



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

Franklin Pacheco-Coello ¹ .

Pilar Nieto ² . ORCID: [HTTP://ORCID.ORG/000](http://ORCID.ORG/000)

¹ Universidad de Carabobo sede Aragua, Escuela de Bioanálisis,
Departamento de Ciencias Básicas, Laboratorio de Metales Pesados y
Solventes Orgánicos, Laboratorio de Desarrollo Biotecnológico
"FITOQUIMICA20" C.A.

² Licenciada en Bioanálisis Laboratorio Clínico BioSalud, Barinas.
0-0002-2765-4069 fpacheco2@uc.edu.ve

Correspondencia: Instituto de Medicina Tropical - Facultad de Medicina -
Universidad Central de Venezuela.

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RESUMEN

Introducción: diariamente estamos expuestos 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 planteó 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 TSH, T3L y T4L en el GE fueron $0,46 \pm 0,08 \mu\text{UI/L}$; $8,34 \pm 0,45 \text{ pg/mL}$ y $1,25 \pm 0,23 \text{ ng/mL}$. 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 $11,54 \pm 0,75 \mu\text{g/dL}$ y $8,43 \pm 0,11 \mu\text{g/g}$ de creatinina, valores por encima de los límites permisibles para estos dos metales y estadísticamente significativos respecto al GC. **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.

ASSOCIATION OF THE THYROID STIMULATING HORMONE, THYROXINE AND TRIIODOTYRONINE WITH THE HEAVY METALS LEAD AND MERCURY IN PATIENTS WITH POSSIBLE HYPERTHYROIDISM

SUMMARY

Introduction: daily we are exposed to various chemical elements through water, food and use of personal hygiene and beauty products that can cause alterations in the various 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 (T3L) and free triiodothyronine (T4L) with the levels of lead (Pb) and mercury (Hg) in patients with possible hyperthyroidism. **Materials and Methods:** The study group (SG) consisted of 20 patients regardless of sex and 20 of a control group (CG) 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, T3L and T4L in the SG were $0.46 \pm 0.08 \mu\text{UI/L}$, $8.34 \pm 0.45 \text{ pg/mL}$ and $1.25 \pm 0.23 \text{ ng/mL}$. Statistical analysis yielded significant difference for TSH and T3L with respect to CG ($p=0.046$ and 0.021). Pb and mercury levels for the SG were $11.54 \pm 0.75 \mu\text{g/dL}$ and $8.43 \pm 0.11 \mu\text{g/g}$ of creatinine, values above the permissible limits for these two metals and statistically significant with respect to the SG. **Conclusions:** this study allows establishing a possible link between Pb and Hg with TSH and T3L alteration, together with other toxicological aspects to which the patients may be associated.

KEY WORDS: Endocrine, Thyroid, Xenobiotics, Toxicology

ASOCIACIÓN DE LA HORMONA ESTIMULANTE DE TIROIDES, TIROXINA Y TRIYODOTIRONINA CON LOS METALES PESADOS PLOMO Y MERCURIO EN PACIENTES CON POSIBLE HIPERTIROIDISMO

INTRODUCTION

The thyroid gland is regulated by the pituitary gland by secreting 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 because they regulate growth and development, cardiac activity and blood pressure and the way in which the body uses and stores energy (Bhakat et al., 2023; Durá-Travé & Gallinas-Victoriano, 2024). One of the most common disorders is hyperthyroidism or thyrotoxicosis, which results from an excess in the synthesis of thyroid hormone (Alexander et al., 2017). 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 (Korevaar, 2017). These patients may present insomnia, irritability, psychomotor agitation, affective lability, memory impairment and sometimes psychotic symptoms (Stagnaro-Green, 2017).

At present 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 (Tang et al., 2021). 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 (Desai et al., 2022).

Based on the problems described above, and that currently specialists in radiology and endocrinology have requested the determination of these metals in this type of patients the present study aimed to relationship 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 possible hyperthyroidism.

MATERIALS AND METHODS

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

a. Inclusion criteria

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

b. Exclusion criteria

4. Individuals suffering from chronic or hematological diseases.
5. Persons occupationally exposed to heavy metals.

Biological sample

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

For the extraction of blood samples, the rules of asepsis and antisepsis were followed. 10 mL of blood was extracted from the antecubital vein with a 12 mL disposable injector and a 21G x 1" needle, then the contents of the injector were slowly deposited into two tubes previously identified with the patient's data. 5 mL of blood was placed in a tube with two drops of ethylenediaminetetraacetic acid (EDTA) for Pb analysis. The other 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, 1994). 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 also 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 (Delanghe & Speeckaert, 2011). 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 (Frank et al., 2029).

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 R^2 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 (Martínez, 2020).

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, where the objectives of the study were explained 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 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 Level

When TSH and T3F levels were associated with Pb and Hg concentration, it was found that there is a significant negative association 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 and 2a-b.

Table 1. Levels of TSH ($\mu\text{UI/L}$) T3F (pg/mL) and T4F (ng/mL) in both groups

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

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

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 American Conference of Governmental Industrial Hygienists Biological (ACGIH, 2015), with Pb values up to $8 \mu\text{g/dL}$ for non-occupationally exposed women and Hg values up to $5 \mu\text{g/g}$ creatinine. Other data of interest are shown in Table 2.

