, muscle soreness, such as running or swimming.

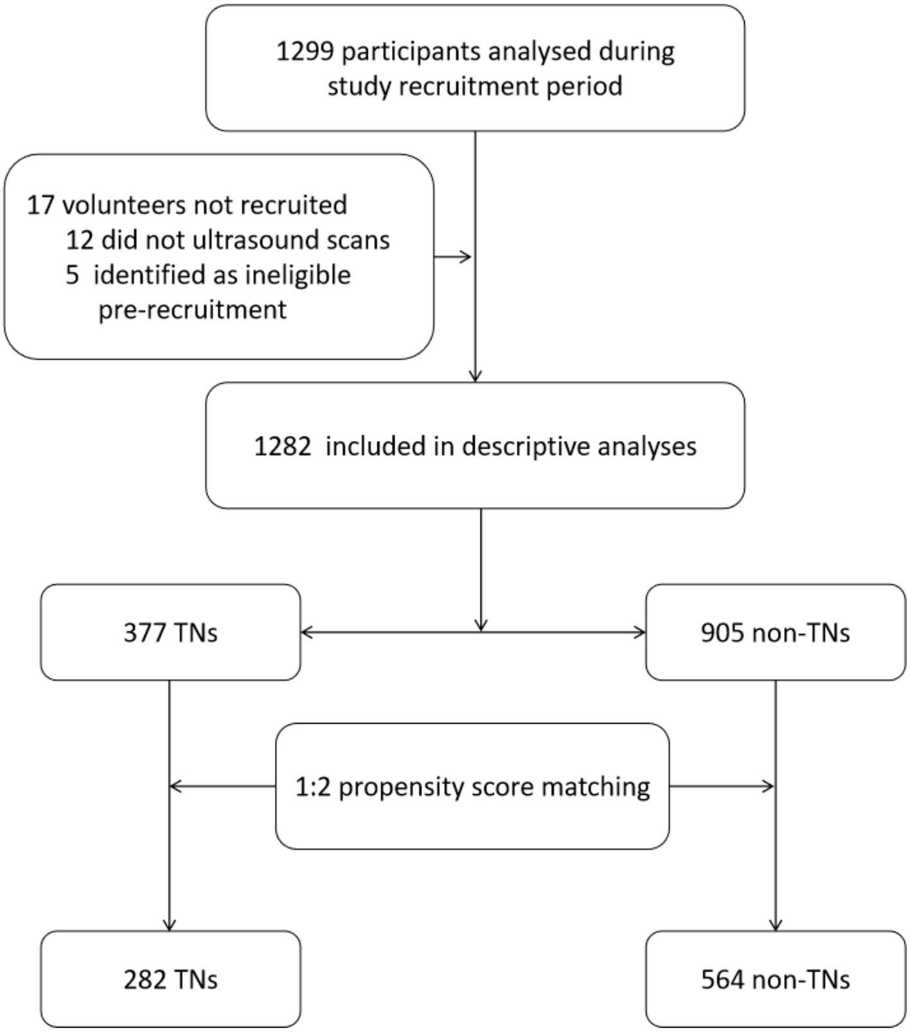

Fig. 1. Participant flow chart. Initial enrollment included 1299 subjects. Of these, 12 participants did not undergo the required ultrasound scan according to the study protocol, and an additional 5 participants were excluded based on meeting the exclusion criteria. Consequently, the final sample consisted of 1282 subjects who completed the questionnaire, underwent an ultrasound scan, and provided at least one fasting biological specimen. TNs, Thyroid nodules.

Groups	N	Level of iodine intake[b], n (%)				*p*-Value[a]
		Insufficient	Adequate	Above requirement	Excessive	
Study-population						0.523
TN	889	291 (32.73)	267 (30.03)	141 (15.86)	190 (21.37)	
Non-TN	371	115 (31.00)	127 (34.23)	57 (15.36)	72 (19.41)	
TN-population						0.163
TI-RADS-Cystic	29	3 (10.34)	18 (62.07)	1 (3.45)	7 (24.14)	
TI-RADS-2	4	1 (25.00)	1 (25.00)	1 (25.00)	1 (25.00)	
TI-RADS-3	272	91 (33.46)	84 (30.88)	44 (16.18)	53 (19.49)	
TI-RADS-4	66	20 (30.30)	24 (36.36)	11 (16.67)	11 (16.67)	

Table 2. Iodine level of urine in different groups. TN, Thyroid nodule. [a]Chi-square tests. [b]Concentrations of the urine iodine less than 99 μg/L were considered as iodine insufficient, 100–199 μg/L were adequate, 200–299 μg/L were above requirement, and more than 300 μg/L were excessive.

differences in age, gender, fertility, marital status and annual household income between groups. The TN group were significantly more frequent in women ($P < 0.001$) and in older ages ($P < 0.001$). Tables 1 and 2 present the lifestyle information and dietary habits, underlying diseases, and the iodine level of urine, respectively. Significant differences were found in drinking, anxiety, vitamin supplement, tea consumption, hypertension and hyperlipidemia, while no significant differences were found in the iodine level of urine between groups.

