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KAZI TANI Latifa Sarra

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THESE

Présentée par

Mlle. KAZI TANI Latifa Sarra

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Approche bio-géochimique et statut en sélénium au cours du cancer de la thyroïde chez la population de l'ouest Algérien

Soutenue le / / 2022, devant le jury composé de :

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Dedicate

To my brother who believed in me, supported me and who encouraged me more than anyone

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To all my cousins, my family, my entourage, and my friends

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To myself for all the challenges, obstacles, and difficulties as well as the happiness that life has given me

"Study, not to learn more, but to learn better."

Sarra.

Abstract

The link between mineral elements and cancer has not yet been proven. Therefore, thanks to a biogeochemical approach, we undertook to enrich, by data not yet recorded the impact of certain elements and their various isotopes on thyroid cancer in Algerian population.

The first objective of this work is to study the profile of the selenium (Se) status in 47 patients of the west Algerian region, affected by thyroid carcinoma and 55 healthy controls. This status includes plasma Se content, the activity of glutathione peroxidases (GPx3 and GPx1) and the plasma content of selenoprotein P (SePP). The second objective is the use of copper isotope fractionation (δ^{65}Cu) as a new diagnostic tool for papillary thyroid carcinoma (PTC). Plasma levels of copper (Cu), zinc (Zn), iron (Fe), and calcium (Ca) were also studied. The last objective is the study of the impact of Se nanoparticles (SeNPs) and selenite ions on the development of spheroids thyroid carcinoma metastasis (MDA-T120) models.

Plasma Cu concentrations were higher (1.35 ± 0.33 vs. 1.06 ± 0.22 mg/L, $p < 0.0001$), and Zn concentrations were lower (0.94 ± 0.20 vs. 1.03 ± 0.15 mg/L, $p < 0.05$) in patients compared with controls. Accordingly, the Cu/Zn ratio was significantly higher in PTC compared with controls ($p < 0.0001$). Plasma δ^{65}Cu levels in patients were significantly lower in PTC patients. Thyroid tumor tissues had high δ^{65}Cu values. The predictive model showed a significant association between low plasma Se level and the risk for PTC (OR = 5.02; 95% CI: 1.51 - 16.73; P = 0.009). Regarding plasma Fe and Ca concentrations, our results show significant differences in patients compared with controls. We found a significant reduction in cell viability in the presence of selenite and SeNP-chitosan, compared to controls with a decrease in reactive oxygen species production. The area under the ROC curve (AUC) obtained for Cu/Zn and δ^{65}Cu was 0.81 and 0.78, respectively ($p < 0.001$), indicating exceptional discrimination in favor of using Cu/Zn and δ^{65}Cu ratio as biomarkers of PTC.

The results of this thesis show that the homeostasis of minerals elements is altered in cancer PTC patients. This study suggests novel biomarkers for thyroid carcinoma diagnostic and the use of Se nanoparticles in the treatment of PTC.

Keywords: Selenium, Copper, Zinc, Iron, Calcium, δ^{65}Cu, Thyroid cancer, Selenium nanoparticles.

Résumé

Le lien entre les éléments minéraux et le cancer n'a pas encore été prouvé. Ainsi, grâce à une approche biogéochimique, nous avons entrepris d'enrichir, par des données non encore répertoriées, l'impact du statut de certains éléments et de leurs différents isotopes sur le cancer de la thyroïde dans la population algérienne.

Le premier objectif de ce travail est d'étudier le profil du statut en sélénium (Se) chez 47 patients de la région ouest algérienne, atteints de carcinome thyroïdien et 55 témoins sains. Ce statut comprend la teneur en Se du plasma, l'activité des glutathion peroxydases (GPx3 et GPx1) et la teneur plasmatique en sélénoprotéine P (SePP). Le deuxième objectif est l'utilisation du fractionnement isotopique du cuivre (δ^{65}Cu) comme nouvel outil de diagnostic du carcinome papillaire de la thyroïde (CPT). Les niveaux plasmatiques de cuivre (Cu), de zinc (Zn), de fer (Fe) et de calcium (Ca) ont également été étudiés. Le dernier objectif est l'étude de l'impact des nanoparticules de Se (SeNPs) et des ions sélénites sur le développement de modèles sphéroïdes de métastases de carcinome thyroïdien (MDA-T120).

Les concentrations plasmatiques de Cu étaient plus élevées (1,35 ± 0,33 vs. 1,06 ± 0,22 mg/L, $p < 0,0001$), et les concentrations de Zn étaient plus faibles (0,94 ± 0,20 vs. 1,03 ± 0,15 mg/L, $p < 0,05$) chez les patients par rapport aux témoins. En conséquence, le rapport Cu/Zn était significativement plus élevé chez les CTP par rapport aux contrôles ($p < 0,0001$). Les taux plasmatiques de δ^{65}Cu étaient significativement plus faibles chez les CTP patients. Les tissus tumoraux thyroïdiens présentaient des valeurs élevées de δ^{65}Cu. Le modèle prédictif a montré une association significative entre un faible taux plasmatique de Se et le risque de CPT (OR = 5,02 ; IC 95 % : 1,51 - 16,73 ; P = 0,009). En ce qui concerne les concentrations plasmatiques de Fe et de Ca, nos résultats montrent des différences significatives chez les patients par rapport aux témoins. Nous avons constaté une réduction significative de la viabilité cellulaire en présence de sélénite et de SeNP-chitosan, par rapport aux contrôles, avec une diminution de la production d'espèces réactives de l'oxygène. L'aire sous la courbe ROC (AUC) obtenue pour Cu/Zn et δ^{65}Cu était de 0,81 et 0,78, respectivement ($p < 0,001$), indiquant une discrimination exceptionnelle en faveur de l'utilisation du rapport Cu/Zn et δ^{65}Cu comme biomarqueurs du CTP.

Les résultats de cette thèse montrent que l'homéostasie des éléments minéraux est altérée chez les patients atteints de CTP, Cette étude propose de nouveaux biomarqueurs pour le diagnostic du carcinome thyroïdien et l'utilisation de nanoparticules de Se dans le traitement du CTP

Mots clés: Sélénium, Cuivre, Zinc, Fer, Calcium, δ^{65}Cu, Cancer de la thyroïde, Nanoparticules de sélénium.

الملخص

لم يتم بعد إثبات الصلة بين العناصر المعدنية والسرطان. لذلك، وبفضل المقاربة البيوجيوكيميائية، تعهدنا بإثراء تأثير بعض العناصر ونظائرها المختلفة على سرطان الغدة الدرقية لدى المجتمع الجزائري من خلال معطيات جديدة.

الهدف الأول من هذا العمل هو دراسة حالة السيلينيوم (Se) في 47 مريضا من منطقة غرب الجزائر، مصابين بسرطان الغدة الدرقية و 55 من. الضوابط تشمل هذه الحالة محتوى البلازما Se ونشاط الجلوتاثيون بيروكسيداز (GPx3 و GPx1) ومحتوى البلازما من بروتين سيلينوبروتين P (SePP). الهدف الثاني هو استخدام تجزئة نظائر النحاس (δ^{65}Cu) كأداة تشخيصية جديدة لسرطان الغدة الدرقية الحليمي (PTC). كما تمت دراسة مستويات البلازما من النحاس (Cu) والزنك (Zn) والحديد (Fe) والكالسيوم (Ca). الهدف الأخير هو دراسة تأثير الجسيمات النانوية للسيلينيوم (Se-NPs) وأيونات السيلينيت على تطور ورم خبيث لسرطان الغدة الدرقية من الأجسام الشبه الكروية (MDA-T120).

كانت تركيزات النحاس في البلازما أعلى (1,35 ± 0,33 مقابل 1,06 ± 0,22 مغ/ ل، $p < 0,0001$)، وكانت تركيزات الزنك أقل (0,94 ± 0,20 مقابل 1,03 ± 0,15 مغ/ ل، $p < 0,05$) في المرضى مقارنة مع الضوابط. وفقًا لذلك، كانت نسبة Zn/Cu أعلى بشكل ملحوظ في PTC مقارنةً بالضوابط ($p < 0,0001$). كانت مستويات البلازما δ^{65}Cu أقل بكثير لدى المرضى. أنسجة ورم الغدة الدرقية كانت لها قيم δ^{65}Cu عالية. أظهر النموذج التنبئي ارتباطًا كبيرًا بين انخفاض مستوى البلازما Se وخطر الإصابة بـ PTC (OR = 5,02 ؛ 95 ٪ IC: 1,51 - 16,73 ؛ P =0,009). فيما يتعلق بتركيزات الحديد والكالسيوم في البلازما، تظهر نتائجنا اختلافات معنوية في المرضى مقارنة مع الضوابط. وجدنا انخفاضًا كبيرًا في قابلية بقاء الخلية في وجود السيلينيت و SeNP-chitosan، مقارنةً بالضوابط مع انخفاض في إنتاج أنواع الأكسجين التفاعلية. كانت المساحة الواقعة تحت منحن ROC (AUC) التي تم الحصول عليها من أجل Zn/Cu و δ^{65}Cu 0,81 و 0,78 على التوالي ($p < 0,001$) ، مما يشير إلى تمييز استثنائي لاستخدام نسبة Zn/Cu و δ^{65}Cu كمؤشرات حيوية لـ.PTC.

تظهر نتائج هذه الأطروحة أن توازن العناصر المعدنية قد تغير لدى مرضى السرطان. تقترح هذه الدراسة مؤشرات حيوية جديدة لتشخيص سرطان الغدة الدرقية واستخدام الجسيمات النانوية ل Se في علاجPTC.

الكلمات المفتاحية: السيلينيوم ، النحاس ، الزنك ، الحديد ، الكالسيوم ، δ^{65}Cu ، سرطان الغدة الدرقية ، جسيمات السيلينيوم النانوية.

Scientific productions

1. Publications

a. Articles

Kazi Tani, L.S., Dennouni-Medjati, N., Toubhans,B., Charlet, L (2020). Selenium Deficiency From Soil to Thyroid Cancer. Applied Sciences. doi:10.3390/app10155368.

Kazi Tani, L.S., Gourlan, A.T., Dennouni-Medjati, N., Telouk, P., Dali-Sahi, M., Harek, Y., Sun, Q., Hackler, J., Belhadj, M., Schomburg, L., Charlet, L (2021). Copper Isotopes and Copper to Zinc Ratio as Possible Biomarkers for Thyroid Cancer. Frontiers in Medecine. doi:10.3389/fmed.2021.698167.

Belhadj, M., **Kazi Tani, L.S.,** Dennouni-Medjati, N., Harek, Y., Dali-Sahi, Majda., Sun, Q., Heller, R., Behar, A., Charlet, L., Schomburg, L (2020). Se Status Prediction by Food Intake as Compared to Circulating Biomarkers in a West Algerian Population. Nutrients. doi :10.3390/nu12123599.

b. Proceeding

Kazi Tani, L.S., Gourland, A., Dennouni Medjati, N., Dali Sahi, M., Harek, Y., Belhadj, M., Telouk, P., Charlet, L. Copper isotope for potential thyroid cancer diagnosis. Goldschmidt2021, 4th – 9 julyth, Lyon, France.

2. Communications

a. International communications

i. Oral communications

Kazi Tani, L.S., Dennouni Medjati, N., Telouk, P., Gourland, A., Toubhans, T., Dali Sahi, M., Charlet, L. Copper Isotope in Serum of Thyroid Cancer Patients. Goldschmidt2019, 18th - 23rd August 2019, Barcelona, Spain.

ii. Posters

Kazi Tani, S.L., Dennouni Medjati, N., Dali Sahi, M. Etude épidémiologique des dysthyroïdies dans la population de l'ouest Algérien. Implication du sélénium dans le métabolisme de la glande thyroïdienne. Colloque international Biosciences, 28rd -29rd October 2018 Oran, Algérie.

Kazi Tani, L.S., Dennouni Medjati, N., Dali Sahi, M., Charlet, L., Harek, Y., Belhad, M. Statut en Cu de patients atteints de carcinome de la glande thyroïdienne. Journées francophones de la nutrition, Couvent des Jacobins, 27rd -29 rd November 2019, Rennes, France.

Belhadj, M., Dennouni-Medjati, N., Dali-Sahi, M., Harek, Y., Behar, A., **Kazi Tani, L.S.** Evaluation de l'apport alimentaire en micronutriments chez une population de cardiopathes. Journées francophones de la nutrition, Couvent des Jacobins, 27rd -29 rd November 2019, Rennes, France.

Dennouni Medjati, N., Dali Sahi, M., Medjahdi, A., Saidi, M., **Kazi Tani, L.S.,** Harek, Y. Épidémiologie du carcinome de la thyroïde dans l'ouest algérien. Journées francophones de la nutrition, Couvent des Jacobins, 27rd -29 rd November 2019, Rennes, France.

Behar, A., Dennouni Medjati, N., Dali-Sahi, M., Belhadj, M., Halfaoui, N.S., **Kazi Tani, L.S.,** Harek, Y. "Le diabète, entre culture et santé publique ". Séminaire International sur la Biodiversité, Environnement et Santé. 21 and 22 September 2021. Tlemcen, Algeria.

b. National communications

i. Oral communications

Kazi Tani, S.L., Dennouni Medjati, N., Behar, A., Belhadj, M., Dali Sahi, M., Harek, Y. La micronutrition, une approche importante dans l'étude du métabolisme de la glande thyroïdienne. Quatrième congrès de Biochimie et de Génétique médicales. 29 - 30 April 2019, Oran, Algeria.

ii. Posters

Behar, A., Dennouni-Medjati, N., Dali-Sahi, M., Harek, Y., **Kazi Tani, L.S.,** Belhadj, M., Kachecouche, Y. Statut en zinc chez des diabétique de type 2. 1er colloque national de Biotoxicologie et Bioactivité. 29 November 2019. Oran, Algeria.

Behar, A., Dennouni-Medjati, N., Dali-Sahi, M., Harek, Y., **Kazi Tani, L.S.,** Belhadj, M., Kachecouche, Y., Benslama, Y., Meziane, F.Z. « Consommation d'antioxydants naturels chez les diabétiques de type 2 de la wilaya de Tlemcen ». 7èmes journées sur le diabète et les maladies vasculaires. SNV Tlemcen. 29 and 30 November 2019. Tlemcen, Algeria.

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List of abbreviations

^{131}I: Iodine

CTP : Cancer Papillaire de la Thyroïde

DIO1: Iodothyronine deiodinase 1

DIO2: Iodothyronine deiodinase 2

DIO3: Iodothyronine deiodinase 3

DIOs: Iodothyronine deiodinase

DNA: Deoxyribonucleic acid

ER: Endoplasmic Reticulum

FAO **:** Food and Agriculture Orgamisation

GHS : Glutathione

GIT : Gastrointestinal tract

GPx1 : Glutathion peroxydase 1

GPx3 : Glutathion peroxydase 3

GPx4 : Glutathion peroxydase 4

GPx6 : Glutathion peroxydase 6

GPxs : Glutathion peroxydases

H_2O_2 ; Hydrogen peroxide

H_2Se : Hydrogen selenide

I^- : Iodide

IDA : Iron Deficency Anemia

IRMS : Isotope Ratio Mass Spectrometry

LIBS **:** Laser Induce Breakdowm Spectroscopy

MC-ICP-MS : Multi-collector inducted couple plasma mass spectrometry

Mt : Mitochondria

NGS : Next Geneation Sequencing

NPC : Nutritional Prevention of Cancer

NPs : Nanoparticles

P : Phosphate

PTC: Papillary Thyroid Carcinoma

RDA: Recommended Dietary Allowance

ROS : Reactive Oxygen Species

rT3 : reverce Triiodothyronine

S : Sulfure

Se^0 : elemental selenium

Se^{2-} : Selenide

SeAlb : Serum albumin,

SeAM : Adenosyl-Se-Met

Se-Cys : Selenocysteine

SEER: Surveillance Epidemiology End Results

SeGalNac : Selenosugar.

SELECT : Selenium and Vitamin E Cancer Prevention Trial

Se-Met : Selenomethionine

Se-NPs: Selenium nanoparticles

SeO_3^{2-} : Selenite

SeO_4^{2-} : Selenate

SePP: Selenoprotein P

SOD1: Superoxide dismutase 1

T2 : Diiodothyronine

T3 : Triiodothyronine

T4 : Thyroxine

TG : Thyroglobuline

TIMS: Thermic ionization

TKIs : Tyrosine kinase inhibitors

TPO: Thyroid peroxidase

TRH : Thyrotropin-releasing hormone

TriMethylSe : Trimethylselenonium,

TRx1: Thioredoxin reductase 1

TRx2: Thioredoxin reductase 2

TRx3: Thioredoxin reductase 3

TRxs: Thioredoxin reductase

TSH : Thyroid Stimulating Hormone

TXRF : Total Reflection X-ray Fluorescence

WHO: World Health Organization

γ-glu-SeMeSeCys :γ–glutamine–Se–MethylselenoCysteine

δ^{65}Cu: $^{65}Cu/^{63}Cu$ ratios

List of annexes

Introduction

Thyroid cancer is an abnormal, uncontrolled, and permanent multiplication of initially normal thyroid gland cells, which eventually result in the formation of a mass termed malignant tumor. It affects follicular epithelial cells and calcitonin-producing para-follicular C cells. It is the most common malignant endocrine tumor, responsible for approximately 90% of endocrine cancers and accounting for ~2% of all diagnosed cancers [1]. There is no systematic screening for thyroid cancer, it most often develops without causing any apparent symptoms and the diagnosis is made accidentally in 25% of cases during the follow-up of another thyroid disease [2].

There are different typologies of thyroid cancer, we can distinguished: differentiated cancers including papillary thyroid cancer (PTC), which affects the follicular cells of the thyroid that are iodine-free (Figure 1), and vesicular cancer, which affects the vesicular cells ; medullary cancers derived from the calcitonin-secreting C cell ; and undifferentiated anaplastic cancers [3].

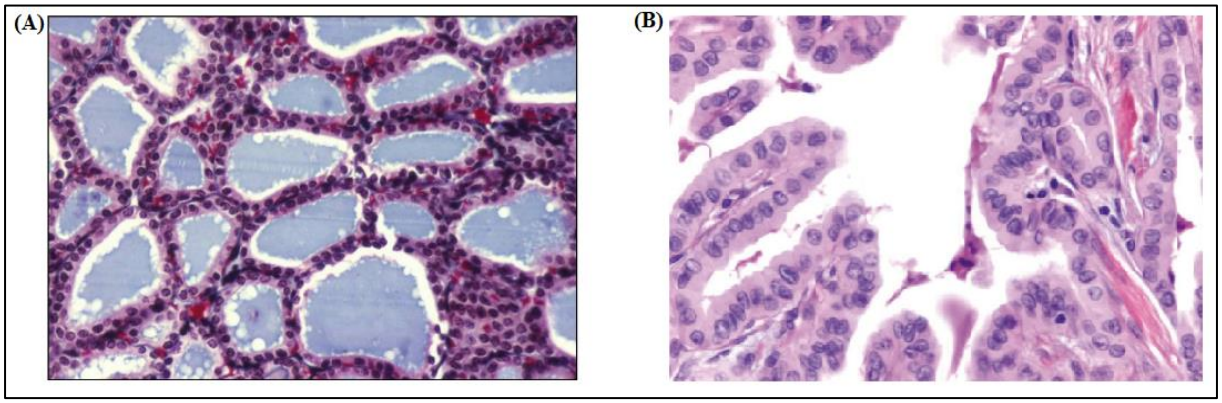

Figure 1. Histological aspect of (A): healthy thyroid cell. (B): papillary thyroid carcinoma [4].

In the last decades, the incidence of thyroid cancer has increased significantly with 567,000 new cases annually worldwide, placing it in the 9th place worldwide in cancer incidence [5]. This increase is mainly due to the rise in the incidence of PTC. This cancer accounts for 84% of new cases, and it is the most common malignant thyroid tumor [6]. It is diagnosed three times more in women (10.2/ 100,000) than in men (3.1/ 100,000) and it is rare in children and teen agers [5].

In the United States, and based on Surveillance, Epidemiology, and End Results (SEER), the thyroid cancer is the 5th most common cancer in women [7], as well as in Europe and Asia [8]. In Africa, thyroid cancer is not considered as one of the 10 most frequent cancers in women [8] (Figure 2.A). However, in Algeria, African country, it occupies the third place after breast and colorectal cancers (Figure 2.B), while it occupied only the 15th place in 1980 then the 5th place in order of frequency in 2006 [9-11]. In 2012, a total of 27,000 cases of thyroid cancer deaths

were observed in women and 13,000 in men, corresponding to mortality rates of approximately 0.6 /100,000 women and 0.3 /100,000 men [3].

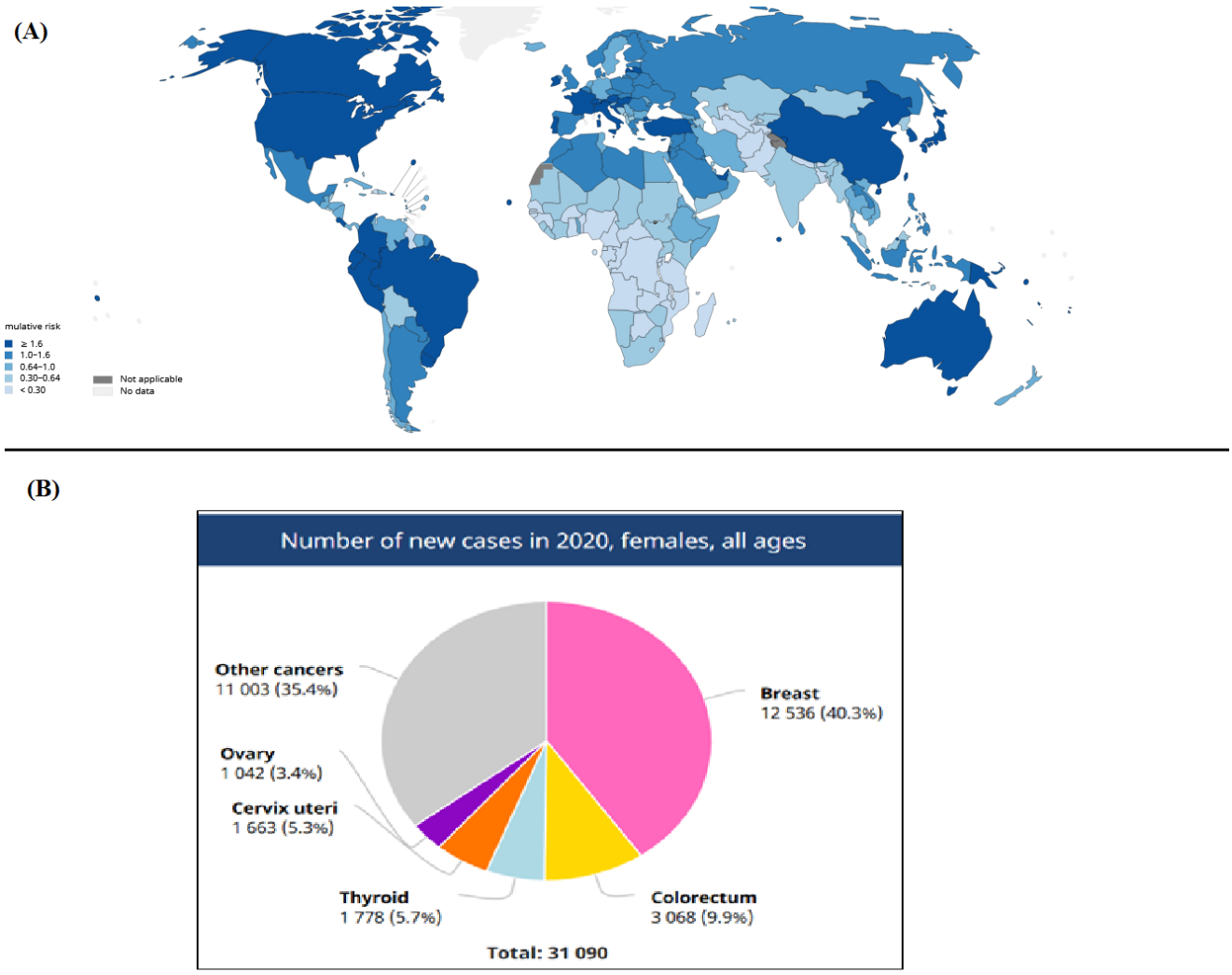

Figure 2. Women thyroid cancer incidence: (A) Worldwide distribution (B) Numbers of new cancers cases in Algerian population [8].

The thyroid is a vascularized and innervated endocrine gland that is composed of chambers called follicles [12-14]. The follicle is the functional and anatomical unit in which thyroid hormone biosynthesis takes place. It is also the only human site where iodine is chemically activated and used for biosynthetic functions [14]. In addition to iodine, other elements (micro and macronutrients e.g., Selenium, Iron, Zinc and Vitamins) are necessary for the proper functioning of the thyroid gland (Figure 3) [15].

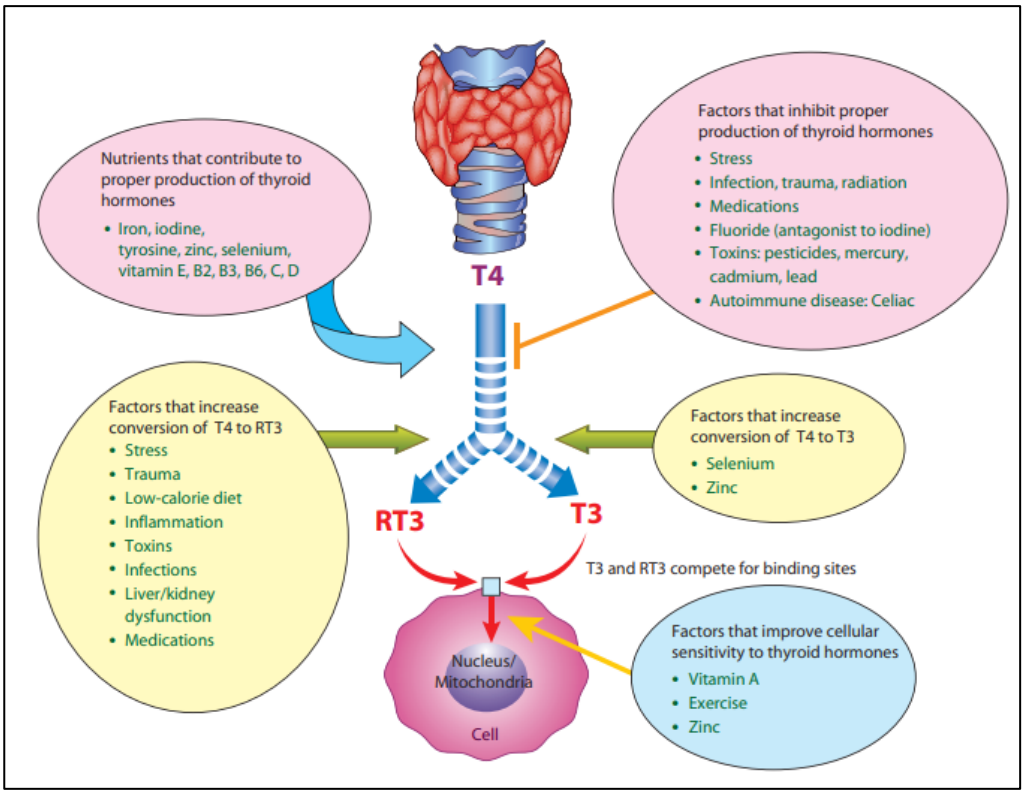

Figure 3. Factors that affect thyroid function and thyroid hormones biosynthesis [15].

Other factors such as stress, trauma, and autoimmune diseases can also negatively influence and inhibit the production of thyroid hormones as illustrated in Figure 3 above [15]. The risk factors for thyroid cancer are not yet all understood. Furthermore, some of them have been established, including exposure to ionizing radiation in childhood, iodine deficiency, hormonal factors, obesity, pollutants and disruptors of thyroid function and socioeconomic factors [16]. Genetic alterations and different mutations observed for thyroid cancers are numerous and vary according to the histological type [17].

The treatments for thyroid cancer vary according to cancer's stage and type by [18] :

- Surgery including total or partial thyroidectomy. It represents the first line therapy and has been proven to be the most effective one, with an increase in overall survival and to decrease recurrence.
- Radioactivity using iodine (^{131}I).
- Tyrosine kinase inhibitors (TKIs).
- External beam radiation or chemotherapy with cisplatin. It remains the least used treatment except when there is resistance of the tumors.

Introduction

Micro-nutrition is an interesting approach that is in line with the sciences of dietetics, particularly nutrition and preventive medicine, and is based on the fact that the disorders or pathologies which may occur are, at least in part, related to an imbalance or micronutrients deficit. In addition to vitamins, trace elements and essential minerals are strongly implicated in cellular dysfunctions that can be at the origin of many pathologies such as diabetes, cardiovascular diseases, or cancer like thyroid cancer. Imbalance of trace elements in metabolism and/or homeostasis (deficiency / excess) could play an important role in thyroid diseases [19].

Selenium (Se) is one of the trace elements essential for human health. It plays a fundamental role in cellular metabolism such as maintaining homeostasis and protection against oxidative stress [20]. Among all human tissues, the thyroid gland is unique because it contains the highest amount of Se per tissue unit mass. Even under Se deficiency conditions, thyroid may express essential selenoproteins like brain, testis and other several endocrine organs. Se is considered to be the second most important component input to thyroid metabolism, after iodine [21-23].

Dietary intake is the main source of Se exposure and food items are characterized by a great diversity in Se concentration. Food Se content depends on a specific geographical area ; underlying rock ; soil type, presence of hydromorphic or not, as well as on the ability of plants to accumulate this element. In addition, other factors such as climatic conditions, cultivation, and food preparation methods also exert some effect [24]. Se in food products is most often linked to protein. Products rich in Se include meat, fish, offal, and cereals, while in contrast fruits and vegetables contain small amounts of Se [24].

Previous work had shown that the general population of Western Algeria had a rather low Se status, as well as Se nutritional intakes well below the recommended intakes [25]. Furthermore, a recent work published by Belhadj *et al.,* [26] mentioned that in the same region, the Se status increased at the normal level according to the international standards of Recommended Dietary Allowance (RDA).

As known, the Se deficiency leads to dysfunction of the internal antioxidant system, which lead to clinical morbidity [27]. However, high Se intake can also lead to intoxication, namely “selenosis”. Randomized trials have shown that high Se body levels increase the risk of cardiovascular disease, diabetes, and specific neoplasms such as skin and prostate cancer [28-29]. The effects of Se follow a U-shaped curve, related to both deficiency and overexposure, with a recommended daily intake in the order of 70 µg/day for men and 60 µg/day for women, at a normal body weight [30].

This (semi) metallic element is genetically integrated within more than 25 selenoproteins as constitutive part of selenocysteine, the 21st amino acid [31]. Selenoproteins mediate most biological roles of Se, and the two most common forms in blood are selenoprotein P (SePP) and glutathione peroxidase 3 (Gpx3) [32]. Most of thyroid Se is contained in thyrocyte functional selenoproteins that are implicated in the proper functioning of the thyroid gland, e.g., iodothyronine 5'-deiodinase (DIOs), glutathione peroxidase (GPxs) and thioredoxin reductase type 1 (TRx1) (Figure 4) [23,33].

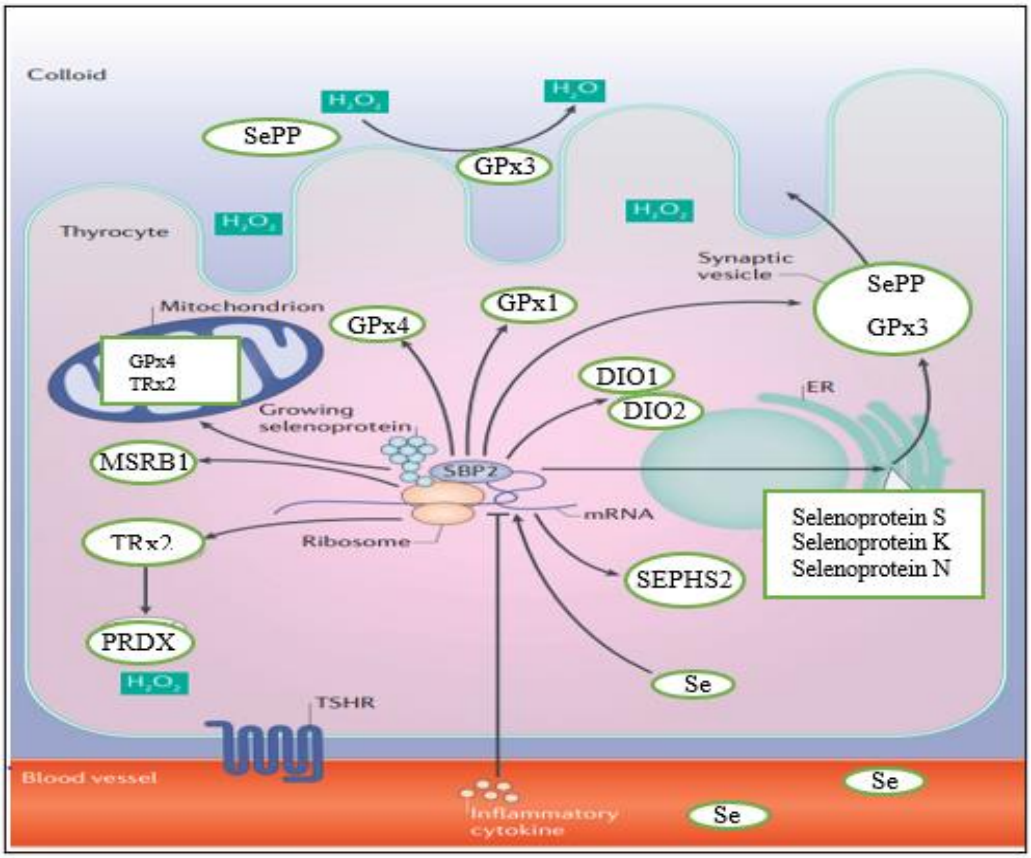

Figure 4. Selenoproteins in thyroid gland [34]. Thyrocytes express several selenoproteins, including iodothyronine deiodinase (DIO1 and DIO2); glutathione peroxidase family (GPx1, GPx3 and GPx4); thioredoxin reductases (TRx1, TRx2 and TRx3); selenoprotein 15 (SELENOF), selenoprotein P (SePP), selenoprotein M and selenoprotein S. SePP and GPx3 are actively secreted from the thyrocyte. DIO1 and DIO2 can convert T4 into T3 by removal of the 5′-iodine. GPxs and TRxs acts as an antioxidant enzyme.

The transmembrane selenoproteins Iodothyronine deiodinases (DIO1 to DIO3), involved in the enzymatic biosynthesis, regulation and circulating of thyroid hormones, DIO1 and DIO2 converts prohormone thyroxine (T4) to active hormone triiodothyronine (T3) by 5'-deiodination or inactive T4 to T3 reverse (rT3) and this contributes to iodine homeostasis and to the circulating pool of T3 [35]. DIO3 inactivates thyroid hormones by removing iodine from the inner ring of iodothyronines. DIOs catalyze specific but complementary reactions which protects sensitive

tissues from excess thyroid hormone stimulation and also act collectively to control serum and local concentrations and activity of thyroid hormones [36].

A single enzyme, thyroid peroxidase (TPO), catalyzes all steps of thyroid hormone biosynthesis. TPO uses hydrogen peroxide (H_2O_2) produced in large quantities by thyroid double oxidases 1 and 2. Thus, in the thyroid, reactive oxygen species (ROS) and free radicals are constantly formed and participate in physiological and pathological processes of the gland as a consequence of its normal physiological activity. The cells have developed a complex set of antioxidant defense mechanisms to limit the action of ROS [37]. The GPx family acts as an effective antioxidant enzyme and is one of the main lines of defense against the aggressions produced by oxygen free radicals. GPx family inhibit the production of highly oxidizing free radicals such as the hydroxyl radical derived from hydrogen peroxide and the alkoxyl radical derived from organic hydroperoxides, eliminating H_2O_2 and thus protecting thyroid cells from oxidative damage [36,38].

Thioredoxin reductase (TRxs) is a recently discovered dimeric selenoprotein located in the cytoplasm of human cells and catalyzes the NADPH-dependent reduction of thioredoxin. Three isoforms (TRx1 to TRx3) play an essential role in antioxidant processes by reducing intramolecular disulfide bridges in proteins and catalyzing the regeneration of the reduced form of substances such as in vitamin C and vitamin E [36,39].

Selenoprotein S is located in the endoplasmic reticulum (ER), and is involved in the regulation of the inflammatory response by retrotranslocation of misfolded proteins from the ER lumen to the cytosol, for degradation by the proteasome [40]. Moreover, Selenoprotein S protects against the transcription of numerous genes encoding pro-inflammatory cytokines that are implicated in pathogenesis of chronic autoimmune thyroiditis [41]. In addition, several others selenoproteins are involved to the adequate functioning of thyroid gland including Selenoprotein N and K that provide the quality and control in endoplasmic reticulum [29].

Selenoprotein P represents the major plasma selenoprotein [42]. It is actively secreted from the thyrocyte [29], and has an essential role to provides the right amount of Se for proper functioning of thyroid gland [43].

The relationship between Se and thyroid cancer is complex. The hierarchy in the distribution of Se protects the thyroid from small fluctuations in Se intake [44]. However, several studies have reported a lower serum level of Se status and a significant association with thyroid cancer patients [45-46]. In addition, Vinciti *et al.*, [47] highlighted the role of Se in helping cells to resist

oxidative damage which is a major cause of cellular damage and is implicated as a key factor in early-stage cancer. The overproduction of free radicals and the consequent increase in oxidative stress led to irreversible cellular damage, frequently associated with diseases such as atherosclerosis, hypertension, diabetic nephropathy, pulmonary fibrosis, Alzheimer's disease and particularly cancer [48].

Iodine and selenium are dietary factors for which there is the most information to play a role in the risk of differentiated thyroid cancer [34]. Although many studies have shown that Se deficiency may be associated with an increased risk of several types of cancer [49-50]. In vitro study showed that Se may cause inhibition of thyroid cancer cell growth accompanied by cell cycle arrest in the S and G2/M phases [51]. In addition, thyroid hormones and selenoproteins are substrates of enzymes encoded by genes whose polymorphisms are suspected to play a role in thyroid cancer risk such as Thr92Ala (rs225014 T/C) polymorphism in the DIO2 gene and Ala234Thr (rs3877899) polymorphism in SePP gene [52-53]. Desoxyribonucleic Acid (DNA) structure chemical changes, usually induced by oxidative stress, have been demonstrated in thyroid cancer [54-55]. Moreover, elevated levels of GPxs and malondialdehyde (a product of lipid peroxidation) have been detected in blood of patients with thyroid cancer [56-57]. Several studies have demonstrated an association between low Se status and thyroid cancer [58]. However, in the absence of direct proof, further studies are needed to confirm and explain this hypothesis.

Selenium supplementation is an important issue. Different methods of Se supplementation have been used including: oral administration of selenite sodium, long-acting injections, Se-enriched yeast and supplementation of the fertilized soil with selenates [24]. Se supplementation has been demonstrated to decrease the incidence of Keshan disease [15].

Certain results of trials on Se supplementation demonstrating positive health effects. It has been reported that patients with Hashimoto’s thyroiditis, Graves’ disease, or Graves’ orbitopathy respond positively to Se supplementation [44]. In addition, several studies have provided some evidence for a beneficial effect of Se on the risk of lung, bladder, colorectal, liver, oesophageal, gastric-cardia, thyroid, and prostate cancers [47]. The two large cancer prevention studies, the Nutritional Prevention of Cancer (NPC) and the Selenium and Vitamin E Cancer Prevention Trial (SELECT), showed positive effects on cancer prevention only in the lowest quintile subject, i.e., in the lowest percentile of all subjects enrolled in the two studies combined [59-60]. Other epidemiological studies and randomized intervention trials have not found a positive effect of Se supplementation on thyroid parameters [15].

Selenium supplementation remains a complex approach and must be considered on a case-by-case basis, despite its beneficial effect in some situations, if it is not controlled or adjusted, can be toxic, harmful, and even cause new pathologies. Unless there is evidence to the contrary, it can be hypothesized that the beneficial effects of Se on health are restricted to subjects and patients who are insufficiently supplied with this essential micronutrient and may be considered Se deficient, needing increased or supplemental Se intake [44]. It is still unclear whether Se supplementation could decreases the risk of thyroid cancer and delays the process of thyroid tumorigenesis, but this are prospects for future research [14].

As seen, the Se effects follow a U-shaped curve, related to both deficiency and overexposure, recent studies have shown an increased risk of certain cancers such as prostate cancer with high levels of Se [61] and the chemotherapeutic effect of Se has not been proven at safe and tolerated doses (<90 µg/day) [30]. Other research has pointed out that the optimal concentration of Se in the diet is narrow and lies between 100 and 200 µg/day [62-63] (Figure 5). High intake, such as 1500 µg/day, can lead to single and double strand DNA breaks that progressively aggravate with rising dose, causing selenosis characterized in the acute phase by necrosis and hemorrhage and, in case of chronic intoxication, by degenerative and fibrotic changes of the liver and skin [64-67]. Se consumption between 200 and 400 µg/day may be protective against liver necrosis and they also observed an increase in GPxs and TRxs levels. This may contribute to the protection against ROS overexpression and leads to the study the supranutritional doses of selenium effect (>200µg) in cancer, doses consumption above 400µg/day demonstrated to have strong inhibitor effect on cancer cells proliferations as well as growth and have hindered cancer development [47, 68-70] (Figure 5).

To overcome the limitations of supplementation and systemic toxicity at high doses, the use of nanomedicine technology has been introduced. The cancer nanomedicine corresponds to a variety of nanomaterials and nanosized biological entities that have been designed and are being to therapeutic nanomaterial applications for the diagnosis and treatment of cancers e.g., antibody drugs [71].

