A follow-up study on factors affecting the rehabilitation of patients with hypothyroidism in different selenium environments

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Abstract

Background

Hypothyroidism is a key manifestation of autoimmune thyroid disease (AITD). Our previous research has found that low selenium (Se) status was linked to an elevated prevalence and incidence of thyroid diseases. We hypothesized that Se status may also influence the restoration of thyroid function. Thus, our study aims to investigate the factors affecting the recovery of thyroid function in patients with (sub-)clinical hypothyroidism, with a specific focus on Se status.

Methods

We conducted a 6-year prospective cohort study comparing different Se concentration regions. Demographic and disease data were collected from 1190 subjects (549 Se-adequate and 641 Se-deficient), who successfully completed the follow-up study in 2019. Additionally, urinary iodine (I) levels, thyroid function, and serum and nail Se levels were measured. We used logistic regression to investigate the relationship between Se deficiency and thyroid function recovery.

Results

Two counties were similar in sex, smoking status. The recovery rate of thyroid function was significantly higher in Se-deficient counties (46.0% vs. 30.6%, P = 0.008). In multivariate analysis, our results show that female sex [OR (95%CI) = 1.977 (1.279–3.055), P = 0.002] and increasing age [OR (95%CI) = 1.027 (1.007–1.047), P = 0.007] were associated with the recovery rate. Additionally, our study revealed that while the Se status was significant in univariate analysis, this association appeared to fade in multivariate analysis.

Conclusions

Female sex and increasing age have unfavorable effects on the recovery of thyroid function in patients with (sub-)clinical hypothyroidism who are over 30 years old.

Introduction

Autoimmune thyroid disease (AITD) is the most common endocrine and autoimmune disease in the general population, characterized by lymphocyte infiltration in the thyroid gland caused by a disordered immune system that attacks thyroid follicular cells, leading to hyperthyroidism or hypothyroidism.\(^1\)–\(^3\) Graves’ disease (GD) is typically characterized by hyperthyroidism, while hypothyroidism is characteristic of Autoimmune thyroiditis (AIT), with some correlation between autoantibody concentrations and disease severity.\(^4\),\(^5\)

The thyroid gland has the highest concentrations of I and selenium (Se) in the human body, both of which are essential for normal thyroid cell function and adequate biosynthesis of thyroid hormones.\(^6\),\(^7\)
The concentration of Se in water and plants in a particular region depends on the Se content of the soil, resulting in varying levels of Se in the blood of individuals living in regions with different Se contents. Observational studies have shown a particular risk of AITD development with insufficient Se intake\textsuperscript{8,9}. However, supplementation studies on AITD have yielded controversial results, especially for AIT, where positive or neutral effects of Se on thyroid peroxidase antibody titers and thyroid gland structure have been reported\textsuperscript{10,11}. The reason for these inconsistent effects is not yet well understood but may be linked to the baseline Se status\textsuperscript{12,13}. Positive effects of Se supplementation have consistently been observed in GD and are included in clinical practice in countries with borderline Se deficiency\textsuperscript{14–18}.

In 2013, we conducted a cross-sectional study of villagers residing in two counties in the Shaanxi Province with different soil Se environments. The data from this study indicated that habitual low Se intake and status were associated with an increased risk of thyroid diseases, particularly a high prevalence of AIT\textsuperscript{19}. In our subsequent follow-up study conducted over six years, we monitored disease incidence and substantiated these findings by revealing an increased incidence of AIT and a high seroconversion rate of thyroid peroxidase antibody (TPO-Ab) in subjects residing in the Se-deficiency area\textsuperscript{20}. Despite this evidence, there is a lack of data on the potential link between selenium intake and thyroid function recovery in patients with AITD. Therefore, we compared patients with AITD in Se-deficient and Se-adequate areas to investigate any potential association.