Associations of trace elements with thyroid nodule

The covariates significantly associated with TN have been adjusted to optimize inter-group comparability using a PSM model. PSM eliminated the effects of all confounding factors and improved the balance on the baseline characteristics with all SMD decreasing to 0.1 between the groups (Fig. 2). After PSM, the final analyzed data were matched in a 1:2 ratio, including 282 TNs and 564 non-TNs (SM Table S2).

In the present study, we detected a total of ten trace elements in the blood samples, including Mo, V, Mn, Fe, Co, Cu, Zn, Se, Mg and Ca. The concentrations of each element were shown in Table 3 an SM Figure S1. Fe and Cu presented significant differences between groups, whereas other elements presented no significant differences. The TN group had higher Cu concentrations ($P = 0.002$) and lower Fe concentrations ($P = 0.019$), respectively. Figure 3 illustrated the associations between individual trace elements and risk of TN in RCS models after adjusting for standardized urine iodine. No significant differences were found in the dose–response relationships between concentrations of trace elements (ln-transformed) and risk of TN in the single-element models (P for nonlinear > 0.05). The joint associations between the trace elements mixture and the risk of TN were shown in Fig. 4. Although no significant difference was found in the BKMR model, there was a U-shaped curve trend. In the examination for exposure–response function of individual element (all of the remaining elements set at their median value) and single-exposure effects (from its 25th to its 75th percentile with all of the remaining elements fixed to 25th, 50th or 75th percentile) between all trace elements in the association with TN, we did not observe any significant differences (Fig. 5a; SM Figure S2a). Besides that, no synergistic interaction was found when we examined potential interaction between trace elements and TN (SM Figure S3a).

Associations of trace elements with thyroid functions

Associations between the trace elements and thyroid functions were estimated by the BKMR models. T4 levels increased significantly with increasing total trace elements mixture levels (Fig. 4c). Similar results were

