Study on the association between trace elements and thyroid nodule in Guangxi, China

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Abstract

In addition to genetic factors, environmental exposures are considered to be one of the most important factors affecting thyroid dysfunction in humans. And some trace elements and metal elements in patients often interact with related proteins and hormones, leading to thyroid dysfunction. This study investigated the relationship of selected elements on thyroid nodule in a population from Guangxi. Blood and urine collected from 96 participants (48 patients with thyroid nodules and 48 controls) were analyzed to determine the hormones in serum as well as the elements in urine using kits as well as inductively coupled mass spectrometry, respectively. The relationship between demographics, clinical characteristics and urinary levels of characteristic thyroid trace elements (T3, T4 and TSH) was investigated using Chi-square test, Pearson correlation analysis, independent sample T-test and Kruskal-Wallis test (K-W test). In the serum, T3/T4 and TSH were lower in the nodule group than in the control group, but there was a correlation between T3 and T4 and the elements in the nodule group. In urine, the concentrations of Cd and Sr were higher and the values of Se, Fe and V were lower in the urine of patients with thyroid nodules compared to controls, and there was a correlation between the various elements. This study showed a correlation between thyroid nodules and exposure to environmental elements, particularly among the various elements in the organism of patients with thyroid nodules.

Introduction

The environment and the thyroid

In recent decades, the incidence of thyroid cancer in China has been rising year by year, especially in the female population. The latest data shows that the incidence of thyroid cancer in women has risen from the seventh to the third highest in China's cancer epidemiological statistics, a 20-fold increase compared to 2000 [1]. The global incidence of thyroid disease is also increasing [2], and some scholars have suggested that this is related to overdiagnosis [3, 4]. The more definitive factor influencing thyroid disease is elemental iodine, which is essential for thyroid hormone synthesis, and its scarcity or oversupply may lead to thyroid dysfunction [5]. As research into thyroid disease continues, we are becoming aware that abnormalities in serum trace elements can also have an impact on the development of thyroid disease.

The environment is awash with elements, and all mineral elements are considered potentially toxic, including heavy metals and trace elements. Previously, a significantly higher incidence of thyroid cancer was reported in populations exposed to low doses of metallic elements in volcanic areas over a long period of time [6]. In fact, the incidence of thyroid cancer is distributed worldwide, and high incidences of thyroid cancer have been reported in some areas with excellent environments. People living in the natural environment for long periods of time are necessarily exposed to a wide range of environmental factors, and the environment is considered to be one of the important factors affecting thyroid cancer in addition to genetic factors [7].

Hormones, Elements And The Thyroid
The thyroid hormone profile includes TSH, T3 and T4. Thyroid-stimulating hormone (TSH) is a known thyroid growth factor and serum TSH levels can be used as a predictor of staging of differentiated thyroid cancer and can be used to predict the likelihood of cancer [8]. Thyroxine (T4) and triiodothyronine (T3) are key enzymes in the synthesis of thyroid hormones [9]. Trace elements are elements that make up just under 0.01% of the total body weight and include Iron (Fe), Zinc (Zn), Copper (Cu), Cadmium (Cd), Selenium (Se), Cobalt (Co) and Fluorine (F). Trace elements are not abundant but are closely related to the health of the human body. In fact, trace elements can act as endocrine disruptors to interfere with the thyroid gland, and there have been studies on the alteration of trace elements in thyroid disease, reporting that certain element ratios can be used as blood markers for thyroid disease [10].

Trace elements have some anti-cancer and cancer prevention, and a randomized study showed that Zn and Magnesium (Mg) supplements may be beneficial in patients with hypothyroidism and in diseases associated with hyperthyroidism [11]. In areas with or without I deficiency, together with adequate iodine intake, adequate intake of Se and Zn may prevent the formation of thyroid nodules [12]. At the same time, excess or deficiency of trace minerals can cause disease, resulting in thyroid dysfunction and even thyroid tumors. Se is a life-essential trace mineral that is the backbone of thyroid function and the active component of thyroid selenium protein [13]. It has been shown that low serum Se concentrations are associated with larger thyroid volumes and a higher prevalence of Goiter [14]. Low Se status is associated with an increased risk of thyroid disease, and increased Se intake may reduce the risk of disease in areas with low Se intake [15]. In this study, we investigated the correlation between environmental element intake and the development of thyroid nodules in a Guangxi population by measuring trace elements in the serum and urine of selected populations for the prevention and treatment of thyroid cancer.