**Material and Methods**

**1. Study design and Participants**

We conducted a six-year prospective observational cohort study following a baseline cross-sectional survey in 2013 that compared the prevalence of thyroid diseases based on Se status. After six years, participants who had been diagnosed with thyroid-related diseases in the baseline study were invited back and re-examined to determine the progression of their disease and how well their thyroid function had recovered. Data of their Serum Se, TPO-Ab, and thyrotropin levels (TSH), thyroxine (T4), and 3,5,3′-triiodothyronine (T3) concentrations were recorded at baseline. We originally identified 1629 patients in the 2013 baseline survey, but due to adjustments in the type of disease and definition in our study, the resulting total cohort included 1284 subjects who were eligible for follow-up. Of these, 1190 (92.7%) successfully completed the follow-up study in 2019, including 549 from the Se-adequate area and 641 from the Se-deficient area (Fig. 1). A detailed description of our inclusion and exclusion criteria can be found in the supplementary (Table S1).

The main topic of our study is the rehabilitation of patients with (sub-)clinical hypothyroidism. Based on previous research findings, we approximated the thyroid function recovery rate of patients residing in counties with different Se levels\textsuperscript{21–23}. We determined that a sample size of 86 to 280 participants would be necessary to achieve a study power of 90% at an \( \alpha \) level of 5%. However, given the possibility that
individuals may become ineligible or choose to withdraw from the study, our sample size target for patients with (sub-)clinical hypothyroidism was set at 350 participants.

Our study was conducted according to the principles of the Declaration of Helsinki and was approved by the Medical Ethics Committee of Xi’an Jiaotong University (ethical approval file number: #2019-874). All participants provided written informed consent prior to enrollment in this study.

2. Data collection

To ensure consistency among personnel who participated in the field surveys, they received uniform training. Semi-structured questionnaires were used to collect data on follow-up subjects through face-to-face interviews. The questionnaire consisted of 35 items that collected basic information on anthropometrics, social and economic situations, lifestyle, and health status. The information recorded included demographic information, such as date of birth, sex, education, marital status, occupation, and more. Life behavior characteristics, such as smoking status, alcohol consumption, frequency of drinking tea, frequency of eating pickles, type of salt, frequency of consuming kelp/seaweed, were also included. Additionally, disease and health conditions, such as a history of thyroid disease, use of drugs affecting thyroid function, disease type, diagnosis time, and recovery status were recorded. Physical examinations performed during visits included assessment of height, weight, heart rate, thyroid palpation, exophthalmos, thyroid function tests, thyroid B-ultrasounds, and additional analyses. Details are provided in the supplementary (Table S2).

3. Sampling

Experienced nurses collected venous blood samples (5 mL) from a local county hospital to prepare serum. A subset of patients was randomly selected to provide additional samples of 5–10 mL of midstream urine and 1 g of fingernails, along with their serum samples. All specimens were frozen until shipment and analysis.

4. Laboratory analyses

Laboratory analyses were carried out to measure serum Se, nail Se, urinary I, and thyroid function indicators. Serum Se levels were quantified using dual-channel hydride generation atomic fluorescence photometry (AFS-2202E; Beijing Haiguang Instrument Co.). This same method was also used to detect Se levels in nail, water, rice, and wheat samples. A standardized arsenic-cerium-catalyzed spectrophotometric assay was employed to detect urinary I concentrations. Thyroid function was assessed through radioimmunoassay and chemiluminescence for serum TSH, T3, T4, and TPO-Ab, conducted in accordance with the manufacturer’s instructions (Weifang Sanwei Bioengineering Group Co., Ltd., Beijing North Institute of BIOTECHNOLOGY Co., Ltd., Siemens Healthcare Diagnostics Limited). The normal reference values were as follows: TPO-Ab: <35 IU/mL (2013), <15 U/mL (2019); TSH: 0.25 ~ 5 µIU/mL; T3: 0.78 ~ 2.20 ng/mL; T4: 4.2 ~ 13.5 µg/dL.

5. Diagnostic criteria
Thyroid function recovery was defined as a negative TPO-Ab and normal levels of T3, T4, and TSH. In addition to the normal reference value of the instructions, a cut-off value for TPO-Ab positivity was established in our laboratory using 300 healthy individuals not involved in the study, resulting in a value of 15 U/mL (radioimmunoassay). The limit of serum Se deficiency was set at 80 µg/L, which is consistent with our previous analyses 19. The diagnostic criteria for thyroid diseases are provided as a supplement (Table S3).