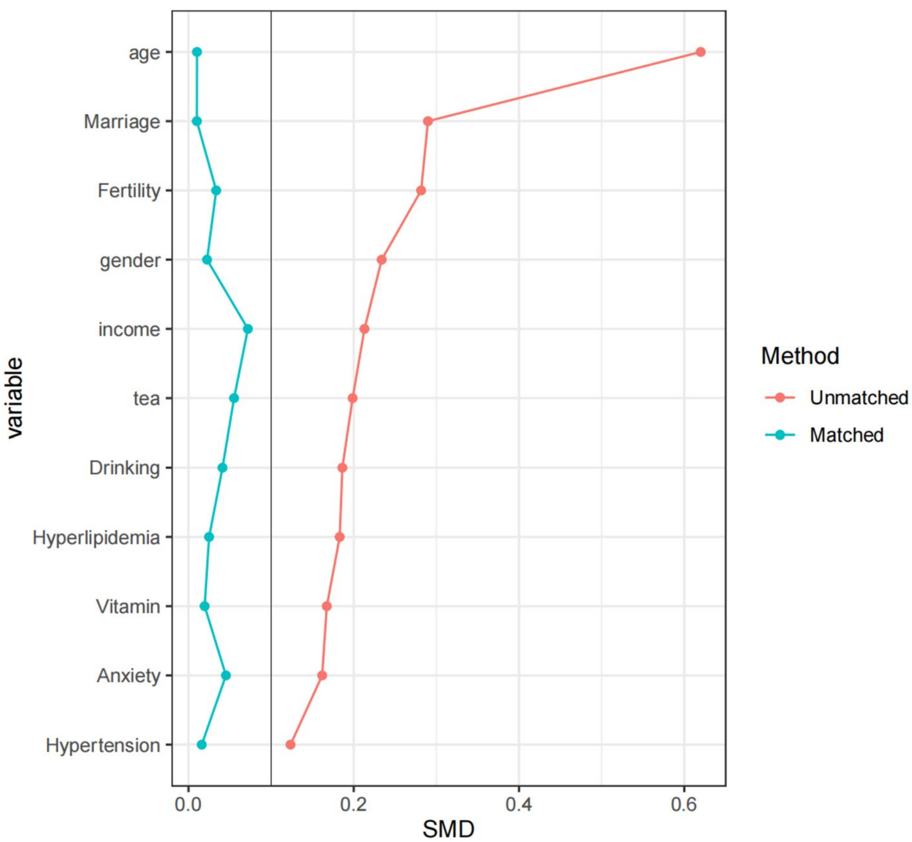

Fig. 2. Point-fold Line Chart of standardized mean differences (SMD) comparing pre- and post-propensity score matching (PSM) results. PSM enhanced the balance of investigated baseline characteristics, with all standardized mean differences (SMD) between groups decreasing to 0.1. After PSM, the final analyzed data were matched in a 1:2 ratio. SMD, standardized mean differences.

Elements	TN (−)				TN (+)			
	Mean (SD)	Percentile 25	Median	Percentile 75	Mean (SD)	Percentile 25	Median	Percentile 75
Mo	1159.39 (433.14)	916.76	1098.37	1315.05	1222.45 (552.87)	34.4	39.85	47.43
V	1171.75 (335.67)	987.56	1183.52	1340.76	1142.52 (318.54)	76.36	156.56	272.35
Mn	1441.20 (556.45)	1187.03	1368.05	1585.34	1444.46 (678.43)	1660.91	1849.69	2073.75
Fe	1602.58 (532.38)	1232.35	1563.76	1885.48	1526.52 (519.90)	1137.43	1350.44	1592.91
Co	846.75 (1414.58)	360.30	469.81	789.95	1002.08 (2318.49)	1198.64	1482.1	1814.05
Cu	921.48 (175.51)	820.27	901.06	1001.43	945.26 (165.24)	373.95	523.14	900.72
Zn	1104.35 (169.71)	986.82	1079.46	1209.09	1087.4 (179.08)	847.33	932.73	1013.4
Se	115.95 (28.01)	100.55	113.37	126.91	116.82 (23.78)	577.56	813.27	1145.82
Mg	24,777.28 (2031.27)	23,389.35	24,781.11	26,153.63	24,846.57 (2266.25)	103.02	115.08	128.17
Ca	95,186.63 (7021.33)	89,945.26	94,912.43	99,822.12	95,496.09 (8216.62)	23,414.14	24,958.64	26,193.37

Table 3. Concentrations of serum trace elements (μg/L) TN, Thyroid nodule; SD, Standard deviation; Mo, Molybdenum; V, Vanadium; Mn, Manganese; Fe, Iron; Co, Cobalt; Cu, Copper; Zn, Zinc; Se, Selenium; Mg, Magnesium; Ca, Calcium.

