Biociencias
ISSN electrónico: 2390-0512

Association of the Thyroid-Stimulating Hormone, Thyroxine and Triiodothyronine with the Heavy Metals Lead and Mercury in Patients with Suspected Hyperthyroidism

Asociación de la hormona estimulante de tiroide, tiroxina y triyodotironina con los metales pesados plomo y mercurio en pacientes con posible hipertiroidismo

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Recibido: 10/06/2024
Aceptado: 16/01/2025

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DOI: https://doi.org/10.18041/2390-0512/biociencias.2.11756

Abstract

Introduction: Humans are exposed daily to various chemical elements through water, food and use of personal hygiene and cosmetic products that can cause alterations in the multiple systems of the human body, including the endocrine system. In this sense, the objective of the study was to associate the concentration of thyroid stimulating hormone (TSH), free thyroxine (T3F) and free triiodothyronine (T4F) with the levels of lead (Pb) and mercury (Hg) in patients with suspected hyperthyroidism. **Materials and Methods:** The study group (SG) consisted of 20 patients regardless of sex and a control group (CG) of 20 individuals with no history of underlying pathologies. Method atomic absorption, coupled to a hydride generator, were used for Pb and Hg analysis, the biological sample being whole blood (Pb) and partial urine (Hg). Hormones were analyzed in serum by chemiluminescence. **Results:** the concentration of TSH, T3F and T4F in the SG were 0.46 ± 0.08 µUI/L, 8.34 ± 0.45 pg/mL and 1.25 ± 0.23 ng/mL. Statistical analysis yielded significant difference for TSH and T3F with respect to CG (p=0.046 and 0.021). Pb and mercury levels for the SG were 11.54 ± 0.75 µg/dL and 8.43 ± 0.11 µg/g of creatinine, values above the permissible limits for these two metals and statistically significant with respect to the SG. **Conclusions:** This study suggests a potential association between Pb and Hg with THS and T3F alteration, together with other toxicological aspects to which the patients may be associated.

Keywords: Endocrine, Thyroid, Xenobiotics, Toxicology.

Resumen

Introducción: los humanos estamos expuestos diariamente a diversos elementos químicos a través del agua, alimentos y uso de productos de aseo y belleza personal que pueden ocasionar alteraciones en los diversos sistemas del cuerpo humano, entre ellos el sistema endocrino. En tal sentido en el estudio se plateó como objetivo asociar la concentración de la hormona estimulante de tiroides (TSH), tiroxina libre (T3L) y triyodotironina libre (T4L) con los niveles de plomo (Pb) y mercurio (Hg) en pacientes con posible hipertiroidismo. **Materiales y Métodos:** El grupo estudio (GE) estuvo conformado por 20 pacientes sin distinción de sexo y 20 de un grupo control (GC) sin historia de patologías de base. Para el análisis de Pb y Hg se empleó absorción atómica , acoplado a generador de hidruros, siendo la muestra biológica sangre total (Pb) y orina parcial (Hg). Las hormonas fueron analizadas en suero por quimioluminiscencia. **Resultados:** la concentración de la THS, T3L y T4L en el GE fueron 0,46 ± 0,08 µUI/L; 8,34 ± 0,45 pg/mL y 1,25 ± 0,23 ng/mL. El análisis estadístico arrojó diferencia significativa para la TSH y T3L respecto al GC (p=0,046 y 0,021). Los niveles de Pb y Hg para el GE fueron de 11,54 ± 0,75 µg/dL y 8,43 ± 0.11 µg/g de creatinina, valores por encima de los límites permisibles para estos dos metales y estadísticamente significativos respecto al GE. **Conclusión:** este estudio permite establecer una posible vinculación del Pb y Hg con la alteración de la TSH y T3L, aunado a otros aspectos toxicológicos a los que pueden estar asociados los pacientes.

Palabras clave: endocrino, tiroides, xenobióticos, toxicología.

Introducción

The thyroid gland is regulated by the pituitary gland which secretes thyroid-stimulating hormone (TSH) to produce thyroxine (T3) and triiodothyronine (T4); when the levels of these are sufficient in the blood the pituitary gland detects the levels and then reduces the secretion of TSH and therefore the levels of T3 and T4 are maintained within normal limits, playing a very important role in the organism as they regulate growth and development, cardiac activity and blood pressure and the way in which the body uses and stores energy (1,2,3). One of the most common disorders is hyperthyroidism or thyrotoxicosis, which results from excessive synthesis of thyroid hormone (4). The prevalence of this pathology in the general population is 0.5%, and it occurs more frequently in women than in men. It has been observed that in women it appears between the third and fourth decade, while in men the highest incidence occurs in the last decades of life (5). These patients may present insomnia, irritability, psychomotor agitation, affective lability, memory impairment and in some cases, psychotic symptoms (6,7).