6. Statistical analysis

For continuous variables, the mean and standard deviation (X ± SD) were used if they followed a normal distribution. If they did not follow a normal distribution, the median and interquartile range (M, IQR) were used instead. Categorical variables were reported as frequencies and percentages. Differences between groups were compared using the chi-square test and rank-sum test (Mann-Whitney U and Kruskal-Wallis tests). Differences between continuous variables with skewed distributions between two groups were compared using the Mann-Whitney nonparametric test. We employed the Wilcoxon signed-rank test to compare the differences in serum Se content between baseline and follow-up. Multivariate regression analysis was conducted to explore the association between demographic characteristics, life behavior characteristics, and the recovery rate of thyroid function, as well as to estimate the odds ratio (OR) and 95% confidence interval (CI). We also used a restricted cubic spline (RCS) to explore the association between age at follow-up and significant thyroid function recovery by sex. Statistical analysis was performed using SPSS version 26.0, GraphPad Prism v.8 (GraphPad Software Inc., San Diego, CA, USA), and R statistical software version 4.2.1 (package rms, ggplot2). Statistical significance was defined as a P value < 0.05 for two-tailed analysis.

Results

1. Study participants and their demographic and lifestyle characteristics by county

The two groups did not significantly differ in terms of sex and smoking status. However, significant between-group differences were observed in age, education, BMI, and alcohol consumption. In the survey, subjects from the Se-deficient county were younger on average than those from the Se-adequate county (median age: 56.0 vs. 61.0, Z = -5.530, P < 0.001). The level of education was higher in the Se-deficient counties in general (P < 0.001). Also, subjects from the Se-deficient county displayed a relatively high consumption of alcohol, with 24.5% drinking occasionally and 8.9% drinking frequently (Table 1).
Table 1
Demographic and lifestyle characteristics of the participants in the two counties

<table>
<thead>
<tr>
<th></th>
<th>Se-adequate</th>
<th>Se-deficient</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(549)</td>
<td>%</td>
<td>N(641)</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Age2019(years)</td>
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<tr>
<td>18–30</td>
<td>3</td>
<td>11</td>
<td>10.872</td>
<td>0.012</td>
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<tr>
<td>31–40</td>
<td>32</td>
<td>42</td>
<td></td>
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<tr>
<td>41–50</td>
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<tr>
<td>&gt;=51</td>
<td>442</td>
<td>470</td>
<td></td>
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<tr>
<td>Gender</td>
<td></td>
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<td></td>
</tr>
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<td>Male</td>
<td>122</td>
<td>173</td>
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<td>0.058</td>
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<tr>
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<td>427</td>
<td>468</td>
<td></td>
<td></td>
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<tr>
<td>Education</td>
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<td></td>
<td>46.777</td>
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<td>395</td>
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<td>70</td>
<td>162</td>
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<td></td>
</tr>
<tr>
<td>High school / technical secondary school</td>
<td>24</td>
<td>52</td>
<td></td>
<td></td>
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<tr>
<td>University and above</td>
<td>17</td>
<td>32</td>
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<td>527</td>
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<td>Occasional smokers</td>
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<tr>
<td>Frequent smokers</td>
<td>67</td>
<td>98</td>
<td></td>
<td></td>
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<tr>
<td>BMI</td>
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<td></td>
<td>25.944</td>
<td>&lt;0.001</td>
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<tr>
<td>&lt;18.5</td>
<td>29</td>
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<td>18.5 ~ 23.99</td>
<td>350</td>
<td>317</td>
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<tr>
<td>24 ~ 27.99</td>
<td>144</td>
<td>222</td>
<td></td>
<td></td>
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<tr>
<td>&gt;=28</td>
<td>26</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td>13.862</td>
<td>0.001</td>
</tr>
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<td>Never</td>
<td>418</td>
<td>427</td>
<td></td>
<td></td>
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<tr>
<td>Occasional</td>
<td>102</td>
<td>157</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent</td>
<td>29</td>
<td>57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
During the follow-up investigation, we randomly selected some participants from both counties to test the levels of Se and urinary I concentrations in their nails. The subjects from the Se-adequate county had higher median Se content in their nails than subjects from the Se-deficient county (sample size: Se-adequate vs. Se-deficient: 47 vs. 74; 627.3 µg/kg vs. 358.0 µg/kg, Z = -8.381, P < 0.001). On the other hand, subjects from the Se-deficient county displayed higher urinary I concentrations (sample size: Se-deficient vs. Se-adequate: 81 vs. 66; 167.6 µg/L vs. 279.2 µg/L, Z = -4.187, P < 0.001). The information on the changes in serum Se between the two investigations was provided as a supplement (Figure S1).