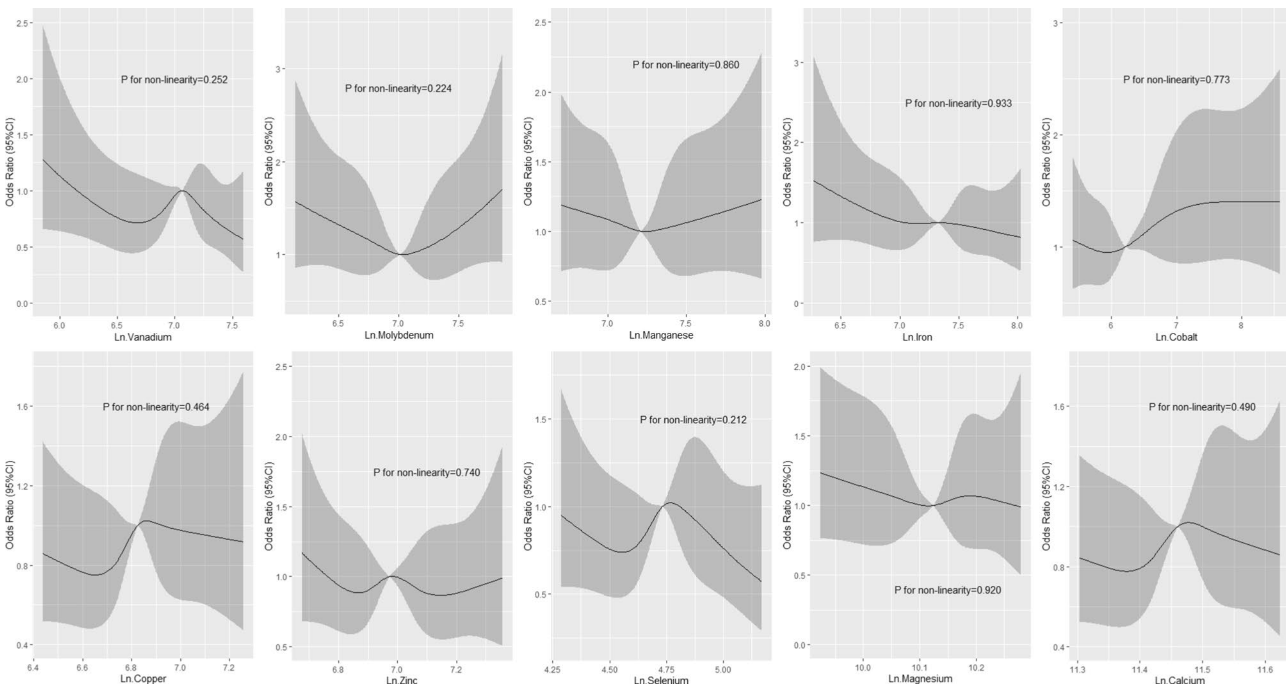

Fig. 3. Nonlinear associations between serum trace elements (ln-transformed) and the risk of thyroid nodule (Odds Ratio) in the single-element models. Nonlinear associations were presented by the restricted cubic splines (RCS) regression, and adjusted by standardized urine iodine in the single-element models. These curves were composed of the odds ratios (ORs) in the logistic regression equation and shading in the plots indicated the confidence interval (95%CI). The reference OR value was considered as 1. The knots in the plots were positioned at 5th, 25th, 50th, 75th and 95th percentile, respectively.

observed between trace elements exposure and FT3, FT4 levels, but not with T3, TSH, TRAb, TGAb and TPO levels (Fig. 4). When all other elements were at their median levels, Cu, Zn showed increasing and Mg showed decreasing associations with T4, whereas other elements presented showed a flat relationship (Fig. 5b). There was a parabola exposure–response relationship between Mn, Fe and FT3, while Mg showed decreasing associations with FT3 (Fig. 5c). Fe showed increasing associations with FT4 with a decrease in the highest concentration (Fig. 5d). We further examined the relationship when a single-element was at the 75th percentile as compared to when that element was at its 25th percentile, where all of the remaining elements were fixed to 25th, 50th or 75th percentile (SM Figure S2). Cu, Zn showed positive associations with T4; Mg showed negative associations with T4 and FT3; Mn, Fe showed positive associations with FT3, and Fe showed a positive association with FT4, regardless of whether all of the remaining elements were fixed at 25%, 50%, or 75%. Furthermore, we did not observe interaction, as no significant differences were found in interaction examination (SM Figure S3) and the trace elements had parallel bivariate exposure–response functions for all pairs (SM Figure S4).