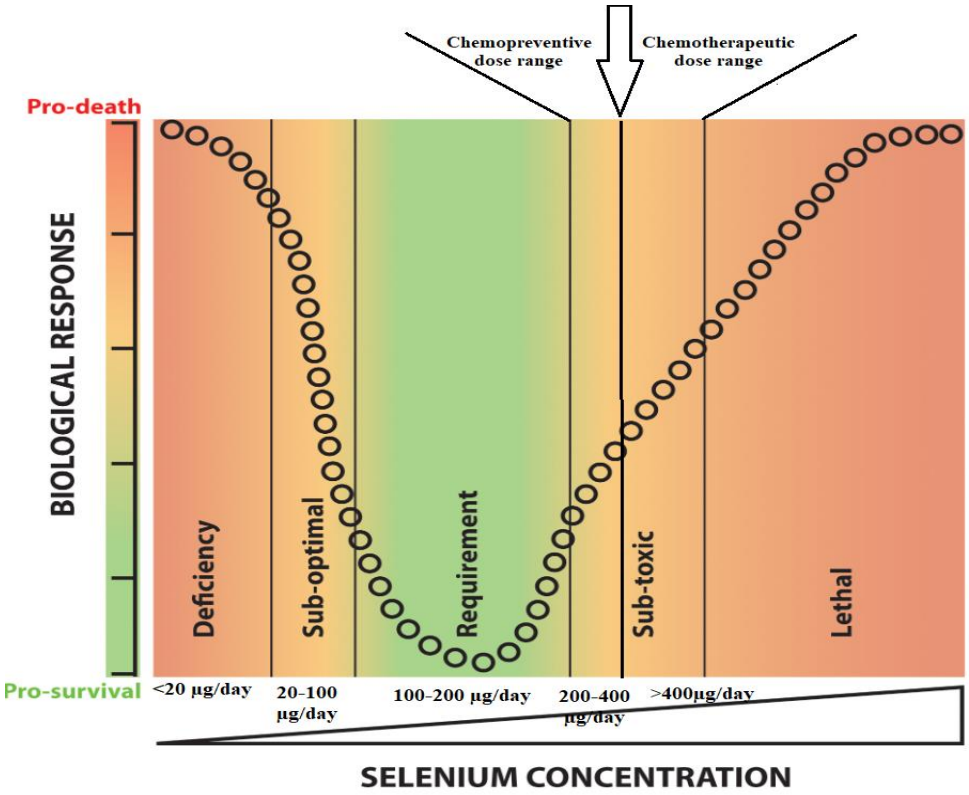

Figure 5. Dietary range of selenium in human diet [30].

Nanoparticles (NPs) have unique characteristics including small size, high surface/volume ratio, surface charge, surface chemistry, solubility and multifunctionality that make them remarkably distinctive [72]. NPs solve many biopharmaceuticals and pharmacokinetic problems related to many drugs in a diverse disease. They boost the therapeutic effect of ionized drugs and increase the absorption of water-soluble compounds, proteins, peptides, vaccines, siRNAs, miRNAs, DNA, and other biological therapeutics in cells [72]. The adaptation of the nanoparticle surface with targeting ligands allows the drug administration system to be highly polyvalent and to be able to selectively deliver the drugs to the target site [72].

Selenium in the form of nanoparticles (SeNPs) has been initiated, with numerous therapeutic benefits including anticancer, antioxidant, anti-inflammatory and anti-diabetic action (Figure 6). The anticancer effect is mainly due to its pro-oxidant properties in cancer cells, triggering the synthesis of ROS, which leads to damage to the mitochondria (Mt) and ER, which in turn leads to DNA damage [73].

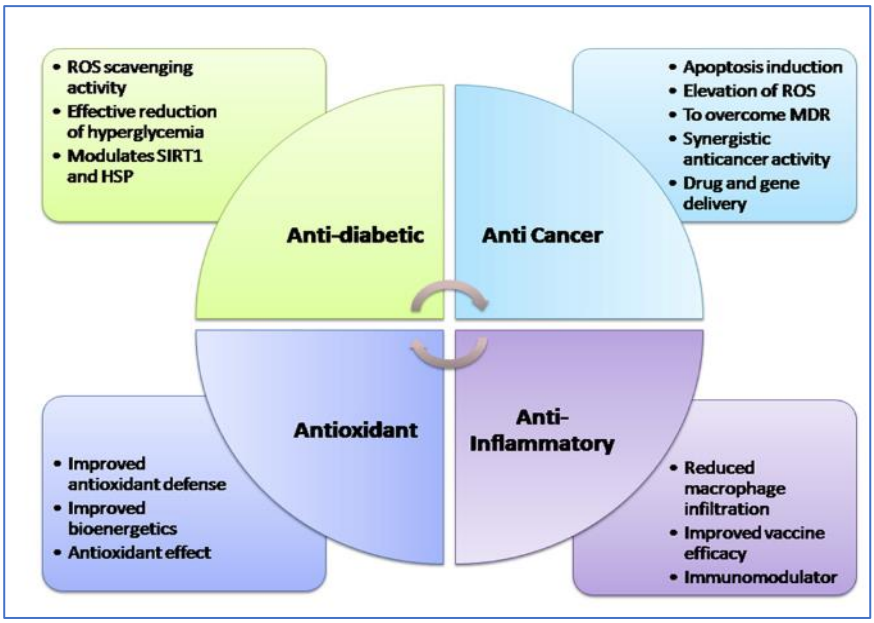

Figure 6. Therapeutic applications of selenium nanoparticles (SeNPs) [73].

The mechanism action of SeNPs in different cancers is not fully elucidated. However, several hypotheses are described in Figure 7 below [73]. SeNPs integrate into cells by endocytosis and mediated by receptors. Tumor cells present an acidic pH environment with a redox imbalance this leads to pro-oxidative conversion of SeNPs and triggers the generation of free radicals which cause disruption of the Mt membrane leading to leakage of Mt proteins and lead to ER stress [74]. The induced damage to the Mt membrane causes leakage of various proteins and triggers apoptosis through activation of caspases. This condition of cellular stress orders the activation of numerous molecular pathways including the PI3K/Akt/mTOR, Wnt/β-catenin, MAPK/Erk, and apoptotic pathways. The PI3K/Akt/mTOR, MAPK/Erk, VEGF, and Wnt/β-catenin pathways are important in oncogenic signaling and their modulation by SeNPs causes alteration of cell proliferation and impairs growth-promoting signaling adjacent to the tumor microenvironment [73,75]. Furthermore, SeNPs could inhibit angiogenic signaling in cancer cells, causing a cessation of cell cycle leading to cell death [73].

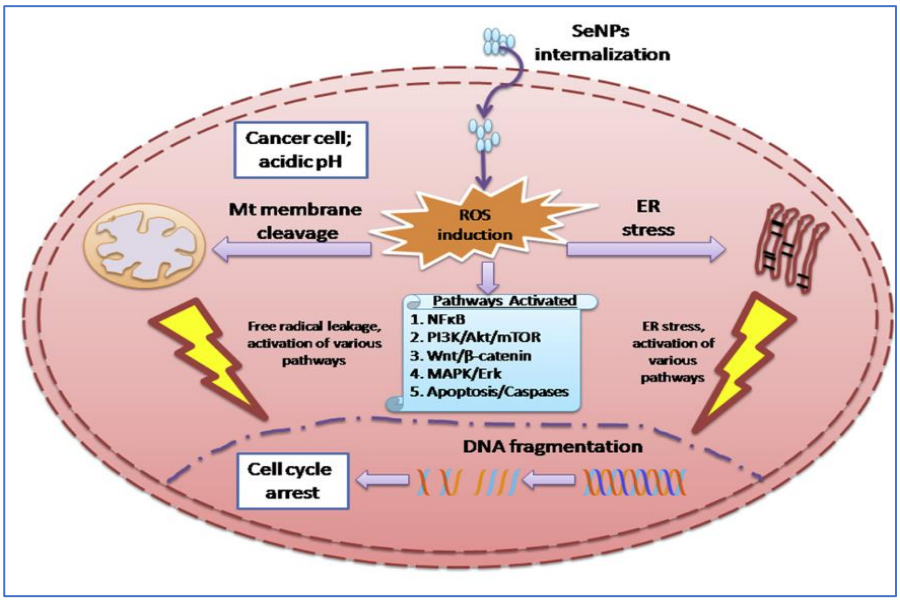

Figure 7. Hypothetical mechanism of anticancer activity of SeNPs [73].

Previous research found a preferential uptake of SeNPs by cancer cells in comparison with normal cells [76-77]. Like the aqueous Se studies, SeNPs provide antioxidant action in cancer cell lines, reducing the incidence of cancer at a lower dose and revealing a cytotoxic effect at higher doses [78]. Studies conducted on a mouse model have shown that SeNPs decreased the toxicity of Se by up to fourfold in comparison with Se in solution and the impact on livers was significantly diminished [79]. SeNPs were demonstrated to exhibit anti-proliferative activity in MDA-MB-231 cells at dose range 10–40 μmol/L and arrested the S phase at 10 μmol/L [80]. A major inconvenience of SeNPs appears to be their poor cellular uptake, which was overcome by conjugation to stabilizing and targeted ligands on the outer surface of the nanoparticles [73].

Another interesting element in this study is Copper (Cu). It is a metal, whose physicochemical properties make it an essential element in low concentrations and toxic in high concentrations for living organisms [81-82]. In humans, it is distributed to cells via the ceruloplasmin in blood [83]. Additionally, Cu is an important dietary micronutrient, even though only small quantities of the metal are required for welfare [84]. Although Cu is the 3rd most abundant trace metal within the body, after iron (Fe) and zinc (Zn), the dietary intake of Cu in human body is merely between 1 to 3 mg/day [85]. And it is present in every tissue of the body, but is stored primarily in the liver, with less amounts found within the heart, brain, kidney, and muscles [86].

In the thyroid gland, Cu interacts with the metabolism of the amino acid tyrosine, which is required for the genesis of thyroid hormones [87]. It regulates the excessive absorption of T4 by controlling calcium (Ca) levels and can eliminate free radicals and reduce some of the damage

caused in cells during the synthesis of thyroid hormones [88]. It is reported that thyroid hormones affect the metabolism of various trace elements such as Cu [89-90]. A significant reduction in Cu levels can lead to impairment of thyroid function [91]. Optimal concentrations of Cu are necessary for normal metabolic functions, but elevated Cu levels can cause oxidative stress [92]. Some studies suggest higher blood levels of Cu in patients with altered thyroid disease compared with the control subjects [58]. Cu is also vital in cell division in both normal and cancer tissues [92]. Several studies found that higher levels of Cu could endorse DNA through toxic free hydroxyl radicals to induce growth, proliferation, and cancer [93]. In different cancers it has been shown that Cu is required for angiogenic processes, stimulating proliferation and migration of endothelial cells. In the liver tissue of colon tumor bearing mice, gene expression the Cu transporters ceruloplasmin; CTR1; and ATP7B was increased significantly, which can explain elevated Cu serum levels and suggesting its potential use as a diagnostic marker of cancer [94].

It is due to the concept of biogeochemistry, which was introduced during the 19th century by the researcher Eville Gorham, and which is in clear development until today, that the analysis of the distribution of elements (major or trace) and their relationship with cancer has been possible [95]. Thus, in recent years, scientists have used the knowledge gained and the various advanced instruments including multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS), thermal ionization mass spectrometry (TIMS) and isotope ratio mass spectrometry (IRMS). These instruments measure small elemental isotopic variations of certain alkaline earth metals such as Ca and Mg and the transition elements Fe, Cu, and Zn, because of their functional role in biology and because their turnover rate in the body is relatively short [96-98].

Copper is naturally present as two ions Cu (I) and Cu (II) in cells and blood [82]. The transport and absorption of these ions are responsible for the selective distribution (fractionation) of the Cu isotopes ^{63}Cu(I/II) and ^{65}Cu(I/II) between cells and blood [99]. The conventional delta value (δ^{65}Cu) is used to report the Cu isotope abundances and corresponds to the relative deviation of ^{65}Cu/^{63}Cu ratios in the measured samples from its value. It is calculated by the formula:

$$\delta^{65}Cu = \left[\frac{(^{65}Cu/^{63}Cu)\ sample}{(^{65}Cu/^{63}Cu)\ IStd} - 1 \right] \times 10\ 000$$

Some studies conducted on patients with breast, prostate, and ovarian cancer observed variations in δ^{65}Cu. It could be linked to change in metabolic processes e.g., oxidative phosphorylation and hypoxia or in angiogenesis, and thus to health and disease [100]. δ^{65}Cu were found to be lower in serum (-0.28‰) and higher in erythrocytes (0.46 to 0.67‰) [101].

Furthermore, δ^{65}Cu is more concentrated in women (-0.24 ± 0.36 ‰) serum vs. men (-0.28 ± 0.40 ‰). The isotopic abundances may vary according to sex, menopause, and metabolic diseases [102]. The potential of Cu isotope variability assay as a new diagnostic tool for cancer detection was evaluated in the serum of ovarian and breast cancer patients [103-104]. They observed a decrease in δ^{65}Cu concentration of cancer patients compared to healthy controls related and in mirror image to increase in tumor δ^{65}Cu concentration [102]. In addition, this change in Cu isotope composition has also been observed in other mammals such as dogs and felines [101].

Like copper, zinc (Zn) is a catalytic cofactor in many enzymatic reactions, including Cu/Zn dependent superoxide dismutase I enzyme (SOD1), with antioxidant defense system that requires adequate Zn and Cu levels to support cellular defense against ROS [105]. The interaction between Cu and Zn that allows the SOD1 to function properly [106]. The disruption of Cu/Zn homeostasis and overproductions of ROS cause damage in DNA and possibly cancer development such as hepatocellular carcinoma [106], and overexpression of SOD1 promotes tumor growth in lung cancer cells and reduce apoptosis [107]. Maintenance of adequate Cu/Zn ratio is important for supporting anti-oxidative functions of SOD1 and protection from DNA damage. Imbalances of Cu/Zn ratio are clinically more sensitive indices of disease than the concentration of any single trace metal alone [106-107].

In the thyroid gland, Zn is an essential micronutrient for normal thyroid homeostasis, it is required for both synthesis and mode of action of thyroid hormones [108-109]. Thyroid hormone binding transcription factors, involved in gene expression, contain Zn bound to cysteine residues [108]. In addition, the transcription factor 2, which interacts with the promoters for the thyroglobulin and thyroperoxidase genes, is a zinc-containing protein [110]. Zn also affects the activity of 5′-deiodinase for the conversion of T4 to T3, Zn is required for the correct metabolism of thyroid hormone [108-109].

It was observed that Zn deficiency might have a negative effect on the activity of normal thyroid, with decrease plasma T3 levels [111]. Zn deficiency appears to have no influence on the effects of iodine deficiency in rats. However, in a more complex study with combined Se, iodine and Zn deficiencies, there was an interaction between Se and Zn deficiencies on thyroid follicle cell [112]. Low Zn level could alter cancer-specific tissue, associated with most tumors and in particular with thyroid carcinoma. Few studies reported a lower level of Zn in thyroid cancer patients [113].

Iron (Fe) is also included in this study. It is an essential element in the body and Fe homeostasis is critical for biological processes in normal cells [114]. In thyroid gland, TPO catalyzes the oxidation of iodide, the organization and coupling of iodinated tyrosyl residues generates iodothyronine, essential for the thyroid hormone synthesis and storage protein thyroglobulin [115]. This enzyme requires an adequate intake of Fe and are Fe-depend. Several studies have indicated that Fe is essential for the efficient utilization of iodine and contributes to thyroid hormone biosynthesis. Iron deficiency is the most common nutritional disorder affecting about 20–25% of the world's population, predominantly children, and women [116]. Iron-deficient women have lower levels of Thyroid Stimulating Hormone (TSH), free T4, and free T3 than the levels of controls, as well as higher risk for isolated hypothyroxinemia [116]. Iron deficiency will impair thyroid hormone synthesis, storage, and secretion even by reducing activity of heme-dependent thyroid peroxidase if iodine intake is adequate [117]. The evidence of the Fe and iodine interaction has led to several randomized clinical trials in populations with elevated prevalence of goiter and iron deficiency anemia [118]. All studies showed greater improvement in thyroid function indices and/or thyroid volume in groups with Fe and iodine treatment compared to groups with iodine treatment alone [119].

Iron and selenium both contribute to necrotic cell death "ferroptosis". Fe promotes ferroptosis through the Fenton reaction and uncontrolled lipid autoxidation and Se through GPx4 which induces phospholipid peroxidation and associated cell death. Ferroptosis is characterized by the accumulation of ROS and Fe [120]. Ferroptosis has been implicated in the pathogenesis of several cancers, but the mechanism of ferroptosis regulation in thyroid cancer remains not fully elucidated [121].

The last element studied is Calcium (Ca) which corresponds to the most abundant minerals in the human body. It is essential for the proper functioning of the organism, it has an effect on systemic physiology, including the thyroid, in health and disease, it was suspected to be a possible environmental goitrogen [122]. Thyroid gland contains also parafollicular neuroendocrine cells called "C cells". These cells, like parathyroid cells, detect serum Ca levels and secrete calcitonin, a peptide hormone, that decreases Ca serum by inhibiting bone resorption [123]. Ca is involved in thyroid hormones synthetizes by activating iodide efflux from the thyroid cells into the follicular lumen [124]. In *in vitro* study, the uptake of iodide in thyroid tissue was found to rise with Ca administration [122,125]. It has also been reported that Ca triggers the secretion of TSH and thyrotropin-releasing hormone (TRH), and has a repressive effect on transcription factors including TTF-1, TTF-2 and the gene expressions of TPO and

Thyroglobulin (TG) [126-127]. Low serum Ca levels were found in patients with Riedel's thyroiditis, a rare chronic fibrosing disorder, characterized by an infiltrative lesion in the thyroid gland [128]. It is also important to know that after thyroidectomy the levels of parathyroid hormone, Ca and albumin should be measured for surveillance reasons [129].

Furthermore, Ca signaling is an essential regulator for proper cell functioning and variations in cytosolic free Ca^{2+} are involved in various processes such as cell proliferation, gene transcription and cell death [130]. In addition, various studies have shown that low Ca intake could represent a risk factor for certain cancers [131].

For the thyroid, micronutrients are important to thyroid hormone metabolism and biosynthesis, interaction between micronutrients maintain homeostasis in cells. However, alterations in the concentration of micronutrients in body fluids can affect the body's oxidative and antioxidant balance. This may affect the endocrine system, causing various thyroid dysfunctions such as hyperthyroidism, hypothyroidism, Graves' disease, goiter, Hashimoto's disease and thyroid cancer [132-135].

The relationship between macro and micro-elements and thyroid cancer has been debated for a long time and has not yet been proven, as well as the epidemiological studies are contradictory, but what is it with the Algerian population? Could a low nutritional status of micronutrients and minerals be a risk factor for thyroid cancer in Western Algeria? It is with the intention of answering this question that the present project was launched.

The first objective of this work is to study the profile of Se status of west Algerian population, affected by thyroid carcinoma. This status includes the plasma Se, the activity of GPxs (GPx1 and GPx3) and the plasma content of SePP.

The second objective is to study the possibility of using Cu isotope fractionation ($\delta^{65}Cu$) as a new tool in the diagnosis of PTC. The plasma levels of Cu, Zn, Fe and Ca were also analyzed.

The last objective is to study the impact of Se nanoparticles (SeNPs) and selenite on the development of spheroids, models of thyroid carcinoma metastasis (MDA-T120).

References

[1] B. Castro et E. Rodrigues, « Molecular biology of papillary thyroid microcarcinomas: What is new? », *Rev. Port. Endocrinol. Diabetes E Metab.*, vol. 11, nº 2, p. 287-295, juill. 2016, doi: 10.1016/j.rpedm.2016.04.003.

[2] K. Kaliszewski, D. Diakowska, B. Wojtczak, et J. Rudnicki, « Cancer screening activity results in overdiagnosis and overtreatment of papillary thyroid cancer: A 10-year experience at a single institution », *PLOS ONE*, vol. 15, nº 7, p. e0236257, juill. 2020, doi: 10.1371/journal.pone.0236257.

[3] M. E. Cabanillas, D. G. McFadden, et C. Durante, « Thyroid cancer », *The Lancet*, vol. 388, nº 10061, p. 2783-2795, déc. 2016, doi: 10.1016/S0140-6736(16)30172-6.

[4] S. L. Asa, « The Current Histologic Classification of Thyroid Cancer », *Endocrinol. Metab. Clin. North Am.*, vol. 48, nº 1, p. 1-22, 2018, doi: 10.1016/j.ecl.2018.10.001.

[5] L. Barrea *et al.*, « Nutritional status and follicular-derived thyroid cancer: An update », *Crit. Rev. Food Sci. Nutr.*, vol. 61, nº 1, p. 25-59, janv. 2021, doi: 10.1080/10408398.2020.1714542.

[6] H. Lim, S. S. Devesa, J. A. Sosa, D. Check, et C. M. Kitahara, « Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013 », *JAMA*, vol. 317, nº 13, p. 1338, avr. 2017, doi: 10.1001/jama.2017.2719.

[7] « Thyroid Cancer — Cancer Stat Facts ». https://seer.cancer.gov/statfacts/html/thyro.html (consulté le 1 novembre 2021).

[8] T. I. A. for R. on Cancer (IARC), « Global Cancer Observatory ». https://gco.iarc.fr/ (consulté le 30 mai 2022).

[9] A. Benouis, Z. Bekkouche, M. S. Merad, L. Loudjedi, H. Khelil, et N. Berber, « Thyroid Cancer in Western Algeria: Histopathological and Epidemiological Study », *J. Cancer Ther.*, vol. 08, nº 07, p. 672-682, 2017, doi: 10.4236/jct.2017.87058.

[10] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, et A. Jemal, « Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries », *CA. Cancer J. Clin.*, vol. 68, nº 6, p. 394-424, nov. 2018, doi: 10.3322/caac.21492.

[11] D. D. Hammouda, « EQUIPE DU REGISTRE »:, p. 17, 2004.

[12] J. Rosai, E. Kuhn, et M. L. Carcangiu, « Pitfalls in thyroid tumour pathology », *Histopathology*, vol. 49, nº 2, p. 107-120, août 2006, doi: 10.1111/j.1365-2559.2006.02451.x.

[13] H. Fagman et M. Nilsson, « Morphogenetics of early thyroid development », *J. Mol. Endocrinol.*, vol. 46, nº 1, p. R33-R42, févr. 2011, doi: 10.1677/JME-10-0084.

[14] L. Schomburg, « Selenium, selenoproteins and the thyroid gland: interactions in health and disease », *Nat. Rev. Endocrinol.*, vol. 8, nº 3, p. 160-171, mars 2012, doi: 10.1038/nrendo.2011.174.

[15] B. McGregor, « The Role of Selenium in Thyroid Autoimmunity: A Review », *J. Restor. Med.*, vol. 4, nº 1, p. 83-92, déc. 2015, doi: 10.14200/jrm.2015.4.0102.

[16] T.-J. Lee, S. Kim, H.-J. Cho, et J.-H. Lee, « The Incidence of Thyroid Cancer Is Affected by the Characteristics of a Healthcare System », *J. Korean Med. Sci.*, vol. 27, nº 12, p. 1491, 2012, doi: 10.3346/jkms.2012.27.12.1491.

[17] P. Hofman, « Apport de la biologie moléculaire en pathologie thyroïdienne », *Rev. Francoph. Lab.*, vol. 2011, nº 428, p. 49-56, janv. 2011, doi: 10.1016/S1773-035X(11)70861-9.

[18] Q. T. Nguyen, E. J. Lee, M. G. Huang, Y. I. Park, A. Khullar, et R. A. Plodkowski, « Diagnosis and Treatment of Patients with Thyroid Cancer », vol. 8, n° 1, p. 11, 2015.

[19] S. El-Fadeli *et al.*, « Effects of Imbalance in Trace Element on Thyroid Gland from Moroccan Children », *Biol. Trace Elem. Res.*, vol. 170, n° 2, p. 288-293, avr. 2016, doi: 10.1007/s12011-015-0485-2.

[20] M. P. Rayman, « Selenium and human health », *The Lancet*, vol. 379, n° 9822, p. 1256-1268, mars 2012, doi: 10.1016/S0140-6736(11)61452-9.

[21] Balázs C. et Rácz K., « The relationship between selenium and gastrointestinal inflammatory diseases », *Orv. Hetil.*, vol. 154, n° 41, p. 1628-1635, oct. 2013, doi: 10.1556/OH.2013.29723.

[22] D. Sakız, A. Kaya, et M. Kulaksizoglu, « Serum Selenium Levels in Euthyroid Nodular Thyroid Diseases », *Biol. Trace Elem. Res.*, vol. 174, n° 1, p. 21-26, nov. 2016, doi: 10.1007/s12011-016-0688-1.

[23] J. Köhrle, « Selenium and the thyroid », *Curr. Opin. Endocrinol. Diabetes Obes.*, vol. 22, n° 5, p. 392-401, oct. 2015, doi: 10.1097/MED.0000000000000190.

[24] M. Kieliszek et S. Błażejak, « Current Knowledge on the Importance of Selenium in Food for Living Organisms: A Review », *Molecules*, vol. 21, n° 5, p. 609, mai 2016, doi: 10.3390/molecules21050609.

[25] N. Dennouni-Medjati, M. Dali-Sahi, H. Harek, et Y. Harek, « Statut en sélénium de la population d'une région côtière de l'Ouest algérien à forte activité anthropique », *Env. Risque Sante*, vol. 18, p. 8, 2019.

[26] M. Belhadj *et al.*, « Se Status Prediction by Food Intake as Compared to Circulating Biomarkers in a West Algerian Population », *Nutrients*, vol. 12, n° 12, p. 3599, nov. 2020, doi: 10.3390/nu12123599.

[27] M. Ventura, M. Melo, et F. Carrilho, « Selenium and Thyroid Disease: From Pathophysiology to Treatment », *Int. J. Endocrinol.*, vol. 2017, p. 1-9, 2017, doi: 10.1155/2017/1297658.

[28] V. Triggiani *et al.*, « Role of Iodine, Selenium and Other Micronutrients in Thyroid Function and Disorders », *Endocr. Metab. Immune Disord. - Drug Targets*, vol. 9, n° 3, p. 277-294, sept. 2009, doi: 10.2174/187153009789044392.

[29] K. H. Winther, M. P. Rayman, S. J. Bonnema, et L. Hegedüs, « Selenium in thyroid disorders — essential knowledge for clinicians », *Nat. Rev. Endocrinol.*, vol. 16, n° 3, p. 165-176, mars 2020, doi: 10.1038/s41574-019-0311-6.

[30] M. Wallenberg, S. Misra, et M. Björnstedt, « Selenium Cytotoxicity in Cancer », *Basic Clin. Pharmacol. Toxicol.*, vol. 114, n° 5, p. 377-386, mai 2014, doi: 10.1111/bcpt.12207.

[31] L. H. Duntas et S. Benvenga, « Selenium: an element for life », *Endocrine*, vol. 48, n° 3, p. 756-775, avr. 2015, doi: 10.1007/s12020-014-0477-6.

[32] J. T. Deagen, J. A. Butler, B. A. Zachara, et P. D. Whanger, « Determination of the Distribution of Selenium between Glutathione Peroxidase, Selenoprotein P, and Albumin in Plasma », *Anal. Biochem.*, vol. 208, n° 1, p. 176-181, janv. 1993, doi: 10.1006/abio.1993.1025.

[33] G. F. Combs *et al.*, « Effects of selenomethionine supplementation on selenium status and thyroid hormone concentrations in healthy adults », *Am. J. Clin. Nutr.*, vol. 89, n° 6, p. 1808-1814, juin 2009, doi: 10.3945/ajcn.2008.27356.

[34] R. Margaret P, « Iodine and Selenium as Antiviral Agents: Potential Relevance to SARS-CoV-2 and Covid-19 », *Arch. Oral Maxillofac. Surg.*, vol. 3, n° 1, nov. 2020, doi: 10.36959/379/357.

[35] A. Orozco, C. Valverde-R, A. Olvera, et C. García-G, « Iodothyronine deiodinases: a functional and evolutionary perspective », *J. Endocrinol.*, vol. 215, nº 2, p. 207-219, nov. 2012, doi: 10.1530/JOE-12-0258.

[36] L. Schomburg, « Selenium, selenoproteins and the thyroid gland: interactions in health and disease », *Nat. Rev. Endocrinol.*, vol. 8, nº 3, p. 160-171, mars 2012, doi: 10.1038/nrendo.2011.174.

[37] I. Szanto, M. Pusztaszeri, et M. Mavromati, « H2O2 Metabolism in Normal Thyroid Cells and in Thyroid Tumorigenesis: Focus on NADPH Oxidases », *Antioxidants*, vol. 8, nº 5, p. 126, mai 2019, doi: 10.3390/antiox8050126.

[38] O. Guillin, C. Vindry, T. Ohlmann, et L. Chavatte, « Selenium, Selenoproteins and Viral Infection », *Nutrients*, vol. 11, nº 9, p. 2101, sept. 2019, doi: 10.3390/nu11092101.

[39] N. Maulik et D. K. Das, « Emerging potential of thioredoxin and thioredoxin interacting proteins in various disease conditions », *Biochim. Biophys. Acta BBA - Gen. Subj.*, vol. 1780, nº 11, p. 1368-1382, nov. 2008, doi: 10.1016/j.bbagen.2007.12.008.

[40] J. E. Curran *et al.*, « Genetic variation in selenoprotein S influences inflammatory response », *Nat. Genet.*, vol. 37, nº 11, p. 1234-1241, nov. 2005, doi: 10.1038/ng1655.

[41] L. R. Santos *et al.*, « A Polymorphism in the Promoter Region of the Selenoprotein S Gene (*SEPS1*) Contributes to Hashimoto's Thyroiditis Susceptibility », *J. Clin. Endocrinol. Metab.*, vol. 99, nº 4, p. E719-E723, avr. 2014, doi: 10.1210/jc.2013-3539.

[42] R. F. Burk et K. E. Hill, « Selenoprotein P—Expression, functions, and roles in mammals », *Biochim. Biophys. Acta BBA - Gen. Subj.*, vol. 1790, nº 11, p. 1441-1447, nov. 2009, doi: 10.1016/j.bbagen.2009.03.026.

[43] R. F. Burk et K. E. Hill, « SELENOPROTEIN P: An Extracellular Protein with Unique Physical Characteristics and a Role in Selenium Homeostasis », *Annu. Rev. Nutr.*, vol. 25, nº 1, p. 215-235, août 2005, doi: 10.1146/annurev.nutr.24.012003.132120.

[44] L. Schomburg, « The other view: the trace element selenium as a micronutrient in thyroid disease, diabetes, and beyond », *Hormones*, vol. 19, nº 1, p. 15-24, mars 2020, doi: 10.1007/s42000-019-00150-4.

[45] A. K. Baltaci, T. K. Dundar, F. Aksoy, et R. Mogulkoc, « Changes in the Serum Levels of Trace Elements Before and After the Operation in Thyroid Cancer Patients », *Biol. Trace Elem. Res.*, vol. 175, nº 1, p. 57-64, janv. 2017, doi: 10.1007/s12011-016-0768-2.

[46] L. S. Kazi Tani, N. Dennouni-Medjati, B. Toubhans, et L. Charlet, « Selenium Deficiency—From Soil to Thyroid Cancer », *Appl. Sci.*, vol. 10, nº 15, p. 5368, août 2020, doi: 10.3390/app10155368.

[47] M. Vinceti *et al.*, « Selenium for preventing cancer », *Cochrane Database Syst. Rev.*, janv. 2018, doi: 10.1002/14651858.CD005195.pub4.

[48] J. D. Lambeth, « Nox enzymes, ROS, and chronic disease: An example of antagonistic pleiotropy », *Free Radic. Biol. Med.*, vol. 43, nº 3, p. 332-347, août 2007, doi: 10.1016/j.freeradbiomed.2007.03.027.

[49] D. J. Hughes *et al.*, « Prediagnostic selenium status and hepatobiliary cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort », *Am. J. Clin. Nutr.*, vol. 104, nº 2, p. 406-414, août 2016, doi: 10.3945/ajcn.116.131672.

[50] D. J. Hughes *et al.*, « Selenium status is associated with colorectal cancer risk in the European prospective investigation of cancer and nutrition cohort: Selenium status is associated with colorectal cancer risk », *Int. J. Cancer*, vol. 136, nº 5, p. 1149-1161, mars 2015, doi: 10.1002/ijc.29071.

[51] M. P. Rayman et L. H. Duntas, « Selenium Deficiency and Thyroid Disease », in *The Thyroid and Its Diseases*, M. Luster, L. H. Duntas, et L. Wartofsky, Éd. Cham: Springer International Publishing, 2019, p. 109-126. doi: 10.1007/978-3-319-72102-6_9.

[52] V. Panicker *et al.*, « Common Variation in the DIO2 Gene Predicts Baseline Psychological Well-Being and Response to Combination Thyroxine Plus Triiodothyronine Therapy in Hypothyroid Patients », *J. Clin. Endocrinol. Metab.*, vol. 94, nº 5, p. 1623-1629, mai 2009, doi: 10.1210/jc.2008-1301.

[53] C. Méplan *et al.*, « Relative Abundance of Selenoprotein P Isoforms in Human Plasma Depends on Genotype, Se Intake, and Cancer Status », *Antioxid. Redox Signal.*, vol. 11, nº 11, p. 2631-2640, nov. 2009, doi: 10.1089/ars.2009.2533.

[54] O. Sobolev *et al.*, « Biological role of selenium in the organism of animals and humans », *Ukr. J. Ecol.*, vol. 8, nº 1, p. 654-665, mars 2018, doi: 10.15421/2018_263.

[55] J. Kochman, K. Jakubczyk, P. Bargiel, et K. Janda-Milczarek, « The Influence of Oxidative Stress on Thyroid Diseases », *Antioxidants*, vol. 10, nº 9, p. 1442, sept. 2021, doi: 10.3390/antiox10091442.

[56] R. Ameziane El Hassani, C. Buffet, S. Leboulleux, et C. Dupuy, « Oxidative stress in thyroid carcinomas: biological and clinical significance », *Endocr. Relat. Cancer*, vol. 26, nº 3, p. R131-R143, mars 2019, doi: 10.1530/ERC-18-0476.

[57] C. Chang, B. L. Worley, R. Phaëton, et N. Hempel, « Extracellular Glutathione Peroxidase GPx3 and Its Role in Cancer », *Cancers*, vol. 12, nº 8, p. 2197, août 2020, doi: 10.3390/cancers12082197.

[58] A. K. Baltaci, T. K. Dundar, F. Aksoy, et R. Mogulkoc, « Changes in the Serum Levels of Trace Elements Before and After the Operation in Thyroid Cancer Patients », *Biol. Trace Elem. Res.*, vol. 175, nº 1, p. 57-64, janv. 2017, doi: 10.1007/s12011-016-0768-2.

[59] A. J. Duffield-Lillico *et al.*, « Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial », *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.*, vol. 11, nº 7, p. 630-639, juill. 2002.

[60] E. A. Klein *et al.*, « Vitamin E and the Risk of Prostate Cancer: The Selenium and Vitamin E Cancer Prevention Trial (SELECT) », *JAMA*, vol. 306, nº 14, p. 1549, oct. 2011, doi: 10.1001/jama.2011.1437.

[61] A. R. Kristal *et al.*, « Baseline Selenium Status and Effects of Selenium and Vitamin E Supplementation on Prostate Cancer Risk », *JNCI J. Natl. Cancer Inst.*, vol. 106, nº 3, mars 2014, doi: 10.1093/jnci/djt456.

[62] F. M. Fordyce, « Selenium Deficiency and Toxicity in the Environment », in *Essentials of Medical Geology*, O. Selinus, Éd. Dordrecht: Springer Netherlands, 2013, p. 375-416. doi: 10.1007/978-94-007-4375-5_16.

[63] B. Caballero, L. Allen, et A. Prentice, *Encyclopedia of Human Nutrition, Four-Volume Set*. Burlington: Elsevier, 2005. Consulté le: 30 mai 2022. [En ligne]. Disponible sur: http://public.eblib.com/choice/publicfullrecord.aspx?p=269866

[64] L. Letavayová, V. Vlčková, et J. Brozmanová, « Selenium: From cancer prevention to DNA damage », *Toxicology*, vol. 227, nº 1-2, p. 1-14, oct. 2006, doi: 10.1016/j.tox.2006.07.017.

[65] C. G. Fraga, « Relevance, essentiality and toxicity of trace elements in human health », *Mol. Aspects Med.*, vol. 26, nº 4-5, p. 235-244, août 2005, doi: 10.1016/j.mam.2005.07.013.

[66] P. D. Whanger, « Selenium and its relationship to cancer: an update », *Br. J. Nutr.*, vol. 91, nº 1, p. 11-28, janv. 2004, doi: 10.1079/BJN20031015.

[67] « Selenosis Definition & Meaning | Merriam-Webster Medical ». https://www.merriam-webster.com/medical/selenosis (consulté le 30 mai 2022).

[68] F. Hazane-Puch, J. Arnaud, C. Trocmé, P. Faure, F. Laporte, et P. Champelovier, « Sodium Selenite Decreased HDAC Activity, Cell Proliferation and Induced Apoptosis in Three Human Glioblastoma Cells », *Anticancer Agents Med. Chem.*, vol. 16, nº 4, p. 490-500, févr. 2016, doi: 10.2174/1871520615666150819095426.

[69] M. Vinceti *et al.*, « Health risk assessment of environmental selenium: Emerging evidence and challenges », *Mol. Med. Rep.*, vol. 15, nº 5, p. 3323-3335, mai 2017, doi: 10.3892/mmr.2017.6377.

[70] J. Mandrioli *et al.*, « Elevated Levels of Selenium Species in Cerebrospinal Fluid of Amyotrophic Lateral Sclerosis Patients with Disease-Associated Gene Mutations », *Neurodegener. Dis.*, vol. 17, nº 4-5, p. 171-180, 2017, doi: 10.1159/000460253.

[71] K. A. Conklin, « Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness », *Integr. Cancer Ther.*, vol. 3, nº 4, p. 294-300, déc. 2004, doi: 10.1177/1534735404270335.

[72] R. A. Sperling et W. J. Parak, « Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles », *Philos. Trans. R. Soc. Math. Phys. Eng. Sci.*, vol. 368, nº 1915, p. 1333-1383, mars 2010, doi: 10.1098/rsta.2009.0273.

[73] A. Khurana, S. Tekula, M. A. Saifi, P. Venkatesh, et C. Godugu, « Therapeutic applications of selenium nanoparticles », *Biomed. Pharmacother.*, vol. 111, p. 802-812, mars 2019, doi: 10.1016/j.biopha.2018.12.146.

[74] B. Yu, Y. Zhang, W. Zheng, C. Fan, et T. Chen, « Positive Surface Charge Enhances Selective Cellular Uptake and Anticancer Efficacy of Selenium Nanoparticles », *Inorg. Chem.*, vol. 51, nº 16, p. 8956-8963, août 2012, doi: 10.1021/ic301050v.

[75] W. Zheng *et al.*, « Multifunctional polyamidoamine-modified selenium nanoparticles dual-delivering siRNA and cisplatin to A549/DDP cells for reversal multidrug resistance », *Acta Biomater.*, vol. 11, p. 368-380, janv. 2015, doi: 10.1016/j.actbio.2014.08.035.

[76] X. Wang, K. Sun, Y. Tan, S. Wu, et J. Zhang, « Efficacy and safety of selenium nanoparticles administered intraperitoneally for the prevention of growth of cancer cells in the peritoneal cavity », *Free Radic. Biol. Med.*, vol. 72, p. 1-10, juill. 2014, doi: 10.1016/j.freeradbiomed.2014.04.003.

[77] T. Chen, Y.-S. Wong, W. Zheng, Y. Bai, et L. Huang, « Selenium nanoparticles fabricated in Undaria pinnatifida polysaccharide solutions induce mitochondria-mediated apoptosis in A375 human melanoma cells », *Colloids Surf. B Biointerfaces*, vol. 67, nº 1, p. 26-31, nov. 2008, doi: 10.1016/j.colsurfb.2008.07.010.

[78] S. Skalickova, V. Milosavljevic, K. Cihalova, P. Horky, L. Richtera, et V. Adam, « Selenium nanoparticles as a nutritional supplement », *Nutrition*, vol. 33, p. 83-90, janv. 2017, doi: 10.1016/j.nut.2016.05.001.

[79] J. Zhang, X. Wang, et T. Xu, « Elemental Selenium at Nano Size (Nano-Se) as a Potential Chemopreventive Agent with Reduced Risk of Selenium Toxicity: Comparison with Se-Methylselenocysteine in Mice », *Toxicol. Sci.*, vol. 101, nº 1, p. 22-31, janv. 2008, doi: 10.1093/toxsci/kfm221.

[80] H. Luo, F. Wang, Y. Bai, T. Chen, et W. Zheng, « Selenium nanoparticles inhibit the growth of HeLa and MDA-MB-231 cells through induction of S phase arrest », *Colloids Surf. B Biointerfaces*, vol. 94, p. 304-308, juin 2012, doi: 10.1016/j.colsurfb.2012.02.006.

[81] S. Lutsenko, « Human copper homeostasis: a network of interconnected pathways », *Curr. Opin. Chem. Biol.*, vol. 14, nº 2, p. 211-217, avr. 2010, doi: 10.1016/j.cbpa.2010.01.003.

[82] G. Brewer, « Copper in medicine », *Curr. Opin. Chem. Biol.*, vol. 7, nº 2, p. 207-212, avr. 2003, doi: 10.1016/S1367-5931(03)00018-8.

[83] F. Martin *et al.*, « Copper-dependent activation of hypoxia-inducible factor (HIF)-1: implications for ceruloplasmin regulation », *Blood*, vol. 105, nº 12, p. 4613-4619, juin 2005, doi: 10.1182/blood-2004-10-3980.

[84] M. Araya, F. Pizarro, M. Olivares, M. Arredondo, M. González, et M. Méndez, « Understanding copper homeostasis in humans and copper effects on health », *Biol. Res.*, vol. 39, nº 1, 2006, doi: 10.4067/S0716-97602006000100020.

[85] M. S. Willis *et al.*, « Zinc-Induced Copper Deficiency: A Report of Three Cases Initially Recognized on Bone Marrow Examination », *Am. J. Clin. Pathol.*, vol. 123, nº 1, p. 125-131, janv. 2005, doi: 10.1309/V6GVYW2QTYD5C5PJ.

[86] J. Osredkar, « Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance », *J. Clin. Toxicol.*, vol. s3, nº 01, 2011, doi: 10.4172/2161-0495.S3-001.

[87] T. G. Kazi *et al.*, « Interaction of Copper with Iron, Iodine, and Thyroid Hormone Status in Goitrous Patients », *Biol. Trace Elem. Res.*, vol. 134, nº 3, p. 265-279, juin 2010, doi: 10.1007/s12011-009-8478-7.

[88] F. Shen, W.-S. Cai, J.-L. Li, Z. Feng, J. Cao, et B. Xu, « The Association Between Serum Levels of Selenium, Copper, and Magnesium with Thyroid Cancer: a Meta-analysis », *Biol. Trace Elem. Res.*, vol. 167, nº 2, p. 225-235, oct. 2015, doi: 10.1007/s12011-015-0304-9.

[89] M. Rezaei, S. Y. Javadmoosavi, B. Mansouri, N. A. Azadi, O. Mehrpour, et S. Nakhaee, « Thyroid dysfunction: how concentration of toxic and essential elements contribute to risk of hypothyroidism, hyperthyroidism, and thyroid cancer », *Environ. Sci. Pollut. Res.*, vol. 26, nº 35, p. 35787-35796, déc. 2019, doi: 10.1007/s11356-019-06632-7.

[90] Y. Liu *et al.*, « Serum Trace Elements Profile in Graves' Disease Patients with or without Orbitopathy in Northeast China », *BioMed Res. Int.*, vol. 2018, p. 1-8, 2018, doi: 10.1155/2018/3029379.

[91] S. Hybsier, C. Höfig, J. Mittag, G. Brabant, et L. Schomburg, « Control of serum copper (Cu) and selenium (Se) status by thyroid hormones », *Exp. Clin. Endocrinol. Diabetes*, vol. 122, nº 03, p. s-0035-1547752, mars 2015, doi: 10.1055/s-0035-1547752.