Methods

Methodology of the study population

The main types of thyroid nodules are nodular goiter, toxic nodular goiter, isolated thyroid nodules, thyroid cancer and thyroid cysts. The majority of patients participating in this study had benign thyroid nodules. The remaining serum levels of triiodothyronine (T3), thyroxine (T4) and serum thyrotropin (TSH) in thyroid patients better reflect the differences in hormone levels between patients and the normal population, while the differences in trace elements metabolized in urine better reflect the effects of environmental factors on both populations. In this regard, to study the effect of environmental factors exposure mainly through elements on thyroid disease, we randomly selected 96 people who participated and could provide accessible data from the Guangxi Key Laboratory on Prevention and Treatment for Thyroid Tumor (located at the Second Affiliated Hospital of Guangxi University of Science and Technology) between September 2021 and April 2022, with an overall mean age of 53.29±13.32 years, divided into is 48 patients with confirmed thyroid nodules and 48 people without thyroid nodules to create separate cohorts of nodule and control groups.

Serum hormone and protein determination methods

In this study, we investigated the correlation between hormones, trace elements and thyroid nodules in the blood of patients with thyroid nodules. We collected blood and urine for serological tests from patients and
control persons who participated in this experiment. High-density lipoprotein (HDL) cholesterol test kits (direct method-catalase scavenging), triglyceride test kits (GPO-PAP method), total cholesterol test kits (CHOD-PAP method), FT3 and FT4 assay kits (chemiluminescence method) were used. T3, TSH and T4 are measured using chemiluminescence, a paramagnetic particle chemiluminescence immunoassay that uses the Access immunoassay system to quantify T3 and T4 levels in human serum and plasma. Access Total T4 is a sample added to a reaction tube containing anti-T4, as well as thyroxine-alkaline phosphatase conjugate, paramagnetic particles encapsulated with goat antibody mouse capture antibody and an extraction reagent that separates T4 from the bound protein. In contrast, TSH is detected by a Chemiluminescent immuno-sandwich assay method in which one anti-TSH monoclonal antibody is labelled with FITC, another TSH monoclonal antibody is labelled with ABEI and a goat anti-FITC antibody is encapsulated in magnetic microspheres.

**Urine trace element determination method**

The method used for this test was the National Standard Method for the Determination of Occupational Exposure in People. Trace elements were determined in the urine samples by ICP-MS. The urine samples were first diluted with 1% nitric acid solution and then the trace elements, especially metals, were determined by inductively coupled plasma spectrometry (ICP-MS), the mass-to-charge ratios of the ionized elements were separated and characterized, the charge ratios of the elements were tested and quantified using an internal standard curve. More than 80% of the measured values of V, Fe, Ni, Cu, Zn, As, Se, Sr, Cd and Cs were above the instrumental detection limits and could be included in the statistical analysis.

**Statistical analysis**

All analyses (including Chi-square test, independent samples T-test, Pearson correlation) and means, standard deviations, medians, and interquartile spacing were performed and calculated using SPSS 26 (IBM SPSS Statistics, ver.26.0). Continuous variables in this study were expressed using means and standard deviations, and categorical variables were expressed using median and interquartile spacing ratios. Chi-square test, Pearson correlation analysis, independent samples T-test and Kruskal-Wallis test (K-W test) were used to examine the relationship between sociodemographic, clinical characteristics and urinary characteristic levels of thyroid trace elements (T3, T4 and TSH), respectively. Table 1 shows the demographics of the nodule group, which were analyzed using a Chi-square test. Table 2 shows the serum assays for the nodule group population, expressed as means and standard deviations, and the independent samples T-test was used to verify differences between the data sets. Table 3 reports the Pearson correlation analysis between serum thyroid hormones and urine concentrations of each metal element in the nodule group population. Table 4 reports the urine elemental tests for the nodule and control groups, using the K-W test for significant differences between the two data sets (Table 5). Table 6 reports the Pearson correlation analysis between the elements in the nodule group.