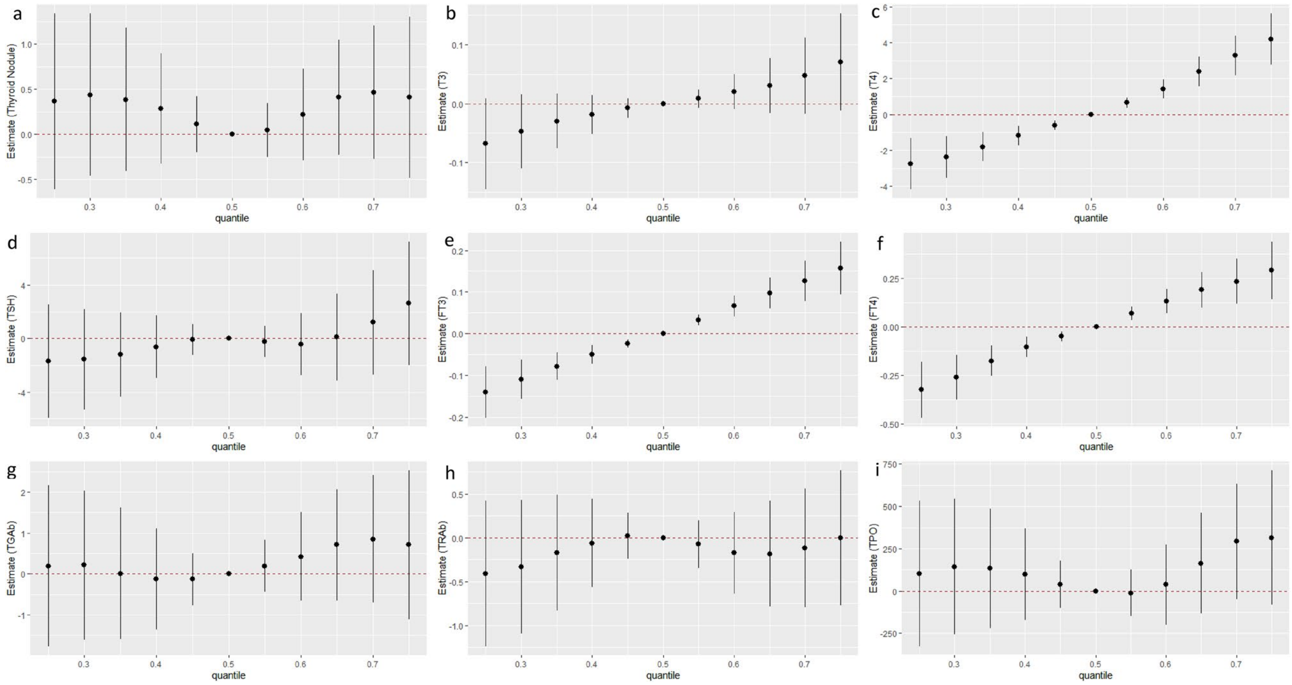

Fig. 4. Joint effects of the mixture on thyroid nodule and thyroid functions. Joint effects (95%CI) of the mixture on thyroid nodule (**a**), T3 (**b**), T4 (**c**), TSH (**d**), FT3 (**e**), FT4 (**f**), TGAb (**g**), TRAb (**h**), TPO (**i**) when all trace elements were at a particular quantile was compared to when they were at their median value. The results were assessed by the BKMR model, adjusted for standardized urine iodine. T3, tri-iodothyronine; T4, tetra-iodothyronine; FT3, free tri-iodothyronine; FT4, free tetra-iodothyronine; TR-Ab, TSH receptor autoantibodies; TG-Ab, thyroglobulin autoantibodies; TPO-Ab, thyroperoxidase autoantibodies.

