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Bioanálisis

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

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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 antecedentes de patologías de base. Para el análisis de Pb y Hg se empleó absorción atómica y fotometría de llama, 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 mercurio para el GE fueron de 11,54 ± 0,75 µg/dL y 8,43 ± 0.11 µg/g 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.

Palabras Claves:Endocrino, Tiroides, Xenobióticos, Toxicología

Title

Association of the thyroid stimulating hormone, thyroxine and triiodotyronine with the heavy metals lead and mercury in patients with possible hyperthyroidism

Abstract

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 (T3F) and free triiodothyronine (T4F) 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) with no history of underlying pathologies. Atomic absorption and flame photometry, 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 THS, T3F and T4L 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 GC (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 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 T3F alteration, together with other toxicological aspects to which the patients may be associated.

Key Word
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 entity 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, the present study aimed 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 (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 with a. Inclusion criteria: persons of both sexes willing to participate in the study voluntarily, over 18 years of age, individuals with good eating habits, non-smokers and low or sporadic alcohol consumption. b. Exclusion criteria**:** individuals suffering from chronic or hematological diseases, 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. Are 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.

Determination of mercury. It was performed by 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% HNO3 (Merck KGaA, Germany) was added. After 5 minutes, 60 mL of deionized water was added and, to reduce the mercury ion Hg^{2+} to its elemental form and initiate the emission of cold vapors, 1 mL of 20% SnCl2 solution prepared from of $SnCl_2 \cdot 2H_2O$ 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.

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 and flame spectrophotometry

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”. 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

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 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).

Table 1. Levels of TSH (µUI/L) free T3 (pg/mL) and free T4 (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 *
T3	8.34 ± 0.45	3.12 ± 0.16	0.021 *
T4	1.25 ± 0.23	1.38 ± 0.12	0.841

(*) Significant if p < 0.05; M = mean; SD = standard deviation. Source: Research 2024

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 µg/dL for non-occupationally exposed women and Hg values up to 5 µg/g creatinine. Other data of interest are shown in Table 2.

Table2. Concentration of lead (µg/dL) and mercury (µg/g 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: Research 2024

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.

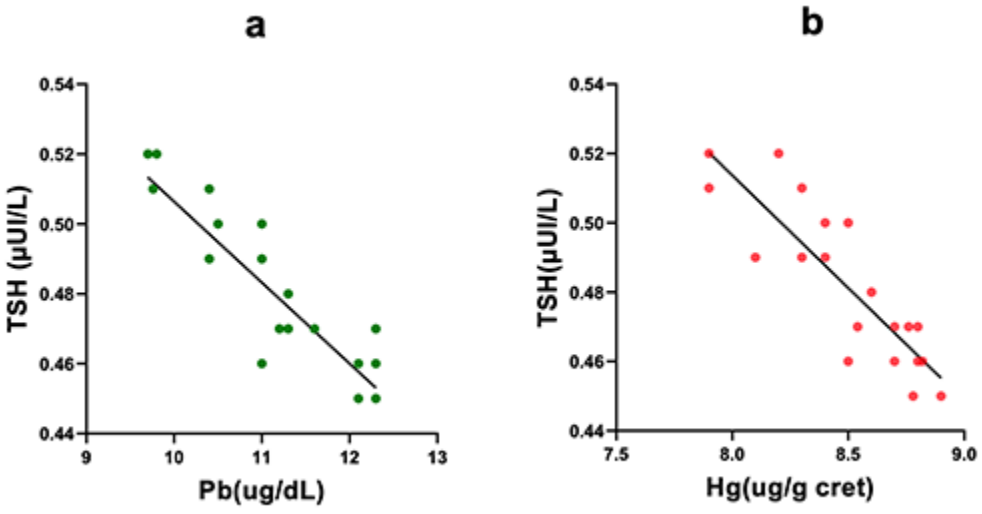

Figure 1. Association of TSH levels with heavy metal concentrations in blood and urine of the study group. Source: Research 2024