Currently,there are a large number of toxic substances to which we are exposed through environmental pollution, which occurs globally. These substances are toxic not only to humans but also to ecosystems and are used indiscriminately mainly for economic reasons (8). Among these toxic agents are lead and mercury, which are heavy metals with well-known toxic effects. These effects occur due to exposure to these elements or to compounds containing them. However, studies on the effect of Pb and Hg on thyroid function and particularly on TSH and T3 free levels are scarce (9)(Figure 1).

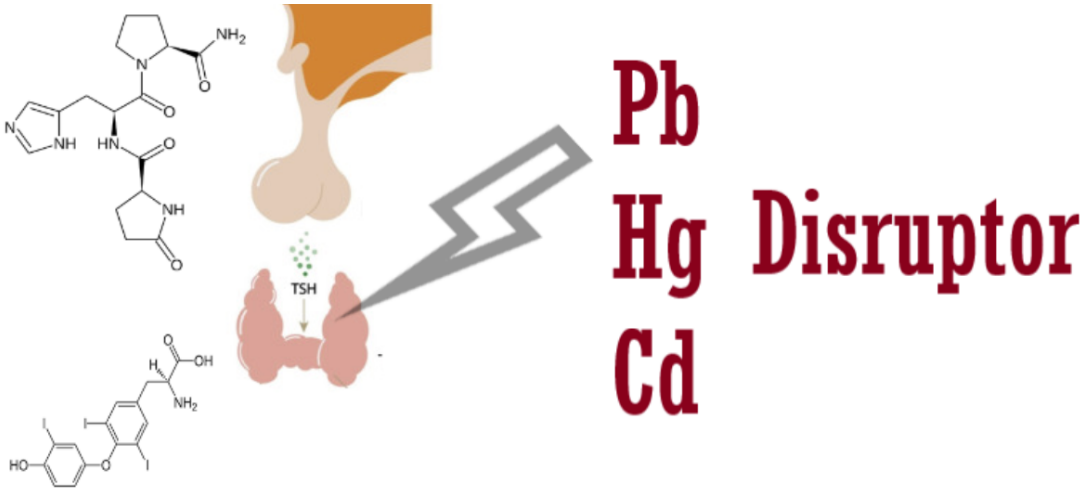

Figure 1. Heavy metals as endocrine disruptors.

Source: own elaboration.

Based on the problems described above, and that currently specialists in cardiology and endocrinology have requested the determination of these metals in this type of patients the present study aimed to evaluate the relationship between the concentration of thyroid stimulating hormone (TSH), free thyroxine (T3F) and free triiodothyronine (T4F) with the levels of lead (Pb) and mercury (Hg) in patients (adults) with suspected hyperthyroidism.

Materials and Methods

The study sample consisted of 20 individuals of both sexes with a diagnostic impression of possible hyperthyroidism and 20 individuals with no underlying pathology (control group). The following criteria were taken into consideration for sample selection:

a. Inclusion criteria

1. Individuals of both sexes willing to participate voluntarily in the study.
2. Over 18 years of age.
3. Non-smokers and low or sporadic alcohol consumption.

b. Exclusion criteria

1. Individuals suffering from chronic or hematological diseases.
2. Persons occupationally exposed to heavy metals.

Biological sample

Each individual participating in the study provided a specific urine sample (first-morning, before starting activities), in clean plastic containers, after instructions for correct collection. Samples were refrigerated between 2°C and 8°C and transported to the FITOQUIMICA20 C.A Laboratory.

For blood sample collection, the rules of asepsis and antisepsis were followed. 10 mL of blood was drawn from the antecubital vein with a 12 mL disposable injector and a 21G x 1" needle. The contents of the injector were then gently transferred into two tubes previously labeled with the patient's data. Five milliliters of blood were placed in a tube with two drops of ethylenediaminetetraacetic acid (EDTA) for Pb analysis. The remaining 5 ml were intended for the analysis of hormones.