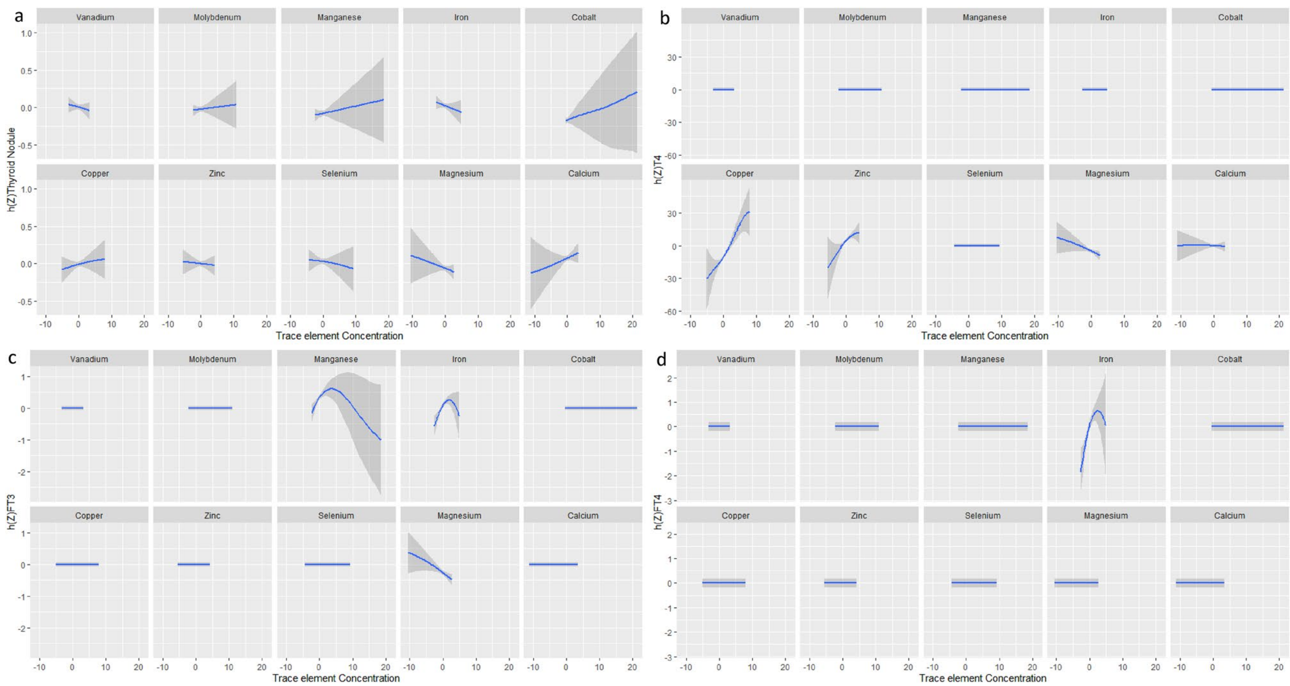

Fig. 5. Univariate exposure–response functions of selected trace elements concentrations. For indicators that have shown statistical significance in joint effects, univariate exposure–response functions (95%CI) between selected trace elements concentrations and thyroid nodule (**a**), T4 (**b**), FT3 (**c**), FT4 (**d**) were estimated when all the other elements were fixed at their median levels. T4, tetra-iodothyronine; FT3, free tri-iodothyronine; FT4, free tetra-iodothyronine.

In TI-RADS-4 TN subjects, TPO-Ab level increased significantly with increasing total trace elements mixture levels, when the concentration of each element was higher than 60th percentile (Fig. 6a). When all other elements were at their median levels, Ca, Zn and Mo showed an association with TPO-Ab, while the other elements did not (Fig. 6b). Ca, Zn, Mo at the 75th percentile showed positive individual effects on TPO-Ab when all of the remaining elements were fixed to a particular quantile (Fig. 6c). Furthermore, the interaction between Ca, Zn and Mo was statistically significant in the high-dose range (Fig. 6d).

Discussion

In the present study, relationships between trace elements and TNs, as well as trace elements and thyroid function indexes were examined in the general population of the Western China adjusting by propensity score matching. The results showed that the incidence of TN was not associated with the concentration of trace elements, whereas the mixture of all elements was positively associated with T4, FT3 and FT4. Although no significant association between single-exposure/overall mixed elements and TNs appeared in the RCS and BKMR models, there was a U-shaped curve trend. Of note, the study population is a mixture of those without previously diagnosed thyroid disease and not taking thyroid medication. Therefore, these associations were found among subjects with normal thyroid function.

Similar to the clear association between iodine and TNs, a U-shaped curve trend was observed between TNs and overall trace elements mixture[34]. The high risk of TNs was associated with both lowest level and highest level of trace elements. However, this association was found to be statistically insignificant in this study. The consistency of non statistically significant results in the RCS and BKMR models reinforced suggestion that it was not necessary to detect trace elements for the clinical management of TNs in general adults. In the past half century, TNs care at times included unnecessary or excessive test, and an increasingly conservative approach to nodule management was widely considered as the most appropriate intervention that led to favourable outcomes[35,36]. To optimize management approach to TNs and maintain the goal of identifying only patients who would benefit from treatment, our results highlighted that additional examination of trace elements for TNs might lead to overdiagnosis. Most notably, TPO-Ab of TI-RADS-4 TN subjects was found to be increased significantly with increasing trace elements mixture levels in the high-dose range. As a clinical marker for the detection of early autoimmune thyroid disease (AITD) such as Graves' disease and Hashimoto's thyroiditis, TPO-Ab plays a key role in thyroid hormone synthesis[37]. Although studies illuminated that 70% of the susceptibility to develop TPO-Ab was due to genetic factors, in present study the significant association between Ca, Zn, Mo and TPO-Ab of