2. Recovery rate of thyroid function by county

After excluding the patient effect of drugs, the recovery of thyroid function in patients with subclinical hypothyroidism residing in Se-deficient areas was significantly better when compared to those in Se-adequate areas (46.0% vs. 30.6%, P = 0.008), and the recovery in all other diseases did not show significant differences between the two areas (Table 2).

Due to the limited number of individuals with clinical hypothyroidism, we combined them with subclinical hypothyroidism patients. We found that subjects residing in Se-deficient areas still had a greater rate of recovery of thyroid function (44.4% vs. 32.7%, \( \chi^2 = 4.613, P = 0.032 \)). Thus, we focused the main topic of our next analysis on the subclinical and clinical hypothyroidism patients, collectively referred to as (sub-)clinical hypothyroidism, based on the recovery data of various disorders.

| Table 2: Recovery rates of thyroid function in various AITD subjects in areas with different Se levels |
|-----------------|---------------|-----------------|--------------|---|---|
|                  | Se-adequate   | Se-deficient    | \( \chi^2 \) | \( P \) |
|                  | N  | recovery % | N  | recovery % |             |
| Subclinical hyperthyroidism | 17 | 8  | 9  | 4  | 0.613\textsuperscript{a} |
| Hypothyroidism    | 12 | 6  | 30 | 9  | 0.749  |
| Subclinical hypothyroidism | 98 | 30 | 285 | 131 | 7.054 | \textbf{0.008} |
| HT + GD           | 36 | 3  | 29 | 2  | 0.047  |
| Single TPO-Ab positive | 73 | 19 | 86 | 28 | 0.809  |
| Total thyroid disorders | 236 | 66 | 439 | 174 | 9.122 | \textbf{0.003} |
| Subjects with positive TPO-Ab at baseline | 131 | 26 | 160 | 39 | 0.851  |

\textsuperscript{a} The \( P \) value came from Fisher’s Exact Test.

\textsuperscript{b} The \( P \) value came from Continuity Correction.
3. Parameters affecting the recovery rate of thyroid function in (sub-)clinical hypothyroidism