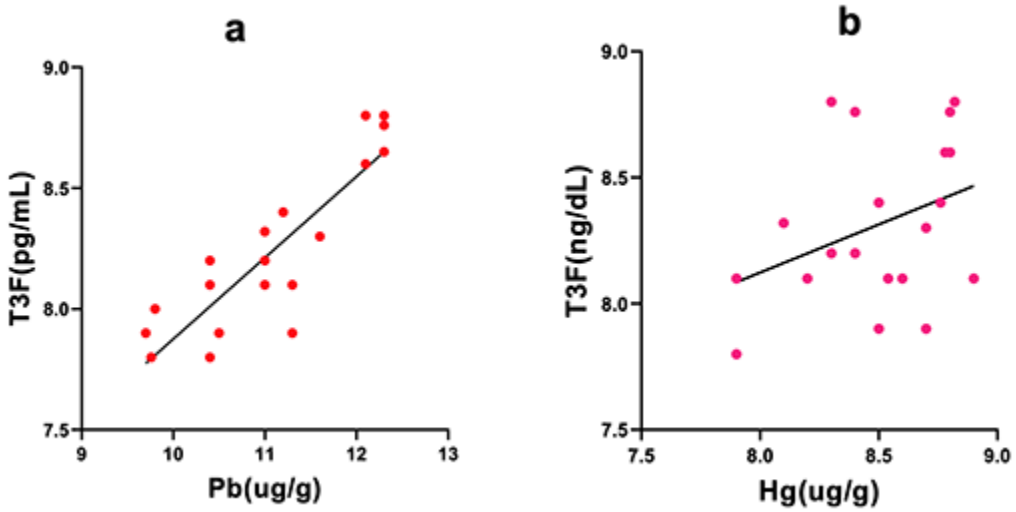

Figure 2. Association of T3F levels with heavy metal concentrations in blood and urine of the study group. Source: Research 2024

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).

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.

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 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. 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.