[92] F. Kosova *et al.*, « Serum copper levels in benign and malignant thyroid diseases », *Bratisl. Med. J.*, vol. 113, nº 12, p. 718-720, 2012, doi: 10.4149/BLL_2012_162.

[93] T. Theophanides et J. Anastassopoulou, « Copper and carcinogenesis », *Crit. Rev. Oncol. Hematol.*, vol. 42, nº 1, p. 57-64, avr. 2002, doi: 10.1016/S1040-8428(02)00007-0.

[94] Y. Nose, B.-E. Kim, et D. J. Thiele, « Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function », *Cell Metab.*, vol. 4, nº 3, p. 235-244, sept. 2006, doi: 10.1016/j.cmet.2006.08.009.

[95] T. S. Bianchi, « The evolution of biogeochemistry: revisited », *Biogeochemistry*, vol. 154, nº 2, p. 141-181, juin 2021, doi: 10.1007/s10533-020-00708-0.

[96] V. Balter *et al.*, « Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients », *Proc. Natl. Acad. Sci.*, vol. 112, nº 4, p. 982-985, janv. 2015, doi: 10.1073/pnas.1415151112.

[97] F. Albarède, « Metal Stable Isotopes in the Human Body: A Tribute of Geochemistry to Medicine », *Elements*, vol. 11, nº 4, p. 265-269, août 2015, doi: 10.2113/gselements.11.4.265.

[98] G. Chamel *et al.*, « Retrospective evaluation of blood copper stable isotopes ratio ^{65}Cu/^{63}Cu as a biomarker of cancer in dogs: ∂CU, a potential biomarker of cancer in dogs », *Vet. Comp. Oncol.*, vol. 15, nº 4, p. 1323-1332, déc. 2017, doi: 10.1111/vco.12273.

[99] A. T. Gourlan, G. Douay, et P. Telouk, « Copper isotopes as possible neoplasia biomarkers in captive wild felids », *Zoo Biol.*, vol. 38, nº 4, p. 371-383, août 2019, doi: 10.1002/zoo.21504.

[100] F. Albarède, P. Télouk, et V. Balter, « Medical Applications of Isotope Metallomics », *Rev. Mineral. Geochem.*, vol. 82, nº 1, p. 851-885, 2017, doi: 10.2138/rmg.2017.82.20.

[101] F. Albarède, P. Telouk, A. Lamboux, K. Jaouen, et V. Balter, « Isotopic evidence of unaccounted for Fe and Cu erythropoietic pathways », *Metallomics*, vol. 3, nº 9, p. 926, 2011, doi: 10.1039/c1mt00025j.

[102] C. N. Maréchal, P. Télouk, et F. Albarède, « Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry », *Chem. Geol.*, vol. 156, nº 1-4, p. 251-273, avr. 1999, doi: 10.1016/S0009-2541(98)00191-0.

[103] B. Toubhans *et al.*, « Cu isotope ratios are meaningful in ovarian cancer diagnosis », *J. Trace Elem. Med. Biol.*, vol. 62, p. 126611, déc. 2020, doi: 10.1016/j.jtemb.2020.126611.

[104] P. Télouk *et al.*, « Copper isotope effect in serum of cancer patients. A pilot study », *Metallomics*, vol. 7, nº 2, p. 299-308, 2015, doi: 10.1039/C4MT00269E.

[105] K. Iwase *et al.*, « Localization of Cu/Zn and Mn superoxide dismutase in various thyroid disorders », *Acta Endocrinol. (Copenh.)*, vol. 129, nº 6, p. 573-578, déc. 1993, doi: 10.1530/acta.0.1290573.

[106] J. Osredkar, « Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance », *J. Clin. Toxicol.*, vol. s3, nº 01, 2011, doi: 10.4172/2161-0495.S3-001.

[107] R. Somwar *et al.*, « Superoxide dismutase 1 (SOD1) is a target for a small molecule identified in a screen for inhibitors of the growth of lung adenocarcinoma cell lines », *Proc. Natl. Acad. Sci.*, vol. 108, nº 39, p. 16375-16380, sept. 2011, doi: 10.1073/pnas.1113554108.

[108] J. B. Barnett *et al.*, « Effect of zinc supplementation on serum zinc concentration and T cell proliferation in nursing home elderly: a randomized, double-blind, placebo-controlled trial », *Am. J. Clin. Nutr.*, vol. 103, nº 3, p. 942-951, mars 2016, doi: 10.3945/ajcn.115.115188.

[109] E. Danforth et A. G. Burger, « The Impact of Nutrition on Thyroid Hormone Physiology and Action », *Annu. Rev. Nutr.*, vol. 9, nº 1, p. 201-227, juill. 1989, doi: 10.1146/annurev.nu.09.070189.001221.

[110] A. Kralik, K. Eder, et M. Kirchgessner, « Influence of Zinc and Selenium Deficiency on Parameters Relating to Thyroid Hormone Metabolism », *Horm. Metab. Res.*, vol. 28, nº 05, p. 223-226, mai 1996, doi: 10.1055/s-2007-979169.

[111] J. S. Severo *et al.*, « The Role of Zinc in Thyroid Hormones Metabolism », *Int. J. Vitam. Nutr. Res.*, vol. 89, nº 1-2, p. 80-88, juill. 2019, doi: 10.1024/0300-9831/a000262.

[112] R. B. Jain, « Thyroid Function and Serum Copper, Selenium, and Zinc in General U.S. Population », *Biol. Trace Elem. Res.*, vol. 159, nº 1-3, p. 87-98, juin 2014, doi: 10.1007/s12011-014-9992-9.

[113] J. Gumulec, M. Masarik, V. Adam, T. Eckschlager, I. Provaznik, et R. Kizek, « Serum and Tissue Zinc in Epithelial Malignancies: A Meta-Analysis », *PLoS ONE*, vol. 9, nº 6, p. e99790, juin 2014, doi: 10.1371/journal.pone.0099790.

[114] G. J. Anderson et G. McLaren, Éd., *Iron physiology and pathophysiology in humans*. New York: Humana, 2009.

[115] S. Y. Hess, M. B. Zimmermann, M. Arnold, W. Langhans, et R. F. Hurrell, « Iron Deficiency Anemia Reduces Thyroid Peroxidase Activity in Rats », *J. Nutr.*, vol. 132, nº 7, p. 1951-1955, juill. 2002, doi: 10.1093/jn/132.7.1951.

[116] M. B. Zimmermann, « The Influence of Iron Status on Iodine Utilization and Thyroid Function », *Annu. Rev. Nutr.*, vol. 26, nº 1, p. 367-389, août 2006, doi: 10.1146/annurev.nutr.26.061505.111236.

[117] M. B. Zimmermann et J. Köhrle, « The Impact of Iron and Selenium Deficiencies on Iodine and Thyroid Metabolism: Biochemistry and Relevance to Public Health », *Thyroid*, vol. 12, nº 10, p. 867-878, oct. 2002, doi: 10.1089/105072502761016494.

[118] J. L. Beard, M. J. Borel, et J. Derr, « Impaired thermoregulation and thyroid function in iron-deficiency anemia », *Am. J. Clin. Nutr.*, vol. 52, nº 5, p. 813-819, nov. 1990, doi: 10.1093/ajcn/52.5.813.

[119] T. G. Kazi *et al.*, « Interaction of Copper with Iron, Iodine, and Thyroid Hormone Status in Goitrous Patients », *Biol. Trace Elem. Res.*, vol. 134, nº 3, p. 265-279, juin 2010, doi: 10.1007/s12011-009-8478-7.

[120] M. Ge *et al.*, « A Ferroptosis-Related Signature Robustly Predicts Clinical Outcomes and Associates With Immune Microenvironment for Thyroid Cancer », *Front. Med.*, vol. 8, p. 637743, avr. 2021, doi: 10.3389/fmed.2021.637743.

[121] M. Conrad et B. Proneth, « Selenium: Tracing Another Essential Element of Ferroptotic Cell Death », *Cell Chem. Biol.*, vol. 27, nº 4, p. 409-419, avr. 2020, doi: 10.1016/j.chembiol.2020.03.012.

[122] A. K. Chandra, H. Goswami, et P. Sengupta, « Dietary calcium induced cytological and biochemical changes in thyroid », *Environ. Toxicol. Pharmacol.*, vol. 34, nº 2, p. 454-465, sept. 2012, doi: 10.1016/j.etap.2012.06.003.

[123] L. D. Quarles, « Extracellular calcium-sensing receptors in the parathyroid gland, kidney, and other tissues »:, *Curr. Opin. Nephrol. Hypertens.*, vol. 12, nº 4, p. 349-355, juill. 2003, doi: 10.1097/00041552-200307000-00002.

[124] J. C. Silveira et P. A. Kopp, « Pendrin and anoctamin as mediators of apical iodide efflux in thyroid cells », *Curr. Opin. Endocrinol. Diabetes Obes.*, vol. 22, nº 5, p. 374-380, oct. 2015, doi: 10.1097/MED.0000000000000188.

[125] Y. R. Gandra et J. G. Coniglio, « Calcium and metabolism of I^{131} in rats and homogenates of rat thyroid », *Am. J. Physiol.-Leg. Content*, vol. 200, nº 5, p. 1023-1026, mai 1961, doi: 10.1152/ajplegacy.1961.200.5.1023.

[126] K. Murao *et al.*, « Role of Calcium-Calmodulin-Dependent Protein Kinase Cascade in Thyrotropin (TSH)-Releasing Hormone Induction of TSH and Prolactin Gene Expression », *Endocrinology*, vol. 145, nº 11, p. 4846-4852, nov. 2004, doi: 10.1210/en.2004-0544.

[127] K. Suzuki *et al.*, « Thyroid Transcription Factor 1 Is Calcium Modulated and Coordinately Regulates Genes Involved in Calcium Homeostasis in C Cells », *Mol. Cell. Biol.*, vol. 18, nº 12, p. 7410-7422, déc. 1998, doi: 10.1128/MCB.18.12.7410.

[128] K. Takeshima *et al.*, « Clinicopathological features of Riedel’s thyroiditis associated with IgG4-related disease in Japan », *Endocr. J.*, vol. 62, nº 8, p. 725-731, 2015, doi: 10.1507/endocrj.EJ15-0175.

[129] B. M. Genovese, S. I. Noureldine, E. M. Gleeson, R. P. Tufano, et E. Kandil, « What Is the Best Definitive Treatment for Graves' Disease? A Systematic Review of the Existing Literature », *Ann. Surg. Oncol.*, vol. 20, nº 2, p. 660-667, févr. 2013, doi: 10.1245/s10434-012-2606-x.

[130] T. A. Stewart, K. T. D. S. Yapa, et G. R. Monteith, « Altered calcium signaling in cancer cells », *Biochim. Biophys. Acta BBA - Biomembr.*, vol. 1848, nº 10, p. 2502-2511, oct. 2015, doi: 10.1016/j.bbamem.2014.08.016.

[131] V. Centeno, G. Díaz de Barboza, A. Marchionatti, V. Rodríguez, et N. Tolosa de Talamoni, « Molecular mechanisms triggered by low-calcium diets », *Nutr. Res. Rev.*, vol. 22, nº 2, p. 163-174, déc. 2009, doi: 10.1017/S0954422409990126.

[132] M. Rezaei, S. Y. Javadmoosavi, B. Mansouri, N. A. Azadi, O. Mehrpour, et S. Nakhaee, « Thyroid dysfunction: how concentration of toxic and essential elements contribute to risk of hypothyroidism, hyperthyroidism, and thyroid cancer », *Environ. Sci. Pollut. Res.*, vol. 26, nº 35, p. 35787-35796, déc. 2019, doi: 10.1007/s11356-019-06632-7.

[133] A. K. Baltaci, T. K. Dundar, F. Aksoy, et R. Mogulkoc, « Changes in the Serum Levels of Trace Elements Before and After the Operation in Thyroid Cancer Patients », *Biol. Trace Elem. Res.*, vol. 175, nº 1, p. 57-64, janv. 2017, doi: 10.1007/s12011-016-0768-2.

[134] S. Hanif, A. Ilyas, et M. H. Shah, « Statistical Evaluation of Trace Metals, TSH and T4 in Blood Serum of Thyroid Disease Patients in Comparison with Controls », *Biol. Trace Elem. Res.*, vol. 183, nº 1, p. 58-70, mai 2018, doi: 10.1007/s12011-017-1137-5.

[135] A. Stojsavljević *et al.*, « Determination of toxic and essential trace elements in serum of healthy and hypothyroid respondents by ICP-MS: A chemometric approach for discrimination of hypothyroidism », *J. Trace Elem. Med. Biol.*, vol. 48, p. 134-140, juill. 2018, doi: 10.1016/j.jtemb.2018.03.020.

Chapter I: Selenium Deficiency—From Soil to Thyroid Cancer

I. Presentation of the article

Selenium is a fascinating trace element for human health. It is essential for many cellular and sub-cellular functions including redox homeostasis and antioxidant defense. Dietary intake is the main source of Se exposure and is closely correlated with soil Se. In the human body, Se is ingested from food, absorbed in the gastrointestinal tract, transported to the liver and kidney for metabolism and distribution to all tissues.

It is genetically incorporated into selenoproteins via a UGA codon in the form of a 21 amino acid "selenocysteine". Thyroid glands contain several selenoproteins including glutathione peroxidases, iodothyronine deiodinases and thioredoxine reductases. They play an important role in the proper functioning of thyroid gland and in thyroid hormones synthetized. The thyroid represents the richest organ in tissue weight of Se and it is the 2nd most important element after iodine.

Selenium remains a complex element and its involvement in human health and disease, particularly cancer, has not been fully elucidated. Recent studies have shown that some selenoproteins suppress tumor cell growth, while others promote it, underscoring the fact that the mechanisms of carcinogenesis related to Se status are not fully understood.

Selenium deficiency leads to a dysfunction of the internal antioxidant system, which can be the cause of clinical morbidity and DNA damage. However, a high intake of Se can lead to intoxication. Se deficiency and overexpression have been correlated with many diseases and have a U-shaped curve effect in human health. The Se/thyroid cancer relationship has been debated for a long time, several studies have found Se deficiency in thyroid cancer patients while others have found no or insignificant effect of this association, therefore the results remain controversial and inconclusive.

In this section and in review article form, we have highlighted the link between Se forms present in the soil, their transfer/absorption by plant and the outcome of the Se after ingestion via vegetable diet. We have also focused on the importance of selenoproteins and their roles in the thyroid gland with an emphasis on thyroid cancer in case of Se deficiency.

We reached to a conclusion that there are different approaches still needed to understand better the link between Se status and thyroid cancer. More genetic association studies, large-scale population-based studies, and other omics-based analysis will be necessary to complete these approaches.

II. Article

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Selenium Deficiency—From Soil to Thyroid Cancer

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Featured Application: The paper underlines the link between soil, food and human health, with a particular emphasis on thyroid cancer in case of Se deficiency, in order to provide a scientific basis to Public Health Recommendations.

Abstract:

Selenium (Se) is an essential micronutrient present in human diet, entering in the composition of selenoproteins as selenocysteine (Se-Cys) amino acid. At the thyroid level, these proteins play an important role as antioxidant and in hormone metabolism. Selenoproteins are essential for the balance of redox homeostasis and antioxidant defense of mammalian organisms, while the corresponding imbalance is now recognized as the cause of many diseases including cancer. The food chain is the main source of Se in human body. Dietary intake is strongly correlated with Se content in soil and varies according to several factors such as geology and atmospheric input. Both Se deficiency and toxicity have been associated with adverse health effects. This review synthesizes recent data on the transfer of Se from soil to humans, Se U-shaped deficiency and toxicity uptake effects and particularly the impact of Se deficiency on thyroid cancer.

Keywords: selenium; food; dietary intake; antioxidant; deficiency; cancer; selenoproteins

1. Introduction

Selenium is one of the most intriguing trace elements of the periodic table. After its discovery in 1818 by the Swedish Berzelius in a sulfuric acid plant, it was used for its electric and photoelectric properties [1]. Its first medical application dates back to 1911, when an unidentified Se compound had caused necrosis and the disappearance of Ehrlich's carcinoma and sarcoma in mice [1]. A year later, a publication by Delbet in 1912 reported the death of patients who received high doses of sodium selenite [2]. However, all therapeutic applications of Se ceased when, in 1943, Nelson *et al.*, had declared the element itself carcinogenic [3]. It was not until 1957 that scientists collected evidence for the other face of the moon (*Selen* in Greek), that is, for the essentiality of the trace element Se for humans and animals [1,4].

Despite the importance of selenium in human health, the major impact of supply, speciation and restricted uptake of this micronutrient, is currently not well understood [5]. Much research is still needed to improve our understanding of optimized requirements, taking into account the very narrow range between the beneficial and toxic effects of the mineral [5].

Selenium is essential for many cellular functions including redox homeostasis and antioxidant defense [6]. In the body, Se is ingested from food, absorbed in the gastrointestinal tract (GIT), transported to the liver and kidney for metabolism and distribution to the body tissues [7].

In humans, Se is integrated into 25 selenoproteins in the form of the amino acid Se-Cys. Some of them such as glutathione peroxidases (GPxs), iodothyronine deiodinases (DIOs) and thioredoxine reductases (TRxs) play an important role in the metabolism of the thyroid gland [8]. Se is present at higher concentrations in the thyroid than in other organs and is an essential element for the biosynthesis of thyroid hormones [9]. Another important role of selenoproteins in the thyroid is the detoxification by GPx of hydrogen peroxide (H_2O_2) massively synthetized in order to oxidize iodide (I^-) and introduce it into thyroglobulin, a precursor of L-3,5,3′,5′-tetraiodothyronine (thyroxine; T4) and L-3,5,3′-triiodothyronine (T3) [10]. Therefore, Se is considered to be the second most important element in thyroid metabolism after iodine [11].

The results of initial epidemiological studies have suggested a significant association between low Se status and cancer, suggesting that Se may have anticarcinogenic effect [12]. Surprisingly, recent studies have shown an increased risk of some cancers such as prostate cancer with high Se levels [13] and the chemotherapeutic effect of Se have not been proven at safe and tolerated doses (<90 µg/day) [14].

However, in the case of thyroid cancer, serum Se deficiency may be considered a risk factor but the results are inconclusive [15-16].

This review synthesizes the speciation-controlled transport of Se from soil to humans via food and its absorption in human gastrointestinal tract. We then focus on the use of Se by thyroid gland and on the relationship between Se and thyroid cancer, according to the latest knowledge produced on this pathology.

2. Selenium from Soil to Food Bowl

2.1. Selenium in Soil and Surface Waters

Selenium is a common element found in nature, in the Earth's atmosphere, lithosphere, biosphere and hydrosphere [17]. Se is present in the soil in inorganic forms, such as selenide (Se^{2-}), elemental selenium ($Se^{(0)}$), selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) and in organic forms (selenocysteine "Se-Cys" and selenomethionine "Se-Met") [18]. The availability of selenium for plant absorption is highly dependent on the chemical and physical conditions of the soil. The concentration of Se in the soil is very different from one country to another and even from one region to another within the same country [19]. Together with atmospheric input, bedrocks are the main source of Se in soil, with varying levels depending on their nature, sedimentary rocks (e.g., shale, limestone and sandstone) being the richest in Se [20]. Thus, the concentration of Se in soils is strongly dependent on the concentrations of the bedrock [21].

In water, Se is present in trace amounts, mainly in the form of selenate and selenite, where Se is present in +VI and +IV oxidation state, respectively [22]. Se is more abundant in groundwater than in seawater, due to the elution of Se from source rocks and over-fertilization of soils with mixtures rich in Se compounds [21,23].

Another source of Se in the soil is due to natural and anthropogenic input from the atmosphere [24]. The combustion of coal, crude oil and the use of agro-technical processes mainly in fertilization contribute enormously to the content of Se in the soil [25]. It has been reported that Se levels are important in people living near processing plants, for example, sulfuric acid production or coking [26-27]. Volcanic gases are also a major source of Se to the atmosphere, together with Se methylation and volatilization by ocean algal bloom [24]. Biomethylation of Se by microorganisms and the decomposition of Se-rich organic matter are additional factors that contribute to the enrichment of the atmosphere with Se [28]. In these

cases, volatile selenium compounds such as dimethylselenium (DMeSe), hydrogen selenide (H_2Se) and selenium oxide (SeO_2) are produced [29].

2.2. Transfer and Absorption of Se from Soil to Plant

Each plant variety has the ability to accumulate Se at the root and shoot level, depending on the concentration of Se in the soil and the ability of the plant species to absorb, accumulate and metabolize Se [30]. There are several types of plants, so-called hyper-accumulators that grow only in soils very rich in Se "seleniferous" and accumulate between 1000 and 15,000 mg kg^{-1}, including species of the genera Astragalus, Stanleya, Morinda, Neptunia, Oonopsis and Xylorhiza. Secondary accumulators such as cereals, some plants of the cruciferous family (rape, broccoli) and Allium species such as garlic and onion grow in soils with variable Se content and can accumulate 30 to 1000 mg kg^{-1}. Non-accumulating plants generally grown on "selenium-poor" soils and cannot accumulate high concentrations of Se in their tissues and contain less than 30 mg kg^{-1}. This is the case for most plants used for food and feed (forage, vegetables, fruits, etc.) [30].

Absorption of organic selenium by plants is more efficient than of inorganic forms such as Se (IV) or Se (VI) [31]. Less than 5% of the Se present in the soil is used by plants [32]. Other forms of Se such as selenide and elemental Se are not absorbed by plants since they are insoluble in water [31] (Figure 1).

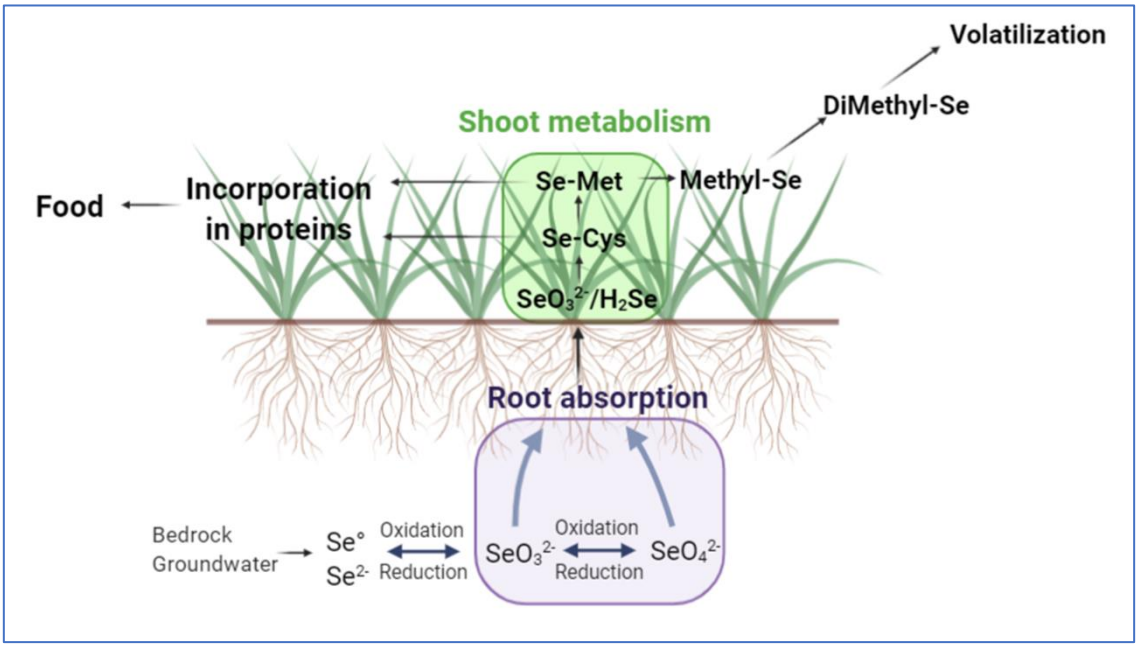

Figure 1. Selenium speciation from soil to plant metabolism (Adapted from Winkel *et al.*, [29] and Lazar *et al.*, [33]). $Se^{(0)}$ = elemental selenium, Se^{2-}= selenide, SeO_4^{2-} = selenate, SeO_3^{2-}= selenite, H_2Se= hydrogen selenide, Se-Cys = selenocysteine, Se-Met = selenomethionine.

The accumulation of Se from soil to plants requires several processes and depends on the speciation of Se in the soil. Sulfate (S) and phosphate (P), present in the soil could affect the absorption of Se by plants. As selenate is structurally and chemically similar to sulfate, both ions follow the same metabolic pathways during the translocation process in plants [34]. The high-affinity H+/sulfate symporters, homologous to sulfate transporter, AtSULTR1;1 and AtSULTR1;2, catalyze the influx of selenate into root cells from the rhizosphere [35-36]. The preference for selenate uptake over sulfate varies from plant to plant and is influenced by various factors for example, soil salinity and pH [37], the two parameters affecting Se(IV) and Se(VI) adsorption on soil particles [31]. In other non-accumulating plants, other transporters behave similarly. In hyperaccumulative plants, the genes of AtSULTR1;1 and AtSULTR1;2 have a high expression [36].

Selenite is absorbed by roots as $HSeO_3^-$ by members of the phosphate transporter family [38] or as H_2SeO_3 by aquaporins [39]. After being absorbed, selenate moves from the root symplast to the stele and is then transferred to the shoot, while selenite is converted into organoselenated compounds [34,39-40]. In the xylem, selenate is the dominant form of Se, however minor amounts of Se-Met and selenomethionine Se-oxide (SeOMet) have been reported. After being delivered to the shoot by the xylem, selenate enters leaf cells by SULTR transporters [35,40-41].

2.3. Metabolism and Se Speciation in Plants

Selenate reduction to selenite then to selenide is coupled to an oxidation of glutathione [35-37]. The Selenide is converted to Se-Cys in a manner analogous to sulfur metabolism. Se-Cys is converted by reverse trans-sulfuration to Se-Met and the two amino acids can be incorporated nonspecifically into proteins in the place of methionine and cysteine [33].

Selenomethionine can be further metabolized to Adenosyl-Se-Met (SeAM), DMeSe and then converted to MeSeCys and γ–glutamine–Se–MethylselenoCysteine (γ-glu-SeMeSeCys) [34,42]. At high levels of Se, MeSeCys becomes the main Se compound, although other compounds are present but at low content [43].

Figure 1 also shows the process of biogenic volatilization of Se from soil and plants, which is in fact a detoxification process [33]. It is used to decontaminate soils rich in Se, such as oil refinery effluent fields [33]. Since some plants can absorb large amounts of Se from the soil, it is therefore very important that plants exhale various volatile Se compounds. The main product

of plant volatilization is DMeSe [33]. Heat-treated food products can lose up to 10% of total Se due to the formation of volatile Se compounds (DMeSe) [23].

Different factors can influence the ability of plants to volatilize Se. First, the concentration of Se in the roots, the Se species as well as the concentration of sulfate compared to selenate (they can compete for particular enzymes for the process of volatilization) [33].

2.4. Selenium in Food and Dietary Intake

Food is the principal source of Se. In many populations, plant foods are the main dietary source of Se (mostly as Se-Met), followed by meat and seafood [44-45]. The concentration of Se in food depends on the type of soil, the geographical area and the capacity of plants to accumulate it [23]. In addition, other factors can influence the Se content in the diet, such as consumption of local food or imported food products [19] for example, USA Se-rich cereals [23,46].

The bioavailability of Se in the diet depends on factors such as proteins, fats and heavy metals. Thus, food rich in protein and in the presence of vitamins A, C, E contain higher levels of Se but heavy metals and sulfur reduce Se [47-48].

Cereals provide 50% of daily Se intake. While meat, poultry and fish group provide about 35%. Fresh water and beverages contribute to the daily intake with only 5%–25% [23]. Fresh and non-heat-treated fruits and vegetables contain small amounts of Se about 11% in a balanced diet; this is due to their low protein content and high water content [23].

Another selenium-rich source is found in sea salt, offal, yeast (selenium-containing yeast), garlic, asparagus, kohlrabi (enriched with Se) [6,47-49]. High concentrations of Se have, exceptionally, also been found in Brassica genus (broccoli, cabbage, cauliflower) and onions [23]. Brazil nuts and mushrooms have extremely high concentrations of this element [6,50].

The reference values for dietary Se intake were estimated on the basis of the intake necessary for saturation of the selenoproteins; GPx in plasma or erythrocyte or selenoprotein P (SePP) in plasma [51-52]. The Recommended Dietary Allowances (RDA) of Se in the United States, based on the activity maximization of GPx are around 55 µg/day in adults [53]. Based on the dietary Se intake required for saturation of plasma SePP, the nutrition societies of Germany, Austria and Switzerland (D-A-CH) have recommended a Se intake reference value of 70 µg/day and 60 µg/day for men and women, respectively, for normal body weight, at a rate of approximately 1 µg of Se per kg of body weight [54]. The World Health Organization

recommends that the intake of Se should not exceed 70 µg/day [55]. A dietary intake above 700 µg/day can be toxic to adults [6]. Se intake in the European population varies from 30 µg/day and 50 µg/day [55].

Furthermore, the biological activities of the several selenium species have not been fully characterized in order to assess their specific toxicity in nutrition, hence defining dietary windows for each of them [14,56–58].

3. Selenium Human Uptake and Distribution

3.1. Gastrointestinal Uptake

Selenium is present in diet in the forms of selenomethionine, selenocysteine, selenite and selenate (Figure 2).

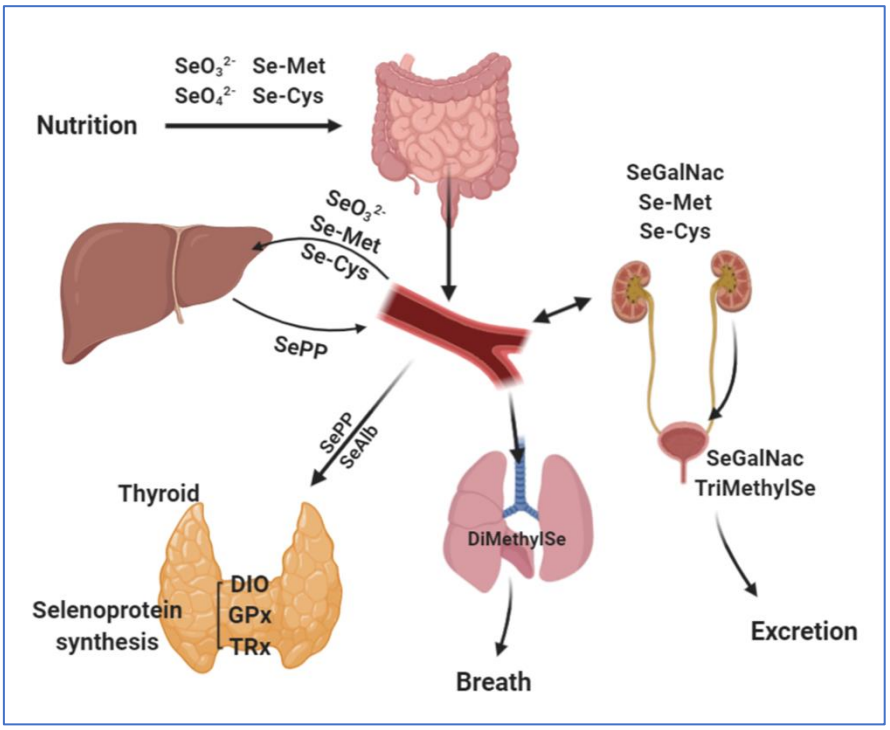

Figure 2. Main selenium species in various human organs and in blood (adapted from Gammelgaard *et al.*, [59]). SeO_3^{2-} = selenite, SeO_4^{2-} = selenate, Se-Met = selenomethionine, Se-Cys = selenocysteine, SePP = selenoprotein P, GPx = glutathione peroxydase, DIO = iodothyronine deiodinase, TRx = thioredoxine reductase, SeAlb = serum albumin, TriMethylSe = trimethylselenonium, SeGalNac = selenosugar.

Selenomethionine represents the main chemical form of Se in the human diet. Once ingested, Se-Met is absorbed in the same sodium transport system as methionine [60]. It is transported to liver where it is metabolized following the same pathway as methionine and can be transformed into Se-Cys following the trans-sulfuration pathway [60]. The concentration of Se-Cys is lower

in plant proteins than Se-Met, while its abundant in diets containing products of animal origin [59]. Se-Cys can also be absorbed in the same way and in completion with its analogue cysteine [61].

The human genome codes for 25 selenoproteins in which the stop codon UGA specifically insert the selenocysteine. This amino acid is not charged as such on its dedicated tRNA but is instead synthesized onto a specific Se-Cys-tRNA$^{[Ser]Se\text{-}Cys}$ from the serine [62].

Selenocysteine is catalyzed by the enzyme Se-Cys β-lyase to elemental Se [63]. The latter is further reduced to H_2Se. The addition of phosphate to Selenide by the selenophosphate 2 synthetase produce a selenophosphate (H_2SePO^{3-}) [64], which can be used as a precursor for selenocysteine synthesis [62].

In the case of excess Se, H_2Se is methylated to DMeSe and trimethylselenonium (TriMethylSe) or converted to selenosugar (SeGalNac) and exhaled or excreted [59-60].

Inorganic selenium such as Selenate passes through the membrane at the edge of the intestinal brush, where absorption is via the sodium-facilitated and energy-dependent system utilized by sulfate, due to their structural analogies and reduced to selenite by the sulfate reduction pathway [33]. Selenite is absorbed by non-mediated passive diffusion. Absorption of selenite ranges from 50 to 90%, being affected by dietary constituents on which it is strongly adsorbed, while selenate absorption is almost complete [65-66].

Despite the use of inorganic forms in Se supplementation their absorption is not as high as that of Se-Met [66]. A supplementation of Se-Met at 37 μg/day increases the activity of GPx in human plasma up to its saturation level of 150 U/L, whereas for sodium selenite, 66 μg/day are necessary to reach the same level [67-68].

3.2. Blood Transfer and Distribution of Se to Tissues via Selenoproteins P

The supply of Se to tissues depends on the plasma Se transporter, SePP or serum albumin (SeAlb) [59] (Figure 2). SePP is synthesized mainly in the liver, which is the key organ for the homeostasis of Se in the body. SePP is a secreted glycoprotein with a C-terminal domain composed of nine Se-Cys residues in mice and humans. It also has another Se-Cys residue in the N-terminal region in a redox active motif [69-70]. In rats, studies have identified four types of SePP isoforms. However, in humans plasma, only two isoforms were identified; a complete 60 kDa form and a smaller 50 kDa form [71]. The 60 kDa isoform serves as a transporter of Se to body tissues and the 50 kDa isoform is involved in redox and signaling reactions [72]. The

SePP knockout mice show a loss of motor coordination and reduced viability. Their survival time is about 15 days after weaning [73].

In the body, there is a hierarchy of Se distribution in different tissues or organs for example, Se level is highly conserved in the brain, thyroid and testes. This hierarchy give priority to the synthesis of the most important selenoproteins for the organ [7,74-75].

In humans, a recent study has shown that SePP gene variations affect levels of selenium biomarkers after intake of food with a high content of selenium. Indeed, in middle-aged Danes, CC homozygotes of the SePP/rs3877899 polymorphism have higher levels of selenoprotein P and whole blood Se compared to T-allele, after consumption of selenium-rich foods [76]. These results emphasize the importance of a more personalized approach to Se requirements [76].

4. Selenium in Thyroid Gland

Selenium is required at all stages of embryological development of the thyroid gland [77-78]. Deficiency of Se can aggravate the abnormalities induced by iodine deficiency [79]. Hence, the influence of Se on thyroid function is closely related to the state of iodine.

During biosynthesis of thyroid hormones, high concentrations of H_2O_2 are generated in order to oxidize iodide (I^-). The iodination of tyrosyl residues on thyroglobulin give rise to the iodine-containing thyroid hormones; T4 and T3 [10]. The DIO selenoprotein family plays an important role in activation or inactivation of thyroid hormones [80]. Excess hydrogen peroxide could be a major source of free radicals and reactive oxygen species (ROS). These molecules can significantly damage the cell and DNA. Indeed, a greater amount of DNA modified by oxidation was observed in follicular cells of the thyroid compared to the spleen, lung and liver [81]. Thus, Se via selenoproteins plays an important role in the detoxification of H_2O_2 as well as ROS and provides antioxidant protection to thyroid gland, against oxidative stress [81].

4.1. Role of Selenoproteins in Thyroid Gland

The DIO family consists of 3 types of DIO (DIO1, DIO2 and DIO3), that are integral membrane dimeric selenoproteins, each having different catalytic properties in specific tissue and developmental expressions [80] (Table 1). DIO convert pro-hormone T4 to active T3 and reverse T3 (rT3) as well as to diiodothyronine (T2) [80]. They contain a thyroredoxin fold that catalyzes the stereospecific and sequential elimination of iodine atoms from tyrosine residues.

Their reactions are complementary and essential to the metabolism and activities of Thyroid hormones [82]. Table 1 summarizes the main functions of selenoproteins in thyroid.

Table 1. Main selenoproteins in thyroid and their functions.

Selenoproteines	Localization	Functions	References
Deiodinases (DIO)			[80]
DIO1	Liver, kidney, thyroid gland, lung, eyes, pituitary, CNS	Conversion of T4 into T3and rT3 and T3 into rT3 or T2	
DIO2	Thyroid gland, pituitary gland, skeletal, heart muscles , brain, fat tissue, spinal cord, placenta	Conversion of T4 into T3 and of rT3 into T2	
DIO3	Gravid uterus, placenta, fetus liver, fetal and neonatal brain, skin	Conversion of T4 into T3 and of rT3 into T2	
Gluthatione peroxidases (GPx)			[82,83]
GPx1	Cytoplasm, ubiquitous	Cytosol Antioxidant	
GPx3	Plasma and thyroid follicle	Plasma and extracellular antioxidant	
GPx4	Mitochondrial membrane	Membrane antioxidant	
Thioredoxin reductase (TRx)			[82,84]
TRx1	Principally cytosolic, ubiquitous	Inhibition of apoptosis, redox state of transcription factors	
TRx2	Mitochondrial, ubiquitous	Reduce basal oxidative stress,	
TRx3	Principally mitochondrial, ubiquitous	Regulation of apoptosis and signaling pathway	
Selenoprotein P (SePP)	Blood and thyroid	Transportation of selenium and storage, endothelial antioxidant	[85]

DIO = iodothyronine deiodinase, T2 = diiodothyronine, T3 = triiodothyronine, rT3 = reverse T3, T4 = thyroxine, GPX = glutathione peroxydase, TRx = Thioredoxin reductase, SePP = selenoprotein P.

Both DIO1 and DIO2 are hosted in the thyrocytes and provide T3 obtained by deiodination of T4. T3 is the main mediator of the effects of thyroid hormones. Extra-thyroid DIO1 is involved in the conversion in the liver of T4 to T3. DIO2 controls the intracellular activation of T4 into T3 in target tissues and into endocrine cells [86]. Sensitive cells are protected from biologically active T3 thyrotoxic concentrations, so DIOs control the regulation of the thyroid axis [87].

The thyroid physiological role of DIO1 and DIO2 expression is not fully understood. The hierarchy that controls the expression of selenoproteins gives priority to the distribution of Se to essential selenoenzymes and to the most physiologically important tissues preserving their metabolism under Se deficiency conditions [80]. In cultured cells and rodent tissues, the activities of DIO are protected against Se deficiency at the expense of other selenoproteins. Similar compensation pathways also function in humans, as there is no report of an association

between limited Se intake and general defects in the central nervous system and in vision or hearing development under otherwise normal conditions [80].

Glutathione peroxidase is the best characterized selenoprotein family in human. Among the selenoproteins, the GPx family constitutes the main components of human antioxidant defense with five isozymes (GPx1–GPx4, GPx6) [83]. The role of the GPx is to reduce H_2O_2 and organic hydroperoxides to protect cells from the effects of reactive oxygen species (ROS) [82].

This group of selenoproteins is largely involved in thyroid gland function [87]. GPx3 is the most actively expressed isoform in thyroid gland and kidneys [87]. Probably its role in thyroid colloid is to degrade the large amount of H_2O_2 produced during the iodination of thyroid hormones [88]. GPx4 isoform is present in mitochondria of thyrocyte, its role is the protection of membrane lipids by reducing phospholipid hydroperoxides while, GPx1 is a cytosolic enzyme [87]. Among the thyroid selenoproteins, GPx1 and GPx3 are the most sensitive to the Se level [89].

The family of TRx play an important role in thyroid metabolism, mainly its members; TRx1 and TRx2 [84]. TRx1 is an intracellular enzyme while TRx2 is mitochondrial ensuring the reduction of oxidative stress in mitochondria [82].

Others selenoproteins are involved in the function of thyroid gland including SePP, Selenoprotein S and K provide quality control in endoplasmic reticulum [82].

The relationship between Se and thyroid diseases is complex. The hierarchy in the distribution of Se protects the thyroid from small fluctuations in Se intake [90]. Two isoforms of Se-Cys-tRNA$^{[Ser]Se\text{-}Cys}$ have been described. One of them is involved in the biosynthesis of the most important selenoproteins (TRx1, TRx2 and GPx4). The second ensures the synthesis of less essential proteins (GPx1, GPx3, selenoprotein S) [91]. Several studies have shown that Se supplementation has given positive results in certain autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease [90].

4.2. Selenium and Thyroid Cancer

Thyroid cancer is the most common endocrine tumor, responsible for more than half a million new cases per year, ranking 9th place in cancers prevalence worldwide [92-93]. It remains rare in children and adolescent, the median age of diagnostic is 45 to 50 years old [94], it is diagnosed three times more often in women than in men [95]. Thyroid cancer includes three main types of tumors: medullary thyroid carcinoma, anaplastic thyroid carcinoma and

differentiated thyroid carcinoma [96]. Differentiated carcinoma alone accounts for about 90% of thyroid cancers. It is derived from the follicular cells of the thyroid, which are responsible for the production of thyroid hormones [95].

The relationship between Se status and cancer has been debated for a long time. Observational studies and randomized controlled trials have shown conflicting results. In a meta-analysis and meta-regression conducted by Cai *et al.*, [97], the results were in favor of a significant association between Se and cancer. High Se exposure may reduce risk of cancer, especially those of: lung, breast, esophagus, stomach and prostate [97]**.** On the other hand, Vinceti *et al.,* [57] and Jablonska and Vinceti [98] published a review that reports the results of various trials suggesting dramatic effects of Se on cancer development.

The Selenium and Vitamin E Cancer Prevention Trial (SELECT) is one of the largest intervention studies, launched in 2001 and involving more than 32,000 American males. The trial investigated the effect of vitamin E and/or L-selenomethionine supplementations, primarily against the development of prostate cancer and other type of cancers, that is, colorectal cancer, lung cancer and bladder cancer [14]. The results of SELECT showed an increase in prostate cancer risk for patients supplemented with the highest Se levels [13]. However, the results of this study should be interpreted taking into account certain limitations. The possibility that the supplements given to men exceeded the adequate doses to prevent prostate cancer and on the other hand, men selected were characterized by a relatively high baseline selenium status, which suggests that selenium only reduces the risk of prostate cancer in selenium-deficient men and not in the general population [14].This may explain why certain clinical trials have not shown any side effects in cancer patients after intravenous administration of sodium selenite [99].