This study included 425 participants with (sub-)clinical hypothyroidism. During the six-year observation period, an exploratory analysis was performed to investigate potential factors affecting the recovery of thyroid function in patients with (sub-)clinical hypothyroidism. The variables significantly correlated with thyroid function recovery included place of residence (whether the county was Se-deficient or Se-sufficient), age, sex, smoking status, and alcohol intake (Table 3). Participants who consumed alcohol in Se-deficient areas showed a higher rate of recovery of thyroid function. Compared to men, women exhibited a lower rate of recovery of thyroid function.
Table 3
Potential parameters affecting the recovery rate of thyroid function in (sub-)clinical hypothyroidism.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Recovery N(176)</th>
<th>Recovery rate (%)</th>
<th>Non-recovery N(249)</th>
<th>Non-recovery rate (%)</th>
<th>χ²/Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se-adequate</td>
<td>36 20.5</td>
<td>29.7</td>
<td>74 29.7</td>
<td>32.7</td>
<td>4.613</td>
<td>0.032</td>
</tr>
<tr>
<td>Se-deficient</td>
<td>140 79.5</td>
<td>70.3</td>
<td>175 70.3</td>
<td>44.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–30</td>
<td>5 2.8</td>
<td>1.6</td>
<td>4 1.6</td>
<td>55.6</td>
<td>2.904</td>
<td>0.407</td>
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<tr>
<td>31–40</td>
<td>13 7.4</td>
<td>7.2</td>
<td>18 7.2</td>
<td>41.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41–50</td>
<td>39 22.2</td>
<td>16.9</td>
<td>42 16.9</td>
<td>48.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=51</td>
<td>119 67.6</td>
<td>74.3</td>
<td>185 74.3</td>
<td>39.1</td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
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<td>-2.707</td>
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<td>Serum Se at baseline</td>
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<td>-0.530</td>
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<tr>
<td>Male</td>
<td>69 39.2</td>
<td>27.3</td>
<td>68 27.3</td>
<td>50.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>107 60.8</td>
<td>72.7</td>
<td>181 72.7</td>
<td>37.2</td>
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<td>65.9</td>
<td>164 65.9</td>
<td>38.9</td>
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<tr>
<td>middle school</td>
<td>47 26.7</td>
<td>22.9</td>
<td>57 22.9</td>
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<td>High school / technical secondary school</td>
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<td>8.0</td>
<td>20 8.0</td>
<td>46.0</td>
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<td>8 3.2</td>
<td>50.0</td>
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<td>Smoking status</td>
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<td>Never</td>
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<td>83.5</td>
<td>208 83.5</td>
<td>38.5</td>
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<td></td>
</tr>
<tr>
<td>Occasional smokers</td>
<td>3 1.7</td>
<td>4.4</td>
<td>11 4.4</td>
<td>21.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent smokers</td>
<td>43 24.4</td>
<td>12.0</td>
<td>30 12.0</td>
<td>58.9</td>
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<tr>
<td>BMI</td>
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<td></td>
<td>6.685</td>
<td>0.083</td>
</tr>
<tr>
<td>&lt; 18.5</td>
<td>9 5.1</td>
<td>10.4</td>
<td>26 10.4</td>
<td>28.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Recovery | Non-recovery | Recovery rate (%) | $\chi^2/Z$ | $P$
---|---|---|---|---
18.5 ~ 23.99 | 91 | 51.7 | 136 | 54.6 | 40.7
24 ~ 27.99 | 62 | 35.2 | 65 | 26.1 | 48.7
>=28 | 14 | 8.0 | 22 | 8.8 | 40.6

Alcohol consumption
Never | 99 | 56.3 | 178 | 71.5 | 35.7
Occasional | 54 | 30.7 | 50 | 20.1 | 51.9
Frequent | 23 | 13.1 | 21 | 8.4 | 52.3

4. Multivariate analysis of parameters affecting the recovery rate of thyroid function in (sub-)clinical hypothyroidism

Based on the results of binary logistic regression analysis, female sex and increasing age were identified as factors hindering thyroid function recovery (Table 4). The area of residence showed significance in the univariate analysis. However, multifactor analysis appeared to exclude its effect, while BMI appeared to be unrelated to the recovery of thyroid function.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>OR 95%CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
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<td>Location</td>
<td>Se-adequate</td>
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</tr>
<tr>
<td></td>
<td>Se-deficient</td>
<td>0.626</td>
<td>0.365–1.073</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
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<tr>
<td></td>
<td>Female</td>
<td>1.977</td>
<td>1.279–3.055</td>
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<tr>
<td>Age</td>
<td>1.027</td>
<td>1.007–1.047</td>
<td>0.007</td>
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<tr>
<td>BMI</td>
<td>18.5 ~ 23.99</td>
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<td></td>
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<tr>
<td></td>
<td>&lt; 18.5</td>
<td>1.934</td>
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<td></td>
<td>24 ~ 27.99</td>
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<td>0.450–1.115</td>
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<td></td>
<td>&gt;=28</td>
<td>1.068</td>
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<td>Serum Se at baseline</td>
<td>0.997</td>
<td>0.991–1.004</td>
<td>0.404</td>
</tr>
</tbody>
</table>

*Dependent variable assignment: Thyroid function turns recovery – 1, others – 2.
We conducted an in-depth analysis to determine how sex and age at follow-up affected research outcomes ($P_{\text{overall}} = 0.0019$). By stratifying the results by sex, we found that the recovery of thyroid function varied with age in subjects of different sexes, with females exhibiting an OR of 1 at age 51 (beyond which the OR significantly increased with age) and males showing the same trend at age 67. When we focused on OR95%CI, we observed a threshold of 55 years old. When less than 55 years old, male sex showed the protective effect, while over 55 years old, female sex showed the risk effect (Fig. 2).