**Results**

**Demographic analysis**
In this demographic analysis, the mean age of the subjects in the control group was 55.6±14.91 years and the mean age of the subjects in the nodal group was 51.0±11.21 years and the Chi-square test was performed on the number of age groups, which showed no significant difference in age. (Table 1)

**Statistical analysis of serum results: protein, hormone and elemental link**

We detected trace elements (Potassium (K), Sodium (Na), Chloride (Cl), Calcium (Ca)) as well as lipids (cholesterol (TC), triglycerides (TG), high density protein (HDL) and low density protein (LDL)) and indicators of thyroid function (TSH, T3, T4 and FT3, FT4) in both groups, using the mean and standard deviation to express the overall picture of the data in both groups. (Table 2)

The TSH level in the thyroid nodule group (2.10±3.25 μIU/mL) was lower than the TSH level in the control group (2.79±3.04 μIU/mL), the T3 level in the thyroid nodule group (1.34±0.28 nmol/L) was significantly lower than the T3 level in the control group (1.78±2.48 nmol/L), while the T4 level in the thyroid nodule group (126.93±29.79 nmol/L) was significantly higher than that in the control group (121.97±31.96 nmol/L). Using independent sample T-test on serum test results from both groups, we found significant differences between the nodule group and the control group for sodium (Na, \( p=0.001 \)), chloride (Cl, \( p=0.001 \)), calcium (Ca, \( p=0.034 \)) and HDL (\( p=0.049 \)) in serum.

To investigate the relationship between thyroid hormones and urinary elements in the nodal group, we used Pearson correlation analysis (Table 3) and found a positive correlation between T3 and V (\( r=0.3055, p=0.0347 \)); a significant positive correlation between T4 and Cd (\( r=0.5438, p=0.0001 \)), while Cu (\( r=0.4564, p=0.0011 \)), Ni (\( r=0.3758, p=0.0085 \)), Se (\( r=0.3749, p=0.0086 \)), Fe (\( r=0.3581, p=0.0124 \)), Zn (\( r=0.3235, p=0.0249 \)) and T4 had a weaker positive correlation.

**Statistical analysis of urine results: correlation between elements**

We tested urine and the final measurements obtained for the various elements were described overall using median and interquartile spacing (Table 4) and the Kruskal-Wallis non-parametric test was performed on the data sets (Table 5). Significant differences were found for Fe (\( p=0.002 \)) and V (\( p=0.015 \)) in the thyroid nodule group, with a significant positive effect on thyroid nodules.

To investigate whether there was an association between the elements, a Pearson correlation analysis was performed for each element (Table 6). In this analysis, we found positive correlations between elements, with high correlations between Vanadium (V) and Cu (\( r=0.6120, p=0.0000 \)) and Cesium (Cs, \( r=0.5104, p=0.0002 \)), and with Se (\( r=0.4632, p=0.0009 \)), Arsenic (As, \( r=0.4456, p=0.0015 \)), Zn (\( r=0.4282, p=0.0024 \)), Ni (\( r=0.3962, p=0.0053 \)), Strontium (Sr, \( r=0.3832, p=0.0072 \)), Fe (\( r=0.3380, p=0.0188 \)), and Cd (\( r=0.3105, p=0.0317 \)) had a weaker correlation.