Determination of mercury

It was performed by method cold vapor atomic absorption spectrophotometry, using the method recommended by the National Institute for Occupational Safety and Health (NIOSH), (10). To 4 mL of uncentrifuged urine, 7 mL of 65% nitric acid (HNO3) (Merck KGaA, Germany) was added. After 5 minutes, 60 mL of deionized water was added and, to reduce the mercury ion Hg2+ to its elemental form and initiate the emission of cold vapors, 1 mL of 20% SnCl2 solution prepared from of SnCl2•2H2O ACS 98% (Sigma-Aldrich Co., USA). The absorbance measurement of the samples at 253.7 nm (maximum absorption at the mercury resonance line) was performed with a Bacharach® MAS-50B cold vapor spectrophotometer. A calibration line was elaborated using a mercury chloride standard in a range of concentrations from 0.2 to 3 µg/dL.

Creatinine determination

Creatinine analysis by the modified Jaffe method is based on reacting the sample with sodium picrate, in an alkaline medium, to form a red chromogen with an absorption maximum at 510 nm (11). Analytical results are frequently expressed in micrograms of mercury per gram of creatinine. The method consists of diluting the urine sample with distilled water (1/100) to a final volume of 5 mL. An aliquot of 0.5 mL of sample was taken, 0.5 mL of distilled water and 2 mL of alkaline picrate were added. The latter reagent was prepared by mixing 20 mL of a saturated aqueous solution of ACS 99% picric acid (Merck KGaA , Germany) and 4 mL of 10% NaOH ACS 97% (Sigma-Aldrich Co., USA).

Determination of lead by atomic absorption

Blood collected in polyethylene tubes with heparin as an anticoagulant is hemolyzed. Lead is complexed with ammonium pyrrolidinedithiocarbamate (APDC) and the complex formed is extracted with methyl isobutyl ketone (MIBK). The lead contained in the organic phase is determined by flame Atomic Absorption Spectrophotometry, at a wavelength of 283.3 nm, using a direct quantification method. To determine analyte concentrations in a sample, the absorbances of standard solutions or standards of known analyte concentrations were first determined (12).

The value of these absorbances was then plotted against the concentrations, thus obtaining the calibration curve (concentration range from 0.1 to 10 µg/dL with an R2 of 0.99 to validate the straight line). Generally, analyte concentrations that have a linear relationship with absorbance are used, becoming known as the absorbance/concentration relationship “Calibration line”. Once the calibration line was established, the readings were taken and the concentration of the analyte was obtained (13).

Determination of TSH, T3 and free T4 in serum

The commercial brand Chroma™ was used, which is a lateral flow chromatography fluorescence immunoassay (FIA) for the quantitative determination of the level of Thyroid Stimulating Hormone (TSH), Free T3 and Free T4 in serum or plasma.

Bioethical considerations

In order to adequately select participants, informed consent was obtained after explaining the objectives to the patients and volunteers. The research adhered to the criteria established in the fifth revision of the Declaration of Helsinki.

Statistical Analysis

Metals, creatinine and thyroid profile were analyzed in triplicate. A descriptive statistical analysis was performed using measures of dispersion and central tendency, such as mean and standard deviation. Likewise, association and comparison tests(t-Student and Pearson correlation) were applied using the statistical program, Statistix10.0 for Windows.

Results

Sociodemographic Aspects and TSH, T3F and T4F Levels

In order to carry out the present investigation, 12 women and 8 men with an average age of 39 years with possible hyperthyroidism participated. The control group consisted of 20 people, 9 women and 11 men with an average age of 41 years, who met the parameters previously established in the research. In relation to the concentration of the hormones, a decrease in TSH and an increase in T3L were observed, being statistically significant with respect to the control for both hormones (Table 1).

Tabla 1. Levels of TSH (µUI/L), T3F (pg/mL), and T4F (ng/mL) in both groups

Test	Study Group (M ± SD)	Control Group (M ± SD)	P
TSH	0.46 ± 0.08	3.56 ± 0.12	0.046 *
T3F	8.34 ± 0.45	3.12 ± 0.16	0.021 *
T4F	1.25 ± 0.23	1.38 ± 0.12	0.841

(*) Significant if $p < 0.05$; M = mean; SD = standard deviation.

Source: own elaboration.

Lead and mercury levels in both groups

With respect to the analysis of Pb in whole blood and Hg in partial urine, it was found that the study group presented values above the biological exposure index or permissible limit (BEIs) established by the American Conference of Governmental Industrial Hygienists Biological (ACGIH), (14), with Pb values up to 8 µg/dL for non-occupationally exposed women and Hg values up to 5 µg/g creatinine. Additional relevant data are presentedin Table 2.

Tabla 2. Concentration of lead (µg/dL) and mercury (µg/g of creatinine)

Metal	Estudy Group (M ± SD)	Control Group (M ± SD)	P
Pb	11.4 ± 0.75	6.08 ± 0.18	0,012 *
Hg	8.43 ± 0.11	3.92 ± 0.23	0,031 *

(*) Significant if p < 0.05; M = mean; SD = standard deviation.