**Discussion**

**1. Complex interrelationship between Se status and hypothyroidism**

This 6-year prospective cohort study aimed to investigate the recovery rate of thyroid function in patients with AITD (especially hypothyroidism) residing in two counties in the Shaanxi Province with different soil Se levels and associated differences in habitual Se intake. The Se concentrations in the nail and serum samples of the participants supported the selection of the areas and revealed a Se status almost two times higher in the Se-adequate county of Ziyang compared to the Se-deficient county of Ningshan, which was consistent with the baseline survey. Notably, the cure rate for thyroid function, particularly (sub-)clinical hypothyroidism, was higher in the Se-deficient counties (Ningshan).

The effects of Se supplementation in patients with thyroid disease may differ based on their baseline levels. Several intervention studies have reported no health benefits from Se intake or any effect on thyroid gland appearance or thyroid autoantibody titters. However, these studies cannot be directly compared to the present analysis due to differences in study design, intervention duration, Se form, and dosage. Nonetheless, some Se supplementation trials have reported positive effects on thyroid ultrasound appearance and autoantibody titters. For instance, a seminal randomized control study using 200 µg of sodium selenite per day for 3 months or later studies testing a similar regimen with varying dosages of selenite or selenomethionine over different periods of time. Notably, most of these studies were conducted in areas with low Se supply, where supplemental Se served as substitution therapy rather than pharmacological therapy. The underlying mechanism likely involves increased biosynthesis of selenoproteins.

Two focused studies have supported the general relevance of selenoproteins in autoimmune disease development and immune system function. These studies highlighted the central role of glutathione peroxidase 4 (GPX4) in preventing ferroptosis of autoimmune-relevant neutrophils and follicular helper T cells’ ability to mount antibody responses to infection and vaccination. Therefore, severe Se deficiency is a relevant and preventable risk factor for the development of autoantibodies and autoimmune disease. This notion was supported by a sufficiently sized randomized controlled study, which showed a significant reduction in postpartum thyroiditis when supplemental Se was given to pregnant women who were healthy but positive for TPO-Ab. Again, this study design is not perfectly...
compatible with our prospective cohort study reported here but clearly highlights the relevant role of Se deficiency in thyroid autoimmunity. While the study design is not entirely comparable with our prospective cohort study, it highlights the significant role of Se deficiency in thyroid autoimmunity. However, our analysis showed that thyroid function recovery rates were not significantly higher in TPO-Ab positive patients residing in Se-deficient areas. Collectively, these findings emphasize a complex but significant relationship between Se status and thyroid diseases.

The higher recovery rate of thyroid function in the Se-deficient area may be related to the tendency of a better Se status in the enrolled participants, and there is a possibility of gender bias in Se-deficient areas, with a higher proportion of males residing in these regions. Our study found that the higher recovery rate of thyroid function in Se-deficient areas could be attributed to increased population mobility, higher living standards, and reduced Se shadowing due to geographic restrictions. The thyroid gland actively accumulates Se and has the highest Se content in the human body. Hence, even a subtle increase in serum Se levels could be associated with a better Se status in the thyroid gland and immune cells. Though the theory is yet to be tested in humans, our longitudinal analysis of thyroid hormone changes suggests that an improved Se status supported the recovery of thyroid disease. For example, the TSH improved to normal in 21.1% of subjects residing in Se-deficient counties compared to -3.2% in the Se-adequate area, while T3 improved in 14.1% of subjects compared to 13.8%, and T4 improved in 7.5% of subjects compared to 5.1%. These findings further support the interpretation of an improved Se status supporting the recovery of thyroid disease.

2. Other factors affecting thyroid function

I plays a crucial role in thyroid hormone synthesis, and its intake has a “U” shaped response on human health. Insufficient or excessive intake can negatively impact health. We found that the urinary I level in Se-deficient counties was significantly higher than that in Se-adequate counties, but both groups were I-sufficient. Previous studies have indicated that the I enhancement policy in China has helped achieve the lowest prevalence of thyroid-related diseases when the median urinary I concentration ranged between 100 and 300 µg/L, which was the case for both groups of subjects in our study. Therefore, this policy likely improved I intake in both counties and did not appear to affect the outcome of our study.