Significant correlations existed between Fe and Ni (\( r=0.7327, p=0.0000 \)), Se (\( r=0.5403, p=0.0001 \)), Cu (\( r=0.5072, p=0.0002 \)) and weaker correlations with Cs (\( r=0.4341, p=0.0021 \)). Whereas there was a high correlation between Ni and Se (\( r=0.6319, p=0.0000 \)), Cu (\( r=0.5886, p=0.0000 \)), Cs (\( r=0.5327, p=0.0001 \)) and a weak correlation with Zn (\( r=0.4310, p=0.0022 \)). There was a high correlation between Cu and Se (\( r=0.7091, p=0.0000 \)), Cs (\( r=0.7044, p=0.0000 \)), Zn (\( r=0.6556, p=0.0000 \)), Cd (\( r=0.6521, p=0.0000 \)), while Sr (\( r=0.4455, p=0.0000 \))
As (r= 0.3616, p=0.0116) were weakly correlated. There was a significant correlation between Zn and Se (r=0.6640, p=0.0000) and a weaker correlation between As and Cd (r=0.3769, p=0.0083). There was a significant correlation between Se and Cs (r=0.7253, p=0.0000). There was a significant correlation between Sr and Cd (r= 0.5434, p=0.0001), while Cd was weakly correlated with Cs (r=0.4092, p=0.0039).

Discussion

The intake of micronutrients such as Iodine (I) was initially used to treat goiter, however, with the consumption of Iodized salt, some scholars suspect that thyroid disease in our country seems to be associated with an increased intake of Iodized salt, and related studies have shown that people who do not consume both Iodized salt and milk have a higher risk than those who consume both Iodized salt and milk [16]. Another study showed that patients with thyroid nodules had a higher incidence in the Iodine-deficient group than in the Iodine-sufficient, Iodine-excess group [17]. In addition to I, Se, Fe, Zn and Cu are also involved in the synthesis of thyroid hormones, particularly Se and Fe, which are factors limiting epidemiological and interference studies in benign thyroid disease [18]. A paper published by Hanif et al [19] showed that Fe levels were significantly increased in the hypothyroid group compared to the control group, suggesting a correlation between Fe and hypothyroidism. Khatiwada et al [20] conducted a cross-sectional study and found a significant negative correlation between TSH and hemoglobin and transferrin saturation. In the present study, there was a significant correlation between elemental Fe in the thyroid nodule group compared to the control population.

Se is an essential element for thyroid hormone metabolism [21]. Winther, K. H. et al [22] noted that Se concentrations in the functioning thyroid gland are generally higher than in other tissues and organs and therefore selenium supplementation is often used as a supplement for thyroid disorders. Some scholars have found that pregnant populations and higher FBG may suggest a risk by thyroid nodules [23]. The increased demand for Se in the pregnant population may also lead to selenium deficiency [24, 25], which may affect thyroid-related hormone levels leading to thyroid disease. In the present study we found that urinary selenium was significantly lower in people with thyroid disease than in controls, and by Pearson correlation analysis we found a positive correlation between Se and T4, consistent with the results obtained by Winther, K. H.

Many metals can cause a variety of reactions in biological systems. Low levels of potentially toxic heavy metals (As, Cu, Cd, Ag) can stimulate cellular activity in vitro [26, 27] and in vivo [28, 29]. Several studies have shown that Cd as a metallic element, although not as a true carcinogen affecting the body's thyroid activity, exposure to higher levels of Cd may be beneficial as a promoter of thyroid cancer initiation [6]. Whereas in a study by Ferrari et al [30], Cd was found to accumulate in the thyroid, pancreas, liver as well as kidneys, and diseases such as multinodular goiter occur in the thyroid in response to chronic Cd toxicity, a portion of the study also indicated that higher Cd and Pb may suggest an association with the development of hypothyroidism [31, 32]. In the current study, we observed that patients in the nodular group had Cd values twice as high as those in the control group and a significant positive correlation with T4 in the thyroid nodular group, consistent with this finding.
The current study also found lower values for the metal elements Fe, Zn and Cu relative to the control group, while Cu is mainly captured in the blood by copper cyanide (CP), and some studies have shown that urinary Cu decreases in patients with hyperthyroidism and increases in patients with hypothyroidism [19, 33]. In the present study, we found a significant correlation between Cu and T4 in the thyroid nodule group, suggesting that elemental Cu levels in patients with thyroid nodules are consistent with those in patients with hyperthyroidism. Zn is generally transported in serum bound to albumin, and in the hyperthyroid state, serum albumin levels decrease, Zn-albumin complexes decrease and Zn metabolism increases [34]. In addition, Zn is required for the receptor activity of thyroid hormone (T3) and for the conversion of thyroxine to triiodothyronine and affects T4 levels by increasing the production of thyroxine-binding protein [35]. In the present study, mean urinary Zn levels were lower in the nodule group than in the control group, yet T4 was higher in the nodule group than in the control group, and correlation study analysis found a correlation between Zn and the nodule group hormone as well, consistent with this finding.