In a review of Murdolo *et al.*, different information was collected to explain the divergence concerning the role of Se in the pathophysiology of cancer [100]. First: the effects of Se may be more effective against the progression of cancer, at advanced stages of the disease rather than at early stages. Its role could therefore be more important in preventing cancer progression than in its development. Second: the effects of Se are observed at concentrations lower or higher than the required concentrations to optimize selenoprotein activities especially GPx and SePP. The effects of selenium status on cancer show a U-shape curve. Third: genetic variability could also be of importance. Several single nucleotide polymorphisms (SNP) of certain selenoprotein genes have been linked to different types of cancer.

A change in the status of Se has been demonstrated in the pathogenesis of thyroid cancer [101-102]. Significantly lower levels of Se in thyroid cancer patients were found compared to the control group in several studies [10,101-104].

In contrast, other studies do not provide evidence of a significant association between Se deficiency and thyroid cancer [105-106]. Table 2 presents some studies and results conducted on Se and/or selenoproteins in serum, tissue, fingernail, and urine of thyroid cancer subjects.

Table 2. Recent clinical studies conducted on the link between Se and thyroid cancer.

Analysis	Sample	Outcome	References
Serum Se and GPx3 concentration.	25 patients with PTC 13 patients with FTC 20 Control	No significant differences in Se and GPx3 concentrations among groups.	[107]
Pre-surgery evaluation of serum Se and vit D3 at different stages of disease.	35 patients with PTC 12 patients with FTC 17 patients with goiter	No significant differences among groups.	[105]
Fingernail Se level	215 patients diagnosed with various thyroid cancer 331 controls	No significant association between fingernail Se levels in patients vs controls, at different thyroid cancer stage	[106]
Serum concentrations of Se, Cu, Mn	Meta-analysis on 1291 subjects performed in Norwegian, Austrian, and Polish populations	Significantly lower levels of Se and Mn and higher level of Cu, in patients with thyroid carcinoma	[108]
Serum Zn and Se concentration before and after surgery or two weeks later, as well as in thyroid tissues.	50 women and men with thyroid cancer	Lower serum Se and Zn concentrations before and after surgery but higher concentrations in thyroid tissue in the 2 groups	[103]
Pre-surgery Se, Cd, Zn serum concentration and correlation to cancer stage	92 Korean women, of PTC	Se, Cd, Zn concentrations were significantly higher in cancer stages III and IV	[109]
Serum and urine concentration in eleven metals and in Se	262 patients with PTC 262 controls	Se concentrations significantly lower in PTC. Urinary Se concentration negatively associated to PTC risk	[110]
GPx1 and TRx1 expression and analysis of free radicals tumor vs. healthy tissues	20 samples of thyroid tumor 20 samples of healthy thyroid tissue	GPx1 and TRx1 in thyroid cancer tissue are lower in patients vs. controls Significant increase in production of free radicals in all thyroid tumor tissue samples vs. healthy tissue	[111]
Se, GPx3, SePP, Cu, Zn serum concentration	Patients with various thyroid pathologies including thyroid cancer (n = 323) 200 Controls	Significantly lower serum Se and Zn levels in patients vs. controls (particular the patients with thyroid malignancy).	[10]

PTC: papillary thyroid carcinoma; FTC follicular thyroid carcinoma; GPx: glutathione peroxydase; Vit = vitamin, TRx = thioredoxine reductase; SePP = selenoprotein P.

In Table 2, we highlight that most studies indicate low Se levels in the patients with thyroid cancer. Se deficiency in various diseases, including cancer, could be related to a high level of

free radicals caused by oxidative stress [95]. Significant increase in production of ROS is observed in thyroid tumor tissue samples vs. healthy tissue [81,103]. Se is present in high concentrations in the thyroid and plays an important role in the elimination of ROS. Therefore, a fluctuation in its level could affect the expression of antioxidant selenoproteins, sensitive to the intake of Se in the thyroid (GPx1 and GPx3). In the review by Olivera *et al.*, studies corroborate the reduction in the activity of selenoproteins in thyroid cancer, in case of Se deficiency [95].

In primary papillary thyroid carcinoma (PTC) samples, reduced or even absent expression of GPx3 has been found in patients and it has been correlated with lymph node metastasis and increased tumor size [112]. In thyroid cancer cell lines TPC-1 and FTC133, GPx3 could inhibit Wnt/β-catenin signaling and thereby suppress metastasis of thyroid cancer [112]. The anticarcinogenic effect of Se during the initiation phase of tumor development is the increased expression of antioxidant selenoproteins. Numerous cohort studies have shown that individuals with plasma selenium levels below 100–120 μg/L might benefit by increasing their selenium intake. This concentration is the amount needed to reach a plateau in SePP level and beyond, an increase of selenium concentrations no longer provides protective effects on the development of cancer [92].

In cancer cells, abnormal redox regulation is observed at different stage of tumor progression [99]. Tumor cells require antioxidant molecules such as selenoproteins to maintain the redox balance [92]. The expression of antioxidant proteins increases in many types of cancer and decreases in others [99]. Indeed, tumor cells present major differences in their selenoprotein expression pattern such as the GPx gene [92]. In colorectal cancer, 15 selenoprotein genes were analyzed in two cohorts. Both selenoproteins TRx3 and GPx2 were upregulated in adenoma and carcinoma, while SePP and selenoprotein S were down regulated [113]. The increased gene expression of GPx2 and TRx3 can be explained by the fact that both are target genes for Wnt signaling. This signaling pathway is activated in most colorectal cancer tissues [114]. However, there are not many studies regarding the overall changes in selenoprotein genes in thyroid cancer. Selenoproteins GPx1 and TRx1 in thyroid cancer tissue are lower in patients versus controls, while DIO3 mRNA levels and activity were increased in PTC [111,115]. This increase in DIO3 mRNA levels was correlated with distant metastasis or lymph nodes. Thus, it appears that some selenoproteins fight the growth of tumor cells, while others support it, which underlines the fact that the carcinogenesis mechanisms linked to the Se status are far from being elucidated [115].

Experiments have suggested that selenoproteins can act to modulate the susceptibility of the malignancy by acting on tumor suppressor gene pathways. It has been observed in breast and prostate cancer that selenoproteins carry on, for example, control in the checkpoint kinase-2 (CHEK2) gene, a suppressor tumor gene which is involved in the signal transduction in cellular response to DNA damage who is associated with thyroid malignancy [116]. Different mutations in the tumor suppressor gene CHEK2, such as 1100delC, IVS2 1 1G > A, del5395 and I157T, are associated with multi-organ cancers including breast and papillary thyroid cancer [117]. In addition, Se could act as an anti-mutagenic agent with toxicity against cancer cells [118]. Se acts by inducing cell death by production of superoxide radicals thus triggering the mitochondrial pathway of apoptosis, while sparing healthy cells [118]. It is clear that Se has anticarcinogenic properties, linked to its valence states. Selenite has the capacity to intervene in redox reactions, while selenate is completely devoid of this ability [119]. It is reported in a review by Kieliszek *et al.,* that only selenite ions react with the –SH groups of proteins and prevent the formation of protein polymers rich in disulfides [119]. Indeed, a barrier made up of blood proteins with fibrin properties, protects the membranes of cancer cells from recognition by the immune system. Sodium selenite inhibits the protein disulfide exchange on the surface of cancer cell membranes and thus makes the tumor sensitive to the destructive activity of phagocytic cells [119].

Selenium supplementation has positive results in autoimmune thyroid disease and may improve thyroid cancer outcome, however the results are not conclusive in the majority of cases [95]. The question that remains is whether a deficiency in this micronutrient is a consequence of thyroid cancer or a risk factor. The example of hypoxia, which can influence selenoproteins biosynthesis, the expression of SePP is reduced noting a decrease in the distribution of Se by hepatocytes causing a general decline in selenoproteins expression [120-122].

To conclude, several studies have highlighted a Se deficiency in thyroid cancer patients. However, in the lack of evidence to this relationship, many studies are needed to confirm and to explain this hypothesis.

5. Conclusions

More than two centuries after the discovery of the trace element selenium, the roles of this mineral in human health and disease have not been fully elucidated. The relationship between Se status and cancer has been debated for a long time and the results of epidemiological studies are contradictory. In thyroid cancer, most studies indicate a significant association between Se

deficiency and the risk of this cancer. However, recent studies have shown that certain selenoproteins fight the growth of tumor cells, while others support it, which underlines the fact that the carcinogenesis mechanisms linked to Se status are not completely understood in thyroid cancer. Different approaches are still needed to clarify the link between Se status and thyroid cancer; genetic association studies, large-scale population-based studies and other omics-based analysis.

6. Future Perspectives

At present, the mechanisms that influence the development of thyroid cancer and the anticancer effects of Se have not been fully elucidated. Therefore, analytical and experimental studies on the role of selenoproteins must be conducted using thyroid cancer stem cells. It is also important to have a better knowledge of selenoproteins polymorphisms and their regulation involved in thyroid cancer.

Further research should focus on studies using data on dietary behavior as well as epidemiological data to better understand the effects of selenium deficiency on thyroid cancer.

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References

1. Schrauzer, G.N.; Surai, P.F. Selenium in human and animal nutrition: Resolved and unresolved issues. A partly historical treatise in commemoration of the fiftieth anniversary of the discovery of the biological essentiality of selenium, dedicated to the memory of Klaus Schwarz (1914–1978) on the occasion of the thirtieth anniversary of his death. *Crit. Rev. Biotechnol.* **2009**, *29*, 2–9.
2. Delbet, P. Tentatives de traitement de cancer par selenium. *Bull. De l'Assoc. Francaise pour l'Etude de Cancer* **1912**, *5*, 121–125.
3. Nelson, A.A. Liver Tumors Following Cirrhosis Caused by Selenium in Rats. *Cancer Res.* **1943**, *3*, 230–236.
4. Rayman, M.P. The importance of selenium to human health. *Lancet* **2000**, *356*, 233–241.
5. Rayman, M.P. Selenium and human health. *Lancet* **2012**, *379*, 1256–1268.
6. Kieliszek, M.; Błażejak, S. Current Knowledge on the Importance of Selenium in Food for Living Organisms: A Review. *Molecules* **2016**, *21*, 609.
7. Kato, T.; Read, R.; Rozga, J.; Burk, R.F. Evidence for intestinal release of absorbed selenium in a form with high hepatic extraction. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1992**, *262*, G854–G858.
8. Duntas, L.H.; Benvenga, S. Selenium: An element for life. *Endocrine* **2015**, *48*, 756–775.
9. Köhrle, J. The trace element selenium and the thyroid gland. *Biochimie* **1999**, *81*, 527–533.
10. Mehl, S.; Sun, Q.; Görlich, C.L.; Hackler, J.; Kopp, J.F.; Renko, K.; Mittag, J.; Schwerdtle, T.; Schomburg, L. Cross-sectional analysis of trace element status in thyroid disease. *J. Trace Elem. Med. Biol.* **2020**, *58*, 126430.
11. Sakız, D.; Kaya, A.; Kulaksizoglu, M. Serum Selenium Levels in Euthyroid Nodular Thyroid Diseases. *Biol. Trace Elem. Res.* **2016**, *174*, 21–26.
12. Novotny, L.; Rauko, P.; Kombian, S.; Edafiogho, I. Selenium as a chemoprotective anti-cancer agent: Reality or wishful thinking? *Neoplasma* **2010**, *57*, 383–391.
13. Kristal, A.R.; Darke, A.K.; Morris, J.S.; Tangen, C.M.; Goodman, P.J.; Thompson, I.M.; Meyskens, F.L.; Goodman, G.E.; Minasian, L.M.; Parnes, H.L.; et al. Baseline Selenium Status and Effects of Selenium and Vitamin E Supplementation on Prostate Cancer Risk. *JNCI J. Natl. Cancer Inst.* **2014**, *106*.
14. Vinceti, M.; Filippini, T.; Del Giovane, C.; Dennert, G.; Zwahlen, M.; Brinkman, M.; Zeegers, M.P.; Horneber, M.; D'Amico, R.; Crespi, C.M. Selenium for preventing cancer. *Cochrane Database Syst. Rev.* **2018**, doi: 10.1002/14651858.
15. Rasmussen, L.B.; Schomburg, L.; Köhrle, J.; Pedersen, I.B.; Hollenbach, B.; Hög, A.; Ovesen, L.; Perrild, H.; Laurberg, P. Selenium status, thyroid volume, and multiple nodule formation in an area with mild iodine deficiency. *Eur. J. Endocrinol.* **2011**, *164*, 585–590.
16. Giray, B.; Arnaud, J.; Sayek, İ.; Favier, A.; Hıncal, F. Trace elements status in multinodular goiter. *J. Trace Elem. Med. Biol.* **2010**, *24*, 106–110.
17. Reich, H.J.; Hondal, R.J. Why Nature Chose Selenium. *ACS Chem. Biol.* **2016**, *11*, 821–841.
18. Hu, T.; Liang, Y.; Zhao, G.; Wu, W.; Li, H.; Guo, Y. Selenium Biofortification and Antioxidant Activity in Cordyceps militaris Supplied with Selenate, Selenite, or Selenomethionine. *Biol. Trace Elem. Res.* **2019**, *187*, 553–561.
19. Dumont, E.; Vanhaecke, F.; Cornelis, R. Selenium speciation from food source to metabolites: A critical review. *Anal. Bioanal. Chem.* **2006**, *385*, 1304–1323.
20. Fernández-Martínez, A.; Charlet, L. Selenium environmental cycling and bioavailability: A structural chemist point of view. *Rev. Env. Sci. Biotechnol.* **2009**, *8*, 81–110.

21. Natasha; Shahid, M.; Niazi, N.K.; Khalid, S.; Murtaza, B.; Bibi, I.; Rashid, M.I. A critical review of selenium biogeochemical behavior in soil-plant system with an inference to human health. *Environ. Pollut.* **2018**, *234*, 915–934.
22. He, Y.; Xiang, Y.; Zhou, Y.; Yang, Y.; Zhang, J.; Huang, H.; Shang, C.; Luo, L.; Gao, J.; Tang, L. Selenium contamination, consequences and remediation techniques in water and soils: A review. *Environ. Res.* **2018**, *164*, 288–301.
23. Kieliszek, M. Selenium–Fascinating Microelement, Properties and Sources in Food. *Molecules* **2019**, *24*, 1298.
24. Wen, H.; Carignan, J. Reviews on atmospheric selenium: Emissions, speciation and fate. *Atmos. Environ.* **2007**, *41*, 7151–7165.
25. Söderlund, M.; Virkanen, J.; Holgersson, S.; Lehto, J. Sorption and speciation of selenium in boreal forest soil. J. Environ. Radioactiv. **2016**, 164, 220-231.
26. Cao, S.; Duan, X.; Zhao, X.; Ma, J.; Dong, T.; Huang, N.; Sun, C.; He, B.; Wei, F. Health risks from the exposure of children to As, Se, Pb and other heavy metals near the largest coking plant in China. *Sci. Total Environ.* **2014**, *472*, 1001–1009.
27. Dennouni-Medjati, N.; Dali-Sahi, M.; Harek, H.; Harek, Y. Statut en sélénium de la population d'une région côtière de l'Ouest algérien à forte activité anthropique. *Environ. Risque Sante* **2019**, *18*, 8.
28. Vriens, B.; Lenz, M.; Charlet, L.; Berg, M.; Winkel, L.H.E. Natural wetland emissions of methylated trace elements. *Nat. Commun.* **2014**, *5*, 3035.
29. Winkel, L.; Vriens, B.; Jones, G.; Schneider, L.; Pilon-Smits, E.; Bañuelos, G. Selenium Cycling Across Soil-Plant-Atmosphere Interfaces: A Critical Review. *Nutrients* **2015**, *7*, 4199–4239.
30. Zhu, Y.-G.; Pilon-Smits, E.A.H.; Zhao, F.-J.; Williams, P.N.; Meharg, A.A. Selenium in higher plants: Understanding mechanisms for biofortification and phytoremediation. *Trends Plant Sci.* **2009**, *14*, 436–442.
31. Manceau, A.; Charlet, L. The Mechanism of Selenate Adsorption on Goethite and Hydrous Ferric Oxide. *J. Colloid Interface Sci.* **1994**, *168*, 87–93.
32. Haug, A.; Graham, R.D.; Christophersen, O.A.; Lyons, G.H. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. *Microb. Ecol. Health Dis.* **2007**, *19*, 209–228.
33. Lazard, M.; Dauplais, M.; Blanquet, S.; Plateau, P. Recent advances in the mechanism of selenoamino acids toxicity in eukaryotic cells. *Biomol. Concepts* **2017**, *8*, 93–104.
34. Sors, T.G.; Ellis, D.R.; Salt, D.E. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynth. Res.* **2005**, *86*, 373–389.
35. White, P.J. Selenium metabolism in plants. *Biochim. Et Biophys. Acta (BBA) Gen. Subj.* **2018**, *1862*, 2333–2342.
36. White, P.J. Selenium accumulation by plants. *Ann. Bot.* **2015**, mcv180.
37. González-Morales, S.; Pérez-Labrada, F.; García-Enciso, E.; Leija-Martínez, P.; Medrano-Macías, J.; Dávila-Rangel, I.; Juárez-Maldonado, A.; Rivas-Martínez, E.; Benavides-Mendoza, A. Selenium and Sulfur to Produce Allium Functional Crops. *Molecules* **2017**, *22*, 558.
38. Zhang, L.; Hu, B.; Li, W.; Che, R.; Deng, K.; Li, H.; Yu, F.; Ling, H.; Li, Y.; Chu, C. OsPT2, a phosphate transporter, is involved in the active uptake of selenite in rice. *New Phytol.* **2014**, *201*, 1183–1191.
39. Zhao, X.Q.; Mitani, N.; Yamaji, N.; Shen, R.F.; Ma, J.F. Involvement of Silicon Influx Transporter OsNIP2;1 in Selenite Uptake in Rice. *Plant Physiol.* **2010**, *153*, 1871–1877.

40. Li, H.-F.; McGrath, S.P.; Zhao, F.-J. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* **2008**, *178*, 92–102.
41. Wang, P.; Menzies, N.W.; Lombi, E.; McKenna, B.A.; James, S.; Tang, C.; Kopittke, P.M. Synchrotron-based X-ray absorption near-edge spectroscopy imaging for laterally resolved speciation of selenium in fresh roots and leaves of wheat and rice. *EXBOTJ* **2015**, *66*, 4795–4806.
42. Van Hoewyk, D. A tale of two toxicities: Malformed selenoproteins and oxidative stress both contribute to selenium stress in plants. *Ann. Bot.* **2013**, *112*, 965–972.
43. Grant, T.D.; Montes-Bayón, M.; LeDuc, D.; Fricke, M.W.; Terry, N.; Caruso, J.A. Identification and characterization of Se-methyl selenomethionine in Brassica juncea roots. *J. Chromatogr. A* **2004**, *1026*, 159–166.
44. Office of Dietary Supplements—Selenium. Available online: https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/ (accessed on 30 May 2020).
45. Bañuelos, G.S.; Freeman, J.; Arroyo, I. Accumulation and speciation of selenium in biofortified vegetables grown under high boron and saline field conditions. *Food Chem. X* **2020**, *5*, 100073.
46. Fraczek, A. Selenium in medicine and treatment. *Journal of elementology.* **2013**, *18*, 145–163.
47. Kieliszek, M.; Błażejak, S. Selenium: Significance, and outlook for supplementation. *Nutrition* **2013**, *29*, 713–718.
48. Fairweather-Tait, S.J.; Collings, R.; Hurst, R. Selenium bioavailability: Current knowledge and future research requirements. *Am. J. Clin. Nutr.* **2010**, *91*, 1484S–1491S.
49. Smrkolj, P.; Pograjc, L.; Hlastanribi, C.; Stibilj, V. Selenium content in selected Slovenian foodstuffs and estimated daily intakes of selenium. *Food Chem.* **2005**, *90*, 691–697.
50. Ariane, M.K.; Maristela, M.; Silmara, M.M.; Renata, H.S.; Karine, S.N.; Helyde, A.M.; Augusto, K.J. Properties of Brazil nuts: A review. *Afr. J. Biotechnol.* **2015**, *14*, 642–648.
51. Hurst, R.; Collings, R.; Harvey, L.J.; King, M.; Hooper, L.; Bouwman, J.; Gurinovic, M.; Fairweather-Tait, S.J. EURRECA—Estimating Selenium Requirements for Deriving Dietary Reference Values. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 1077–1096.
52. Kipp, A.P.; Strohm, D.; Brigelius-Flohé, R.; Schomburg, L.; Bechthold, A.; Leschik-Bonnet, E.; Heseker, H. Revised reference values for selenium intake. *J. Trace Elem. Med. Biol.* **2015**, *32*, 195–199.
53. *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*; Institute of Medicine (U.S.), *Panel on Dietary Antioxidants and Related Compounds;* Ed.; National Academy Press: Washington, DC, USA, 2000; ISBN 978-0-309-06949-6.
54. Referenzwerte für die Nährstoffzufuhr. Available online: https://www.dge.de/wissenschaft/referenzwerte/ (accessed on 13 June 2020).
55. World Health Organization; Food and Agricultural Organisation. *Vitamin and Mineral Requirements in Human Nutrition*; World Health Organization: Geneva, Switzerland, 2004; ISBN 978-92-4-154612-6.
56. Hazane-Puch, F.; Arnaud, J.; Trocmé, C.; Faure, P.; Laporte, F.; Champelovier, P. Sodium Selenite Decreased HDAC Activity, Cell Proliferation and Induced Apoptosis in Three Human Glioblastoma Cells. *ACAMC* **2016**, *16*, 490–500.
57. Vinceti, M.; Filippini, T.; Cilloni, S.; Bargellini, A.; Vergoni, A.V.; Tsatsakis, A.; Ferrante, M. Health risk assessment of environmental selenium: Emerging evidence and challenges. *Mol. Med. Rep.* **2017**, *15*, 3323–3335.

58. Mandrioli, J.; Michalke, B.; Solovyev, N.; Grill, P.; Violi, F.; Lunetta, C.; Conte, A.; Sansone, V.A.; Sabatelli, M.; Vinceti, M. Elevated Levels of Selenium Species in Cerebrospinal Fluid of Amyotrophic Lateral Sclerosis Patients with Disease-Associated Gene Mutations. *Neurodegener. Dis.* **2017**, *17*, 171–180.
59. Gammelgaard, B.; Jackson, M.I.; Gabel-Jensen, C. Surveying selenium speciation from soil to cell—Forms and transformations. *Anal. Bioanal. Chem.* **2011**, *399*, 1743–1763.
60. Schrauzer, G.N. Selenomethionine: A Review of Its Nutritional Significance, Metabolism and Toxicity. *J. Nutr.* **2000**, *130*, 1653–1656.
61. Nickel, A.; Kottra, G.; Schmidt, G.; Danier, J.; Hofmann, T.; Daniel, H. Characteristics of transport of selenoamino acids by epithelial amino acid transporters. *Chem. Biol. Interact.* **2009**, *177*, 234–241.
62. Vindry, C.; Ohlmann, T.; Chavatte, L. Translation regulation of mammalian selenoproteins. *Biochim. Et Biophys. Acta (BBA) Gen. Subj.* **2018**, *1862*, 2480–2492.
63. Esaki, N.; Karai, N.; Nakamura, T.; Tanaka, H.; Soda, K. Mechanism of reactions catalyzed by selenocysteine β-Lyase. *Arch. Biochem. Biophys.* **1985**, *238*, 418–423.
64. Cupp-Sutton, K.; Ashby, M. Biological Chemistry of Hydrogen Selenide. *Antioxidants* **2016**, *5*, 42.
65. Mangels, A.R.; Moser-Veillon, P.B.; Patterson, K.Y.; Veillon, C. Selenium utilization during human lactation by use of stable-isotope tracers. *Am. J. Clin. Nutr.* **1990**, *52*, 621–627.
66. Ha, H.Y.; Alfulaij, N.; Berry, M.J.; Seale, L.A. From Selenium Absorption to Selenoprotein Degradation. *Biol. Trace Elem. Res.* **2019**, *192*, 26–37.
67. Burk, R.F. Effects of Chemical Form of Selenium on Plasma Biomarkers in a High-Dose Human Supplementation Trial. *Cancer Epidemiol. Biomark. Prev.* **2006**, *15*, 804–810.
68. Xia, Y.; Hill, K.E.; Byrne, D.W.; Xu, J.; Burk, R.F. Effectiveness of selenium supplements in a low-selenium area of China. *Am. J. Clin. Nutr.* **2005**, *81*, 829–834.
69. Burk, R.F.; Hill, K.E. Selenoprotein P—Expression, functions, and roles in mammals. *Biochim. Et Biophys. Acta (BBA) Gen. Subj.* **2009**, *1790*, 1441–1447.
70. Burk, R.F.; Hill, K.E. SELENOPROTEIN P: An Extracellular Protein with Unique Physical Characteristics and a Role in Selenium Homeostasis. *Annu. Rev. Nutr.* **2005**, *25*, 215–235.
71. Méplan, C.; Nicol, F.; Burtle, B.T.; Crosley, L.K.; Arthur, J.R.; Mathers, J.C.; Hesketh, J.E. Relative Abundance of Selenoprotein P Isoforms in Human Plasma Depends on Genotype, Se Intake, and Cancer Status. *Antioxid. Redox Signal.* **2009**, *11*, 2631–2640.
72. Baclaocos, J.; Santesmasses, D.; Mariotti, M.; Bierła, K.; Vetick, M.B.; Lynch, S.; McAllen, R.; Mackrill, J.J.; Loughran, G.; Guigó, R.; et al. Processive Recoding and Metazoan Evolution of Selenoprotein P: Up to 132 UGAs in Molluscs. *J. Mol. Biol.* **2019**, *431*, 4381–4407.
73. Hill, K.E.; Zhou, J.; McMahan, W.J.; Motley, A.K.; Atkins, J.F.; Gesteland, R.F.; Burk, R.F. Deletion of Selenoprotein P Alters Distribution of Selenium in the Mouse. *J. Biol. Chem.* **2003**, *278*, 13640–13646.
74. Burk, R.F.; Hill, K.E. Regulation of Selenium Metabolism and Transport. *Annu. Rev. Nutr.* **2015**, *35*, 109–134.
75. Berry, M.J. Insights into the hierarchy of selenium incorporation. *Nat. Genet.* **2005**, *37*, 1162–1163.
76. Méplan, C.; Crosley, L.K.; Nicol, F.; Beckett, G.J.; Howie, A.F.; Hill, K.E.; Horgan, G.; Mathers, J.C.; Arthur, J.R.; Hesketh, J.E. Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium

supplementation in a gender-specific manner (the SELGEN study). *FASEB J.* **2007**, *21*, 3063–3074.

77. Valea, A.; Georgescu, C.E. Selenoproteins in human body: Focus on thyroid pathophysiology. *Hormones* **2018**, *17*, 183–196.
78. Lacka, K.; Szeliga, A. Significance of selenium in thyroid physiology and pathology. *Pol. Merkur. Lek.* **2015**, *38*, 348–353.
79. Köhrle, J. Selenium and the thyroid. *Curr. Opin. Endocrinol. Diabetes Obes.* **2015**, *22*, 392–401.
80. Orozco, A.; Valverde-R, C.; Olvera, A.; García-G, C. Iodothyronine deiodinases: A functional and evolutionary perspective. *J. Endocrinol.* **2012**, *215*, 207–219.
81. Krohn, K.; Maier, J.; Paschke, R. Mechanisms of Disease: Hydrogen peroxide, DNA damage and mutagenesis in the development of thyroid tumors. *Nat. Rev. Endocrinol.* **2007**, *3*, 713–720.
82. Schomburg, L. Selenium, selenoproteins and the thyroid gland: Interactions in health and disease. *Nat. Rev. Endocrinol.* **2012**, *8*, 160–171.
83. Guillin, O.M.; Vindry, C.; Ohlmann, T.; Chavatte, L. Selenium, Selenoproteins and Viral Infection. *Nutrients* **2019**, *11*, 2101.
84. Hu, S.; Rayman, M.P. Multiple Nutritional Factors and the Risk of Hashimoto's Thyroiditis. *Thyroid* **2017**, *27*, 597–610.
85. Kurokawa, S.; Berry, M.J. Selenium. Role of the Essential Metalloid in Health. *Met. Ions Life Sci.* 2013, 13, 499–534.
86. Dayan, C.M.; Panicker, V. Novel insights into thyroid hormones from the study of common genetic variation. *Nat. Rev. Endocrinol.* **2009**, *5*, 211–218.
87. Köhrle, J.; Gärtner, R. Selenium and thyroid. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 815–827.
88. Schmutzler, C.; Mentrup, B.; Schomburg, L.; Hoang-Vu, C.; Herzog, V.; Köhrle, J. Selenoproteins of the thyroid gland: Expression, localization and possible function of glutathione peroxidase 3. *Biol. Chem.* **2007**, *388*.
89. Brigelius-Flohé, R. Glutathione peroxidases and redox-regulated transcription factors. *Biol. Chem.* **2006**, *387*.
90. Schomburg, L. The other view: The trace element selenium as a micronutrient in thyroid disease, diabetes, and beyond. *Hormones* **2020**, *19*, 15–24.
91. Schomburg, L.; Schweizer, U. Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium. *Biochim. Et Biophys. Acta (BBA) Gen. Subj.* **2009**, *1790*, 1453–1462.
92. Kipp, A.P. Selenium in colorectal and differentiated thyroid cancer. *Hormones* **2020**, *19*, 41–46.
93. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012: Globocan 2012. *Int. J. Cancer* **2015**, *136*, E359–E386.
94. Schlumberger, M. Cancer papillaire et folliculaire de la thyroïde. *Ann. D'endocrinologie* **2007**, *68*, 120–128.
95. de Oliveira Maia, M.; Batista, B.A.M.; Sousa, M.P.; de Souza, L.M.; Maia, C.S.C. Selenium and thyroid cancer: A systematic review. *Nutr. Cancer* **2019**, 1–9.
96. Cabanillas, M.E.; McFadden, D.G.; Durante, C. Thyroid cancer. *Lancet* **2016**, *388*, 2783–2795.

97. Cai, X.; Wang, C.; Yu, W.; Fan, W.; Wang, S.; Shen, N.; Wu, P.; Li, X.; Wang, F. Selenium Exposure and Cancer Risk: An Updated Meta-analysis and Meta-regression. *Sci. Rep.* **2016**, *6*, 19213.
98. Jablonska, E.; Vinceti, M. Selenium and Human Health: Witnessing a Copernican Revolution? *J. Environ. Sci. Healthpart C* **2015**, *33*, 328–368.
99. Wallenberg, M.; Misra, S.; Björnstedt, M. Selenium Cytotoxicity in Cancer. *Basic Clin. Pharm. Toxicol.* **2014**, *114*, 377–386.
100. Murdolo, G.; Bartolini, D.; Tortoioli, C.; Piroddi, M.; Torquato, P.; Galli, F. Selenium and Cancer Stem Cells. In *Advances in Cancer Research*; Elsevier: Amsterdam, The Netherlands, 2017; Volume 136, pp. 235–257 ISBN 978-0-12-812016-3.
101. Kucharzewski, M.; Braziewicz, J.; Majewska, U.; Gózdz, S. Concentration of Selenium in the Whole Blood and the Thyroid Tissue of Patients with Various Thyroid Diseases. *BTER* **2002**, *88*, 25–30.
102. Moncayo, R.; Kroiss, A.; Oberwinkler, M.; Karakolcu, F.; Starzinger, M.; Kapelari, K.; Talasz, H.; Moncayo, H. The role of selenium, vitamin C, and zinc in benign thyroid diseases and of selenium in malignant thyroid diseases: Low selenium levels are found in subacute and silent thyroiditis and in papillary and follicular carcinoma. *BMC Endocr. Disord.* **2008**, *8*, 2.
103. Baltaci, A.K.; Dundar, T.K.; Aksoy, F.; Mogulkoc, R. Changes in the Serum Levels of Trace Elements Before and After the Operation in Thyroid Cancer Patients. *Biol. Trace Elem. Res.* **2017**, *175*, 57–64, doi:10.1007/s12011-016-0768-2.
104. Kucharzewski, M.; Braziewicz, J.; Majewska, U.; Góźdź, S. Copper, Zinc, and Selenium in Whole Blood and Thyroid Tissue of People with Various Thyroid Diseases. *BTER* **2003**, *93*, 9-18.
105. Jonklaas, J.; Danielsen, M.; Wang, H. A Pilot Study of Serum Selenium, Vitamin D, and Thyrotropin Concentrations in Patients with Thyroid Cancer. *Thyroid* **2013**, *23*, 1079–1086.
106. Ren, Y.; Kitahara, C.M.; de Gonzalez, A.B.; Clero, E.; Brindel, P.; Maillard, S.; Cote, S.; Dewailly, E.; Rachedi, F.; Boissin, J.-L.; et al. Lack of Association between Fingernail Selenium and Thyroid Cancer Risk: A Case-Control Study in French Polynesia. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 5187–5194.
107. Przybylik-Mazurek, E.; Zagrodzki, P.; Kuźniarz-Rymarz, S.; Hubalewska-Dydejczyk, A. Thyroid Disorders—Assessments of Trace Elements, Clinical, and Laboratory Parameters. *Biol. Trace Elem. Res.* **2011**, *141*,65–75.
108. Shen, F.; Cai, W.S.; Li, J.L.; Feng, Z.; Cao, J.; Xu, B. The Association Between Serum Levels of Selenium, Copper, and Magnesium with Thyroid Cancer: A Meta-analysis. *Biol. Trace Elem. Res.* **2015**, *167*, 225–235, doi:10.1007/s12011-015-0304-9.
109. Chung, H.-K.; Nam, J.S.; Ahn, C.W.; Lee, Y.S.; Kim, K.R. Some Elements in Thyroid Tissue are Associated with More Advanced Stage of Thyroid Cancer in Korean Women. *Biol. Trace Elem. Res.* **2016**, *171*, 54–62.
110. Zhang, C.; Wu, H.B.; Cheng, M.X.; Wang, L.; Gao, C.B.; Huang, F. Association of exposure to multiple metals with papillary thyroid cancer risk in China. *Environ. Sci. Pollut. Res.* **2019**, *26*, 20560–20572, doi:10.1007/s11356-019-04733-x.
111. Metere, A.; Frezzotti, F.; Graves, C.E.; Vergine, M.; De Luca, A.; Pietraforte, D.; Giacomelli, L. A possible role for selenoprotein glutathione peroxidase (GPx1) and thioredoxin reductases (TrxR1) in thyroid cancer: Our experience in thyroid surgery. *Cancer Cell Int.* **2018**, *18*, 7.

112. Zhao, H.; Li, J.; Li, X.; Han, C.; Zhang, Y.; Zheng, L.; Guo, M. Silencing GPX3 Expression Promotes Tumor Metastasis in Human Thyroid Cancer. *CPPS* **2015**, *16*, 316–321.
113. Hughes, D.; Kunická, T.; Schomburg, L.; Liška, V.; Swan, N.; Souček, P. Expression of Selenoprotein Genes and Association with Selenium Status in Colorectal Adenoma and Colorectal Cancer. *Nutrients* **2018**, *10*, 1812.
114. Kipp, A.P.; Müller, M.F.; Göken, E.M.; Deubel, S.; Brigelius-Flohé, R. The selenoproteins GPx2, TrxR2 and TrxR3 are regulated by Wnt signalling in the intestinal epithelium. *Biochim. Et Biophys. Acta (BBA) Gen. Subj.* **2012**, *1820*, 1588–1596.
115. Romitti, M.; Wajner, S.M.; Zennig, N.; Goemann, I.M.; Bueno, A.L.; Meyer, E.L.S.; Maia, A.L. Increased Type 3 Deiodinase Expression in Papillary Thyroid Carcinoma. *Thyroid* **2012**, *22*, 897–904.
116. Gupta, S.; Jaworska-Bieniek, K.; Lubinski, J.; Jakubowska, A. Can selenium be a modifier of cancer risk in CHEK2 mutation carriers? *Mutagenesis* **2013**, *28*, 625–629.
117. Siołek, M.; Cybulski, C.; Gąsior-Perczak, D.; Kowalik, A.; Kozak-Klonowska, B.; Kowalska, A.; Chłopek, M.; Kluźniak, W.; Wokołorczyk, D.; Pałyga, I.; et al. *CHEK 2* mutations and the risk of papillary thyroid cancer: Genetic association between thyroid and breast cancer. *Int. J. Cancer* **2015**, *137*, 548–552.
118. Weekley, C.M.; Jeong, G.; Tierney, M.E.; Hossain, F.; Maw, A.M.; Shanu, A.; Harris, H.H.; Witting, P.K. Selenite-mediated production of superoxide radical anions in A549 cancer cells is accompanied by a selective increase in SOD1 concentration, enhanced apoptosis and Se–Cu bonding. *J. Biol. Inorg. Chem.* **2014**, *19*, 813–828.
119. Kieliszek, M.; Lipinski, B.; Błażejak, S. Application of Sodium Selenite in the Prevention and Treatment of Cancers. *Cells* **2017**, *6*, 39.
120. Becker, N.-P.; Martitz, J.; Renko, K.; Stoedter, M.; Hybsier, S.; Cramer, T.; Schomburg, L. Hypoxia reduces and redirects selenoprotein biosynthesis. *Metallomics* **2014**, *6*, 1079–1086.
121. Dreher, I.; Jakobs, T.C.; Köhrle, J. Cloning and Characterization of the Human Selenoprotein P Promoter: Response of selenoprotein p expression to cytokines in liver cells. *J. Biol. Chem.* **1997**, *272*, 29364–29371, doi:10.1074/jbc.272.46.29364.
122. Renko, K.; Hofmann, P.J.; Stoedter, M.; Hollenbach, B.; Behrends, T.; Köhrle, J.; Schweizer, U.; Schomburg, L. Down-regulation of the hepatic selenoprotein biosynthesis machinery impairs selenium metabolism during the acute phase response in mice. FASEB J. 2009, 23, 1758–1765. [CrossRef]

Chapter II: Selenium, Iron and Calcium Status as Biomarkers for Papillary Thyroid Carcinoma

I. Presentation of the article

During the last decades, the incidence of thyroid cancer has increased significantly, which places it in the 9th position in worldwide cancer frequency. Unfortunately, Algeria is not an exception as this cancer is in the 3rd position in women after breast and colorectal cancer. Thus, to clarify the causes responsible for this increase, we must investigate and understand the factors that influence thyroid function. Several studies have suggested a link between trace elements and different diseases, such as type 2 diabetes, cardiovascular disease, and cancer. The imbalance of trace elements in metabolism may play an important role in these diseases.

The present initiative is a contribution to a better understanding of the factors favoring the incidence of thyroid cancer, including micronutrients and minerals. We conducted a cross sectional study with 47 patients of the west Algerian region, and 55 healthy controls, we measured and studied the relationships between trace elements: Se, Fe and Ca as well as the functional markers of Se (SePP, GPx3 and GPx1) by assessing their levels in plasma or pellet (erythrocyte) and their roles with PTC.

The detailed results are presented in the article bellow. We found significant lower plasma Se as well as GPx3 in patients compared to healthy controls, conversely, GPx1 activity was significantly higher in patients. We also found that Fe and Ca were significantly lower in patients compared to controls.

The relationship between plasma Se, SePP, GPx3 and GPx1 activities and PTC was studied. The results indicated an acceptable degree of accurate diagnosis and prediction for plasma Se and for GPx3 to discriminate between controls and PTC patients.

We don't discuss enough on how micronutrients are important for human health. The roles of trace elements and minerals in disease have not been fully elucidated. The assessment of Se, Fe and Ca in plasma levels showed a significant difference between PTC patients and healthy controls. The relationship between Se status and cancer has been debated for a long time and the results of epidemiological studies are contradictory. In thyroid cancer, some studies indicate a significant association between Se, Fe and Ca deficiency and the risk of this cancer. More molecular research can give a new perspective to these links.

II. Article

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Selenium, Iron and Calcium Status as Biomarkers for Papillary Thyroid Carcinoma

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Abstract:

Thyroid cancer is the most common endocrine cancer. There is no systematic screening for such cancer, and the current challenge is to find potential biomarkers to facilitate an early diagnosis. Selenium (Se), Iron (Fe), and Calcium (Ca) are essential trace elements involved in the proper functioning of the thyroid gland, and changes in their plasma concentrations have been correlated with the development of cancer. In the present cross-sectional study, including 47 Algerian patients with papillary thyroid carcinoma (PTC) and 55 healthy controls, we measured Se, Fe and Ca by assessing their levels in plasma using total reflection X-ray fluorescence (TXRF), as well as the functional markers of Se, selenoprotein P (SePP) and glutathione peroxidase (GPx3 and GPx1 activities). Significant lower concentrations were observed in patients than in controls, with values of plasma Se: 81.8 ± 22.6 µg/L vs. 94.7 ± 19.5 µg/L ($P < 0.01$) and GPx3: 200.6 ± 42.7 U/L vs. 239.2 ± 36.7 U/L ($P < 0.0001$), respectively, and we found significant correlation between Se, SePP and GPx3 ($P < 0.05$). Conversely, GPx1 activity was significantly higher in patients (177.7 ± 52 Ug/Hb) compared to controls (116.1 ± 40.2 Ug/Hb). The predictive model showed a significant association between low plasma Se and the risk of PTC (OR = 5.02, 95% CI: 1.51 - 16.73: $P = 0.009$). Fe and Ca plasma levels were significantly lower in patients compared to controls. These results support the hypothesis that there is a diagnostic link between trace elements plasma levels and thyroid cancer.

Keywords: Selenium, Iron, Calcium, Selenoprotein P, Glutathione Peroxidase, Papillary Thyroid Carcinoma.

1. Introduction

In last decades, the incidence of thyroid cancer has known a significant increase worldwide with 567.000 new cases annually, rating the ninth place in cancer incidence worldwide. It is mainly due to the increase in the papillary thyroid carcinoma (PTC) incidence [1]. PTC represent 84% of new thyroid cancer cases, and it is the most frequent thyroid malignancy [2]. Women are 3 times more affected than men corresponding to ratio 10.2/100,000 vs. 3.1/100,000 [3-4]. In the USA, thyroid cancer is the 5th most frequent cancer in women [5-6], but in Algeria, it occupies the third place after breast and colorectal cancer [7-8]. Thus, to clarify the causes responsible for this increase, we must investigate and understand the factors that influence thyroid function.