Table2. Concentration of lead ($\mu\text{g/dL}$) and mercury ($\mu\text{g/g}$) of creatinine)

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

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

TSH and T3F levels were associated with Pb and Hg concentration

When TSH and T3F levels were associated with Pb and Hg concentration, it was found that there is a significant negative association 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 and 2a-b.

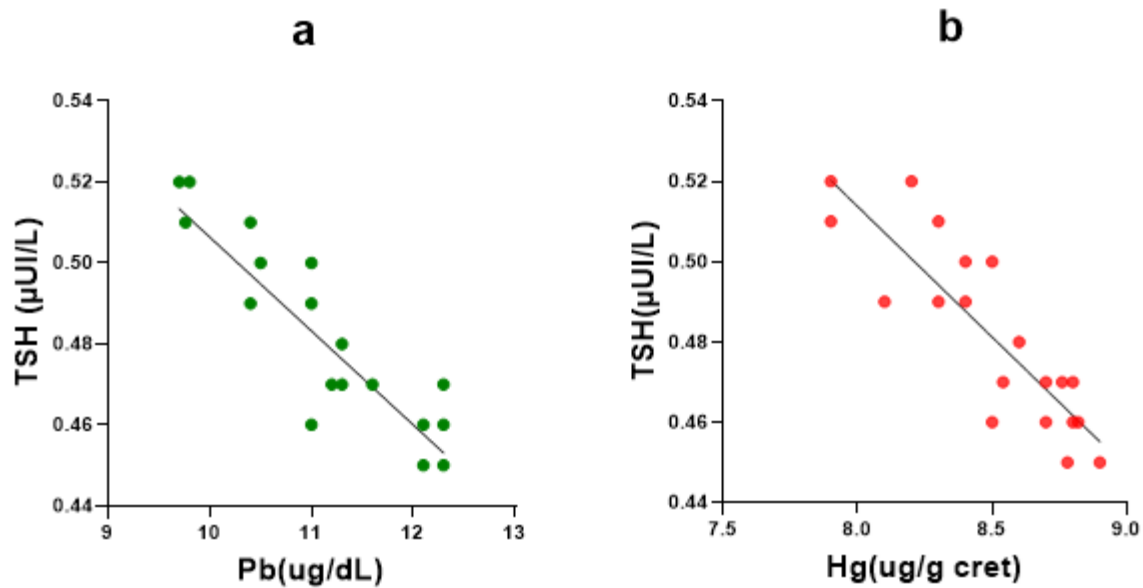


Figure 1. Relationship of TSH levels with heavy metal concentrations in blood and urine of the study group

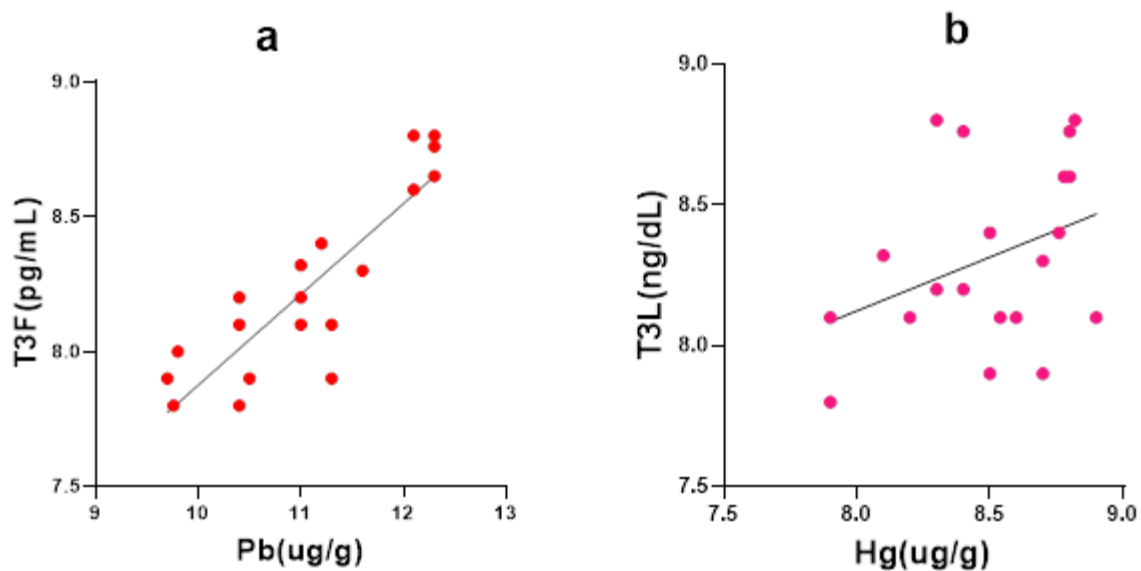


Figure 2. Relationship of T3F levels with heavy metal concentrations in blood and urine of the study group.

DISCUSSION AND CONCLUSIONS

The Agency for Toxic Substances and Disease Registry (ATSDR, 2020) 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 (Javorac et al., 2023, Pacheco et al., 2024). Some of the work that has been published includes 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) (Chen et al., 2013)

To understand what has been expressed above, it is essential to know that the toxic action of heavy metals on living beings 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. (2023), 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, FT3, anti-TPO and anti-Tg. Thus, they emphasize that the dose-dependent effects were the increase in T4 and FT4, 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. (2017) 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. (2020), 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 adrenocorticotrophic 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. Finally, a pilot study conducted by Nascimento et al. (2018), 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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