In this study, we found that serum TSH and T3/T4 were significantly higher in the control group than in the thyroid nodule group. TSH is a serum thyrotropin, a hormone secreted by the pituitary gland in the hypothalamic-pituitary-thyroid axis that has the function of promoting thyroid secretion hormones and is generally used clinically as an indirect response to thyroid function. Some reports have shown that T4 levels in mice are reduced after 4 weeks of exposure to higher levels of As compounds, and that they are carcinogenic to mice. However, after 8 weeks of exposure, T4 levels in mice increased significantly and T4 was also shown to inhibit As accumulation in mice [36]. In the present study, both T4 and FT4 levels were higher in the thyroid nodule group than in the control group, while As levels were slightly lower in the nodule group than in the control group, but Pearson correlation analysis showed that there was no correlation between As and thyroid-related hormones in the nodule group, which may be due to differences in species.

A point worth noting is that most of the literature we referenced was in English and most of the population indicators referenced were some data from other countries. In addition, the current study has several limitations. Firstly, the population sample size is too small and there is still a large gap for the overall population, and many statistics may have a large bias; secondly, the amount of information collected is not sufficient and comprehensive, apart from the analysis of the population serum and urine sample data, some participants did not measure the effects of other hormones on thyroid hormones, and it was not possible to measure patients’ BMI, FBG, and estrogen and progesterone in patients with thyroid nodules. Thus I think we need to refine this area a little more.

**Conclusion**

The incidence of thyroid disease is currently increasing worldwide year by year, and although many studies have been done on the factors influencing thyroid disease, describing a correlation between concentrations of various metal elements and thyroid dysfunction, the exact mechanism of action has not been confirmed by definitive evidence, but the effect of environmental exposure, harmful substances such as metal elements on thyroid disease is one of the more definite factors that have been associated. We found in this study of the thyroid nodule population that there are also links between elements such as Fe, Zn, Cu, Cd and Se and thyroid-related hormones, and between elements, and that the relationship between hormones and elements
and elements in the organism is complex, suggesting that environmental factors and elemental content between different regions have some influence on the organism, and we believe that further research can continue in this direction.

Declarations

Author Contribution XZ L and CL W: project development, data collection, data analysis, and manuscript writing; ML H, MM H: data collection and manuscript writing. LT C, JJ L, XH X: contributed to data acquisition. N L, MT Q and XL L: contribute to sample testing. All authors read and approved the final manuscript.

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Ethics Approval This study was approved by the Ethics Committee of the Second Affiliated Hospital of Guangxi University of Science and Technology.

Consent to Participate All participants were informed and agreed to provide information for this study.

Conflict of interest The authors declare that they have no conflict of interest.

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**Tables**

**Table 1** Demographic analysis of nodal groups
| Variables                                      | Thyroid nodule | Chi-square test | Controls |
|-----------------------------------------------|----------------|-----------------|----------|
|                                               | N=48           | 100% (total)    | N=48     | 100% (total) |
| Gender                                        | Male           | 4 8.33          | 25 52.08 |
|                                               | Female         | 44 91.67        | 23 47.92 |
| Age                                           | <20years       | 0 0             | 1 2.08   |
|                                               | 21-30years     | 3 6.25          | 1 2.08   |
|                                               | 31-40years     | 7 14.58         | 6 12.5   |
|                                               | 41-50years     | 10 20.83        | 6 12.5   |
|                                               | 51-60years     | 20 41.67        | 15 31.25 |
|                                               | >60years       | 8 16.67         | 19 39.58 |
| Thyroid                                       | No             | 0 0             | 48 100   |
|                                               | Thyroid nodule | 46 95.83        | 0 0      |
|                                               | Thyroid nodule and hyperthyroidism | 1 2.08 | 0 0 |
|                                               | Thyroid nodule and nail cancer | 1 2.08 | 0 0 |