References

1. Alexander, E. K., Pearce, E. N., Brent, G. A., Brown, R. S., Chen, H., Dosiou, C., Grobman, W. A., Laurberg, P., Lazarus, J. H., Mandel, S. J., Peeters, R. P., & Sullivan, S. (2017). 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum.*Thyroid : official journal of the American Thyroid Association*, *27*(3), 315–389. https://doi.org/10.1089/thy.2016.0457
2. Agency for Toxic Substances and Disease Registry (ATSDR) (2020). Division Of Toxicology and Environmental Medicine. https://www.atsdr.cdc.gov/es/phs/es_phs3.h tml
3. American Conference of Governmental Industrial Hygienists. (2015). Biological Exposure Indices (BIEs). Cincinnati, Ohio: ACGIH. https://www.acgih.org/science/tlv-beiguidelines/biological-exposure-indices-beiintroduction/
4. Bhakat, B., Pal, J., Das, S., Charaborty, S. K., SircarMedical, N. R., Kolkata, RGKar, NorthBengal, & Siliguri (2023). A Prospective Study to Evaluate the Possible Role of Cholecalciferol Supplementation on Autoimmunity in Hashimoto's Thyroiditis.*The Journal of the Association of Physicians of India*,*71*(1), 1.
5. Castiello, F., Olmedo, P., Gil, F., Molina, M., Mundo, A., Romero, R. R., Ruíz, C., Gómez-Vida, J., Vela-Soria, F., & Freire, C. (2020). Association of urinary metal concentrations with blood pressure and serum hormones in Spanish male adolescents.*Environmental research*,*182*, 108958. https://doi.org/10.1016/j.envres.2019.108958
6. Delanghe, J. R., & Speeckaert, M. M. (2011). Creatinine determination according to Jaffe-what does it stand for?.*NDT plus*,*4*(2), 83–86. https://doi.org/10.1093/ndtplus/sfq211
7. Desai, G., Niu, Z., Luo, W., Frndak, S., Shaver, A. L., & Kordas, K. (2021). Low-level exposure to lead, mercury, arsenic, and cadmium, and blood pressure among 8-17-year-old participants of the 2009-2016 National Health and Nutrition Examination Survey.*Environmental research*,*197*, 111086. https://doi.org/10.1016/j.envres.2021.111086.
8. Durá-Travé, T., & Gallinas-Victoriano, F. (2024). Autoimmune Thyroiditis and Vitamin D.*International journal of molecular sciences*,*25*(6), 3154. https://doi.org/10.3390/ijms25063154
9. Frank, J. J., Poulakos, A. G., Tornero-Velez, R., & Xue, J. (2019). Systematic review and meta-analyses of lead (Pb) concentrations in environmental media (soil, dust, water, food, and air) reported in the United States from 1996 to 2016.*The Science of the total environment*,*694*, 133489. https://doi.org/10.1016/j.scitotenv.2019.07.295
10. Javorac, D., Anđelković, M., Repić, A., Tatović, S., Djordjevic, A. B., Miljaković, E. A., Batinić, B., Boričić, N., Đukić-Ćosić, D., Antonijević, B., & Bulat, Z. (2022). Comprehensive insight into the neurotoxic mechanisms of low dose Pb exposure in Wistar rats: Benchmark dose analysis.*Chemico-biological interactions*,*360*, 109932. https://doi.org/10.1016/j.cbi.2022.109932
11. Korevaar T. I. (2017). Evidence-Based Tightrope Walking: The 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum.*Thyroid : official journal of the American Thyroid Association*,*27*(3), 309–311. https://doi.org/10.1089/thy.2017.29040.tko
12. Martínez,(2020). Aplicación de la ley de Lambert-Beer en espectroscopia UV-visible https://m.riunet.uvp.es/handle/10251/16360.
13. Nascimento, S., Göethel, G., Gauer, B., Sauer, E., Nardi, J., Cestonaro, L., Correia, D., Peruzzi, C., Mota, L., Machry, R. V., Furlanetto, T. W., Saint' Pierre, T., Gioda, A., Arbo, M. D., & Garcia, S. C. (2018). Exposure to environment chemicals and its possible role in endocrine disruption of children from a rural area.*Environmental research*,*167*, 488–498. https://doi.org/10.1016/j.envres.2018.07.039
14. National Institute for Occupational Safety and Health. (1994). Manual of analytical methods NMAM. Mercury: method 6009, issue 2. 4th ed, pp. 5.
15. Pacheco, F., Florentino, D., García, J., y Ali, N. (2023). Alteraciones hematológicas y bioquímicas por exposición a benceno en trabajadores de, y cercanos a las estaciones de servicio. *Salud de los trabajadores, 31(2)*, 179-191

16. Stagnaro-Green A. (2017). Clinical guidelines: Thyroid and pregnancy - time for universal screening?.*Nature reviews. Endocrinology*,*13*(4), 192–194. https://doi.org/10.1038/nrendo.2017.17
17. Tang, J., Zhu, Q., Xu, Y., Zhou, Y., Zhu, L., Jin, L., Wang, W., Gao, L., Chen, G., & Zhao, H. (2022). Total arsenic, dimethylarsinic acid, lead, cadmium, total mercury, methylmercury and hypertension among Asian populations in the United States: NHANES 2011-2018.*Ecotoxicology and environmental safety*,*241*, 113776.
18. Vukelić, D., Djordjevic, A. B., Anđelković, M., Baralić, K., Ćurčić, M., Đukić-Ćosić, D., Antonijević, B., & Bulat, Z. (2023). In vivo and in silico approach in revealing the influence of lead (Pb) on thyroid gland function.*Environmental research*,*237*(Pt 2), 117035. https://doi.org/10.1016/j.envres.2023.117035