Several studies have suggested a link between trace elements and different diseases, such as type 2 diabetes, cardiovascular disease, and cancer. The imbalance of trace elements in metabolism and/or homeostasis, deficiency or excess, may play an important role in these diseases [9]. Selenium (Se) in physiological amount is an essential trace element to which a high number of health benefits have been attributed e.g., antioxidant defense and production of thyroid hormones [10]. This non-metal element is required for selenocysteine synthesis and is essential to produce selenoproteins [11]. Selenoproteins mediate most biological roles of Se, and the two most common forms in blood are selenoprotein P (SePP) and Glutathione peroxidase 3 (GPx3), with 60% and 20% plasma Se, respectively [12]. SePP reflects Se nutritional status and is considered as Se indicator [13]. Both SePP and Gpx3 participate in antioxidant defense protecting cells from oxidative damage. Moreover, the thyroid gland is the organ with the highest Se content, as selenoproteins including iodothyronine 5' deiodinase (DIOs) essential in thyroid hormones synthesis and GPxs which is part of the antioxidant defense mechanism [14]. Several studies reported a lower serum level of Se status significantly associated with thyroid cancer patients. Furthermore, Vinciti *et al.*, [15] evidenced the role of Se for assisting cells to resist oxidative damage that is a major cause of cellular damage and is implicated as a key factor in the early stage of cancer.

Like Se, Iron (Fe) is also implicated in the regulation of thyroid hormones [14]. Some studies reported an interaction between Fe and iodine on thyroid function, however, the exact pathways are still unclear [16-17]. Fe is required for efficient iodine utilization and thyroid hormone production [17]. For instance the thyroid peroxidase (TPO), essential for the synthesis of thyroid hormones, is Fe-dependent [17].

Iron is required for cellular respiration via Fe-containing cytochrome proteins and for redox reactions catalyzed by ribonucleotide reductases, Fe dependent enzymes that play a role in DNA damage [18]. It is also implicated in catalase and peroxidase functions that protect cells from free radical production [19]. Iron concentration should be closely regulated because any excess of free Fe is toxic, and its deficiency induces hypoxia related to anemia [18]. Lamy *et al.*, [20] described a significant association between Fe body levels and different cancers.

During the synthesis of thyroid hormones other essential elements are involved in this process such as calcium (Ca). This element plays role in activating the efflux of iodide from the thyroid cells into the follicular lumen [21]. Also, several studies found that low Ca intake could be a risk factor for some cancers [22].

Based on these considerations, the aims of this work are to determine and investigate within case/controls study, from west Algerian population, the relationships between trace elements: Se, Fe and Ca as well as the functional markers of Se (SePP, GPx3 and GPx1 activities) by assessing their levels in plasma or pellet (erythrocyte) and their roles with PTC.

2. Materiel et methods

2.1 Human samples

We conducted a cross-sectional study with 47 PTC patients and 55 healthy controls. Patient recruitment was carried in western Algeria at University Hospital Tlemcen and Oran Centers, from June 2018 to May 2019. The blood sampling was done before the thyroidectomy and collected using a venipuncture in heparinized tubes and was immediately centrifuged using a Sigma 2-16P centrifuge and at relative centrifugal force of 1100x g for 10 min. The plasma and pellet were recovered in 500 µl aliquots and then stored at −80 °C freezer, until manipulation. We also conducted face-to-face interviews with each participant, and collected, using a detailed questionnaire, the different anthropometric and socio-cultural parameters. We excluded any participant with other malignancies or taking a chronic drug therapy.

2.2 Ethics approval and consent of the study

This research was approved by the Scientific Ethics Deontology Committee of Tlemcen University for collection of blood samples of PTC patients and healthy controls, and conducted according with Algerian law (25/2006, resolution N◦. 387). All participants were informed of the objectives of the research study, and their formal written consent was requested and signed previously. Individuals refusing to participate were excluded from this study. The thyroid

cancer diagnosis was confirmed by an independent pathologist after post-surgical pathohistological analysis of thyroid tissues.

2.3 Selenium, iron and calcium assessments

Total Se, Fe and Ca concentration in plasma was determined by total reflection X-ray fluorescence (TXRF) analysis using a spiked gallium solution as standard and a benchtop TXRF analyser (S2 Picofox, Bruker nano GmbH, Berlin, Germany), tested for accuracy by using a Seronorm serum standard (Sero AS, Billingstad, Norway) as described by Combs *et al.,* [23].

2.4 Selenoprotein P and glutathione peroxidase 3 assessments

Plasma SePP was quantified by a validated commercial SePP-specific ELISA (selenOtestTM, selenOmed GmbH, Berlin, Germany) as described by Hybsier *et al.,* [24]. Enzymatic activity of plasma GPx3 was determined by a coupled enzymatic test, monitoring the consumption of NADPH at 340 nm [25].

2.5 Erythrocyte glutathione peroxidase 1 activity assessments

Glutathione Peroxidase 1 activity was analyzed in the pellet erythrocyte, according to a protocol described by Gunzler *et al.,* [25]. The amount of glutathione oxidized by tert-butyl hydroperoxide (t-Bu-OOH), specific to selenodependent GPx is measured following the enzymatic kinetics absorption of NADPH at 340 nm under the conditions of 25°C and pH = 7 on a double beam spectrophotometer SPECORD® 210 plus (Analytik Jena Germany) [25].

2.6 Statistical analysis

All statistical analyses were performed with IBM SPSS Statistic software (version 25, IBM Corporation, NC, USA) and all figures designed using GraphPad Prism 9.1.2. Normal distribution of the data was assessed by Kolmogorov–Smirnov–Test. Results are presented as median or mean ± Standard Deviation (SD) for continuous variables or percentages (%) for categorical variables. Comparison of quantitative variables was done using T test for normal distribution or Mann Whitney test for non-normal distribution. The binary logistic regression study was used for adjusted predictive model of Se and PTC disease. The Receiving Operating Characteristics (ROC) curve was plotted and the area under curve (AUC) calculated to evaluate the probability of novel biomarkers model for PTC. The correlations were performed using Pearson correlation for normal distributed or Spearman's test for non-normal distribution. Significant differences were designated when $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****) and no significant difference (ns) was designated when $P > 0.05$.

3. Results

A total of 102 subjects were included in this study, 47 patients with PTC and 55 healthy controls from western Algeria. The general characteristics are presented in Table 1. The sampled population was predominantly female. The average age of cases and controls was 41 ± 14 years and 44 ± 13 years, respectively. The patients presented an overweight profile (28.31 ± 5.85 kg/m^2) compared to the controls (25.93 ± 4.17 kg/m^2) with a $P < 0.05$, however the distribution between international classes remained no significant with $P > 0.05$. There was no significant difference between cases and controls in all socioeconomic characteristics except in the distribution of education levels, which was significantly different ($P < 0.01$).

Table 1. Demographics and anthropometric characteristics of study populations.

Characteristics	Patients	Controls	P values
Number of participants	47	55	
Gender (%)			0.20
Women	90%	84%	
Men	10%	16%	
Age (years)			0.20
mean ± SD	41 ± 14	44 ± 13	
			0.25
<50 years (%)	72%	79%	
>50 years (%)	28%	21%	
BMI (kg/m²)			0.02
mean ± SD	28.31 ± 5.85	25.93 ± 4.17	
			0.85
Underweight (BMI < 18.5)	2%	4%	
Ideal weight (BMI 18.5-24.9)	33%	33%	
Overweight (BMI 25.0-29.9)	31%	33%	
Obese (BMI ≥ 30.0)	33%	31%	
Marital status (%)			0.56
Married	83%	86%	
Single	17%	14%	
Education levels (%)			0.001
< High school	23%	16%	
High school	62%	36%	
> High school	15%	48%	
Smoking (%)			0.27
Yes	10%	13%	
No	90%	87%	

SD: Standard Deviation; BMI: Body Mass Index

Table 2 includes the hematological profile and thyroid parameters of our patients. 25% and 30% of the patients have, respectively, a hemoglobin and hematocrit level below normal values and 20% of the population has an anemic profile. Regarding thyroid parameters, we note that

most of our patients have Thyroid-stimulating hormone (TSH) and Thyroglobulin (TG) levels above references values.

Table 2. Hematological levels and thyroid parameters of PTC patients.

Parameters	Patients	References values
Hematological levels		
Red blood cell (10^6/µl)	4.67 ± 0.69	[4.5 – 5.5]
Hemoglobin (g/dl)	12.95 ± 1.60	[12 – 16]
Hematocrit (%)	39.47%	[37 – 46]
Platelet (10^3/µl)	266.41 ± 9.56	[150 – 400]
White blood cell (10^3/ µl)	6639 ± 2277	[4000 – 10000]
Lymphocytes (10^3/µl)	35.45 ± 9.36	[15 – 40]
Thyroid parameters		
TSH (µIU/mL)	82.89 ± 21.88	[0.3 - 5.0]
TPO-ab (Ul/mL)	18.67 ± 9.84	< 34
TG (ng/mL)	109.50 ± 243	[3.0 – 40]

TSH: Thyroid Stimulating Hormone; TPO-ab: anti-Thyroid Peroxidase antibodies; TG: Thyroglobulin.

We then compared the plasma concentration of plasma Se and biomarkers SePP, GPx3 and GPx1 activities between PTC patients and healthy controls (Figure 1). Our results showed that plasma Se as well as GPx3 were significantly lower in patients compared to controls with values of Se: 81.8 ± 22.6 µg/L vs. 94.7 ± 19.5 µg/L ($P < 0.01$) and GPx3: 200.6 ± 42.7 U/L vs. 239.2 ± 36.7 U/L ($P < 0.0001$), respectively. Conversely, GPx1 activity was significantly higher in patients (177.7 ± 52 Ug/Hb) compared to controls (116.1 ± 40.2 Ug/Hb). Even though the SePP values were higher in the cases (median = 5.66 mg/L) than in the controls (median = 4.90 mg/L), the difference was not significant ($P > 0.05$).

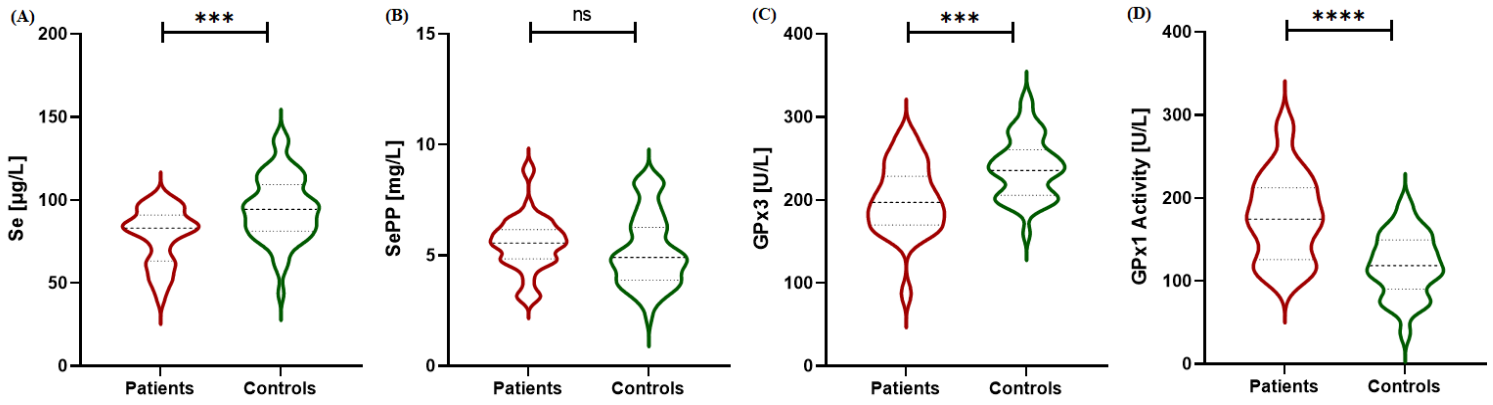

Figure1. Comparison of Se, SePP, GPx3 and GPx1 activities in PTC patients versus healthy controls. Significant lower concentration was observed between patients and controls in (A) Se plasmatic and (C) GPx3 and significant higher concentration for (D) GPx1 Activity. No significant difference was observed for (B) SePP. T test was used for variables with normal distribution and Mann Whitney test for non-normal distribution; ns: non-significant; $p < 0.001$ (***) and $p < 0.0001$ (****).

Correlation between Se status and two additional selenoproteins biomarkers, SePP and GPx3 were analyzed (Figure 2). All these analyses yielded positive and linear correlations, indicating that neither SePP nor GPx3 have reached saturating expression levels. Since there are no significant correlations between GPx1 and the other settings (plasma Se, SePP, GPx3) we did not illustrate it in the Figure 2 above.

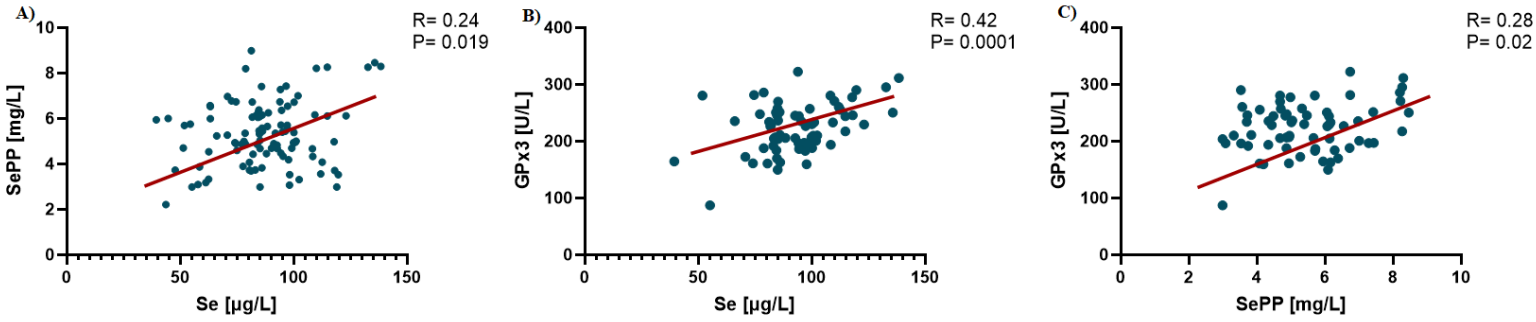

Figure 2. Interrelation of different Se status biomarkers, i.e., plasma Se, plasma SePP concentrations and GPx3 activity. Comparison of (A) plasma Se vs. SePP concentrations (B) Plasma Se vs. GPx3 activity and comparison of (C) plasma SePP concentration vs. GPx3 activity indicates linear correlations over the full range of concentration.

Table 3 represents the distribution of plasma Se as well as the minimum requirements of SePP, GPx3 and GPx1 for optimal activity. We observed that 30% of the patients had Se levels below normal value (70 µ/L) compared to the healthy control which had only 7% below normal value. In addition, the distribution of plasma Se for saturation of SePP, GPx3 and GPx1 activities remained significantly lower in the patient cases compared to controls ($P = 0.0001$)

and thus patients had insufficient plasma Se concentration for optimal SePP, GPx3 and GPx1 activities to occur.

Table 3. Prevalence and distribution of plasma Se in PTC patients and healthy controls.

References Values	Patients	Controls	P- Values	References
Plasma Se			**0.0001**	[26]
< 70 µg/L	30 %	7 %		
≥ 70 µg/L	70 %	93 %		
Plasma Se required for SePP saturation			**0.0001**	[27]
< 100 µg/L	97%	64%		
≥ 100 µg/L	3%	36%		
Plasma Se required for optimal GPx (1 and 3) activities (µg/L)			**0.0001**	[28]
≥ 90 µg/L	76%	42%		
<90 µg/L	24%	58%		

We also used the ROC curve to analyze and test the relationship between Se, SePP, GPx3, GPx1 activities and PTC risk. The results indicated an acceptable degree of accurate diagnosis and prediction for plasma Se concentration with an AUC = 68.8% ($P < 0.001$) and AUC = 0.78% for GPx3 ($P < 0.01$), to discriminate between controls and PTC patients, respectively (Figure 3). SePP and GPx1 activity did not show significant results ($P > 0.05$). Plasma Se as well as GPx3 may constitute possible biomarkers of PTC.

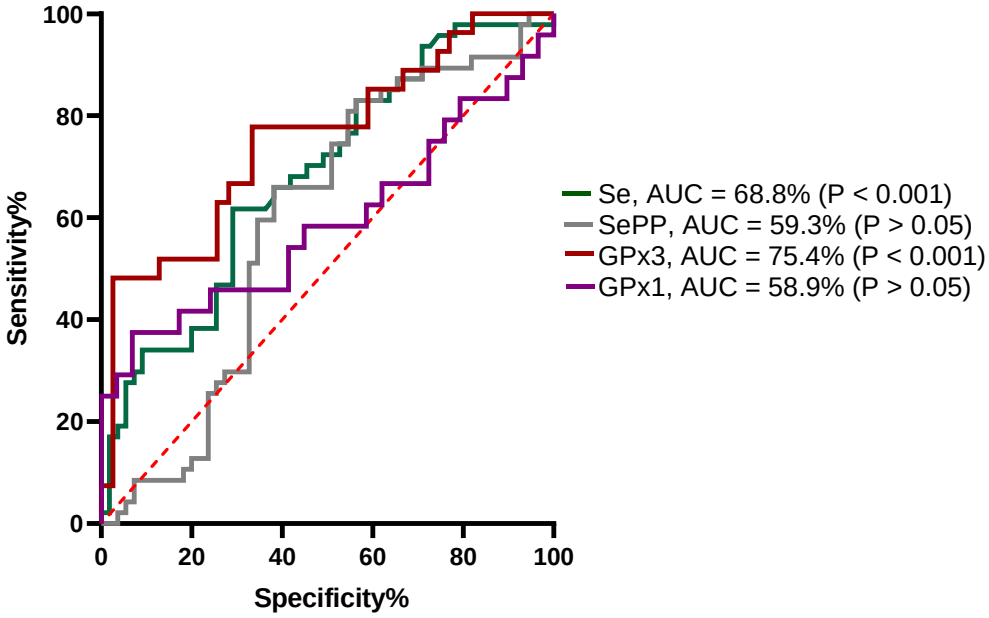

Figure 3. Receiver Operating Characteristics (ROC) curve for Se plasma, SePP, GPx3 and GPx1 activities. We place the True Positive decision on the ordinate axis and False Positive rate on the abscissa to form the ROC diagram. The area under the ROC curve (AUC) obtained for the present data was significant for Se and GPx3 with 68.8% and 75.4%, respectively ($p < 0.001$). This supports the hypothesis that Se and GPx3 are potential PTC biomarker.

In adjusted binary logistic regression, model 1 for Se, we divided the sampled population into two groups, group 1 with plasma Se less than 70 µg/L and group 2 with more than 70 µg/L (group 2 was used as reference). Then we adjusted model 2 according to SePP and model 3 according to gender, age and BMI, these results are presented in Table 4. No significant results were obtained for GPx3 and GPx1.

The results obtained by binary logistic regression showed a significant association between plasma Se and PTC occurrence. In addition, model 1 of the regression showed an increased risk of PTC associated with low Se level (OR = 5.02; P = 0.009). For model 2 and model 3, we found a significant influence of SePP concentration level (OR = 6.24 and p = 0.004) and gender; age and BMI (OR = 8.75, p = 0.001). These results support the hypothesis that plasma Se level could be a novel biomarker of PTC.

Table 4. Odds ratios and 95% CLs of the different models of binary logistic regression.

Models	Coefficients	Wald	Group 2	Group 1	P-value
Model 1 (unadjusted OR)				**OR (CI 95%)**	
	1.61	2.63	1.00 (reference)	5.02 (1.51 – 16.73)	0.009
Model 2 (OR adjusted to SePP)	1.83	2.85	1.00 (reference)	6.24 (1.77 – 21.94)	0.004
Model 3 (OR adjusted to gender, age, and BMI)	2.16	3.19	1.00 (reference)	8.75 (2.31 – 33.15)	0.001

BMI: Body Mass Index
Group 1: subject with Se value bellow 70 µg/L; Groupe 2: subject with Se above 70 µg/L

Using Mann Whitney test, we compared the micronutrients Fe and Ca between patients and healthy controls, the results are presented in Figure 4. Our results showed significant low differences in patients vs. healthy controls with respective values of Fe: 1207.4 µg/L (873, 1778) vs. 1474.6 4 µg/L (1036, 2400) and for Ca: 108.3 mg/L (100.7, 117.2) vs. 117.7 mg/L (105.4, 127.3).

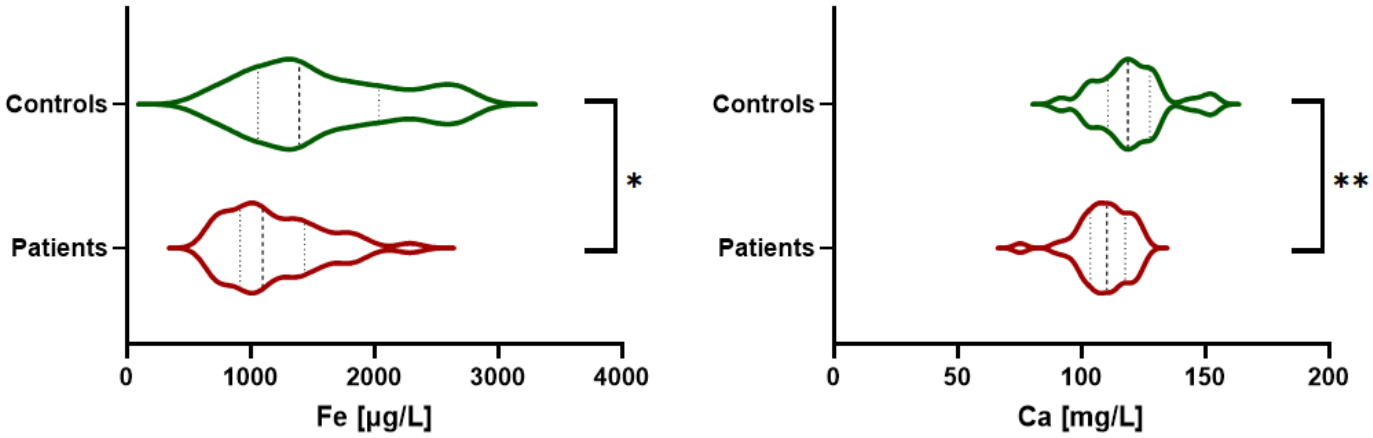

Figure 4. Mann Whitney comparison of Fe and Ca in PTC patients versus healthy controls. Significant lower concentration was observed between patients and controls in (A) Fe and (B) Ca. $P < 0.05$ (*) and $P < 0.01$ (**).

We also examined the correlation of trace elements: Se, Fe and Ca as well as the functional markers of Se (SePP, GPx3 and GPx1 activities) and thyroid parameters (Figure 5). We found an interesting positive and significant correlation between Se, SePP and GPx3 and a negative correlation between GPx1 activity and anti-Thyroid Peroxidase antibodies (TPO-ab).

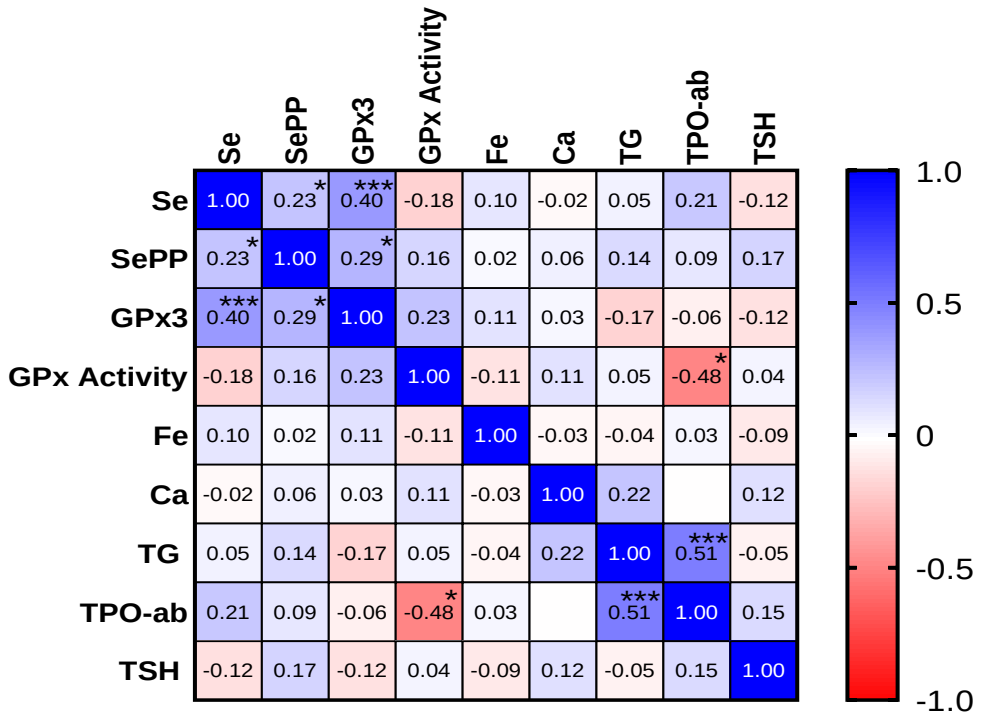

Figure 5. Coefficients correlation between Se levels (Se, SePP, GPx3 and GPx1 Activities), Fe, Ca, and thyroid parameters in all population. $P < 0.05$ (*) and $P < 0.001$ (***).

Figure 6 represent the relationship between Fe and hemoglobin as well as the hematocrit, responsible for anemia in a few patients. We found a positive correlation between Fe and the anemic biomarkers of our patients ($P < 0.05$).

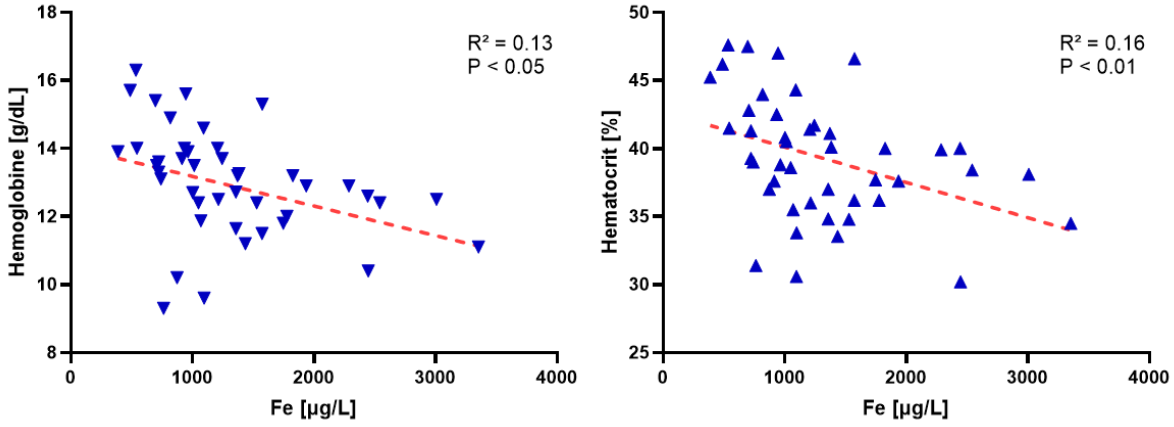

Figure 6. Linear regression between Fe and A) hemoglobin and B) hematocrit.

Finally, we studied the relationship between Fe and Se, as these two micronutrients interact in the thyroid gland and in different processes such as ferroptosis [29].

The distribution of Fe/Se ratios in patients and controls are represented in Table 5. We found a significant difference between the two groups with a $P < 0.05$. Then, we separated the ratio according to the quartiles and we did not find significant differences in the ratio repartition.

Table 5. Ratios between Fe and Se in PTC patients and healthy controls.

Fe/Se ratio	Patients	Controls	P - value
Median (IQR)	15.08 (5.13 – 40.19)	15.04 (3.08 – 37.86)	0.016
Q1: 3.08 - 11.09	22.73%	26.42%	0.28
Q2: 11.09 - 15.04	27.27%	24.53%	
Q3: 15.04 - 23.77	20.45%	28.30%	
Q4: 23.77- 40.19	29.55%	20.75%	

The correlation between Fe and Fe/Se ratio in the PTC patients and in the healthy controls are presented in Figure 7. We found a significant and positive correlation between Fe and Fe/Se in the two groups, we observed a clear correlation reflecting regulation and association between Fe and Se levels in the body (x/y = Se) with $P < 0.0001$.

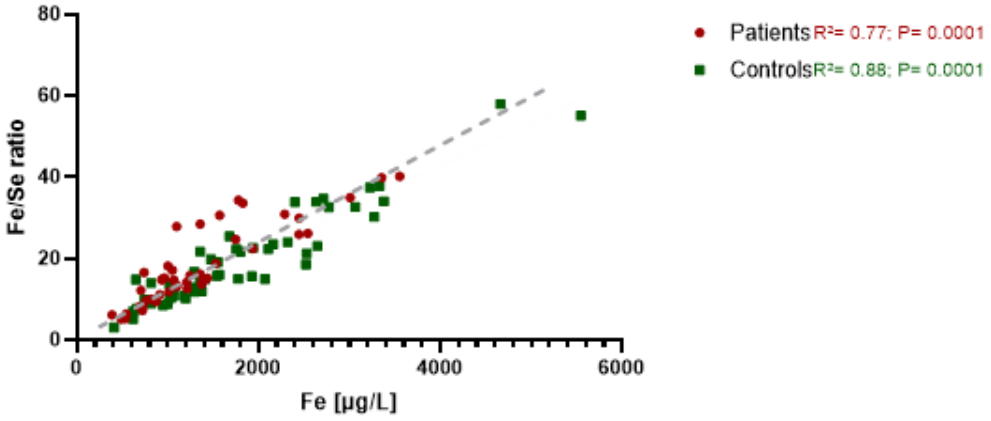

Figure 7. Correlation analysis between Fe and Fe/Se ratio in plasma of PTC patients and healthy controls. The two group expressed a significant and a positive correlation ($P < 0.0001$).

4. Discussions

In the present cross-sectional study, the population is predominantly female (ratio 9:1), representing the typical gender in the prevalence of thyroid cancer [30]. We observed that most of our patients had an overweight or obese BMI. A clinical study by Han *et al.*, [31] found a significant association between obesity and thyroid cancer. Obesity is considered as a risk factor

for PTC [32]. We also found that the bulk of our patients were illiterate or had an intermediate level of education, these results are similar to the findings established in other studies [33-34].

We noticed that 20% of our patients had anemic disease. Anemia impacts a quarter of the world's population and is centered in children and women, making it a global public health problem [35]. It is defined by a hemoglobin and hematocrit level below normal values, it can be caused by Fe deficiency, called Iron Deficiency Anemia (IDA), or not, and seems to be associated with thyroid dysfunction e.g., hypothyroidism [35]. Anemia is frequent in patients with cancer, about > 40% of cases, and it negatively influences the life quality of cancer patients, as it may contribute to cancer induced fatigue [36]. Moreover, we noted that the thyroid parameters, TSH and TG, of PTC patients were unstable, it is important to know that TSH, TG and TPO-Ab were done before ablation unfortunately we do not have these parameters after ablation to follow-up. Patients with thyroid cancer have a prolonged increase in TSH levels resulting in altered thyroid activity [37], and increased levels of TG in the blood represent one of the most indicative biological tests for thyroid cancer and can be associated with recurrence of thyroid cancer following thyroid removal [38]. TPO-Ab, could predict the incidence of thyroid cancer [39], however, in our population TPO-Ab means were in the normal range.

Considering that proper functioning of the thyroid gland depends on various trace elements e.g., Se and Fe, an imbalance of these elements may have carcinogenic, mutagenic and/or deregulatory effects [40]. The relationship between Se, Fe and Ca trace elements and PTC risk has been carefully examined in this study.

Selenium represent one of essential trace element required in maintenance of various functions of the body e.g., synthetizes and functions of thyroid hormones, protective effect against oxidative lipid damage and regulation of immunity functions [41]. In our study, we found significant lowest plasma Se concentration in PTC patients compared to healthy controls ($P < 0.001$). This may reflect the increased risk of PTC in patients with low plasma Se levels and confirm the results of other studies. The result obtained in a random-effects meta-analysis and search indicated low Se levels in serum [30, 42-43] , urine [44] and tissues [45] for thyroid cancer patients compared to controls. In contrast, other studies found no significant differences on serum [46] and fingernail [47] between thyroid cancer patients and controls, it displays contradictory results for the link between low Se status and thyroid cancer risk. However, the pathways relating Se deficiency to thyroid disease seem to involve the GPxs family implicated in antioxidants defense, the DIOs family responsible for the activation and inactivation of

thyroid hormones, and the immune selenoproteins monitoring the inflammatory response [14,48].

Many studies have shown that there is an association between Se diet and cancer risk [49-50]. Se nutritional supplements may reduce incidence of cancer [33]. Jonklaas *et al.,* [50] have shown that serum Se concentrations were inversely correlated with thyroid cancer stage. Although the specific Se anticarcinogenic mechanisms are not yet fully known, multiple mechanisms have been proposed to explain these Se characteristics. Actually, antioxidant properties of selenoenzymes are relevant in carcinogenesis and tumor progression [34]. Selenium is present in GPxs, which protects the DNA and main cellular components from the damage of the free radicals by decreasing ROS generation; in this way, levels of dietary antioxidant vitamins and carotenoids that affect antioxidant selenoproteins modify the effect of Se on cancer risk [33]. In addition, Se enhances tumor-suppressor protein p53 activates that inhibits proliferation, increases DNA repair, and promotes apoptosis [38-39].

Regarding plasma SePP, we have noticed high levels in patients than in the controls. However, this difference remains non-significant. SePP was present at high concentrations in plasma, and the majority of subjects were within the reference range 5-6 mg/L [51]. Also Persson-Moschos *et al.,* [52] found similar results for patients with different cancers. Recent study has underlined the genetic effect of variation in SePP gene. This leads to different responses in the expression of selenoproteins as well as the whole blood Se following the consumption of selenium-rich foods e.g., rs3877899 is functional polymorphism of SePP. These results confirmed the importance of individuals genotypes [49].

Glutathione peroxidases family are present in almost all cells and react very quickly to an alteration of Se status [53]. In the thyroid, GPx3 is produced by the thyroid follicular cells and discharged into the colloid space to protect the thyrocytes from oxidative damage and excess H_2O_2 [54]. Low Se levels could diminished GPx3 expression in cancer patients and increase the probability of cancer developing [55]. Loss or diminution of GPx3 increases proliferation and decreases apoptosis in tumor tissues [56]. It is suggested that Se and glutathione usage by tumor tissue depletes systemic levels and impacts GPx3 protein translations and activity in plasma [57]. In several studies low expression of GPx3 was associated in cancer patients types e.g., lung [58], uterine [59], and colorectal [60] cancer compared to healthy controls, this corroborates with the results obtained in our study for PTC. In another study, conducted on patients with hepatocellular carcinoma, they found an association between low plasma GPx3 and increase tumor recurrence [61]. The anti-tumor properties of GPx3 have been widely

correlated with a loss of oxidant scavenging, increases in oxidative stress and pro-tumorigenic changes involving genomic instability, as well as redox dependent signaling [62]

Zhao *et al*'s, study [63], on thyroid cancer patients, demonstrated that in most cases GPx3 methylation was significantly associated with tumor size and regional lymph node metastasis. The absent or reduced expression of GPx3 was frequently found in primary PTC samples and was associated with hypermethylation of the promoter region [63]. Furthermore, GPx3 polymorphisms rs3805435 heterozygote G allele and rs3828599 heterozygote T allele could have a protective effect for differentiated thyroid cancer, conversely, the rs8177412 heterozygote C allele is related with increased risk for this cancer [64].

Plasma Se is significantly correlated with plasma SePP and GPx3, full expression of GPx3 and SePP are usually observed at whole blood Se concentrations of around 90 µg/L and 100 µg/L, respectively [27-28]. It is indicating that neither SePP nor GPx3 have reached saturating expression levels in the PTC groups and rather deficient in this essential trace element, these results corroborate with other studies [30,65]. In literature, two isoforms of Se-Cys-tRNA$^{[Ser]Se\text{-}Cys}$ have been reported. The first one is involved in the biosynthesis of the most important selenoproteins like TRxs or GPx4. The second one ensures the synthesis of less essential proteins (GPx1, GPx3, selenoprotein R or selenoprotein T) [53]. This may explain our results and the unsaturation of some selenoproteins.

We found an increased levels of GPx1 activity in PTC patients compared to healthy controls. It was reported that the overexpression of GPx1 may promote invasion, migration and proliferation in colon, breast, lung and oral adenoid cystic carcinomas [64-65]. However, others trials carried out on prostate [66] and thyroid [44] cancer have found results opposite to ours, i.e. low level of GPx1 was inversely associated with cancer risk. The effect of Se on GPx1 enzyme dependent on genotype [67]. The influence of dietary Se intake on GPx1 activity will change differently in individuals with different GPx1 genotype, i.e., the relative increase in enzyme activity should be higher in individuals with at least one GPx1 198Pro allele than in those with both GPx1 198Leu alleles [67]. Other studies on breast cancer found that GPx1 activity was significantly low for the Leu allele compared to the Pro allele [68-69]. In addition, several studies indicate an association between GPx1 Pro198Leu polymorphism and an increased risk of cancer [68-70].

Our results showed significant Fe concentration decrease in PTC patients vs. healthy controls. Reddy *et al.,* [71] and Maeda *et al.,* [72] found lower Fe concentrations in carcinoma

thyroid tissues compared to the normal thyroid. Hu *et al.*, [73] observed a significant correlation between high urinary levels of Fe and decreased risk of large tumor size (>1 cm), capsular invasion, and advanced T stage (T3/4a/4b), and they conclude that Fe deficiency could be correlated as risk factor for PTC aggressiveness. On the contrary, Bibi et Shah [74] analyzed 16 metals, including Fe, in thyroid cancer patients and healthy controls, found that Fe concentration in plasma was higher in cases (26,661 μg/dL) than in controls (23,850 μg/dL). Rezaei *et al.*, [75] evaluate the plasma levels of Fe and others trace metals in healthy individuals and patients with thyroid disease (hyperthyroidism, hypothyroidism, and cancerous), they found highest concentration of Fe in cancerous groups. However, in the two studies the differences remained non-significant ($P > 0.05$). This fluctuation can be explained by the fact that trace element levels are dependent on many factors, including the region of the thyroid from which the sample was taken, age, gender, ethnicity, gland mass and stage of cancer [76].

The exact mechanisms by which Fe and iodine mutually impact thyroid function are not well understood. Fe is necessary for effective iodine utilization and Fe deficiency alters thyroid hormone synthesis, storage, and secretion, despite by reducing TPO activity even with adequate iodine intake [77-78]. In addition, we found a positive and significant correlation between Fe and hematocrit or hemoglobin ($P < 0.05$), this suggests that anemia of our patients may be a consequence of Fe deficiency. Anemia may alter thyroid metabolism through reduced oxygen transport [77]. In vivo experimental studies have proposed various mechanisms by which Fe influences thyroid function and iodine utilization [77-80]. In animals' studies, Fe deficiency alters the systemic control of thyroid metabolism, decreases the affinity of T3 for hepatocyte receptors, reduces oxygen transport, and reduces TPO activity [81-83]. Due to the crucial role of Fe in TPO activity, Fe deficiency could decrease TPO function and thus interfere with iodine utilization and thyroid metabolism [77].

Human studies showed that Fe deficiency also impairs thyroid metabolism. Patients with IDA or Fe deficiency without anemia have lower T3 levels than healthy controls [84]. Another study compared women with mild IDA to women with severe Fe deficiency, found that serum T3 and T4 were significantly decreased and TSH significantly increased in the Fe deficient groups, these studies suggest that Fe deficiency decreases thyroid hormone utilization [85]. Fe deficiency could reduce TPO activity and interfere with thyroid hormone synthesis [86]. Research has shown that TPO activity is significantly reduced in IDA individuals, suggesting that Fe status is strongly related to thyroid metabolism [83].

Iron has an impact on epigenetics, whereas Fe homeostasis is affected by epigenetic regulation, which may occur in various forms, such as DNA methylation, histone modification, post-transcriptional control, and some transcription factors that work cooperatively [87]. The HAMP gene, which encodes hepcidin, is regulated by the E4BP4/G9a/SOSTDC1/hepcidin pathway, which causes cellular Fe dysfunction and is a key element in hepcidin regulation and there is a critical link with thyroid cancer growth [88].

Selenium and Iron deficiency may act in tandem with iodine deficiency to affect thyroid metabolism and change the response of iodine [79,83,89]. We found a significant differences in Fe/Se ratio between PTC patients and controls, its suggested that imbalance of these two elements may lead disturbance in thyroid gland functioning.

In addition, Se and Fe represents key trace elements in the ferroptosis mechanism. Ferroptosis is new form of cells death recently demonstrated, characterized by unique morphologies, reduced cell size, and increased mitochondrial membrane density. It is a new form of regulated iron-dependent cell death, the glutathione depletion or inactivation of GPx4 can lead to metabolic imbalance, thus inducing ferroptosis of cancer cells and has shown great potential in cancer therapy [29]. However, ferroptosis can be reversed indirectly by Fe chelation, thus corroborating that an excess of Fe is involve in the initiation of this type of cell death [90].

We also investigated the relation between Ca and PTC risk. We found a significant decrease in Ca levels of PTC patients compared to healthy controls. Similarly, other studies on breast cancer [91] and prostate cancer [92], found lowest Ca concentrations in cancerous tissues patients. As known cellular functions are dependent on maintaining the extracellular Ca concentration within a narrow range and perturbations in this narrowly regulated homeostatic system resulting to a disturbances of Ca metabolism that have predictable effects on cellular functions [93]. The thyroid and parathyroids regulate blood Ca, while abnormal thyroid function profoundly alters Ca metabolism e.g., hypercalcemia can result in hyperthyroidism and create a thyroid disorder [94]. Thyroid hormones are suspected to influence Ca metabolism and have a direct impact on tubular Ca reabsorption in addition to its many influences on blood flow, glomerular filtration rate, and tubular sodium transport [93-94]. Ca is also involved in thyroid hormone synthesis, a recent studies has associated the Ca-activated anion channel anoctamine 1 with apical iodine efflux, and are supposed to allow the transport of iodide across the apical plasma membrane [95-96].

The relationship between Ca and thyroid remains ambiguous and poorly studied, however it is recommended after thyroidectomy to measure parathyroid hormone, Ca and albumin for surveillance purposes [97].

The importance of micronutrients has been demonstrated on several occasions. For this purpose, the World Health Organization (WHO) has published recommendations to reduce the risk of developing cancer, notably to reduce alcohol consumption and smoking, exposure to sunlight and radiation and to have a healthy and balanced diet. Diet plays an important role in preventing the development of cancer, which is known to have a multifactorial origin.

5. Conclusion

The various roles of Se in human health and cancer disease have not been fully elucidated, and the relationship between Se status and cancer has been debated for a long time with contradictory results of epidemiological studies. The assessment of Se, Fe and Ca in plasma levels showed a significant difference between PTC patients and healthy controls. In addition, we found strong and positive correlation between Se, SePP and GPx3 in PTC patients. Our study supports the hypothesis that trace elements concentrations have a high link with papillary thyroid carcinoma. Further molecular studies are needed for a better comprehension of the mechanisms involved in these processes.