In our final multivariable analysis, age and sex were found to be related to the rate of thyroid function recovery, which is consistent with previous studies. As shown in Fig. 2, there is a significant gender difference at the age of 55, with men under 55 acting as a protective factor (OR < 1) and women over 55 as a risk factor (OR > 1), which might be linked to changes in human sex hormones. Previous research reported that the prevalence of thyroid disease increases in postmenopausal women. Furthermore, a study stratified by menopause exploring the relationship between polybrominated diphenyl ethers (PBDEs) (an environmental toxicant that disrupts both thyroid hormones and estrogenic activity) and thyroid disease in women suggested that altered estrogen levels during menopause may enhance the disruption of thyroid signaling by PBDEs. These findings demonstrate that sex hormone presence in the body can influence thyroid function.
3. Limitations

This was a large longitudinal cohort spanning six years, testing the rarely addressed issue of an environmental factor affecting the recovery of thyroid function in subjects with (sub-)clinical hypothyroidism. The relatively long follow-up duration and high follow-up rate strengthened the analysis and improved the reliability of our findings. Among the relevant limitations of our study is the study type, an observational study does not allow firm causal inferences, and we cannot guarantee that the same group of observed subjects is always at the same exposure level, which may have an impact on the final outcomes.

Our study is a large longitudinal cohort study spanning six years, addressing the rarely explored issue of environmental factors affecting the recovery of thyroid function in subjects with (sub-)clinical hypothyroidism. The relatively long follow-up duration and high follow-up rate reinforce the analysis and improve the reliability of our findings. Among the relevant limitations of our study is the observational study design, which does not allow firm causal inferences. Additionally, we cannot ensure that the same group of observed subjects is always at the same exposure level, which might impact the final outcomes. At the same time, due to financial constraints, we are unable to detect the urinary iodine concentration of all subjects, which may lead to some bias.

Moreover, we have made some adjustments to the type of disease and study population definition to include more patients, resulting in some loss of follow-up. Therefore, there are some data differences between this study and the baseline cross-sectional study\(^{19}\). Nevertheless, it is essential to note that these limitations do not diminish the significance of our findings.

Conclusions

Our six-year follow-up study revealed that female sex and increasing age in individuals over 30 years old have negative effects on the recovery of thyroid function in patients with (sub-)clinical hypothyroidism, with menopause potentially being an essential period. However, no significant effect of Se on the recovery of patients with (sub-)clinical hypothyroidism was observed, indicating Se’s complex manifestation that requires further exploratory research.

Abbreviations
Abbreviations | Meaning
---|---
AITD | Autoimmune thyroid disease
GD | Graves’ disease
AIT | Autoimmune thyroiditis
TPO-Ab | Thyroid peroxidase antibody
HT | Hashimoto thyroiditis
TSH | Thyrotropin levels
T4 | Thyroxine
T3 | 3,5,3′-triiodothyronine
GPX4 | Glutathione peroxidase 4
PBDEs | Polybrominated diphenyl ethers
Se | Selenium
I | Iodine
OR | Odds ratio
CI | Confidence interval
RCS | Restricted cubic spline

**Declarations**

**Ethics approval and consent to participate** Our study was conducted according to the principles of the Declaration of Helsinki and was approved by the Medical Ethics Committee of Xi’an Jiaotong University (ethical approval file number: #2019-874). All participants provided written informed consent prior to enrollment in this study.

**Consent for publication** Not applicable.

**Availability of data and materials** The datasets generated and/or analysed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

**Competing interests** We affirm that the research described is original and has not been previously published, nor is it being considered for publication elsewhere, either in whole or in part. All authors have approved the submission of this manuscript and declare no conflicts of interest.

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References


Figures
Figure 1

Study process flowchart including number of participants and laboratory analyses.
Figure 2

Association between follow-up age and significant thyroid function recovery by gender.

**Supplementary Files**

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- FigureS1.tiff
- Appendices.docx