**Table 2** Thyroid hormone levels in the study population

**Table 3** Correlation analysis of thyroid hormones and elements in the nodal group
| Serological Assays | Thyroid nodule | Control group |
|-------------------|---------------|---------------|
|                   | $N=48$        | $N=48$        |
| K (mmol/L)        | 3.97±11.21    | 3.99±0.34     |
| Na (mmol/L)       | 143.54±1.65$^a$ | 140.67±4.47   |
| Cl (mmol/L)       | 106.41±2.24$^a$ | 103.65±5.71   |
| Ca (mmol/L)       | 2.33±0.09$^a$  | 2.30±0.12     |
| TC (mmol/L)       | 4.99±1.12     | 4.85±1.12     |
| TG (mmol/L)       | 1.38±0.26     | 1.73±1.62     |
| HDL (mmol/L)      | 1.42±1.26$^a$ | 1.29±0.42     |
| LDL (mmol/L)      | 2.98±0.89     | 2.78±0.88     |
| TSH (μIU/mL)      | 2.10±3.25     | 2.79±3.04     |
| T3 (nmol/L)       | 1.34±0.28     | 1.78±2.48     |
| T4 (nmol/L)       | 126.93±29.79  | 121.97±31.96  |
| FT3 (pmol/L)      | 4.64±0.51     | 4.60±0.55     |
| FT4 (pmol/L)      | 20.83±14.85   | 18.25±3.05    |

$^a$: implies a significant difference between the two sets of data
Table 4 Elemental concentrations (ng/ml) in the urine of the study population

| Element | T3    | T4    | TSH   | FT3   | FT4   |
|---------|-------|-------|-------|-------|-------|
| V       | 0.3055b | 0.0538 | -0.2067 | 0.2641 | -0.0109 |
| P       | 0.0347 | 0.7166 | 0.1586 | 0.0697 | 0.9412 |
| Fe      | 0.2032 | 0.3581b | -0.0881 | 0.0976 | 0.0077 |
| p       | 0.1660 | 0.0124 | 0.5515 | 0.5092 | 0.9587 |
| Ni      | 0.1703 | 0.3758b | -0.0543 | 0.0136 | -0.0782 |
| p       | 0.2473 | 0.0080 | 0.7142 | 0.9267 | 0.5972 |
| Cu      | 0.1939 | 0.4564b | -0.1891 | -0.1309 | -0.1016 |
| p       | 0.1867 | 0.0011 | 0.1980 | 0.3752 | 0.4920 |
| Zn      | -0.1068 | 0.3235b | -0.1767 | -0.1462 | 0.0048 |
| p       | 0.4701 | 0.0249 | 0.2296 | 0.3216 | 0.9742 |
| As      | 0.1519 | 0.1983 | 0.0180 | 0.0134 | -0.0712 |
| p       | 0.3026 | 0.1767 | 0.9036 | 0.9280 | 0.6308 |
| Se      | -0.0072 | 0.3749b | -0.2306 | -0.1266 | -0.0144 |
| p       | 0.9611 | 0.0086 | 0.1149 | 0.3911 | 0.9228 |
| Sr      | 0.2285 | 0.2195 | -0.1088 | 0.0523 | -0.0935 |
| p       | 0.1183 | 0.1340 | 0.4617 | 0.7241 | 0.5275 |
| Cd      | 0.0606 | 0.5438a | -0.0637 | -0.2672 | -0.0759 |
| p       | 0.6827 | 0.0001 | 0.6673 | 0.0664 | 0.6083 |
| Cs      | 0.0971 | 0.1889 | -0.1021 | -0.0943 | -0.0429 |
| p       | 0.5114 | 0.1985 | 0.4901 | 0.5239 | 0.7724 |