AUTHOR CONTRIBUTIONS

LK, LC, and ND-M designed the study. MB, LK, and AB collected the sample and data via face-to-face interviews. LS did the Se, Fe, Ca SePP and GPx3 measurements. LK, HY performed GPx1 assessment. LK performed the statistical analyses. LK, and ND-M prepared the manuscript. LC and LS corrected the final version. All authors have read and agreed to the published version of the manuscript.

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References

[1] T. Sakai *et al.*, « Active Surveillance for T1bN0M0 Papillary Thyroid Carcinoma », *Thyroid*, vol. 29, nº 1, p. 59-63, janv. 2019, doi: 10.1089/thy.2018.0462.

[2] H. Lim, S. S. Devesa, J. A. Sosa, D. Check, et C. M. Kitahara, « Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013 », *JAMA*, vol. 317, nº 13, p. 1338, avr. 2017, doi: 10.1001/jama.2017.2719.

[3] J. Yu *et al.*, « Lymph node metastasis prediction of papillary thyroid carcinoma based on transfer learning radiomics », *Nat. Commun.*, vol. 11, nº 1, p. 4807, déc. 2020, doi: 10.1038/s41467-020-18497-3.

[4] G. Oler, C. P. Camacho, F. C. Hojaij, P. Michaluart, G. J. Riggins, et J. M. Cerutti, « Gene Expression Profiling of Papillary Thyroid Carcinoma Identifies Transcripts Correlated with *BRAF* Mutational Status and Lymph Node Metastasis », *Clin. Cancer Res.*, vol. 14, nº 15, p. 4735-4742, août 2008, doi: 10.1158/1078-0432.CCR-07-4372.

[5] M. E. Cabanillas, D. G. McFadden, et C. Durante, « Thyroid cancer », *The Lancet*, vol. 388, nº 10061, p. 2783-2795, déc. 2016, doi: 10.1016/S0140-6736(16)30172-6.

[6] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, et A. Jemal, « Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries », *CA. Cancer J. Clin.*, vol. 68, nº 6, p. 394-424, nov. 2018, doi: 10.3322/caac.21492.

[7] « Global Cancer Observatory ». https://gco.iarc.fr/ (consulté le 1 novembre 2021).

[8] A. Benouis, Z. Bekkouche, M. S. Merad, L. Loudjedi, H. Khelil, et N. Berber, « Thyroid Cancer in Western Algeria: Histopathological and Epidemiological Study », *J. Cancer Ther.*, vol. 08, nº 07, p. 672-682, 2017, doi: 10.4236/jct.2017.87058.

[9] M. Cabral *et al.*, « Trace element profile and incidence of type 2 diabetes, cardiovascular disease and colorectal cancer: results from the EPIC-Potsdam cohort study », *Eur. J. Nutr.*, vol. 60, nº 6, p. 3267-3278, sept. 2021, doi: 10.1007/s00394-021-02494-3.

[10] R. F. Burk et K. E. Hill, « Regulation of Selenium Metabolism and Transport », *Annu. Rev. Nutr.*, vol. 35, nº 1, p. 109-134, juill. 2015, doi: 10.1146/annurev-nutr-071714-034250.

[11] M. P. Rayman, « Selenium and human health », *The Lancet*, vol. 379, nº 9822, p. 1256-1268, mars 2012, doi: 10.1016/S0140-6736(11)61452-9.

[12] J. T. Deagen, J. A. Butler, B. A. Zachara, et P. D. Whanger, « Determination of the Distribution of Selenium between Glutathione Peroxidase, Selenoprotein P, and Albumin in Plasma », *Anal. Biochem.*, vol. 208, nº 1, p. 176-181, janv. 1993, doi: 10.1006/abio.1993.1025.

[13] R. F. Burk et K. E. Hill, « Selenoprotein P—Expression, functions, and roles in mammals », *Biochim. Biophys. Acta BBA - Gen. Subj.*, vol. 1790, nº 11, p. 1441-1447, nov. 2009, doi: 10.1016/j.bbagen.2009.03.026.

[14] J. Köhrle, « Selenium and the thyroid », *Curr. Opin. Endocrinol. Diabetes Obes.*, vol. 22, nº 5, p. 392-401, oct. 2015, doi: 10.1097/MED.0000000000000190.

[15] M. Vinceti *et al.*, « Health risk assessment of environmental selenium: Emerging evidence and challenges », *Mol. Med. Rep.*, vol. 15, nº 5, p. 3323-3335, mai 2017, doi: 10.3892/mmr.2017.6377.

[16] J. Luo, M. Hendryx, P. Dinh et K. He « Association of Iodine and Iron with Thyroid Function », *Biol Trace Elem Res.*, Feb. 2017, doi: 10.1007/s12011-017-0954-x.

[17] M. B. Zimmermann, « The Influence of Iron Status on Iodine Utilization and Thyroid Function », *Annu. Rev. Nutr.*, vol. 26, nº 1, p. 367-389, août 2006, doi: 10.1146/annurev.nutr.26.061505.111236.

[18] P.-J. Lamy, A. Durigova, et W. Jacot, « Iron homeostasis and anemia markers in early breast cancer », *Clin. Chim. Acta*, vol. 434, p. 34-40, juill. 2014, doi: 10.1016/j.cca.2014.04.011.

[19] G. J. Anderson et G. McLaren, Éd., « Iron physiology and pathophysiology in humans ». New York: Humana, 2009.

[20] P.-J. Lamy, A. Durigova, et W. Jacot, « Iron homeostasis and anemia markers in early breast cancer », *Clin. Chim. Acta*, vol. 434, p. 34-40, juill. 2014, doi: 10.1016/j.cca.2014.04.011.

[21] N. Stathatos, « Anatomy and Physiology of the Thyroid Gland », in *The Thyroid and Its Diseases*, M. Luster, L. H. Duntas, et L. Wartofsky, Éd. Cham: Springer International Publishing, 2019, p. 3-12. doi: 10.1007/978-3-319-72102-6_1.

[22] V. Centeno, G. Díaz de Barboza, A. Marchionatti, V. Rodríguez, et N. Tolosa de Talamoni, « Molecular mechanisms triggered by low-calcium diets », *Nutr. Res. Rev.*, vol. 22, nº 2, p. 163-174, déc. 2009, doi: 10.1017/S0954422409990126.

[23] G. F. Combs *et al.*, « Determinants of selenium status in healthy adults », *Nutr. J.*, vol. 10, nº 1, p. 75, déc. 2011, doi: 10.1186/1475-2891-10-75.

[24] S. Hybsier *et al.*, « Sex-specific and inter-individual differences in biomarkers of selenium status identified by a calibrated ELISA for selenoprotein P », *Redox Biol.*, vol. 11, p. 403-414, avr. 2017, doi: 10.1016/j.redox.2016.12.025.

[25] L. Flohé et W. A. Günzler, « Assays of glutathione peroxidase », in *Methods in Enzymology*, vol. 105, Elsevier, 1984, p. 114-120. doi: 10.1016/S0076-6879(84)05015-1.

[26] G. F. Combs, Jr., « Biomarkers of Selenium Status », *Nutrients*, vol. 7, nº 4, p. 2209-2236, mars 2015, doi: 10.3390/nu7042209.

[27] L. B. Rasmussen *et al.*, « Serum selenium and selenoprotein P status in adult Danes – 8-year followup », *J. Trace Elem. Med. Biol.*, vol. 23, nº 4, p. 265-271, nov. 2009, doi: 10.1016/j.jtemb.2009.03.009.

[28] C. D. Thomson, « Assessment of requirements for selenium and adequacy of selenium status: a review », *Eur. J. Clin. Nutr.*, vol. 58, nº 3, p. 391-402, mars 2004, doi: 10.1038/sj.ejcn.1601800.

[29] M. Ge *et al.*, « A Ferroptosis-Related Signature Robustly Predicts Clinical Outcomes and Associates With Immune Microenvironment for Thyroid Cancer », *Front. Med.*, vol. 8, p. 637743, avr. 2021, doi: 10.3389/fmed.2021.637743.

[30] S. Mehl *et al.*, « Cross-sectional analysis of trace element status in thyroid disease », *J. Trace Elem. Med. Biol.*, vol. 58, p. 126430, mars 2020, doi: 10.1016/j.jtemb.2019.126430.

[31] J. M. Han *et al.*, « Obesity is a risk factor for thyroid cancer in a large, ultrasonographically screened population », *Eur. J. Endocrinol.*, vol. 168, nº 6, p. 879-886, juin 2013, doi: 10.1530/EJE-13-0065.

[32] A. Matrone, F. Ferrari, F. Santini, et R. Elisei, « Obesity as a risk factor for thyroid cancer », *Curr. Opin. Endocrinol. Diabetes Obes.*, vol. 27, nº 5, p. 358-363, oct. 2020, doi: 10.1097/MED.0000000000000556.

[33] T.-J. Lee, S. Kim, H.-J. Cho, et J.-H. Lee, « The Incidence of Thyroid Cancer Is Affected by the Characteristics of a Healthcare System », *J. Korean Med. Sci.*, vol. 27, nº 12, p. 1491, 2012, doi: 10.3346/jkms.2012.27.12.1491.

[34] D. W. Chen *et al.*, « Disparities in risk perception of thyroid cancer recurrence and death », *Cancer*, vol. 126, nº 7, p. 1512-1521, avr. 2020, doi: 10.1002/cncr.32670.

[35] A. T. Soliman, V. De Sanctis, M. Yassin, M. Wagdy, et N. Soliman, « Chronic anemia and thyroid function », *Acta Bio Medica Atenei Parm.*, vol. 88, nº 1, avr. 2017, doi: 10.23750/abm.v88i1.6048.

[36] M. Dicato, L. Plawny, et M. Diederich, « Anemia in cancer », *Ann. Oncol.*, vol. 21, p. vii167-vii172, oct. 2010, doi: 10.1093/annonc/mdq284.

[37] J. Lukas, J. Drabek, D. Lukas, L. Dusek, et J. Gatek, « The epidemiology of thyroid cancer in the Czech Republic in comparison with other countries », *Biomed. Pap.*, vol. 157, nº 3, p. 266-275, sept. 2013, doi: 10.5507/bp.2012.086.

[38] F. C. Holsinger *et al.*, « Measuring the Extent of Total Thyroidectomy for Differentiated Thyroid Carcinoma Using Radioactive Iodine Imaging: Relationship With Serum Thyroglobulin and Clinical Outcomes », *JAMA Otolaryngol. Neck Surg.*, vol. 140, nº 5, p. 410, mai 2014, doi: 10.1001/jamaoto.2014.264.

[39] Y. A. Cho *et al.*, « Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: a nested case–control study », *BMC Cancer*, vol. 14, nº 1, p. 873, déc. 2014, doi: 10.1186/1471-2407-14-873.

[40] K. Lossow, K. Renko, M. Schwarz, L. Schomburg, T. Schwerdtle, et A. P. Kipp, « The Nutritional Supply of Iodine and Selenium Affects Thyroid Hormone Axis Related Endpoints in Mice », *Nutrients*, vol. 13, nº 11, p. 3773, oct. 2021, doi: 10.3390/nu13113773.

[41] M. Kieliszek, I. Bano, et H. Zare, « A Comprehensive Review on Selenium and Its Effects on Human Health and Distribution in Middle Eastern Countries », *Biol. Trace Elem. Res.*, vol. 200, nº 3, p. 971-987, mars 2022, doi: 10.1007/s12011-021-02716-z.

[42] A. K. Baltaci, T. K. Dundar, F. Aksoy, et R. Mogulkoc, « Changes in the Serum Levels of Trace Elements Before and After the Operation in Thyroid Cancer Patients », *Biol. Trace Elem. Res.*, vol. 175, nº 1, p. 57-64, janv. 2017, doi: 10.1007/s12011-016-0768-2.

[43] H. Al-Sayer *et al.*, « Serum changes in trace elements during thyroid cancers », *Mol. Cell. Biochem.*, vol. 260, nº 1, p. 1-5, mai 2004, doi: 10.1023/B:MCBI.0000026027.20680.c7.

[44] A. Metere *et al.*, « A possible role for selenoprotein glutathione peroxidase (GPx1) and thioredoxin reductases (TrxR1) in thyroid cancer: our experience in thyroid surgery », *Cancer Cell Int.*, vol. 18, nº 1, p. 7, déc. 2018, doi: 10.1186/s12935-018-0504-4.

[45] M. Kucharzewski, J. Braziewicz, U. Majewska, et S. Gózdz, « Concentration of Selenium in the Whole Blood and the Thyroid Tissue of Patients with Various Thyroid Diseases », *Biol. Trace Elem. Res.*, vol. 88, nº 1, p. 25-30, 2002, doi: 10.1385/BTER:88:1:25.

[46] E. Przybylik-Mazurek, P. Zagrodzki, S. Kuźniarz-Rymarz, et A. Hubalewska-Dydejczyk, « Thyroid Disorders—Assessments of Trace Elements, Clinical, and Laboratory Parameters », *Biol. Trace Elem. Res.*, vol. 141, nº 1-3, p. 65-75, juin 2011, doi: 10.1007/s12011-010-8719-9.

[47] Y. Ren *et al.*, « Lack of Association between Fingernail Selenium and Thyroid Cancer Risk: A Case-Control Study in French Polynesia », *Asian Pac. J. Cancer Prev.*, vol. 15, nº 13, p. 5187-5194, juill. 2014, doi: 10.7314/APJCP.2014.15.13.5187.

[48] A. Orozco, C. Valverde-R, A. Olvera, et C. García-G, « Iodothyronine deiodinases: a functional and evolutionary perspective », *J. Endocrinol.*, vol. 215, nº 2, p. 207-219, nov. 2012, doi: 10.1530/JOE-12-0258.

[49] L. S. Kazi Tani, N. Dennouni-Medjati, B. Toubhans, et L. Charlet, « Selenium Deficiency—From Soil to Thyroid Cancer », *Appl. Sci.*, vol. 10, nº 15, p. 5368, août 2020, doi: 10.3390/app10155368.

[50] J. Jonklaas, M. Danielsen, et H. Wang, « A Pilot Study of Serum Selenium, Vitamin D, and Thyrotropin Concentrations in Patients with Thyroid Cancer », *Thyroid*, vol. 23, nº 9, p. 1079-1086, sept. 2013, doi: 10.1089/thy.2012.0548.

[51] R. F. Burk, K. E. Hill, et A. K. Motley, « Plasma selenium in specific and non-specific forms », *BioFactors*, vol. 14, nº 1-4, p. 107-114, 2001, doi: 10.1002/biof.5520140115.

[52] M. E. K. Persson-Moschos, L. Stavenow, B. Åkesson, et F. Lindgärde, « Selenoprotein P in Plasma in Relation to Cancer Morbidity in Middle-Aged Swedish Men », *Nutr. Cancer*, vol. 36, nº 1, p. 19-26, janv. 2000, doi: 10.1207/S15327914NC3601_4.

[53] L. Schomburg et U. Schweizer, « Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium », *Biochim. Biophys. Acta BBA - Gen. Subj.*, vol. 1790, nº 11, p. 1453-1462, nov. 2009, doi: 10.1016/j.bbagen.2009.03.015.

[54] L. Schomburg et J. Köhrle, « On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health », *Mol. Nutr. Food Res.*, vol. 52, nº 11, p. 1235-1246, nov. 2008, doi: 10.1002/mnfr.200700465.

[55] V. Diwadkar-Navsariwala et A. M. Diamond, « The Link between Selenium and Chemoprevention: A Case for Selenoproteins », *J. Nutr.*, vol. 134, nº 11, p. 2899-2902, nov. 2004, doi: 10.1093/jn/134.11.2899.

[56] S.-N. Chang, J. M. Lee, H. Oh, et J.-H. Park, « Glutathione Peroxidase 3 Inhibits Prostate Tumorigenesis in TRAMP Mice: GPx3 Inhibits Tumorigenesis in TRAMP Prostate », *The Prostate*, vol. 76, nº 15, p. 1387-1398, nov. 2016, doi: 10.1002/pros.23223.

[57] C. Chang, B. L. Worley, R. Phaëton, et N. Hempel, « Extracellular Glutathione Peroxidase GPx3 and Its Role in Cancer », *Cancers*, vol. 12, nº 8, p. 2197, août 2020, doi: 10.3390/cancers12082197.

[58] I.-J. Oh *et al.*, « Diagnostic value of serum glutathione peroxidase 3 levels in patients with lung cancer: GPx3 as a biomarker for lung cancer », *Thorac. Cancer*, vol. 5, nº 5, p. 425-430, sept. 2014, doi: 10.1111/1759-7714.12113.

[59] A. Kumar, S. Sharma, C. S. Pundir, et A. Sharma, « Decreased plasma glutathione in cancer of the uterine cervix », *Cancer Lett.*, vol. 94, nº 1, p. 107-111, juill. 1995, doi: 10.1016/0304-3835(95)03832-H.

[60] D. Psathakis, N. Wedemeyer, E. Oevermann, F. Krug, C.-P. Siegers, et H.-P. Bruch, « Blood selenium and glutathione peroxidase status in patients with colorectal cancer », *Dis. Colon Rectum*, vol. 41, nº 3, p. 328-335, mars 1998, doi: 10.1007/BF02237487.

[61] X. Qi *et al.*, « Clinical significance and therapeutic value of glutathione peroxidase 3 (GPx3) in hepatocellular carcinoma », *Oncotarget*, vol. 5, nº 22, p. 11103-11120, nov. 2014, doi: 10.18632/oncotarget.2549.

[62] Y. P. Yu *et al.*, « Glutathione Peroxidase 3, Deleted or Methylated in Prostate Cancer, Suppresses Prostate Cancer Growth and Metastasis », *Cancer Res.*, vol. 67, nº 17, p. 8043-8050, sept. 2007, doi: 10.1158/0008-5472.CAN-07-0648.

[63] H. Zhao *et al.*, « Silencing GPX3 Expression Promotes Tumor Metastasis in Human Thyroid Cancer », *Curr. Protein Pept. Sci.*, vol. 16, nº 4, p. 316-321, avr. 2015, doi: 10.2174/1389203716041504291548 40.

[64] J.-C. Lin *et al.*, « Glutathione peroxidase 3 gene polymorphisms and risk of differentiated thyroid cancer », *Surgery*, vol. 145, nº 5, p. 508-513, mai 2009, doi: 10.1016/j.surg.2008.12.008.

[65] M. Belhadj *et al.*, « Se Status Prediction by Food Intake as Compared to Circulating Biomarkers in a West Algerian Population », p. 12, 2020.

[66] D. Ekoue *et al.*, « GPX1 Localizes to the Nucleus in Prostate Epithelium and its Levels are not Associated with Prostate Cancer Recurrence », *Antioxidants*, vol. 7, nº 11, p. 167, nov. 2018, doi: 10.3390/antiox7110167.

[67] E. Jablonska *et al.*, « Association between GPx1 Pro198Leu polymorphism, GPx1 activity and plasma selenium concentration in humans », *Eur. J. Nutr.*, vol. 48, nº 6, p. 383-386, sept. 2009, doi: 10.1007/s00394-009-0023-0.

[68] Y. J. Hu et A. M. Diamond, « Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium », *Cancer Res.*, vol. 63, nº 12, p. 3347-3351, juin 2003.

[69] G. Ravn-Haren *et al.*, « Associations between GPX1 Pro198Leu polymorphism, erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study », *Carcinogenesis*, vol. 27, nº 4, p. 820-825, avr. 2006, doi: 10.1093/carcin/bgi267.

[70] O. Raaschou-Nielsen *et al.*, « GPX1 Pro198Leu polymorphism, interactions with smoking and alcohol consumption, and risk for lung cancer », *Cancer Lett.*, vol. 247, nº 2, p. 293-300, mars 2007, doi: 10.1016/j.canlet.2006.05.006.

[71] S. B. Reddy *et al.*, « Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method », *Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. At.*, vol. 196, nº 3-4, p. 333-339, nov. 2002, doi: 10.1016/S0168-583X(02)01292-2.

[72] K. Maeda, Y. Yokode, Y. Sasa, H. Kusuyama, et M. Uda, « Multielemental analysis of human thyroid glands using particle induced X-ray emission (PIXE) », *Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. At.*, vol. 22, nº 1-3, p. 188-190, mars 1987, doi: 10.1016/0168-583X(87)90323-5.

[73] M.-J. Hu *et al.*, « Associations between essential microelements exposure and the aggressive clinicopathologic characteristics of papillary thyroid cancer », *BioMetals*, vol. 34, nº 4, p. 909-921, août 2021, doi: 10.1007/s10534-021-00317-w.

[74] K. Bibi et M. H. Shah, « Appraisal of Metal Imbalances in the Blood of Thyroid Cancer Patients in Comparison with Healthy Subjects », *Biol. Trace Elem. Res.*, vol. 198, nº 2, p. 410-422, déc. 2020, doi: 10.1007/s12011-020-02088-w.

[75] M. Rezaei, S. Y. Javadmoosavi, B. Mansouri, N. A. Azadi, O. Mehrpour, et S. Nakhaee, « Thyroid dysfunction: how concentration of toxic and essential elements contribute to risk of hypothyroidism, hyperthyroidism, and thyroid cancer », *Environ. Sci. Pollut. Res.*, vol. 26, nº 35, p. 35787-35796, déc. 2019, doi: 10.1007/s11356-019-06632-7.

[76] « Trace Element Contents in Thyroid Cancer Investigated by Energy Dispersive X-Ray Fluorescent Analysis », *Am. J. Cancer Res. Rev.*, 2018, doi: 10.28933/ajocrr-2017-12-2801.

[77] M. B. Zimmermann, « The Influence of Iron Status on Iodine Utilization and Thyroid Function », *Annu. Rev. Nutr.*, vol. 26, nº 1, p. 367-389, août 2006, doi: 10.1146/annurev.nutr.26.061505.111236.

[78] S. Y. Hess, « The impact of common micronutrient deficiencies on iodine and thyroid metabolism: the evidence from human studies », *Best Pract. Res. Clin. Endocrinol. Metab.*, vol. 24, nº 1, p. 117-132, févr. 2010, doi: 10.1016/j.beem.2009.08.012.

[79] M. B. Zimmermann et J. Köhrle, « The Impact of Iron and Selenium Deficiencies on Iodine and Thyroid Metabolism: Biochemistry and Relevance to Public Health », *Thyroid*, vol. 12, nº 10, p. 867-878, oct. 2002, doi: 10.1089/105072502761016494.

[80] Hess et Zimmermann, « The Effect of Micronutrient Deficiencies on Iodine Nutrition and Thyroid Metabolism », *Int. J. Vitam. Nutr. Res.*, vol. 74, nº 2, p. 103-115, mars 2004, doi: 10.1024/0300-9831.74.2.103.

[81] J. L. Beard, D. E. Brigham, S. K. Kelley, et M. H. Green, « Plasma Thyroid Hormone Kinetics Are Altered in Iron-Deficient Rats », *J. Nutr.*, vol. 128, nº 8, p. 1401-1408, août 1998, doi: 10.1093/jn/128.8.1401.

[82] S. M. Smith, P. E. Johnson, et H. C. Lukaski, « In vitro hepatic thyroid hormone deiodination in iron-deficient rats: Effect of dietary fat », *Life Sci.*, vol. 53, nº 8, p. 603-609, janv. 1993, doi: 10.1016/0024-3205(93)90268-8.

[83] S. Y. Hess, M. B. Zimmermann, M. Arnold, W. Langhans, et R. F. Hurrell, « Iron Deficiency Anemia Reduces Thyroid Peroxidase Activity in Rats », *J. Nutr.*, vol. 132, nº 7, p. 1951-1955, juill. 2002, doi: 10.1093/jn/132.7.1951.

[84] C. Martinez-Torres *et al.*, « Effect of exposure to low temperature on normal and iron-deficient subjects », *Am. J. Physiol.-Regul. Integr. Comp. Physiol.*, vol. 246, nº 3, p. R380-R383, mars 1984, doi: 10.1152/ajpregu.1984.246.3.R380.

[85] J. L. Beard, M. J. Borel, et J. Derr, « Impaired thermoregulation and thyroid function in iron-deficiency anemia », *Am. J. Clin. Nutr.*, vol. 52, nº 5, p. 813-819, nov. 1990, doi: 10.1093/ajcn/52.5.813.

[86] R. F. Hurrell, « Bioavailability of iodine », *Eur. J. Clin. Nutr.*, vol. 51 Suppl 1, p. S9-12, janv. 1997.

[87] Y. Wang, L. Yu, J. Ding, et Y. Chen, « Iron Metabolism in Cancer », *Int. J. Mol. Sci.*, vol. 20, nº 1, p. 95, déc. 2018, doi: 10.3390/ijms20010095.

[88] Q. Zhou, J. Chen, J. Feng, et J. Wang, « E4BP4 promotes thyroid cancer proliferation by modulating iron homeostasis through repression of hepcidin », *Cell Death Dis.*, vol. 9, nº 10, p. 987, oct. 2018, doi: 10.1038/s41419-018-1001-3.

[89] M. Zimmermann, P. Adou, T. Torresani, C. Zeder, et R. Hurrell, « Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Côte d'Ivoire », *Am. J. Clin. Nutr.*, vol. 71, nº 1, p. 88-93, janv. 2000, doi: 10.1093/ajcn/71.1.88.

[90] C. Moreau *et al.*, « Nouvelle stratégie de neuroprotection basée sur la chélation conservatrice du fer dans la maladie de Parkinson », *Bull. Académie Natl. Médecine*, vol. 203, nº 6, p. 415-423, sept. 2019, doi: 10.1016/j.banm.2019.04.019.

[91] Y. Zheng *et al.*, « Accelerated Bone Resorption, Due to Dietary Calcium Deficiency, Promotes Breast Cancer Tumor Growth in Bone », *Cancer Res.*, vol. 67, nº 19, p. 9542-9548, oct. 2007, doi: 10.1158/0008-5472.CAN-07-1046.

[92] A. Sapota, A. Daragó, J. Taczalski, et A. Kilanowicz, « Disturbed homeostasis of zinc and other essential elements in the prostate gland dependent on the character of pathological lesions », *BioMetals*, vol. 22, nº 6, p. 1041-1049, déc. 2009, doi: 10.1007/s10534-009-9255-y.

[93] N. S. Memon *et al.*, « Evaluation of calcium and lead interaction, in addition to their impact on thyroid functions in hyper and hypothyroid patients », *Environ. Sci. Pollut. Res.*, vol. 23, nº 1, p. 878-886, janv. 2016, doi: 10.1007/s11356-015-5325-6.

[94] S. Begic-Karup *et al.*, « Serum calcium in thyroid disease », *Wien. Klin. Wochenschr.*, vol. 113, nº 1-2, p. 65-68, janv. 2001.

[95] J. C. Silveira et P. A. Kopp, « Pendrin and anoctamin as mediators of apical iodide efflux in thyroid cells », *Curr. Opin. Endocrinol. Diabetes Obes.*, vol. 22, nº 5, p. 374-380, oct. 2015, doi: 10.1097/MED.0000000000000188.

[96] K. Brix, M. Qatato, J. Szumska, V. Venugopalan, et M. Rehders, « "Thyroglobulin Storage, Processing and Degradation for Thyroid Hormone Liberation" », in *The Thyroid and Its Diseases*, M. Luster, L. H. Duntas, et L. Wartofsky, Éd. Cham: Springer International Publishing, 2019, p. 25-48. doi: 10.1007/978-3-319-72102-6_3.

[97] J. H. Li, N. Kasid, et J. V. Hennessey, « Graves' Disease », in *The Thyroid and Its Diseases*, M. Luster, L. H. Duntas, et L. Wartofsky, Éd. Cham: Springer International Publishing, 2019, p. 307-321. doi: 10.1007/978-3-319-72102-6_22.

Chapter III: Copper Isotopes and Copper to Zinc Ratio as Possible Biomarkers for Thyroid Cancer

I. Presentation of the article

Thyroid cancer is the most frequent malignancy of the endocrine system. There is no systematic detection for this cancer, and the diagnosis is made incidentally in 25% of cases during the follow-up of another thyroid disease. Therefore, a current challenge lies in identifying suitable biomarkers for supporting a fast diagnosis of thyroid cancer.

In recent years, several studies have focused on the importance and use of metals, especially Cu and Zn, present in blood and their isotopic fractionation as novel diagnostic tools for certain diseases and particularly in cancerology. It also appears that imbalances between the two metals (Cu/Zn) are more clinically sensitive indices of disease than the concentration of a single metal.

Combining biology and geochemistry has allowed to expand the existing knowledge and to develop new analytical techniques with high sensitivity that can measure very small isotopic variations of non-traditional isotopes (e.g., Cu) such as the Multi-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS). Cu isotopes are present as two ions Cu(I) and Cu(II) in cells tissues, and blood. The uptake and selective distribution of these two ions is the basis for the isotopic fractionation of $^{65}Cu/^{63}Cu$ ratio ($\delta^{65}Cu$). Cu isotopes are related to various biological functions so the ratio between these isotopes are determined by MC-ICP-MS and the delta $\delta^{65}Cu$ value obtained is used to measure the ratio of Cu isotopic abundances defined by the formula bellow:

$$\delta^{65}\mathrm{Cu} = \left[\frac{(^{65}\mathrm{Cu}/^{63}\mathrm{Cu})\ \mathrm{sample}}{(^{65}\mathrm{Cu}/^{63}\mathrm{Cu})\ \mathrm{IStd}} \text{-} 1 \right] \times 10\ 000$$

Several studies conducted on patients with breast, colorectal and ovarian cancer have shown for serum a significant decrease of $^{65}Cu/^{63}Cu$ ratio ($\delta^{65}Cu$) compared to healthy controls and in a mirror image result, a higher value of this ratio in tumor tissue relative to healthy tissue. These results support the hypothesis that Cu isotopes have the strong potential to be markers of cancer.

In this chapter we measured the plasma levels of Cu and Zn for the whole population and then we compared between cases and controls, we found very significantly high results for Cu and significantly low for Zn, we also found that the Cu/Zn ratio was disturbed in patients compared to the healthy control. We tested the sensitivity of the Cu isotope fractionation in plasma and biopsy. We found very conclusive results which are structured in the article below. The plasma of the controls was enriched in ^{65}Cu, however that of patients was enriched in ^{63}Cu, conversely and in mirror image biopsies of patients were enriched in ^{65}Cu. These changes can

be explained by different biological processes such as angiogenesis, cell proliferation and cell mass formation.

In the article presented below, we support the hypothesis that trace element concentrations (Cu and Zn) and the isotopic composition of Cu have a high potential to be used as additional biomarkers in the detection of thyroid cancer.

It is preliminary work proving the efficacy of isotopic measurements which can be use as complementary to cancer investigation. However, a large cellular and subcellular studies will be required to define δ^{65}Cu thresholds that would be indicative of the presence of the disease.

II. Article

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Copper Isotopes and Copper to Zinc Ratio as Possible Biomarkers for Thyroid Cancer

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Abstract

Thyroid cancer is the most common endocrine cancer. There is no systematic screening for such cancer, and the current challenge is to find potential biomarkers to facilitate an early diagnosis. Copper (Cu) and zinc (Zn) are essential micronutrients involved in the proper functioning of the thyroid gland, and changes in their concentrations have been observed in the development of cancer. Previous studies have highlighted the potential of $^{65}Cu/^{63}Cu$ ratio ($\delta^{65}Cu$) to be a cancer biomarker, and this study tests its sensitivity on plasma samples (n=46) of Algerian patients with papillary thyroid carcinoma and a set of corresponding biopsies (n=11). The $\delta^{65}Cu$ ratio in blood and tumor samples was determined using multi collector inductively coupled plasma-mass spectrometry (MC-ICP-MS), and their corresponding Cu and Zn plasma total concentrations using total reflection X-ray fluorescence (TXRF). Plasma concentrations of Cu were significantly higher (1346.1 ± 328.3 vs. 1060.5 ± 216.1 µg/L, $p < 0.0001$), and Zn significantly lower (942.1 ± 205.2 vs. 1027.9 ± 151.4 µg/L, $p < 0.05$) in thyroid cancer patients as compared to healthy controls (n=50). Accordingly, the Cu/Zn ratio was significantly different between patients and controls (1.5 ± 0.4 vs. 1.0 ± 0.3, $p < 0.0001$). Furthermore, the $\delta^{65}Cu$ plasma levels of patients were significantly lower than healthy controls ($p < 0.0001$), whereas thyroid tumor tissues presented high $\delta^{65}Cu$ values. These results support the hypothesis that Cu isotopes and plasma trace elements may serve as suitable biomarkers of thyroid cancer diagnosis.

Keywords: Copper, Isotopes, Zinc, Biomarkers, Thyroid Cancer.

Introduction

Thyroid cancer is the most frequent malignancy of the endocrine system, responsible for almost 90% of endocrine cancers (1-2), and accounting for the Surveillance, Epidemiology, and End Results (SEER) Stat Fact 4% of all new cancer cases in the US (3). Its incidence is increasing with 567.000 new cases annually, ranking it the 9th place worldwide. It is diagnosed three times more in woman (10.2 per 100,000) than in men (3.1 per 100,000) (4). In Algeria, thyroid cancer incidence is increasing, taking now the 3rd place in women after breast and colorectal cancer, while in 2006 it occupied the 5th position in frequency (5-6). Four types of thyroid cancer are histologically distinguished. The most common is papillary thyroid carcinoma (PTC) representing 80 to 90% of new thyroid cancer cases (7). There is no systematic detection of thyroid cancer, and the diagnosis is made incidentally in 25% of cases during the follow-up of another thyroid disease (2,8). Therefore, a current challenge lies in identifying suitable biomarkers for supporting a fast diagnosis of thyroid cancer.

The link between metal trace elements and cancer has not been yet proofed nor much studied, especially in Algeria. Copper (Cu) is an essential metallic trace element for living organisms (9). Changes in Cu metabolism have been observed concomitantly with the development of cancer (10). However, Cu is not a carcinogen, but an increased Cu bioavailability may lead to an increased production of ATP, which is used by cancer cells during proliferation (10). Thus, Cu contributes to the proliferation of transformed cells by providing the energy required for cell cycle progression (11). As Cu has a short residence time in the bulk human body (< 6weeks), it is a potential indicator for rapidly evolving diseases such as cancers (12). Like Zinc (Zn), Cu is a catalytic cofactor in Cu/Zn superoxide dismutase I enzyme (SOD1), and it is the interaction between Cu and Zn that allows the enzyme to function properly (13). This enzyme has a very important role in antioxidant defense system of cells, as it converts superoxide into oxygen and hydrogen peroxide. A disturbance of its expression is associated with various cancers such as hepatocellular carcinoma (14), and overexpression of SOD1 promotes tumor growth in lung cancer cells and reduce apoptosis (15). Although significantly elevated concentrations of Cu and Cu/Zn ratio have been observed in the plasma of patients with breast cancer (16-17), it seems that trace metal imbalances (Cu/Zn) are clinically more sensitive indices of disease than the concentration of any single trace metal alone (13).

Copper naturally has two stable isotopes: ^{63}Cu (69.2%) and ^{65}Cu (30.8%) (18). The isotopic abundances of copper in human serum can vary according to sex, menopause, and metabolic

diseases (19-20). The conventional delta value ($\delta\ ^{65}Cu$) is used to report the Cu isotope abundances and corresponds to the relative deviation of $^{65}Cu/^{63}Cu$ ratios in the measured samples from its value in reference material NIST SRM 976. Some studies observed variations in $\delta^{65}Cu$ and indicated an altered metabolism of cancer cells compared to normal cells (12,21). The differences (corresponding to the isotopic fractionation) are still poorly understood. Several steps such as Cu reduction, Cu transport across the membrane or Cu binding to organic ligands have the potential to generate fractionation and different metabolic processes such as hypoxia and angiogenesis (22). The *ab initio* calculations can predict quantitively the variation of Cu isotope abundance, and can provide an overview into a biological process in biological reactions: in tumor, high $\delta^{65}Cu$ values represent a reserve of the circulating ^{63}Cu isotope, due to absorption by tumor cells, conversely low values indicating an enrichment of the lighter ^{63}Cu isotope in the serum (12,21). Recent studies on patients with different types of cancer demonstrate that Cu present in the blood is enriched in light isotopes relative to healthy controls (12,21,23). Consequently, Cu isotopes have strong potential to constitute meaningful markers of cancer detection. For the moment, this type of studies was only applied for breast, ovarian, and colorectal cancer (12,21,23). In this study, its suitability for thyroid cancer is tested by comparing the abundance of ^{63}Cu and ^{65}Cu in plasma and tissue biopsies of thyroid cancer patients, along with an assessment of total plasma Cu and Zn concentrations. Our results support the notion that Cu isotopes and total trace elements may support early thyroid cancer detection and diagnosis.

Materials and Methods

Blood and Tissues Samples

The recruitment of patients was done at University Hospital Centers of TLEMCEN and ORAN (Western Algeria) from June 2018 to May 2019. Forty-six patients with PTC and fifty control subjects were randomly and consecutively selected for the study without applying specific exclusion criteria. All participants had been informed of the purpose of this study, their informed consent had been requested and signed in advance, and people refusing to participate were excluded from the study.

The blood samples, before ablation, of the participants were collected using a venipuncture into 4 mL heparinized tubes and centrifuged at 1100x g (relative centrifugal force) for 10 min using a Sigma 2-16P centrifuge, the plasma was recovered in aliquots of 500 µl. A total number

of 11 investigated tissues with thyroid cancer were collected after surgery (thyroidectomy). A diagnosis of thyroid cancer disease was confirmed by an independent pathologist after postoperative pathohistological analysis of thyroid tissues. Plasma of the participants and thyroid tissues samples were stored and kept on −80 °C freezer until further manipulation.

A questionnaire was filled, via a face-to-face interview, with every participant considering the anthropometric and socio-cultural parameters. Patients presenting other types of malignancies, chronic diseases, or taking a chronic drug therapy were excluded. This study was conducted in accordance with Algerian law (25/2006, resolution N◦. 387) and approved by the Scientific Ethics Deontology Committee of Tlemcen University.

Copper and Zinc Status Assessment

Total plasma Cu and Zn concentrations were determined by total reflection X-ray fluorescence (TXRF) using a benchtop analyzer (S2 Picofox, Bruker nano GmbH, Berlin, Germany), essentially as described (24-25). All samples were supplemented with a gallium standard for calibration. Aliquots were applied to cleaned and polished quartz glass slides and dried, before being analyzed by X-ray fluorescence. A seronorm serum standard (Sero AS, Billingstad, Norway) served as control and was included in all assay runs. The concentrations determined for total Cu and Zn were within the specified range of the standard, and the inter-assay coefficient of variation (CV) was below 15% during the analysis.

Copper Isotope Assessment

Plasma (200 µl) and thyroid tumor biopsies (200 µg) were mineralized on a hot plate in a mixture of nitric acid and hydrogen peroxide. Cu was isolated from the other elements using quartz columns following the protocol described in Albarede *et al.,* (2011) (26-27). The δ^{65}Cu were determined using a Nu Plasma MC-ICP-MS (Nu Instruments, Wrexham, UK) of ENS-Lyon. As MC-ICP-MS suffers from a stable bias, a correction was systematically applied for each isotopic ratio using a constant and known standard of Zn which was added for each sample and standard. Typical external reproducibility on δ^{65}Cu determined from multiple replicates of samples is ~ 0.05‰ for each session. The conventional delta values, δ^{65}Cu, measured for each sample is defined by the formula below:

$$\delta^{65}\mathrm{Cu} = \left[\frac{(^{65}\mathrm{Cu}/^{63}\mathrm{Cu})\ \mathrm{sample}}{(^{65}\mathrm{Cu}/^{63}\mathrm{Cu})\ \mathrm{IStd}} - 1\right] \times 10\,000$$

Procedural blanks were below 0.06 ng on average and can be neglected regarding the quantities of Cu isolated.

Statistical Analysis

Normality of the data was tested using Kolmogorov–Smirnov test. All analysis (Chi-square test, two-samples *t*-test, Mann–Whitney U, Kruskal–Wallis, Spearman correlation, linear regression, and Receiver Operating Characteristics "ROC") were performed using IBM SPSS Statistic software version 23 (IBM Corporation, USA). The results of continuous variables are provided in mean (95% K & Sr peaks CI) and median (IQR) and of categorical variables in percentages (%). When the P-value was < 0.05 the result was considered as statistically significant, and the degree of the differences are marked as: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****). All figures were created with GraphPad Prism 9.0.1.

Results

The study population included 96 individuals, 46 cases with PTC and 50 healthy controls from western Algeria. This population was predominantly female with a ratio of 9:1, which reflects the difference between the sexes in the prevalence of thyroid cancer (28). The average age of the group was 42 ± 13 years. The characteristics of the study population are described in Table 1. No significant differences have been observed between cases and controls in the anthropometric parameters. The thyroid parameters were compared with international standards. A value was considered as abnormal when it was outside the standard range: Thyroid Stimulating Hormone (TSH) $0.3 < TSH < 5.0$ μIU/mL, anti-thyroid peroxidase antibodies (TPO-ab) < 34 UI/mL and thyroglobulin (TG) levels $3.0 < TG < 40$ ng/mL. We observed that thyroid biomarkers are highly perturbed in the individuals with thyroid cancer compared to the international standard.

Table 1. Characteristics of the study population.

Variables	PTC patients	Healthy Controls	P-value
Number of participants	46	50	
Number of biopsy tissues	11	0	
Sex (%)			0.19
Women	91%	85%	
Men	09%	15%	
Age (years)			0.27
median (IQR)	39 (29, 50)	42 (36, 48)	
mean (95% CI)	40 (36, 49)	43 (39, 46)	
			0.19
>50 years (%)	28 %	20 %	
<50 years (%)	72 %	80 %	
BMI (kg/m²)			0.09
median (IQR)	27.8 (23.3, 32.2)	25.9 (23.2, 30.5)	
mean (95% CI)	28.3 (26.6, 30.0)	26.4 (25.2, 27.7)	
Smoking (%)			0,27
Yes	09%	14%	
No	91%	86%	
TSH (µIU/mL)		/	
median (IQR)	92.4 (70.9, 101.0)		
mean (95% CI)	82.9 (75.6, 90.0)		
TPO-ab (Ul/mL)		/	
median (IQR)	16.4 (14.7, 21.0)		
mean (95% CI)	38.7 (15.5, 21.9)		
TG (ng/mL)		/	
median (IQR)	11.9 (4.7, 87.9)		
mean (95% CI)	109.5 (32.8, 186.24)		

BMI: Body Mass Index; TSH: Thyroid Stimulating Hormone; TPO-ab/ anti-Thyroid Peroxidase antibodies; TG: Thyroglobulin.

The trace element status of Cu and Zn as well as the Cu/Zn ratio were compared between PTC patients and healthy controls (Figure 1). Significant higher differences were observed in plasma of Cu levels and Cu/Zn ratio between cases and controls ($P < 0.0001$). The average Cu levels was 1346.1 ± 328.3 µg/L in PTC patients and 1060.5 ± 216.1 µg/L in controls. Conversely, significant lower Zn plasma levels were observed ($P < 0.05$) between the two groups, with average 942.1 ± 205.2 µg/L and 1027.9 ± 151.4 µg/L in PTC and healthy subjects, respectively.