Source: own elaboration.

Association Between TSH, T3F Levels, and Pb and Hg Concentrations When TSH and T3F levels were associated with Pb and Hg concentration, a significant negative association was observed for TSH (r=0.997 and r= 0.993), while for T3F there is a tendency to increase the levels of this hormone as the concentration of Pb Hg increases (r=0.997 and 0.976). The above is shown in Figures 1a-b, 2a-b, 3a-b.

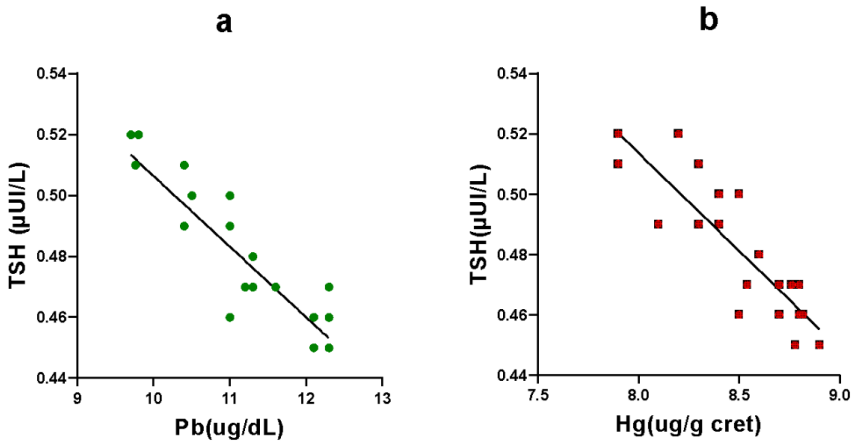

Figure 1. Relationship of TSH levels with heavy metal concentrations in blood and urine of the study group.

Source: own elaboration.

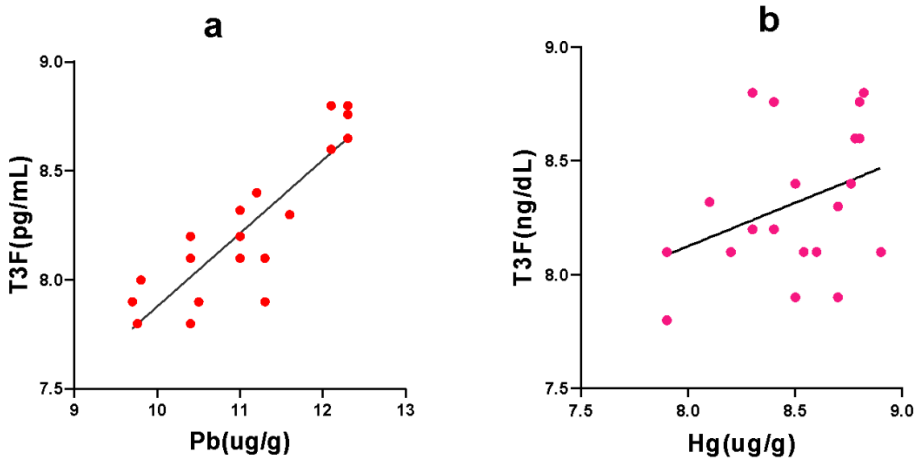

Figure 2. Relationship of T3F levels with heavy metal concentrations in blood and urine of the study group.

Source: own elaboration.

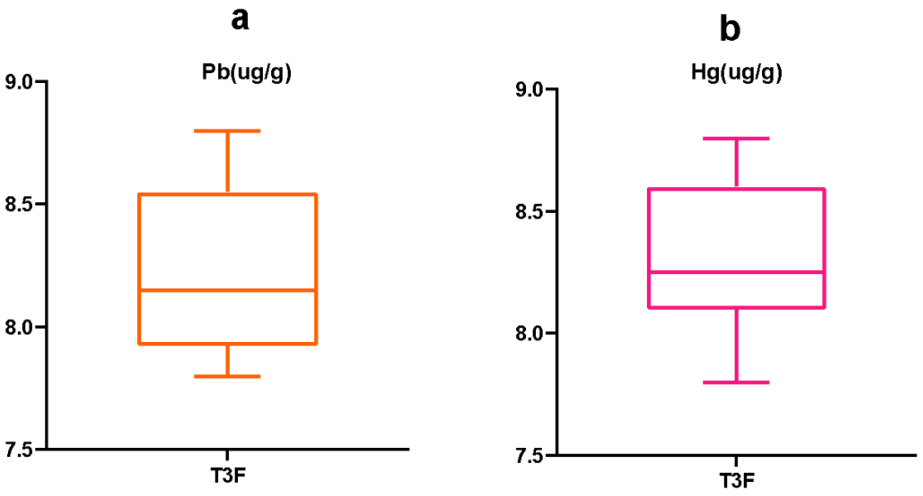

Figure 3. Distribution of T3F levels with heavy metal concentrations in blood and urine of the study group.