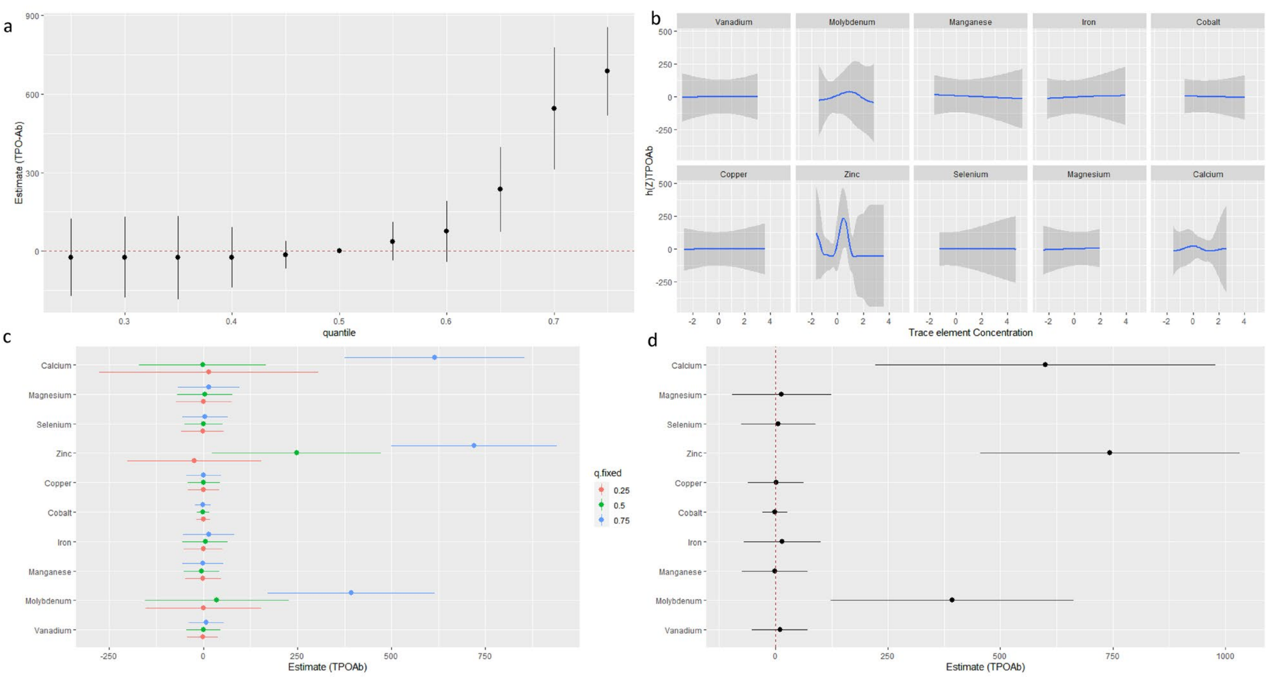

Fig. 6. BKMR model results on TPO-Ab for subjects with TI-RADS-4 thyroid nodule. (**a**): Joint effects (95% CI) of the mixture on TPO-Ab when all trace elements were at a particular quantile was compared to all of them were at their median value. (**b**): Univariate exposure–response functions (95% CI) between trace elements concentrations and TPO-Ab were estimated when all the other elements were fixed at their median levels. (**c**): Individual effects of each trace element were estimated by subtracting the mean outcome value when the single element was at the 25th percentile with the mean value of the outcome when that element was at its 75th percentile, where all of the remaining elements were fixed to a particular quantile (25th, 50th and 75th percentile). (**d**): The single element risks (associated with a change from its 25th to 75th percentile) when the remaining elements were fixed at their 25th percentile was compared to when they were fixed at their 75th percentile. TPO-Ab, thyroperoxidase autoantibodies.

TI-RADS-4 TN subjects cleared that the exploration for environmental factors should be reinforced[38]. Hypercalcemia has been widely reported in cases of thyrotoxicosis and mainly caused by Graves' disease, accounting for approximately 73%[39]. Zn in thyroid autoimmune patients was also positively correlated with thyroid autoantibodies. Experimental mouse models found that chronic exposure to certain levels of Mo can cause histological changes and accelerated malignant transformation to the thyroid follicular cells[40]. Furthermore, the interactions in mixed exposures found in present study showed that Ca, Zn and Mo have a significant impact on the exposure effects of other elements at higher concentrations. The mechanism of interaction need to be further explored.

Multiple associations between trace elements and thyroid function indicators were statistically significant within the normal range observed in general adults. Zn, Mn, Mg, Fe and Cu were associated with altered FT3 or T4 (total or free), but not with TSH, suggestive of secondary effects[41]. There were various ways in which trace elements might interfere with the synthesis and regulation of thyroid hormones[42,43]. Zn is a necessary trace element for the thyrotropin-releasing hormone (TRH) synthesis and acts a fundamental role in protein synthesis, which was involved in thyroid hormone binding transcription[44,45]. Studies indicated that serum Zn levels were high in hyperthyroidism and low in hypothyroidism[46]. Cu and Zn are well-known components of antioxidant defense. Because higher Cu levels were considered to be involved in the pathogenesis of TNs by Cu-MEK1 interaction, detection of Cu element for the clinical management of TNs had been highlighted in some radical studies[12]. However, the present study only supports the detection of Cu element in altered T4 states. The initial step in thyroid hormone synthesis are catalyzed by heme-dependent protein and severe Fe deficiency can reduce TPO activity and interfere with thyroid hormones[40]. A large number of animal and human studies have found that thyroid dysfunction is associated with Fe deficiency anemia by altering Fe utilization and metabolism, whereas excess Fe can induce oxidative stress and lipid peroxidation due to exaggerate autoimmune processes, leading to demyelination of certain autoimmune diseases[47,48]. The essential trace elements are known to influence the thyroid functions at level of action, but very little is known about the interrelationships between Mn and thyroid hormones. FT3 indicated that the high level of Mn may trigger hyperthyroidism[49], and the results of the present study supported this association. Mn might indirectly modulate thyroid hormones by regulating deiodinases or dopaminergic-system. Most notably, compared with other trace elements positive effects, the negative association between Mg and thyroid hormones was shown to be unique. Studies indicated that magnesium antagonised the effect of thyroxine on oxidation in the cells, and the oldest report on the treatment of hyperthyroidism with Mg could be retraced to 1939[50].