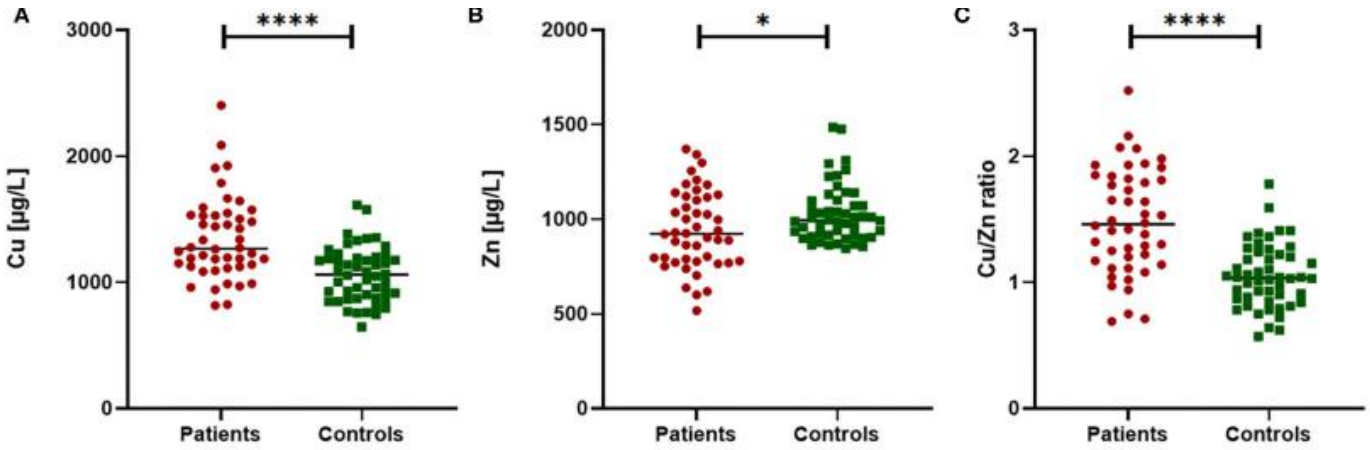

Figure 1. Comparison of Cu, Zn and Cu/Zn ratio in PTC patients versus healthy controls. Significant differences were observed in plasma Cu concentration (A), Zn concentration (B) and in the Cu/Zn ratio (C). Mann Whitney test for non-normal distribution or T test for variables normally distributed; $p < 0.05$ (*), and $p < 0.0001$ (****).

We also evaluated, using Spearman's correlation test, the association between trace metals (Cu, Zn) and thyroid parameters (TSH, TPO-ab, TG). However, no significant associations were observed in PTC patients ($P > 0.05$).

Copper and Zn concentrations were classified into 3 classes according to international reference values (Table 2). The Cu value (median 1060 µg/L) of our control group was close to the corresponding reference ranges from other countries, such as Germany (1020 µg/L, with a range of 804–1620 µg/L) (29). In comparison to the healthy controls, more than 16% of patients with PTC had elevated Cu concentrations. Cu levels in the majority of the studied population was within the reference range. In our patients, Zn concentrations were lower than in our controls, but most of the values were still within the reference range [785-1046] µg/L (28,30). However, the distribution of Cu and Zn concentrations in the classes of the two groups remained significantly different ($P < 0.0001$).

Table 2. Prevalence of elevated Cu and deficient Zn concentrations in PTC patients and healthy controls in comparison to international reference ranges.

Reference values	PTC patients	Halthy Controls	P value	Reference
Cu [µg/L]			0.0001	(29)
n % (Cu ≤ 804 µg/L)	0%	14%		
n % (804 < Cu < 1620 µg/L)	84%16%	86%		
n % (Cu ≥ 1620 µg/L)		0%		
Zn [µg/L]			0.0001	(30)
n % (Zn ≤ 785 µg/L)	26%	0%		
n % (785 < Zn < 1046 µg/L)	46%	68%		
n % (Zn ≥ 1046 µg/L)	28%	32%		

SD: standard deviation

The correlation between Cu and Cu/Zn ratio in the PTC patients (in red, in Figure 2) and in the healthy controls (in green, in Figure 2) was studied. They were widely used as an indicator in breast cancer (12). In the control group, we observed a clear correlation reflecting a strict regulation of Zn levels in the body (x/y = Zn) compared to the cases (p = 0.001). The plasma of PTC showed a high degree of deregulation in Cu and Zn.

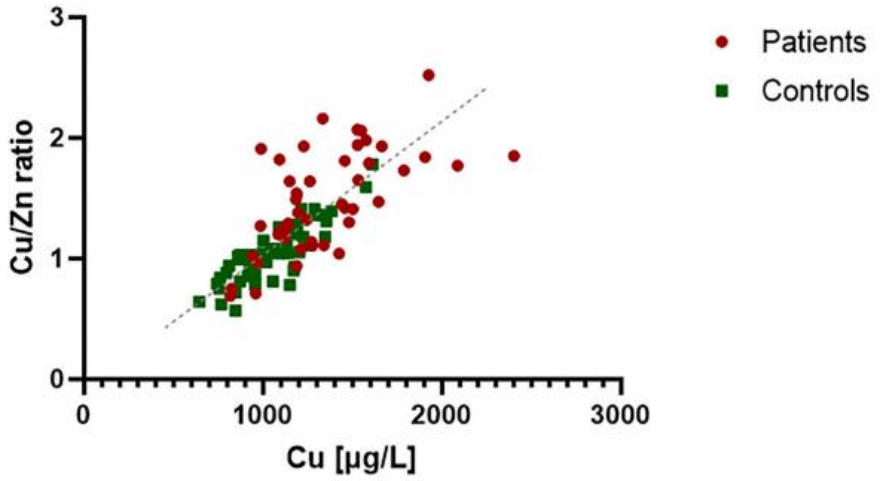

Figure 2. Correlation analysis between Cu and Cu/Zn ratio in plasma of PTC patients and healthy controls. The control group expressed a strong correlation ($R^2 = 0.85$), which reflect Zn regulation in the body (x/y = Zn). Zn concentration in PTC plasma seems to be less regulated and variable compared to controls ($R^2 = 0.39$).

We treated each δ^{65}Cu plasma measurement for each patient, tissue, and control as an independent measurement (Figure 3). PTC plasma samples had a Cu isotope ratio ranking from -1.38 to -0.56‰ (median -0.86‰, mean -0.90 ± 0.24‰) and remained significantly lower (p <

0.0001) compared to the healthy controls (between -0.2 to -0.9 ‰, median -0.60‰, mean -0.61 ± 0.21‰).

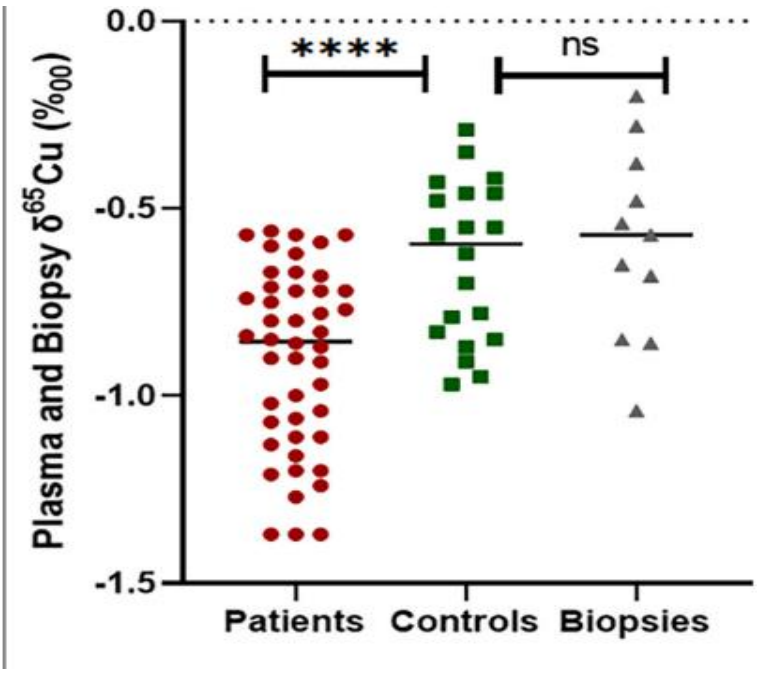

Figure 3. Comparison of $\delta^{65}Cu$ values between healthy controls plasma and thyroid cancer patients' plasma and biopsy. **** = $p \leq 0.0001$.

Nevertheless, there was no significant difference between the $\delta^{65}Cu$ measured in controls and the $\delta^{65}Cu$ measured in the thyroid tissue biopsies ($p = 0.163$). We then compared the mean $\delta^{65}Cu$ of patients and thyroid biopsies and found that there was a significant difference as a mirror effect ($p < 0.05$). We also compared plasma $\delta^{65}Cu$ values with age, sex and thyroid parameters but no association was found in PTC patients ($p > 0.05$).

Finally, we analyzed Cu/Zn ratio and $\delta^{65}Cu$ data with Receiver Operating Characteristics (ROC) curve analysis (Figure 4). The ROC curve allows us to evaluate the probability that a result obtained is a true positive or a false positive, and the area under the curve (AUC) indicates the probability to get a positive value in front of a negative one. AUC vary between 0.5 (pure chance) and 1.0 (totally reliable). In our case, we evaluated the use of Cu/Zn ratio and $\delta^{65}Cu$ as novel biomarkers in diagnostic tool for thyroid cancer. The results indicate an acceptable degree of accurate diagnosis and prediction, with an AUC = 0.81 for Cu/Zn axis ($p < 0.0001$) and an AUC=0.78 for $\delta^{65}Cu$ of axis ($P < 0.0001$), respectively, to discriminate between controls and cancer patients (Figure 4).

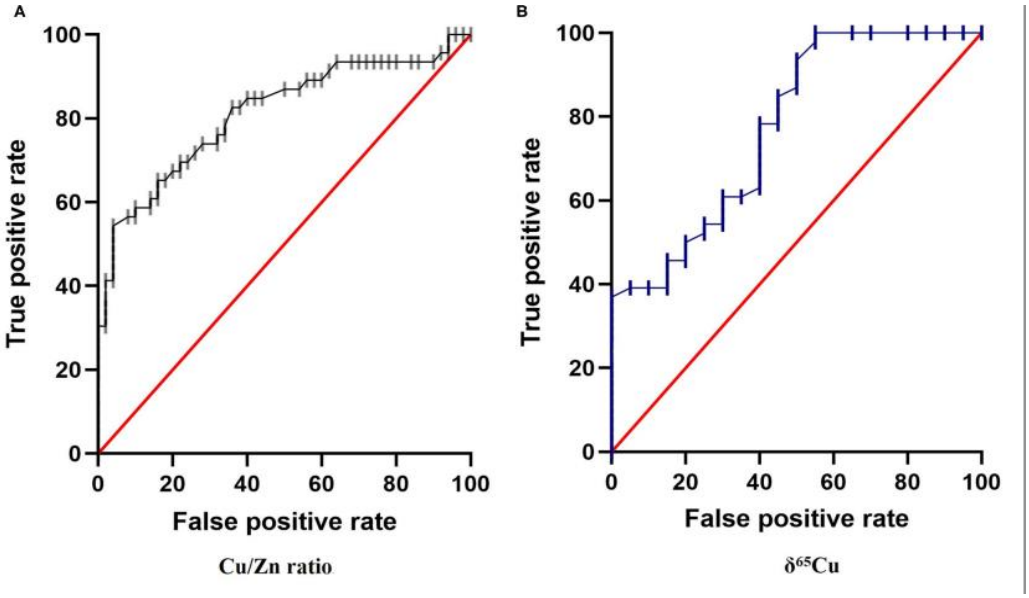

Figure 4. Receiver Operating Characteristics (ROC) curve for: A) Cu/Zn Ratio and B) δ^{65}Cu in the plasma of papillary thyroid carcinoma. We place the True Positive decision on the ordinate axis and False Positive rate on the abscissa to form the ROC diagram. The area under the ROC curve (AUC) obtained for the present data were 0.81 and 0.78, respectively ($p < 0.001$) supports the hypothesis that Cu/Zn ration and δ^{65}Cu have a strong potential to be a novels biomarker in diagnosis for thyroid cancer.

Discussion

In this study, we present a comparison of trace elements in Algerian healthy subjects and patients with a diagnosis of PTC. Our results indicate that both total plasma Cu and Zn concentrations with the Cu/Zn ratio as well as the δ^{65}Cu marker yield fast, low-cost and meaningful insights and may support a thyroid cancer diagnosis. However, the nature of our study is explorative, and a single sample per patient only was available for analysis. For these reasons, the data need to be interpreted with the due caution until the results have been verified in larger study cohorts. Nevertheless, the measurements were conducted by scientists blinded to the clinical phenotypes, and the study groups were of sufficient size to support further research on these novel biomarkers of thyroid cancer.

4.1 Metals Concentrations and Ratios

We observed that blood Cu concentrations were significantly higher and Zn concentrations significantly lower in thyroid cancer patients compared to healthy controls. Cu and Zn are important for thyroid gland function, in metabolism and in synthesis of thyroid hormones (31). Such changes in concentration of trace metal can affect the balance between oxidant and antioxidant in the body and thus the endocrine system and can result in different thyroid diseases such as hyperthyroidism, hypothyroidism, Hashimoto's disease, and cancer (31-32).

First, Copper interact with tyrosine amino acid metabolism which is necessary for production of thyroid hormones (33). Cu regulates the excessive absorption of thyroxine (T4) by monitoring the calcium levels and can eliminate free radicals and reduce some damage caused in the cells during the synthesis of thyroid hormones (34). It is reported in some studies, that high levels of Cu may cause an oxidative stress that can alter normal thyroid function (31,33,35,36). Some studies reported high Cu blood levels in cancer, and they correlated with grade and therapies response (10,37). Ishida *et al.,* (2011) (11) using a mouse model genetically modified of human cervical carcinoma reported a high expression of Cu transporter Ctr1 in the cancer cells, and thus tumors might have a higher dependence for Cu. Cu has the particularity to act as antioxidant and pro-oxidant (34,38). Cu is considered as switch activating the angiogenesis process of tumoral cells. Abnormally high concentration of Cu can facilitate the proliferation of tumors by damaging DNA with toxic free hydroxyl radicals. In addition, high serum Cu concentrations are correlated with patients having different types of cancer and in most cancers, it is considered as hallmark of cancer cells (39). But for the moment, the effect of Cu mechanism in angiogenesis is still unclear (10). Cu is actively involved in the process of tumor progression, particularly during angiogenesis and metastasis (40). Clinical studies in rodent models show that Cu supplementation significantly increases tumor growth in breast, pancreatic and lung cancer (41). Cu regulates the expression and the secretion of certain angiogenic factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF) and interleukin-1alpha (IL-1α). Therefore, Cu indirectly participates in angiogenesis and tumor nutrition (42).

In addition, during the inflammatory response, Cu is mobilized and accumulates in inflamed tissues, contributing to the defense against various infections. However, chronic inflammation is a risk factor in the development of cancer. Certain inflammatory cytokines, such as IL-6 and IL-17, are diagnostic markers for early phase of inflammation and they interact in the promotion of tumors and cancer progression and they act in the regulation of Cu uptake mechanisms, thus contributing to the accumulation of Cu in cancer cells (24,43).

Secondly, Zinc is a micronutrient required for the biosynthesis of thyroid hormones, and Zn affects the activity of 5′-deiodinase for the conversion of T4 to triiodothyronine (T3) (44-45). In addition, Zn is necessary for the synthesis of TSH from pituitary and TRH from hypothalamus (46-47). Kralik *et al.,* (1996) (48) showed Zn to be required for the correct metabolism of thyroid hormone, and Zn deficiency might have a negative effect on the activity of normal thyroid. Meta-analysis conducted by Gumulec *et al.,* (2014) (49) provided evidence

for Zn level to alter cancer-specific tissue, with low Zn concentration associated with most tumors and in particular with thyroid carcinoma.

Few studies reported a lower levels of Zn and relatively higher levels of Cu in thyroid cancer patients (46). The study of Lin *et al.,* (2006) (50) found significantly lower concentration of Zn (0.73 vs. 1.01 mg/L) with significantly higher levels of Cu (1.16 mg/L vs. 0.92 mg/L) in patients with hepatocellular carcinoma compared to controls. Baltaci *et al.,* (2017) (46) found a negative correlation between lower levels of Zn and higher levels of Cu in the same patients. There is an antagonistic relationship between Cu and Zn; a diet rich in zinc (overdose) may lead to disruption of Cu absorption, and conversely a high dose of Cu may decrease Zn absorption (51-52). This could explain the results obtained in our patients showing a perturbation in their Cu/Zn ratio. Alternatively, the altered Cu/Zn ratio may reflect the ongoing inflammation during thyroid cancer, which may set in early. In general, Cu is upregulated as positive acute phase reactant, whereas Zn tends to decline in response to inflammation. This inverse response to cytokines may contribute and synergize with the antagonistic changes of trace element absorption mentioned above.

Furthermore, during the synthesis of thyroid hormones, free radicals (ROS) are produced (53). It has been reported that SOD enzymes are involved in the thyroid gland functioning (54). Through cellular protection mechanisms, SOD enzymes eliminate excess ROS and prevent tumor progression through cellular protection mechanisms. On the other hand, the disturbance of the Cu-Zn homeostasis and an overproduction of ROS can lead to DNA damage, protein modification and eventually to the development of cancer (10). This may also explain the Cu/Zn imbalance observed in our thyroid cancer patient cohorts.

We found higher Cu/Zn ratio in PTC patients compared to healthy controls, this result was similar to previous studies conducted on breast, prostate, colorectal, and uterine cervical cancer (55-56). The Cu/Zn ratio is considered as a potential biomarker for cancer detection (24). Our results support its potential value for PTC detection, thereby expanding its potential use as screening and diagnosis tool to the thyroid gland.

Some studies reported the influence of thyroid hormones in Cu and Zn metabolism (36,57). It was observed that thyroid hormones can influence the expression of Cu transport proteins (ATP7A, ATP7B) and regulate serum Cu levels by controlling the production of the Cu-transport protein ceruloplasmin (58). In addition, it was found a significant correlation between

TSH and Zn levels in hyperthyroidism (59). However, in our study we found no significant association between trace metals and thyroid parameters.

4.2 Copper Isotopic Composition

With the development of new sensitive techniques such as MC-ICP-MS a search for non-invasive low-cost biomarkers has emerged, aiming at the development of early disease diagnosis. In this study, the δ^{65}Cu marker is used and based on the determination of two stable Cu isotopes concentration ratio (^{65}Cu/ ^{63}Cu). We evaluated the Cu fractionation in blood and in tissues samples of patients with PTC and found significantly lower plasma value of δ^{65}Cu in patients with PTC compared to healthy controls, and in a mirror image, significant higher values of δ^{65}Cu in thyroid tumor tissue. Studies conducted in breast, colorectal and ovarian cancer found similar results, and have shown lighter enrichment in ^{63}Cu isotope in serum and heavier enrichment in the ^{65}Cu isotope in tissue, due to a preferential uptake of the heavy isotope by tumor cells (12,21).

We used ROC curve analysis (Figure 4.B) to evaluate the use of Cu isotope as diagnostic biomarker for thyroid cancer. The ROC curve is a widely used tool in diagnostic medicine. It represents the ability of a test to discriminate between the patient and non-patient population (60). The ROC curve represents on the ordinate the proportion of positive tests among the patient's population (the sensitivity), in our case the δ^{65}Cu values of the thyroid cancer patients, versus the proportion of positive tests among the non-diseased population. The reliability of the results is assessed by the AUC which ranges from 0.5 (pure chance) to 1.0 (totally reliable test). The ROC curve represents all the points calculated for each δ^{65}Cu sample value. In the present case of thyroid cancer diagnosis, the AUC is equal to 0.78 and were similar to that observed in breast cancer (12). The use of δ^{65}Cu as a potential diagnostic tool for thyroid cancer seems very promising.

The isotopic variability can be influenced by different mechanisms such menopause (61-62) or biological sex (20). δ^{65}Cu values seem also to vary according to diet. In fact, Jaouen *et al.,* (2013) (20) demonstrated that Cu isotope composition of healthy Yakut's serum are significantly different (lighter) than observed in reference panel of Japanese and European population and supposed to be related to the isotope composition of the diet. The isotopic fractionation in diet can be generated during the intestinal absorption or excretion of food inducing differences in the isotopic composition of the human body or reflect different biogeochemical origins of the food items. Furthermore, it has been observed that metabolic

reactions can also cause isotopic fractionation. In several diseases, such as cancer, this metabolic change can be measured (63).

As previously highlighted, the cause of isotopic fractionation in the case of cancer is not well understood but it has been suggested by Telouk *et al.,* (2015) (12), that the hypoxic environment in tumor cells would be at the origin of the decrease of δ^{65}Cu in the serum and the enrichment of δ^{65}Cu in the tissues. It has been reported that in solid tumors there is a preferential chelation of the heavy ^{65}Cu isotope in the tumor tissues and the release of the light ^{63}Cu isotope into the blood (12,19). However some studies on hematological cancer found different δ^{65}Cu values in tumor patients versus controls, but at the same time similar isotopic fractionation in plasma and solid tumors, suggesting that the mechanism responsible for this distribution was not related to preferential absorption and release from the tumor but was caused by the proliferation of tumor cells (23). The mechanism of isotopic fractionation is thus not yet fully understood, and further studies in cellular and subcellular range are needed to elucidate this variation.

Conclusion

The assessment of Cu and Zn plasma levels showed higher Cu and lower Zn concentrations in PTC patients compared to healthy controls, yielding a highly significant difference in the plasma Cu/Zn ratio. In addition, the Cu isotopic composition was strongly different in the thyroid cancer, patients as compared to healthy controls. Notably, the δ^{65}Cu values differed in opposite directions in plasma versus the thyroid tumor tissue samples. Our study supports the hypothesis that trace element concentrations and isotopic composition have a high potential to be used as additional biomarkers in the detection of thyroid cancer. Further molecular studies are needed for a better comprehension of the mechanisms involved in these processes.

Conflict of Interest: *The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*

Author Contributions: A.T.G., L.S.K.T., L.C., N.D.M. designed the study; M.B., L.S.K.T., M.D.S, collected the sample and data via face-to-face interviews; L.S.K.T., A.T.G., P.T. performed copper isotope assessment; L.S., Q.S., J.H., did the Cu and Zn measurements; L.S.K.T, H.Y. performed the statistical analyses, L.S.K.T., A.T.G, N.D.M prepared the manuscript, L.C. and, L.S., corrected the final version. All authors have read and agreed to the published version of the manuscript.

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References

1. Curado MP, Edwards B, Shin HR, Storm H, Ferlay J, Heanue M, et al. Cancer Incidence in Five Continents Volume IX. Curado MP, International Agency for Research on Cancer. Lyon Vol. 9 (2008). 837 p.
2. Castro B, Rodrigues E. Molecular biology of papillary thyroid microcarcinomas: What is new? Endocrinol Diabetes E Metab. (2016); 11(2) :287-95. doi: 10.1016/j.rpedm.2016.04.003.
3. Thyroid Cancer — Cancer Stat Facts (2020). https://seer.cancer.gov/statfacts/html/thyro.html [Accessed 15 avr 2021].
4. Barrea L, Gallo M, Ruggeri RM, Giacinto PD, Sesti F, Prinzi N, et al. Nutritional status and follicular-derived thyroid cancer: An update. Crit Rev Food Sci Nutr. (2021); 61(1):25-59. doi: 10.1080/10408398.2020.1714542.
5. International Agency for Research on Cancer. GLOBO-CAN 2018: estimated cancer incidence, mortality and prevalence in Algeria in 2018. IARC 2019. http://globocan.iarc.fr/ [Accessed 15 avr 2021].
6. Benouis A, Bekkouche Z, Merad MS, Loudjedi L, Khelil H, Berber N. Thyroid Cancer in Western Algeria: Histopathological and Epidemiological Study. J Cancer Ther. (2017); 08(07):672-82. doi: 10.4236/jct.2017.87058.
7. Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. The Lancet. (2016); 388(10061):2783-95. doi: 10.1016/S0140-6736(16)30172-6.
8. Kaliszewski K, Diakowska D, Wojtczak B, Rudnicki J. Cancer screening activity results in overdiagnosis and overtreatment of papillary thyroid cancer: A 10-year experience at a single institution. PLOS ONE. (2020); 15(7):e0236257. doi: 10.1371/journal.pone.0236257.
9. Linder M. Biochemistry of Copper. Springer Science & Business Media. (2013); 526 p.
10. Ishida S, Andreux P, Poitry-Yamate C, Auwerx J, Hanahan D. Bioavailable copper modulates oxidative phosphorylation and growth of tumors. Proc Natl Acad Sci. (2013); 110(48):19507-12. doi: 10.1073/pnas.1318431110.
11. Ishida S, McCormick F, Smith-McCune K, Hanahan D. Enhancing Tumor-Specific Uptake of the Anticancer Drug Cisplatin with a Copper Chelator. Cancer Cell. (2010); 17(6):574-83. doi: 10.1016/j.ccr.2010.04.011.
12. Télouk P, Puisieux A, Fujii T, Balter V, Bondanese VP, Morel A-P, et al. Copper isotope effect in serum of cancer patients. A pilot study. Metallomics. (2015); 7(2):299-308. doi: 10.1039/C4MT00269E.
13. Osredkar J, Susta N. Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. J Clin Toxicol. (2011); S3:001. doi:10.4172/2161-0495.S3-00
14. Asaduzzaman K M, Tania M, Zhang D, Chen H. Antioxidant enzymes and cancer. Chin J Cancer Res. (2010); 22(2):87-92. doi: 10.1007/s11670-010-0087-7.
15. Somwar R, Erdjument-Bromage H, Larsson E, Shum D, Lockwood WW, Yang G, et al. Superoxide dismutase 1 (SOD1) is a target for a small molecule identified in a screen for inhibitors of the growth of lung adenocarcinoma cell lines. Proc Natl Acad Sci U S A. (2011); 108(39):16375-80. doi: 10.1073/pnas.1113554108.
16. Yücel I, Arpaci F, Özet A, Döner B, Karayilanoĝlu T, Sayar A, et al. Serum copper and zinc levels and copper/zinc ratio in patients with breast cancer. Biol Trace Elem Res. (1994); 40(1):31-8. doi: 10.1007/BF02916818.

17. Cui Y, Vogt S, Olson N, Glass AG, Rohan TE. Levels of Zinc, Selenium, Calcium, and Iron in Benign Breast Tissue and Risk of Subsequent Breast Cancer. Cancer Epidemiol Biomarkers Prev. (2007); 16(8):1682-5. doi: 10.1158/1055-9965.EPI-07-0187.
18. Bertinato J. Copper: Physiology. In: Encyclopedia of Food and Health. Elsevier. (2016); p. 321-6.
19. Balter V, Nogueira da Costa A, Bondanese VP, Jaouen K, Lamboux A, Sangrajrang S, et al. Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. Proc Natl Acad Sci. (2015); 112(4):982-5. doi: 10.1073/pnas.1415151112.
20. Jaouen K, Gibert M, Lamboux A, Telouk P, Fourel F, Albarède F, et al. Is aging recorded in blood Cu and Zn isotope compositions? Metallomics. (2013); 5(8):1016-24. doi: 10.1039/C3MT00085K.
21. Toubhans B, Gourlan AT, Telouk P, Lutchman-Singh K, Francis LW, Conlan RS, et al. Cu isotope ratios are meaningful in ovarian cancer diagnosis. J Trace Elem Med Biol. (2020); 62:126611. doi: 10.1016/j.jtemb.2020.126611.
22. Bondanese VP, Lamboux A, Simon M, Lafont JE, Albalat E, Pichat S, et al. Hypoxia induces copper stable isotope fractionation in hepatocellular carcinoma, in a HIF-independent manner. Metallomics. (2016); 8(11):1177-84. doi: 10.1039/C6MT00102E.
23. Hastuti AAMB, Costas-Rodríguez M, Matsunaga A, Ichinose T, Hagiwara S, Shimura M, et al. Cu and Zn isotope ratio variations in plasma for survival prediction in hematological malignancy cases. Sci Rep. (2020); 10(1):16389. doi: 10.1038/s41598-020-71764-7.
24. Wisniewska M, Cremer M, Wiehe L, Becker N-P, Rijntjes E, Martitz J, et al. Copper to Zinc Ratio as Disease Biomarker in Neonates with Early-Onset Congenital Infections. Nutrients. (2017); 9(4):343. doi: 10.3390/nu9040343.
25. Stepien M, Hughes DJ, Hybsier S, Bamia C, Tjønneland A, Overvad K, et al. Circulating copper and zinc levels and risk of hepatobiliary cancers in Europeans. Br J Cancer. (2017); 116(5):688-96. doi: 10.1038/bjc.2017.1.
26. Albarède F, Telouk P, Lamboux A, Jaouen K, Balter V. Isotopic evidence of unaccounted for Fe and Cu erythropoietic pathways. Metallomics. (2011); 3(9):926. doi: 10.1039/c1mt00025j.
27. Maréchal CN, Télouk P, Albarède F. Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. Chem Geol. (1999); 156(1-4):251-73. doi: 10.1016/S0009-2541(98)00191-0.
28. Mehl S, Sun Q, Görlich CL, Hackler J, Kopp JF, Renko K, et al. Cross-sectional analysis of trace element status in thyroid disease. J Trace Elem Med Biol. (2020); 58:126430. doi: 10.1016/j.jtemb.2019.126430.
29. Heitland P, Köster HD. Biomonitoring of 37 trace elements in blood samples from inhabitants of northern Germany by ICP–MS. J Trace Elem Med Biol. (2006); 20(4):253-62. doi: 10.1016/j.jtemb.2006.08.001.
30. Gammoh N, Rink L. Zinc in Infection and Inflammation. Nutrients. (2017); 9(6):624. 10.3390/nu9060624.
31. Rezaei M, Javadmoosavi SY, Mansouri B, Azadi NA, Mehrpour O, Nakhaee S. Thyroid dysfunction: how concentration of toxic and essential elements contribute to risk of hypothyroidism, hyperthyroidism, and thyroid cancer. Environ Sci Pollut Res. (2019); 26(35):35787-96. doi: 10.1007/s11356-019-06632-7.
32. Liu Y, Liu S, Mao J, Piao S, Qin J, Peng S, et al. Serum Trace Elements Profile in Graves' Disease Patients with or without Orbitopathy in Northeast China. BioMed Res Int. (2018); 2018:1-8. doi: 10.1155/2018/3029379.

33. Kazi TG, Kandhro GA, Afridi HI, Kazi N, Baig JA, Arain MB, et al. Interaction of Copper with Iron, Iodine, and Thyroid Hormone Status in Goitrous Patients. Biol Trace Elem Res. (2010); 134(3):265-79. doi: 10.1007/s12011-009-8478-7.
34. Shen F, Cai W-S, Li J-L, Feng Z, Cao J, Xu B. The Association Between Serum Levels of Selenium, Copper, and Magnesium with Thyroid Cancer: a Meta-analysis. Biol Trace Elem Res. (2015); 167(2):225-35. doi; 10.1007/s12011-015-0304-9.
35. Giray B, Arnaud J, Sayek İ, Favier A, Hıncal F. Trace elements status in multinodular goiter. J Trace Elem Med Biol. (2010); 24(2):106-10. doi: 10.1016/j.jtemb.2009.11.003.
36. Hybsier S, Höfig C, Mittag J, Brabant G, Schomburg L. Control of serum copper (Cu) and selenium (Se) status by thyroid hormones. Exp Clin Endocrinol Diabetes. (2015); 122(03):s-0035-1547752. doi: 10.1055/s-0035-1547752.
37. Linder MC, Moor JR, Wright K. Ceruloplasmin assays in diagnosis and treatment of human lung, breast, and gastrointestinal cancers. J Natl Cancer Inst. (1981); 67(2):263-75.
38. Theophanides T, Anastassopoulou J. Copper and carcinogenesis. Crit Rev Oncol Hematol. (2002); 42(1):57-64. doi: 10.1016/S1040-8428(02)00007-0.
39. Brem N. Angiogenesis and Cancer Control: From Concept to Therapeutic Trial. Cancer Control J Moffitt Cancer Cent. (1999); 6(5):436-58.
40. Brem SS, Zagzag D, Tsanaclis AM, Gately S, Elkouby MP, and BrienBrem SE. Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by penicillamine and the depletion of copper, an angiogenic cofactor. (1990); Am J Pathol 137(5):1121–1142.
41. Skrajnowska D, Bobrowska-Korczak B, Tokarz A, Bialek S, Jezierska E, Makowska J. Copper and resveratrol attenuates serum catalase, glutathione peroxidase, and element values in rats with DMBA-induced mammary carcinogenesis. Biol Trace Elem Res. (2013); 156: 271-8.
42. Denoyer D, Masaldan S, La Fontaine S, Cater MA. Targeting copper in cancer therapy: 'Copper That Cancer'. Metallomics. (2015); 7(11):1459-76.
43. Liao Y, Zhao J, Bulek K, Tang F, Chen X, Cai G, et al. Inflammation mobilizes copper metabolism to promote colon tumorigenesis via an IL-17-STEAP4-XIAP axis. Nat Commun. (2020);11(1):900. doi: 10.1038/s41467-020-14698-y.
44. Barnett JB, Dao MC, Hamer DH, Kandel R, Brandeis G, Wu D, et al. Effect of zinc supplementation on serum zinc concentration and T cell proliferation in nursing home elderly: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr. (2016); 103(3):942-51. doi: 10.3945/ajcn.115.115188.
45. Danforth E, Burger AG. The Impact of Nutrition on Thyroid Hormone Physiology and Action. Annu Rev Nutr. (1989); 9(1):201-27.
46. Baltaci AK, Dundar TK, Aksoy F, Mogulkoc R. Changes in the Serum Levels of Trace Elements Before and After the Operation in Thyroid Cancer Patients. Biol Trace Elem Res. (2017); 175(1):57-64. doi: 10.1007/s12011-016-0768-2.
47. Suzuki S, Kasai K, Yamauchi K. Characterization of little skate (Leucoraja erinacea) recombinant transthyretin: Zinc-dependent 3,3′,5-triiodo-l-thyronine binding. Gen Comp Endocrinol. (2015); 217-218:43-53. doi: 10.1016/j.ygcen.2015.04.006.
48. Kralik A, Eder K, Kirchgessner M. Influence of Zinc and Selenium Deficiency on Parameters Relating to Thyroid Hormone Metabolism. Horm Metab Res. (1996); 28(05):223-6. doi: 10.1055/s-2007-979169.
49. Gumulec J, Masarik M, Adam V, Eckschlager T, Provaznik I, Kizek R. Serum and Tissue Zinc in Epithelial Malignancies: A Meta-Analysis. PLoS ONE. (2014); 9(6):e99790. doi: 10.1371/journal.pone.0099790.

50. Lin CC, Huang JF, Tsai LY, Huang YL. Selenium, Iron, Copper, and Zinc Levels and Copper-to-Zinc Ratios in Serum of Patients at Different Stages of Viral Hepatic Diseases. Biol Trace Elem Res. (2006); 109(1):015-24. doi: 10.1385/BTER:109:1:015.
51. Kaslow JE. Copper/Zinc Imbalance. Medical Board of California. (2011).
52. Balay KS, Hawthorne KM, Hicks PD, Chen Z, Griffin IJ, Abrams SA. Low Zinc Status and Absorption Exist in Infants with Jejunostomies or Ileostomies Which Persists after Intestinal Repair. Nutrients. (2012); 4(9):1273-81. doi: 10.3390/nu4091273.
53. Sugawara M. Reactive Oxygen Species and Thyroid Diseases. Sys Biol of Free Rad and Anti. (2014); 3521–3538. doi:10.1007/978-3-642-30018-9_150.
54. Iwase K, Nagasaka A, Kato K, Ohtani S, Tsujimura T, Inagaki A, et al. Localization of Cu/Zn and Mn superoxide dismutase in various thyroid disorders. Acta Endocrinol (Copenh). (1993); 129(6):573-8. doi: 10.1530/acta.0.1290573.
55. Adaramoye OA, Akinloye O, Olatunji IK. Trace elements and vitamin E status in Nigerian patients with prostate cancer. Afr Health Sci. (2010); 10(1):2-8.
56. Cunzhi H, Jiexian J, Xianwen Z, Jingang G, Shumin Z, Lili D. Serum and Tissue Levels of Six Trace Elements and Copper/Zinc Ratio in Patients with Cervical Cancer and Uterine Myoma. Biol Trace Elem Res. (2003); 94(2):113-22. doi: 10.1385/BTER:94:2:113.
57. Hanif S, Ilyas A, Shah MH. Statistical Evaluation of Trace Metals, TSH and T4 in Blood Serum of Thyroid Disease Patients in Comparison with Controls. Biol Trace Elem Res. (2018); 183(1):58-70. doi: 10.1007/s12011-017-1137-5.
58. Mittag J, Behrends T, Nordström K, Anselmo J, Vennström B, Schomburg L. Serum copper as a novel biomarker for resistance to thyroid hormone. Biochem J. (2012); 443(1):103-9. doi: 10.1042/BJ20111817.
59. Sinha S, Kar K, Dasgupta A, Basu S, Sen S. Correlation of Serum zinc with TSH in hyperthyroidism. Asian J Med Sci. (2015); 7(1):66-9. doi: 10.3126/ajms.v7i1.12895.
60. Delacour H, Servonnet A, Perrot A, Vigezzi JF, Ramirez JM. La courbe ROC (receiver operating characteristic): principes et principales applications en biologie clinique. Ann Biol Clin. (2005); 63:10.
61. Van Heghe L, Deltombe O, Delanghe J, Depypere H, Vanhaecke F. The influence of menstrual blood loss and age on the isotopic composition of Cu, Fe and Zn in human whole blood. J Anal Spectrom. (2014); 29(3):478-82. doi: 10.1039/C3JA50269D.
62. Jaouen K, Balter V. Menopause effect on blood Fe and Cu isotope compositions: Menopause Effect on Blood Fe and Cu Isotopes. Am J Phys Anthropol. (2014); 153(2):280-5. doi: 10.1002/ajpa.22430.
63. Jaouen K, Pons M-L. Potential of non-traditional isotope studies for bioarchaeology. Archaeol Anthropol Sci. (2017); 9(7):1389-404. doi: 10.1007/s12520-016-0426-9.

Chapter IV: Relationship between mineral elements and papillary thyroid carcinoma

In this chapter we presented the combined results obtained in the previous chapters II and III, with a deeper statistical study. We investigated the relationship between the different micronutrients and macronutrients: Se, Cu, Zn, Fe and Ca and their direct or indirect implications in thyroid cancer.

In this project, we conducted a cross sectional study, with 102 participants, including 47 patients with PTC and 55 healthy controls from western Algeria. Characteristics of the participants are presented in chapter II and III. The general population was predominantly female, with average age of patients 41 ± 14 years and controls 44 ± 13 years. The patients presented an overweight profile (28.31 ± 5.85 kg/m^2) compared to the controls (25.93 ± 4.17 kg/m^2) with a $P < 0.05$. There was no significant difference between cases and controls in marital status and smoking habit, however the distribution of education levels was significantly different ($P < 0.01$).

First, we investigated the association between anthropometric and socioeconomic status with the different micro and macronutrients. We studied the distribution of Se, Cu, Zn, Fe and Ca concentrations among the four international Body Mass Index (BMI) classes [Underweight (BMI < 18.5) ; Ideal weight (BMI 18.5-24.9) ; Overweight (BMI 25.0-29.9) and Obese (BMI ≥ 30.0)]. The results are grouped in Figure 1. Regarding Cu, we found a significant difference between PTC patients and controls in its distribution in all the 4 classes. For Se and Ca, the differences observed are in the classes: underweight, ideal weight and overweight ($P < 0.05$). Fe showed significant differences only in the first two classes and finally for Zn only the underweight class showed a significant difference between the two groups.

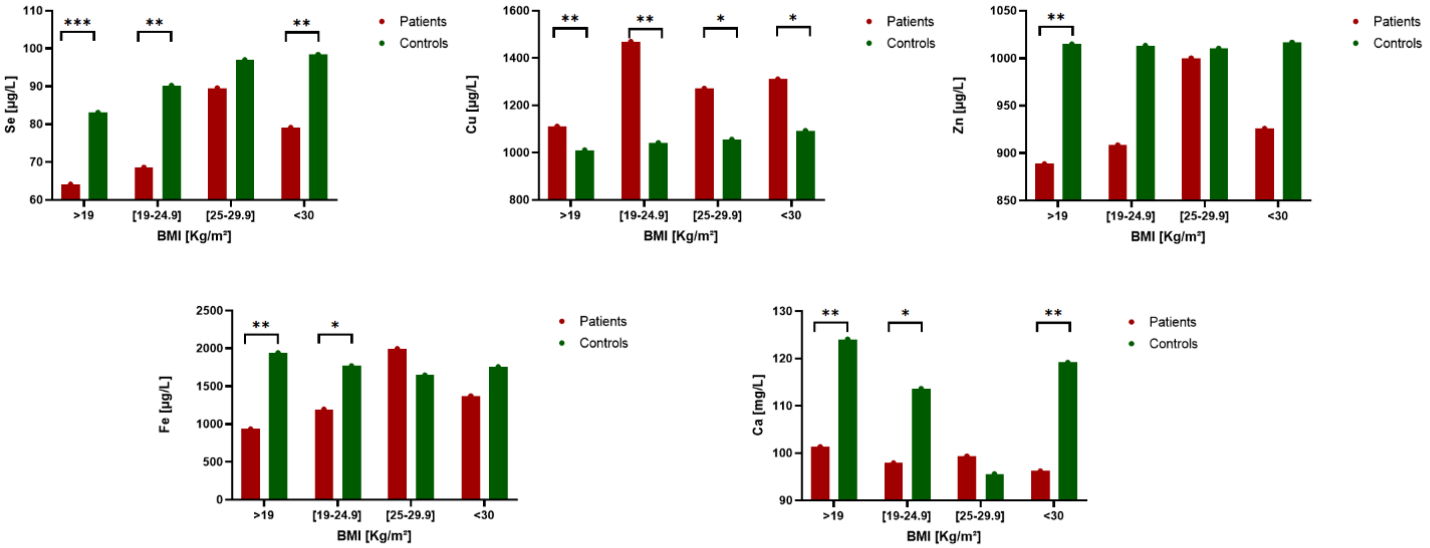

Figure 1. Plasma trace elements distribution in the four international BMI classes. Underweight (BMI < 18.5) ; Ideal weight (BMI 18.5-24.9) ; Overweight (BMI 25.0-29.9) and Obese (BMI ≥ 30.0). T test was used for variables with normal distribution and Mann Whitney test for non-normal distribution $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

Figure 2 illustrates the results of trace element in age distribution. No significant difference was observed between patients and controls. We then separated the patients and controls according to age (< 55 and > 55 years), which represents the average age of menopause, we found significant differences for Se, Cu, Zn and Ca between cases and controls in the < 55 years groups (Figure 2).