Source: own elaboration.

Discussion

The Agency for Toxic Substances and Disease Registry (ATSDR), (15), indicates that there is evidence that certain heavy metals such as Pb and Hg can affect the endocrine system as an endocrine disruptor, affecting the physiological value of hormones and causing an endocrine imbalance. In the present study, it was shown that the free TSH, T3 hormones were outside the reference values, which is associated with what has been reported by various epidemiological studies that relate exposure to Pb and Hg with alteration of thyroid function, as well as alteration of cortisol and insulin levels (16, 17). Previous publications include retrospective human studies that have correlated exposure to Pb, Hg and even Cd with alterations in thyroid hormone function, finding a positive correlation between blood and urine concentrations of these metals with all thyroid hormones, as well as thyroglobulin (Tg) (18). According to Kim et al. (1), Disrupted thyroid homeostasis plays a role in neurocognitive dysfunction and metabolic disorders. Since individuals are exposed to multiple metals simultaneously, it is important to assess the effects of metal mixtures on thyroid hormone status.

To understand what has been expressed above, it is essential to know that the toxic action of heavy metals in living organisms occurs through the blocking of biological activities, that is, enzymatic inactivation by the formation of bonds between the metal and sulfhydryl groups (-SH) and other functional groups of proteins and enzymes, causing irreversible damage in different organisms, displacing other metal ions or modifying the active conformation of biological molecules.

Vukelić et al. (19), in their study, explored the influence of Pb on thyroid hormones and thyroid-related antibody levels in the rat model and revealed that low doses of Pb cause an increase in thyroid hormones (T4, FT4 and TSH) in rats after subacute exposure, while they had no impact on T3, T3F, anti-TPO and anti-Tg. Thus, they emphasize that the dose-dependent effects were the increase in T4 and T4F, where in addition, the in silico toxicogenomic data analysis showed that the main molecular pathways related to Pb-induced hyperthyroidism are connected to 14 genes. A study by Nie et al. (20), which included 5628 Chinese adults, showed the relationship of heavy metal levels in blood and serum antibodies against thyroid proteins and thyroid dysfunction reflected by total T3, total T4 and TSH levels.

Endocrine disruption has become a major human health problem, but it is difficult to study outside the laboratory for several reasons, including the multiplicity of exposures, the difficulty in assessing each exposure, and the variety of possible outcomes among human populations. In this regard, Castiello et al. (21), examined the association of urinary concentrations of arsenic (As), cadmium (Cd), mercury (Hg), nickel (Ni), lead (Pb), manganese (Mn) and chromium (Cr) with blood pressure (BP) and serum hormone levels in male adolescents in Spain, showing significant associations between Hg and increased testosterone and luteinizing hormone (LH) and decreased thyroid-stimulating hormone (TSH); between the combination of As and Hg and

increased LH and insulin-like growth factor 1; between Cr and decreased TSH; and between Cd and increased adrenocorticotropic hormone, concluding that these findings suggest that combined exposure to toxic metals, especially As and Cd, may contribute to elevated BP in male adolescents and that exposure to Hg, As, Cd and Cr may affect their hormone levels. Also considering that the world population is increasing and therefore more pregnancies, Gustin et al. (22), highlights that exposure to Cd and Hg, at levels globally prevalent through the diet, may affect thyroid function during pregnancy, independently of iodine and selenium levels. Further studies on potential implications for maternal and child health are warranted.Finally, a pilot study conducted by Nascimento et al. (23), investigated the possible association between exposure to these xenobiotics and thyroid dysfunction in children living in a rural community in southern Brazil, and found that elevated levels of these metals, along with higher levels of Pb and Hg, were associated with alterations in thyroid hormones.

Conclusions

Statistically significant differences were found in the average concentrations of TSH, T3F, lead and mercury in the study group. These results allow establishing a possible association of the aforementioned metals with hyperthyroidism, leaving then for the medical evaluation the definitive diagnosis and to consider within its profile the analysis of heavy metals.

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