Iodine is an important consideration in interpreting study results because its antioxidant effect inhibits the production of hydrogen peroxide (H2O2), which introduce the trace elements into thyroglobulin, finally giving rise to the iodine-containing thyroid hormone[11]. The inconsistent results of studies linking trace elements and TNs may be due to the neglect of iodine concentration at the individual level of the study subjects. In our present study, urine iodine was included as a covariate in the models fitting before and after PSM. This is important because studies report a non-linear relationship between iodine and TNs[34], and disbalances between trace elements supply, iodine utilization, thyroid hormones synthesis have been observed both in humans and animal experimental models[51].

Propensity score matching method, a cornerstone of confounding adjustment in observational studies[52], was adopted for causal inference in a manner similar to randomised experiments. Before PSM, we observed that TNs were more common in females and in the elderly and that they were also frequently associated with fertility, marital status, annual household income, drinking, anxiety, vitamin supplement, tea consumption, hypertension and hyperlipidemia. Study population were foreseeable subject to selection bias. By using PSM, the present study was able to compare the effect of trace elements between groups with similar background characteristics and was more comparable than unadjusted studies.

A number of limitations of this study should be considered. First, we were not able to rule out reverse causality in this cross-sectional study given that exposure and outcome were measured simultaneously. Cross-sectional studies can identify associations, but they cannot determine causality[53]. The lack of long-term follow-up means that the long-term impact of trace element exposure on the development of thyroid nodules and changes in thyroid function cannot be assessed. Thyroid nodules may affect the levels of trace elements, not just the levels of trace elements affecting the occurrence of thyroid nodules. Second, the concentration of trace elements may vary over time, and a single measurement may not accurately reflect an individual's long-term exposure level. This measurement error could lead to inaccurate estimation of the exposure–response relationship. Seeing that TN was a disorder developed over a long time, measurement error of exposure could exist because elemental concentrations collected at a single timepoint only reflected recent exposures, although this error was expected to be non-differential[54]. Third, potential confounding or unmeasured confounding such as other chemical substances may have acted as confounders affecting the observed association. The bioavailability and toxicity of trace elements may vary due to individual differences (such as nutritional status, disease state) and environmental factors (such as the content of trace elements in soil and water sources)[55]. Despite the use of propensity score matching to adjust for known confounding factors, there may still be unmeasured or inadequately controlled confounding factors. Finally, the exposure–response evaluation may have been limited by the sample size and range of exposure levels, which needs to be further explored. Trace elements interact with each other, and these interactions may affect their impact on thyroid nodules and thyroid function. The current study attempted to assess these interactions, but more complex models may be required to fully understand these relationships[56].

Conclusion

This study provides insights into the relationships between trace elements and TNs so as to optimize management approach to TNs. Our results showed that the detection of trace elements for TNs in general adults may be of no significance, but once individuals classified as TI-RADS-4 TNs are detected with abnormal TPO-Ab, Ca, Zn and Mo level are recommended to measure. The substantive association still needs to continue to be further explored.

Data availability

The datasets used during the present study are available from the corresponding author on reasonable request.

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Author contributions
Y.L. developed the study conceptualization and methodology. Y.R.L., P.S. and Y.Y.L. did the hospital-level investigation, supervision, and project administration. J.G.L., Y.F.Z., Y.N.W. and Z.L. conducted laboratory testing. Q.X. and D.D.Z. prepared the original draft and all authors critically reviewed this. Y.L. and L.Z. verified and had full access to the underlying data reported in this manuscript.

Competing interests
The authors declare no competing interests.

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