a: greater than or equal to 0.5 indicates high correlation
b: between 0.3 and 0.5 indicates a low correlation
| Element | N=48 | M(Q1, Q3 ) |
|---------|------|------------|
| V       | Thyroid nodule | 0.11(0.08, 0.16) \(^a\) |
|         | Controls      | 0.16(0.11, 0.22) |
| Fe      | Thyroid nodule | 21.68(18.84, 29.75) \(^a\) |
|         | Controls      | 29.28(20.53, 50.77) |
| Ni      | Thyroid nodule | 2.26(1.61, 5.71) |
|         | Controls      | 3.38(2.17, 4.97) |
| Cu      | Thyroid nodule | 12.30(7.33, 14.52) |
|         | Controls      | 13.05(8.88, 16.83) |
| Zn      | Thyroid nodule | 403.66(189.52, 643.48) |
|         | Controls      | 508.70(301.88, 734.13) |
| As      | Thyroid nodule | 22.49(16.87, 33.62) |
|         | Controls      | 24.62(15.35, 36.70) |
| Se      | Thyroid nodule | 21.97(11.55, 32.98) |
|         | Controls      | 23.95(14.30, 36.04) |
| Sr      | Thyroid nodule | 104.59(60.87,150.80) |
|         | Controls      | 96.14(44.70, 142.63) |
| Cd      | Thyroid nodule | 2.27(1.04, 4.03) |
|         | Controls      | 1.70(0.88, 3.75) |
| Cs      | Thyroid nodule | 7.72(5.14, 12.27) |
|         | Controls      | 6.80(4.57, 11.30) |

\(^a\): implies a significant difference between the two sets of data.

Table 5  Kruskal-Wallis test for trace elements in the nodal group

|     | V   | Fe  | Ni   | Cu   | Zn   | As   | Se   | Sr   | Cd   | Cs   |
|-----|-----|-----|------|------|------|------|------|------|------|------|
| K-W | 9.928 | 5.954 | 3.197 | 1.392 | 1.701 | 0.048 | 1.192 | 0.310 | 0.735 | 0.735 |
| Sig.(p) | 0.002\(^a\) | 0.015\(^a\) | 0.074 | 0.238 | 0.192 | 0.826 | 0.275 | 0.578 | 0.391 | 0.391 |

\(^a\): significant at \(p < 0.05\) in contrast to the control.
Table 6 Correlation analysis between nodal group elements

|       | V   | Fe   | Ni   | Cu   | Zn   | As   | Se   | Sr   | Cd   |
|-------|-----|------|------|------|------|------|------|------|------|
| Fe    | 0.3380\(^b\) | 0.0188 |      |      |      |      |      |      |      |
| Ni    | 0.3962\(^b\) | 0.7327\(^a\) |      |      |      |      |      |      |      |
| Cu    | 0.6120\(^a\) | 0.5072\(^a\) | 0.5886\(^a\) |      |      |      |      |      |      |
| Zn    | 0.4282\(^b\) | 0.2334 | 0.4310\(^b\) | 0.6556\(^a\) |      |      |      |      |      |
| As    | 0.4456\(^b\) | 0.2482 | 0.2587 | 0.3616\(^b\) | 0.2850 |      |      |      |      |
| Se    | 0.4632\(^b\) | 0.5403\(^a\) | 0.6319\(^a\) | 0.7091\(^a\) | 0.6640\(^a\) | 0.2116 |      |      |      |
| Sr    | 0.3832\(^b\) | 0.1296 | 0.1078 | 0.4455\(^b\) | 0.3700\(^b\) | 0.2735 | 0.2490 |      |      |
| Cd    | 0.3105\(^b\) | 0.2224 | 0.1780 | 0.6521\(^a\) | 0.4780\(^b\) | 0.3769\(^b\) | 0.3154\(^b\) | 0.5434\(^a\) |      |
| Cs    | 0.5104\(^a\) | 0.4341\(^b\) | 0.5327\(^a\) | 0.7044\(^a\) | 0.4630\(^b\) | 0.2627 | 0.7253\(^a\) | 0.2084 | 0.4092\(^b\) |
| p     | 0.0002 | 0.0021 | 0.0001 | 0.0000 | 0.0009 | 0.0712 | 0.0000 | 0.1552 | 0.0039 |

\(a\): greater than or equal to 0.5 indicates high correlation

\(b\): between 0.3 and 0.5 indicates a low correlation