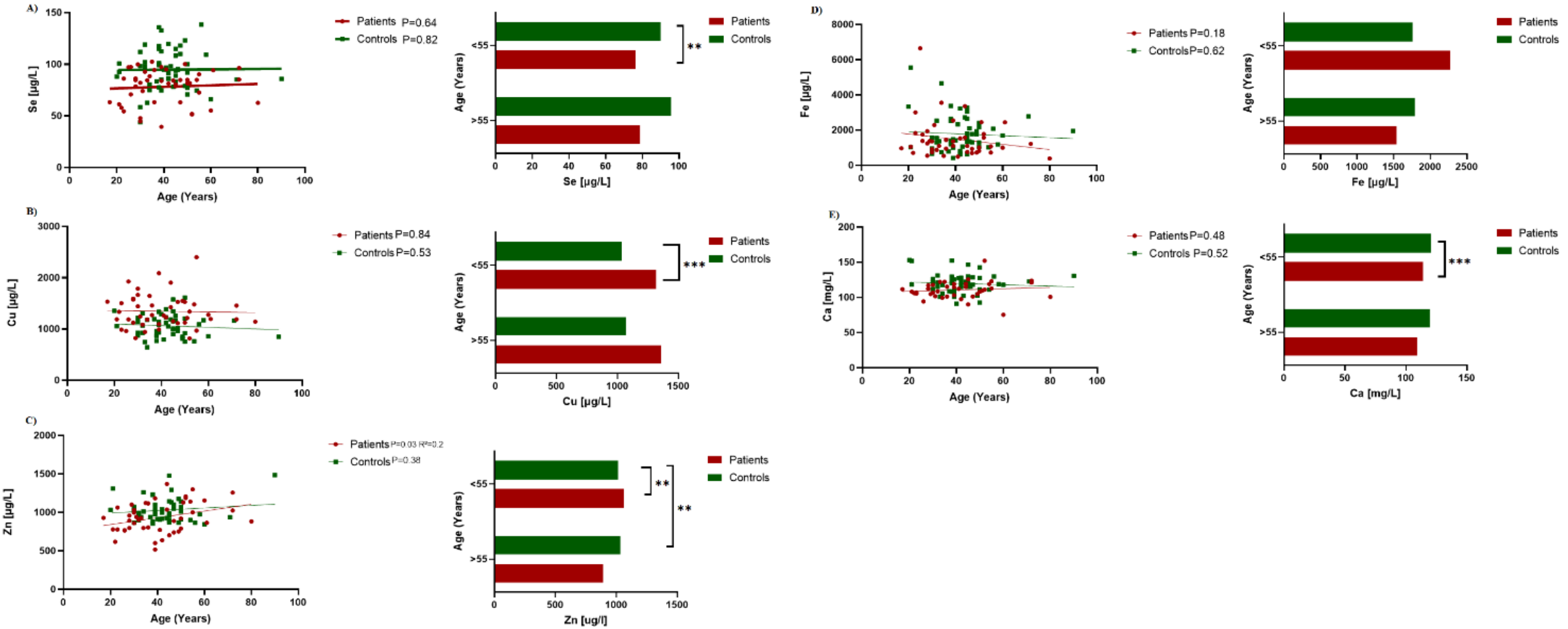

Figure 2. Plasma trace element concentrations in general age and in two age groups (< 55 and > 55 years old) of PTC patients and controls. Illustrations represent the distribution of plasma Se (A), Cu (B), Zn (C), Fe (D) and Ca (E) concentrations. T test was used for variables with normal distribution and Mann Whitney test for non-normal distribution $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

We also examined the correlation between trace elements: Se, Cu, Zn, Fe and Ca in general population, including patients and controls, (Figure 3). We found an interesting positive and significant correlation between Se-Cu, Se-Zn, Se-Ca, Zn-Fe, Zn-Ca and Fe-Ca.

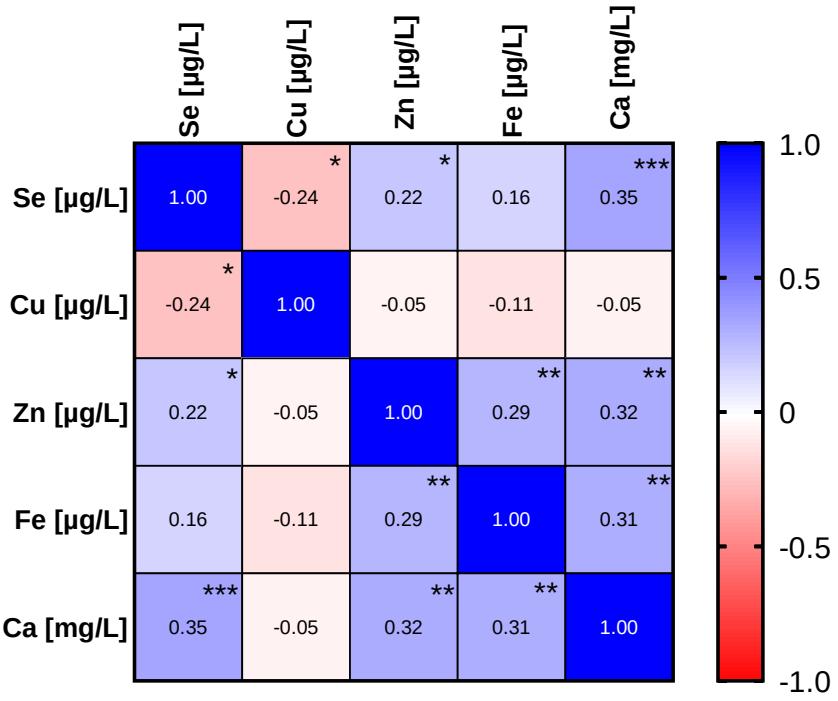

Figure 3. Coefficients correlation between trace elements (Se, Cu, Zn, Fe and Ca) in general population. P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***).

We explored ratios of any two trace element levels (Figure 4). Compared with controls, significantly increased Cu/Se, Cu/Ca, Cu/Zn, Cu/Fe and Ca/Se ratios were detected in patients, conversely, Fe/Se and Fe/Ca were decreased.

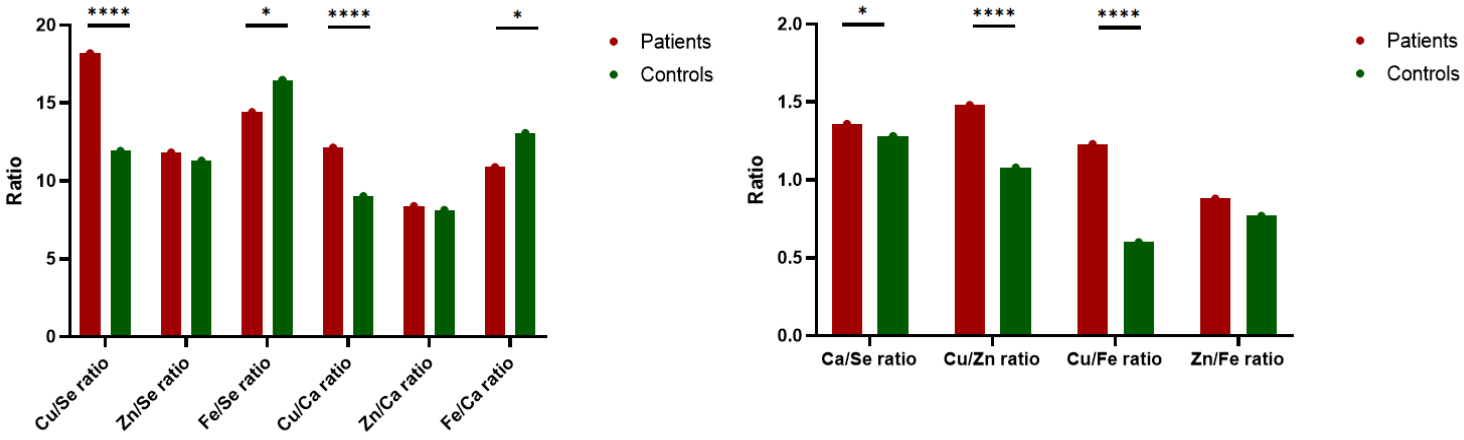

Figure 4. Comparison of trace elements ratios in PTC patients versus healthy controls. Significant lower concentration was observed between patients and controls in Cu/Se, Cu/Ca, Cu/Zn, Cu/Fe and Ca/Se ratios and significant higher for Fe/Se and Fe/Ca. T test was used for variables with normal distribution and Mann Whitney test for non-normal distribution; p < 0.05 (*), p < 0.001 (***) and p < 0.0001 (****).

Finally, we studied the relation between $\delta^{65}Cu$ variation and socioeconomics parameters in PTC patients and controls. Figure 5 represent the distribution of plasma $\delta^{65}Cu$ in the BMI international classes. We observed significant differences in the classes: underweight, overweight and obesity.

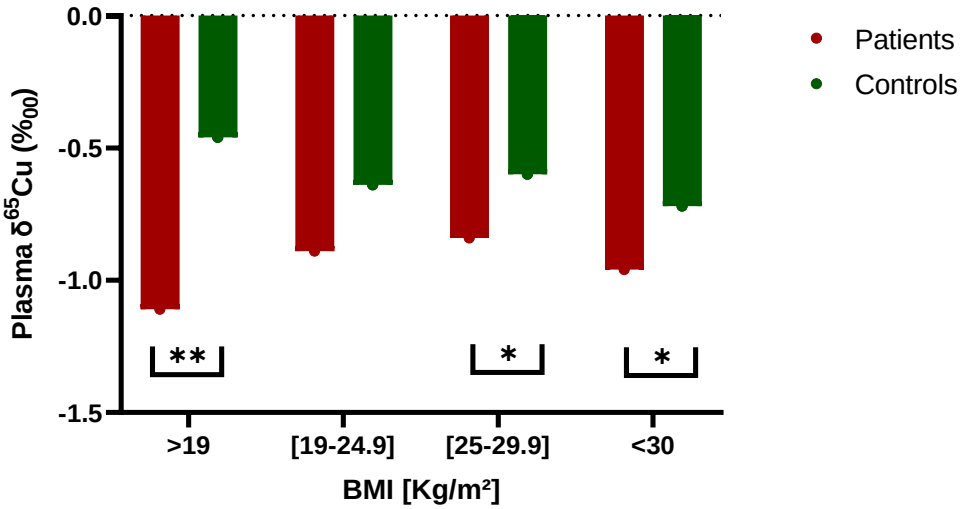

Figure 5. $\delta^{65}Cu$ distribution in the four international BMI classes. Underweight (BMI < 18.5) ; Ideal weight (BMI 18.5-24.9) ; Overweight (BMI 25.0-29.9) and Obese (BMI ≥ 30.0). T test was used for variables with normal distribution. $P < 0.05$ (*) and $P < 0.01$ (**).

Concerning age, no correlation between $\delta^{65}Cu$ and age was observed. We then separated the patients and controls according to age (55 years), which represents the average age of menopause, we found significant differences only in patients' groups (Figure 6).

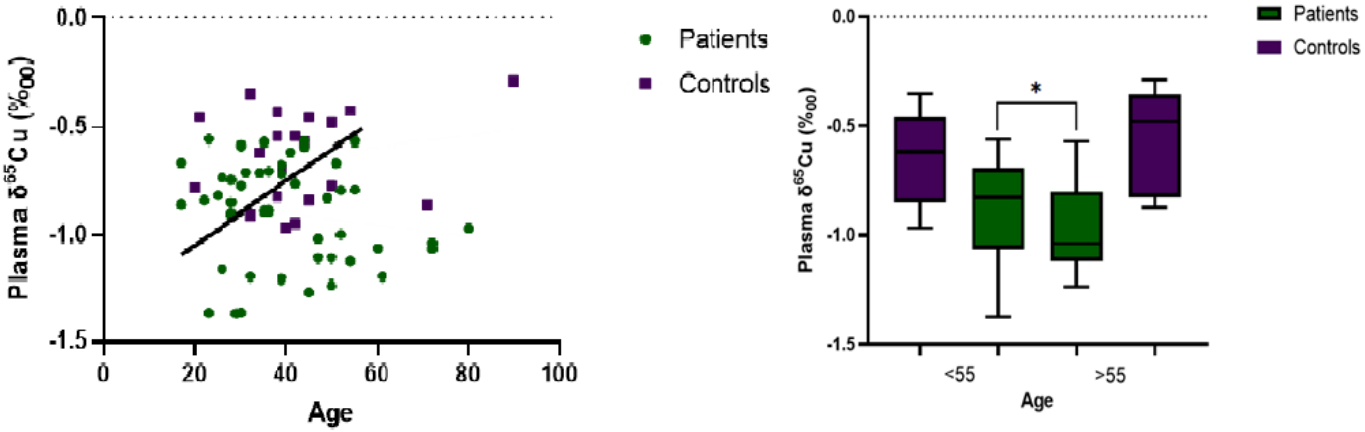

Figure 6. $\delta^{65}Cu$ concentrations in general age and in two age groups (< 55 and > 55 years old) of PTC patients and controls. T test was used for variables with normal distribution and Mann Whitney test for non-normal distribution. $P < 0.05$ (*).

Discussion

In the last decades, the incidence of thyroid cancer has increased significantly around the world and varies greatly from country to country [1]. The highest incidence rates are found in USA, Europe and Asia countries, while the lowest rates are found in Africa [2]. Despite the low incidence rate in Africa, the Maghreb region has a high incidence rate similar to that observed in Europe [2]. In Morocco and Tunisia, thyroid cancer is the 4th most common cancer in women, while in Algeria, this cancer is the 3rd most frequent cancer in women after breast and colorectal cancer [2]. The rising incidence of thyroid cancers affects all races and ethnic groups [3].

Our patients present PTC form. PTC represent the most common subtype, corresponding to 80%–90% of all thyroid cancers cases [4]. The gender distribution obtained in our results corroborates the studies reporting a female predominance [5]. A similar study on 1376 cases carried out in 2017 at the C.H.U (University Hospital Center) of Tlemcen, Algeria, showed a female predominance (94.75%) [6]. Another study conducted on Moroccan neighbors cases states that the predominance is still female (76%) [7], and it is reported that women are 3 times more affected than men [8].

It is appears from different studies that the average age of discovery of the disease is 30 to 50 years with a peak around 40 years [9-11]. For 73% of our patients, the average age of discovery is around 30 to 50 years old. We also found some significant differences in plasma minerals content by age. Several studies have also described significant differences in the distribution of micronutrients according to age including in Parkinson's Disease [12], and cancer [13]. Isotope fractionation also presented a significant difference in PTC patients between the < 55 and > 55 age groups. These isotopic differences could be due to a metabolic response to menstrual discharge [14].

Regarding the impact of anthropometrics factors, only few studies have been interested in finding a possible link with PTC. Nevertheless we noticed that the average BMI of our patients is approximately equal to 28.31 ± 5.85 Kg/m^2 corresponding to overweight classes, and it is similar to the results obtained in another Algerian study [15]. In addition, others search reported a positive relation between obesity and thyroid cancer risk in women [16-17]. Overweight and obesity constitute a risk factors for thyroid cancer [17].

We found a significant differences in the distribution of Se, Cu, Zn, Fe and Ca concentrations among the four international BMI classes between patients and controls. Dietary micronutrient

intake is associated with blood micronutrient concentrations, and the micronutrient deficiencies of individuals could be contributing to the increase in obesity rates [18-19]. Additionally, diet may influence the isotopic variability of individual [20]. Van Hedge *et al.,* [21] found significant differences between vegetarian and omnivorous individuals. Jouen *et al.,* [22] also found significant differences in the distribution of the Cu isotope between the Yakut population and the European and Japanese populations. In our case, the change in isotope values with BMI can be explained by different diets or metabolic variation of individuals.

In our results we did not find a significant association between smoking habit and the risk of PTC. It should be noted that our population is predominantly female, as previously reported, and no women were reported to be smokers. However, in the pooled study conducted by Kitahara *et al.,* [23] suggest that both smoking and alcohol consumption are associated with a reduced risk of thyroid cancer.

Our results reveals that a relatively poor education level was associated with a high risk of PTC. In an epidemiological study, using National Cancer Database from 2000-2013, points out that low education, low income, no insurance and a far distance from one's treatment facility presented a smallest increased in incidence of thyroid cancer [24]. Regarding marital status, previous studies have demonstrated that it influences the prognosis of patients with different types of cancer [25-26] including in thyroid cancer cases [27]. However, in this research we did not find any significant association between marital status and PTC risk.

In thyroid cancer, the simultaneous coexistence of multiple homeostatic disturbances of minerals elements has rarely been studied. Various minerals elements are involved in thyroid hormone synthetizes and are required for the proper functioning of the thyroid gland, some of them are directly implicated e.g., iodine, Se, Cu, Zn and Fe and other are indirectly implicated e.g., Ca [28]. The link between trace elements and thyroid cancer is not yet well defined. In this study we focused on the link between minerals elements: Se, Cu, Zn, Fe and Ca in PTC.

In the present study, we reported a significant diminution in plasma levels of the elements Se, Zn, Fe and Ca, conversely, Cu plasma was found to be elevated in the PTC patients compared to the healthy controls. Interestingly, various studies have reported high levels of Cu with low levels of Zn, Fe and Se in serum cancer patients [29-32].

We found a disturbance in minerals elements ratios with increased for Cu/Se, Cu/Ca, Cu/Zn, Cu/Fe and Ca/Se ratios and decreased Fe/Se and Fe/Ca ratios in PTC patients. It seems to be relative with high levels of Cu and low levels of Se, Zn, Fe and Ca in PTC patient’s vs. healthy

controls. In literature, only few studies described changes in ratios of minerals elements. In prostate adenocarcinoma studies, Sapota *et al.*, [33] found lower Zn/Cu, Zn/Se, Ca/Zn and Cu/Se ratios in cancerous patients compared to benign forms and Singh *et al.*, [34] also found higher Fe/Se and Ca/Se ratios with lower Zn/Fe ratio in prostate cancerous patients. In Hashimoto Thyroiditis study, Cu/Zn and Cu/Se ratios were significantly increased in patients vs. healthy controls [35]. The Cu/Zn ratio was found to be informative for oxidative stress in autoimmune diseases [36] and cancer [37-38] while the Cu/Se ratio was proved to be valuable for thyroid hormone signaling [39]. Indeed, Cu/Zn, Cu/Fe, and Cu/Se ratios seems to be much better indicators of cancer development than Cu, Zn, Fe, or Se levels alone [29].

Selenium and zinc are critical micronutrients which play an important part in the reduction of oxidative stress and the protection of DNA from ROS attack. Se and Zn have been shown to contribute into DNA repair system efficiency [40]. Se generated deficiency in Zn homeostasis, most probably, has a significant impact on DNA stability of cells and could be the causes in cancer development [40]. Furthermore, Se can interfere with Zn and inhibit the DNA repair process by oxidizing Zn fingers of DNA repair genes as well as transcriptional regulators of DNA repair genes. Depending on the concentration of Se and Zn the homeostasis can be disrupted resulting in oxidative DNA damage and cancer [40].

In addition, several studies revealed the association between iodine, Zn, and Se in thyroid cancer risk, but the findings are still controversial [41-43]. However, Zn could interact with or alter the function of Se in thyroid physiology [44]. Therefore, it is extremely important to control the balance between these two trace elements for optimal thyroid gland functioning.

Copper is a catalytic and structural cofactor of many important enzymes. It is also involved in the formation of hemoglobin in the blood, facilitating the absorption and utilization of Fe for red blood cells to deliver oxygen into the tissues [45-46]. Mladenka *et al.*, [47] speculated that the reducing effects of Fe and Cu may be appropriate for a chelator designed for cancer treatment. In addition, Cu interacts with proteins containing Fe-sulfur complexes and can displace other metals such as Zn from metalloproteins, inhibiting their activity [29].

Copper/zinc (Cu/Zn) in superoxide dismutase I enzyme (SOD1), are critical components of antioxidant enzymes [44]. It was reported that Cu deficiency directly affects the function of proteins with antioxidant functions, such as SOD1, ceruloplasmin, metallohtionein process, and indirectly affects glutathione (GSH) peroxidase activity [48]. GSH and metallothioneins bind to intracellular Cu to decrease the discharge of excess Cu [49-50]. In several studies on rodents,

Cu deficiency leads to decrease in GPxs activities [51-52]. Furthermore, in chicks [53] and in human colonic carcinoma (HT-29) cells line studies, Cu is capable to reverse selenite-induced cytotoxicity [54]. Schwarz *et al.*, [55] in their study, exposed hepatocarcinoma cells (HepG2) to different concentrations of Cu and Se. They found that Cu downregulated GPxs and TRxs activities, removed selenoproteins mRNA of GPx1 and selenoprotein W, and reduced the efficiency of UGA recoding in the cells. They demonstrated an interrelation between Cu and Se status, and Cu impacts negatively the expression and activity of selenoproteins. This link may also interfere in the Cu/Se ratio. Increased Cu concentration downregulated selenoproteins expression and affect negatively cellular redox homeostasis contributing to disease processes [55].

It has been demonstrated on rats studies, that Fe deficiency reduce GPxs activity in various rat tissues [56], T4 and T3 were also decreased [57]. In addition, Iron Deficiency Anemia (IDA) caused a decrease in the activity of hepatic DIOs, responsible for the conversion of T4 to T3 [58-59]. This decrease was higher in several anemic and iron-deficient rats form (72%) than in those with a less several form (25%). The mechanisms that control hepatic DIOs activity (enzyme synthesis and regulation of enzyme activity) are directly affected by iron deficiency, independently of thyroid hormone status [58-59]. In human study, It has been reported an altered efficiency of thyroid hormone synthesis in children and adults presented goiter disease and have Fe deficiency, suggesting an adequate Fe intake is necessary for efficient thyroid hormone synthesis with adequate iodine concentration [60-59].

Calcium has been shown to affect Fe absorption [61]. The addition of 150 mg of Ca to bread reduced Fe absorption by 50%. It is suggested that the interaction takes place in the mucosal cells, as both heme and non-heme Fe are affected [62]. Ca does not appear to have a direct effect on Zn absorption [63], nutritional studies suggest that Ca enhances Zn absorption from phytate-containing foods [63-64].

In our statistical study we found significant correlations between different mineral elements (Se, Cu, Zn, Fe and Ca). The limitation of our work is the number of participants. In fact, a study with a larger sample size can provide more solid statements, however we consider our project as a pilot study with very interesting results, which demonstrated that the homeostasis of the mineral elements is disturbed in PTC patients. Moreover, these elements can be directly or indirectly related to each other. Our results suggest that mineral elements can be considered as risk factors and may constitute a novels biomarker for early detection of papillary thyroid cancer.

References

[1] J. Lukas, J. Drabek, D. Lukas, L. Dusek, et J. Gatek, « The epidemiology of thyroid cancer in the Czech Republic in comparison with other countries », *Biomed. Pap.*, vol. 157, nº 3, p. 266-275, sept. 2013, doi: 10.5507/bp.2012.086.

[2] « Global Cancer Observatory ». https://gco.iarc.fr/ (consulté le 1 novembre 2021).

[3] J. A. Sipos et E. L. Mazzaferri, « Thyroid Cancer Epidemiology and Prognostic Variables », *Clin. Oncol.*, vol. 22, nº 6, p. 395-404, août 2010, doi: 10.1016/j.clon.2010.05.004.

[4] C. Xiu, « Strong Correlation of Abnormal Serum and Urinary Iodine Levels with Papillary Thyroid Cancer: A Case-control Study », *Biomed Env. Sci*, p. 6.

[5] S. Mehl *et al.*, « Cross-sectional analysis of trace element status in thyroid disease », *J. Trace Elem. Med. Biol.*, vol. 58, p. 126430, mars 2020, doi: 10.1016/j.jtemb.2019.126430.

[6] A. Benouis, Z. Bekkouche, M. S. Merad, L. Loudjedi, H. Khelil, et N. Berber, « Thyroid Cancer in Western Algeria: Histopathological and Epidemiological Study », *J. Cancer Ther.*, vol. 08, nº 07, p. 672-682, 2017, doi: 10.4236/jct.2017.87058.

[7] M. M. Touati *et al.*, « Les carcinomes de la thyroïde: profils épidémiologique, clinique et thérapeutique, à propos de 102 cas », *Pan Afr. Med. J.*, vol. 21, 2015, doi: 10.11604/pamj.2015.21.59.5688.

[8] M. Colonna, F. D. Maria, C. Schvartz, et P. Grosclaude, « Analyse descriptive de l'incidence du cancer de la thyroïde à partir des données des registres des cancers sur la période 1982-2012 en france », p. 8.

[9] A. H. N. Rakotoarisoa *et al.*, « Les cancers de la thyroïde à Madagascar », *Bull. Société Pathol. Exot.*, vol. 103, nº 4, p. 233-237, oct. 2010, doi: 10.1007/s13149-010-0063-y.

[10] N. Boumansour, H. Beldjilali, F. Agag, Ouhaj, R. Tidjani, et N. Midoun, « Profil épidémiologique des cancers thyroïdiens à l'Ouest algérien », *Rev. DÉpidémiologie Santé Publique*, vol. 62, p. S214, sept. 2014, doi: 10.1016/j.respe.2014.06.137.

[11] C. McIntyre, T. Jacques, F. Palazzo, K. Farnell, et N. Tolley, « Quality of life in differentiated thyroid cancer », *Int. J. Surg.*, vol. 50, p. 133-136, févr. 2018, doi: 10.1016/j.ijsu.2017.12.014.

[12] H.-W. Zhao *et al.*, « Assessing Plasma Levels of Selenium, Copper, Iron and Zinc in Patients of Parkinson's Disease », *PLoS ONE*, vol. 8, nº 12, p. e83060, déc. 2013, doi: 10.1371/journal.pone.0083060.

[13] J. D. Brooks *et al.*, « Plasma selenium level before diagnosis and the risk of prostate cancer development », *J. Urol.*, vol. 166, nº 6, p. 2034-2038, déc. 2001, doi: 10.1016/S0022-5347(05)65500-0.

[14] K. Jaouen et M.-L. Pons, « Potential of non-traditional isotope studies for bioarchaeology », *Archaeol. Anthropol. Sci.*, vol. 9, nº 7, p. 1389-1404, oct. 2017, doi: 10.1007/s12520-016-0426-9.

[15] N. Meziani-Bettayeb et M. Semrouni, « Évaluation des paramètres osseux du traitement par les hormones thyroïdiennes dans les cancers différenciés de la thyroïde », *Ann. Endocrinol.*, vol. 75, nº 5-6, p. 498, oct. 2014, doi: 10.1016/j.ando.2014.07.777.

[16] É. Cléro *et al.*, « Pooled Analysis of Two Case–Control Studies in New Caledonia and French Polynesia of Body Mass Index and Differentiated Thyroid Cancer: The Importance of Body Surface Area », *Thyroid*, vol. 20, nº 11, p. 1285-1293, nov. 2010, doi: 10.1089/thy.2009.0456.

[17] T. Pappa et M. Alevizaki, « Obesity and Thyroid Cancer: A Clinical Update », *Thyroid*, vol. 24, nº 2, p. 190-199, févr. 2014, doi: 10.1089/thy.2013.0232.

[18] A. J. Alberg, « The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients », *Toxicology*, vol. 180, nº 2, p. 121-137, nov. 2002, doi: 10.1016/S0300-483X(02)00386-4.

[19] O. P. García, K. Z. Long, et J. L. Rosado, « Impact of micronutrient deficiencies on obesity », *Nutr. Rev.*, vol. 67, nº 10, p. 559-572, oct. 2009, doi: 10.1111/j.1753-4887.2009.00228.x.

[20] A. T. Gourlan, G. Douay, et P. Telouk, « Copper isotopes as possible neoplasia biomarkers in captive wild felids », *Zoo Biol.*, vol. 38, nº 4, p. 371-383, août 2019, doi: 10.1002/zoo.21504.

[21] L. Van Heghe, E. Engström, I. Rodushkin, C. Cloquet, et F. Vanhaecke, « Isotopic analysis of the metabolically relevant transition metals Cu, Fe and Zn in human blood from vegetarians and omnivores using multi-collector ICP-mass spectrometry », *J. Anal. At. Spectrom.*, vol. 27, nº 8, p. 1327, 2012, doi: 10.1039/c2ja30070b.

[22] K. Jaouen, M.-L. Pons, et V. Balter, « Iron, copper and zinc isotopic fractionation up mammal trophic chains », *Earth Planet. Sci. Lett.*, vol. 374, p. 164-172, juill. 2013, doi: 10.1016/j.epsl.2013.05.037.

[23] C. M. Kitahara *et al.*, « Cigarette smoking, alcohol intake, and thyroid cancer risk: a pooled analysis of five prospective studies in the United States », *Cancer Causes Control*, vol. 23, nº 10, p. 1615-1624, oct. 2012, doi: 10.1007/s10552-012-0039-2.

[24] E. Olson, G. Wintheiser, K. M. Wolfe, J. Droessler, et P. T. Silberstein, « Epidemiology of Thyroid Cancer: A Review of the National Cancer Database, 2000-2013 », *Cureus*, févr. 2019, doi: 10.7759/cureus.4127.

[25] G. Inverso, B. A. Mahal, A. A. Aizer, R. B. Donoff, N. G. Chau, et R. I. Haddad, « Marital status and head and neck cancer outcomes: Marital Status and Head and Neck Cancer », *Cancer*, vol. 121, nº 8, p. 1273-1278, avr. 2015, doi: 10.1002/cncr.29171.

[26] A. A. Aizer *et al.*, « Marital Status and Survival in Patients With Cancer », *J. Clin. Oncol.*, vol. 31, nº 31, p. 3869-3876, nov. 2013, doi: 10.1200/JCO.2013.49.6489.

[27] R. Shi, N. Qu, Z. Lu, T. Liao, Y. Gao, et Q. Ji, « The impact of marital status at diagnosis on cancer survival in patients with differentiated thyroid cancer », *Cancer Med.*, vol. 5, nº 8, p. 2145-2154, août 2016, doi: 10.1002/cam4.778.

[28] S. M. O'Kane, M. S. Mulhern, L. K. Pourshahidi, J. J. Strain, et A. J. Yeates, « Micronutrients, iodine status and concentrations of thyroid hormones: a systematic review », *Nutr. Rev.*, vol. 76, nº 6, p. 418-431, juin 2018, doi: 10.1093/nutrit/nuy008.

[29] D. Denoyer, S. Masaldan, S. La Fontaine, et M. A. Cater, « Targeting copper in cancer therapy: 'Copper That Cancer' », *Metallomics*, vol. 7, nº 11, p. 1459-1476, 2015, doi: 10.1039/C5MT00149H.

[30] H. R. Mali, S. A. Siddiqui, M. Garg, R. K. Singh, et M. L. B. Bhatt, « Changes in serum copper levels in patients with malignant diseases undergoing radiotherapy », *Indian J. Clin. Biochem.*, vol. 13, nº 1, p. 36-40, janv. 1998, doi: 10.1007/BF02873441.

[31] M. Zowczak, M. Iskra, L. Torliński, et S. Cofta, « Analysis of Serum Copper and Zinc Concentrations in Cancer Patients », *Biol. Trace Elem. Res.*, vol. 82, nº 1-3, p. 001-008, 2001, doi: 10.1385/BTER:82:1-3:001.

[32] S. K. Gupta, V. K. Shukla, M. P. Vaidya, S. K. Roy, et S. Gupta, « Serum and tissue trace elements in colorectal cancer », *J. Surg. Oncol.*, vol. 52, nº 3, p. 172-175, mars 1993, doi: 10.1002/jso.2930520311.

[33] A. Sapota, A. Daragó, J. Taczalski, et A. Kilanowicz, « Disturbed homeostasis of zinc and other essential elements in the prostate gland dependent on the character of pathological

lesions », *BioMetals*, vol. 22, nº 6, p. 1041-1049, déc. 2009, doi: 10.1007/s10534-009-9255-y.

[34] B. P. Singh *et al.*, « Status and Interrelationship of Zinc, Copper, Iron, Calcium and Selenium in Prostate Cancer », *Indian J. Clin. Biochem.*, vol. 31, nº 1, p. 50-56, janv. 2016, doi: 10.1007/s12291-015-0497-x.

[35] F. B. Serinkan Cinemre *et al.*, « Hashimoto Tiroiditli Hastalarda Bakır, Demir, Çinko, Selenyum Düzeyleri ve Cu/Se, Cu/Zn, Fe/Se, Fe/Zn Oranlarının Araştırılması », *Sak. Med. J.*, vol. 8, nº 2, p. 285-291, juin 2018, doi: 10.31832/smj.424877.

[36] M. Wacewicz *et al.*, « Selenium, zinc, copper, Cu/Zn ratio and total antioxidant status in the serum of vitiligo patients treated by narrow-band ultraviolet-B phototherapy », *J. Dermatol. Treat.*, vol. 29, nº 2, p. 190-195, févr. 2018, doi: 10.1080/09546634.2017.1357797.

[37] K. Zabłocka-Słowińska, A. Prescha, S. Płaczkowska, I. Porębska, M. Kosacka, et K. Pawełczyk, « Serum and Whole Blood Cu and Zn Status in Predicting Mortality in Lung Cancer Patients », *Nutrients*, vol. 13, nº 1, p. 60, déc. 2020, doi: 10.3390/nu13010060.

[38] W. Yao *et al.*, « Recombinant protein transduction domain-Cu/Zn superoxide dismutase alleviates bone cancer pain via peroxiredoxin 4 modulation and antioxidation », *Biochem. Biophys. Res. Commun.*, vol. 486, nº 4, p. 1143-1148, mai 2017, doi: 10.1016/j.bbrc.2017.04.017.

[39] J. Mittag, T. Behrends, K. Nordström, J. Anselmo, B. Vennström, et L. Schomburg, « Serum copper as a novel biomarker for resistance to thyroid hormone », *Biochem. J.*, vol. 443, nº 1, p. 103-109, avr. 2012, doi: 10.1042/BJ20111817.

[40] A. Yildiz, Y. Kaya, et O. Tanriverdi, « Effect of the Interaction Between Selenium and Zinc on DNA Repair in Association With Cancer Prevention », *J. Cancer Prev.*, vol. 24, nº 3, p. 146-154, sept. 2019, doi: 10.15430/JCP.2019.24.3.146.

[41] H.-K. Chung, J. S. Nam, C. W. Ahn, Y. S. Lee, et K. R. Kim, « Some Elements in Thyroid Tissue are Associated with More Advanced Stage of Thyroid Cancer in Korean Women », *Biol. Trace Elem. Res.*, vol. 171, nº 1, p. 54-62, mai 2016, doi: 10.1007/s12011-015-0502-5.

[42] J. Jonklaas, M. Danielsen, et H. Wang, « A Pilot Study of Serum Selenium, Vitamin D, and Thyrotropin Concentrations in Patients with Thyroid Cancer », *Thyroid*, vol. 23, nº 9, p. 1079-1086, sept. 2013, doi: 10.1089/thy.2012.0548.

[43] L. Zhang *et al.*, « A case-control study of urinary levels of iodine, perchlorate and thiocyanate and risk of papillary thyroid cancer », *Environ. Int.*, vol. 120, p. 388-393, nov. 2018, doi: 10.1016/j.envint.2018.08.024.

[44] V. Triggiani *et al.*, « Role of Iodine, Selenium and Other Micronutrients in Thyroid Function and Disorders », *Endocr. Metab. Immune Disord. - Drug Targets*, vol. 9, nº 3, p. 277-294, sept. 2009, doi: 10.2174/187153009789044392.

[45] T. Theophanides et J. Anastassopoulou, « Copper and carcinogenesis », *Crit. Rev. Oncol. Hematol.*, vol. 42, nº 1, p. 57-64, avr. 2002, doi: 10.1016/S1040-8428(02)00007-0.

[46] F. Kosova *et al.*, « Serum copper levels in benign and malignant thyroid diseases », *Bratisl. Med. J.*, vol. 113, nº 12, p. 718-720, 2012, doi: 10.4149/BLL_2012_162.

[47] P. Mladěnka *et al.*, « Interaction of 2,6,7-Trihydroxy-Xanthene-3-Ones with Iron and Copper, and Biological Effect of the Most Active Derivative on Breast Cancer Cells and Erythrocytes », *Appl. Sci.*, vol. 10, nº 14, p. 4846, juill. 2020, doi: 10.3390/app10144846.

[48] A. A. Alturfan *et al.*, « Investigation of zinc and copper levels in methimazole-induced hypothyroidism: relation with the oxidant-antioxidant status », *Folia Biol. (Praha)*, vol. 53, nº 5, p. 183-188, 2007.

[49] J. H. Freedman, M. R. Ciriolo, et J. Peisach, « The Role of Glutathione in Copper Metabolism and Toxicity », *J. Biol. Chem.*, vol. 264, nº 10, p. 5598-5605, avr. 1989, doi: 10.1016/S0021-9258(18)83589-X.

[50] J. Jiang, C. M. St. Croix, N. Sussman, Q. Zhao, B. R. Pitt, et V. E. Kagan, « Contribution of Glutathione and Metallothioneins to Protection against Copper Toxicity and Redox Cycling: Quantitative Analysis Using MT+/+ and MT−/− Mouse Lung Fibroblast Cells », *Chem. Res. Toxicol.*, vol. 15, nº 8, p. 1080-1087, août 2002, doi: 10.1021/tx020022u.

[51] K. L. Olin, R. M. Walter, et C. L. Keen, « Copper deficiency affects selenoglutathione peroxidase and selenodeiodinase activities and antioxidant defense in weanling rats », *Am. J. Clin. Nutr.*, vol. 59, nº 3, p. 654-658, mars 1994, doi: 10.1093/ajcn/59.3.654.

[52] Y. Chen, J. T. Saari, et Y. J. Kang, « Weak antioxidant defenses make the heart a target for damage in copper-deficient rats », *Free Radic. Biol. Med.*, vol. 17, nº 6, p. 529-536, déc. 1994, doi: 10.1016/0891-5849(94)90092-2.

[53] L. S. Jensen, « Modification of a Selenium Toxicity in Chicks by Dietary Silver and Copper », *J. Nutr.*, vol. 105, nº 6, p. 769-775, juin 1975, doi: 10.1093/jn/105.6.769.

[54] R. L. Davis, J. E. Spallholz, et B. C. Pence, « Inhibition of selenite-induced cytotoxicity and apoptosis in human colonic carcinoma (HT-29) cells by copper », *Nutr. Cancer*, vol. 32, nº 3, p. 181-189, janv. 1998, doi: 10.1080/01635589809514738.

[55] M. Schwarz *et al.*, « Copper interferes with selenoprotein synthesis and activity », *Redox Biol.*, vol. 37, p. 101746, oct. 2020, doi: 10.1016/j.redox.2020.101746.

[56] J. Köhrle, « Thyrotropin (TSH) action on thyroid hormone deiodination and secretion: one aspect of thyrotropin regulation of thyroid cell biology », *Horm. Metab. Res. Suppl. Ser.*, vol. 23, p. 18-28, 1990.

[57] S. Villette, G. Bermano, J. R. Arthur, et J. E. Hesketh, « Thyroid stimulating hormone and selenium supply interact to regulate selenoenzyme gene expression in thyroid cells (FRTL-5) in culture », *FEBS Lett.*, vol. 438, nº 1-2, p. 81-84, oct. 1998, doi: 10.1016/S0014-5793(98)01280-0.

[58] D. E. Brigham et J. L. Beard, « Effect of thyroid hormone replacement in iron-deficient rats », *Am. J. Physiol.-Regul. Integr. Comp. Physiol.*, vol. 269, nº 5, p. R1140-R1147, nov. 1995, doi: 10.1152/ajpregu.1995.269.5.R1140.

[59] Hess et Zimmermann, « The Effect of Micronutrient Deficiencies on Iodine Nutrition and Thyroid Metabolism », *Int. J. Vitam. Nutr. Res.*, vol. 74, nº 2, p. 103-115, mars 2004, doi: 10.1024/0300-9831.74.2.103.

[60] M. B. Zimmermann et J. Köhrle, « The Impact of Iron and Selenium Deficiencies on Iodine and Thyroid Metabolism: Biochemistry and Relevance to Public Health », *Thyroid*, vol. 12, nº 10, p. 867-878, oct. 2002, doi: 10.1089/105072502761016494.

[61] L. Hallberg, M. Brune, M. Erlandsson, A. S. Sandberg, et L. Rossander-Hultén, « Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans », *Am. J. Clin. Nutr.*, vol. 53, nº 1, p. 112-119, janv. 1991, doi: 10.1093/ajcn/53.1.112.

[62] L. Hallberg, L. Rossander-Hulthèn, M. Brune, et A. Gleerup, « Inhibition of haem-iron absorption in man by calcium », *Br. J. Nutr.*, vol. 69, nº 2, p. 533-540, mars 1993, doi: 10.1079/BJN19930053.

[63] B. Sandström, « Micronutrient interactions: effects on absorption and bioavailability », *Br. J. Nutr.*, vol. 85, nº S2, p. S181, mai 2001, doi: 10.1079/BJN2000312.

[64] D. S. Petterson, B. SandstrÖm, et Å. Cederblad, « Absorption of zinc from Iupin (Lupinus angustifolius)-based foods », *Br. J. Nutr.*, vol. 72, nº 6, p. 865-871, déc. 1994, doi: 10.1079/BJN19940091.

Chapter V: Impact of Selenium Nanoparticles in thyroid cancer: a pilot study

I. Presentation of the article

Selenium is receiving increasing interest as a potential cancer prevention agent, especially in populations with low Se intakes. While Se is toxic at doses slightly in excess to dietary requirements, clinical trials of selenite as an anti-cancer therapy have demonstrated its protective effect against liver cancer. SeNPs have increased potential compared to aqueous Se, as it allows to deliver high Se concentrations without toxic effects to the general body, and they could be used specifically against cancer cells.

Indeed, SeNPs were found to induce cell death mechanisms in ovarian and breast cancers. Additionally, Se has been demonstrated to influence the epigenome and influencing selenoprotein expression. High doses of Se treatment have been shown to inhibit DNA methyltransferase activity and expression; however, low doses of Se, may also reduction DNA methylation.

In the present study, we have tested the cytotoxicity, using different concentrations, of SeNPs and selenite on the spheroid developments, a model of human woman papillary thyroid carcinoma metastases (MDA-T120).

We observed a significant reduction in cell viability treated with SeNPs and selenite compared to controls. However, MDA-T120 cell spheroids were more sensitive to selenite than SeNPs. We also found a decrease in ROS production.

We support the idea that SeNPs should be further investigated for using as a non-invasive and therapeutic option in papillary thyroid cancer treatments, but before that, one should better understand action mechanisms involved.

II. Article

This paper is in preparation and will be submitted to Academia on 30th June

Impact of Selenium Nanoparticles in thyroid cancer: a pilot study

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Abstract:

Cancer treatments are accompanied by undesirable side effects for patients, and thyroid cancer treatment is not an exception. Currently, scientists are working on developing less invasive and effective therapies against tumor proliferation. Selenium (Se) is an essential micronutrient for human health, a daily consumption above 400µg were found to have an inhibitor impact on cancer cells growth, however Se in selenite form is toxic for human health. The development of new Se forms, nanoparticles of Se (SeNPs), could be the key to increase the doses administered to obtain the desired therapeutic effect. Our objective is to study, for the first time, the cytotoxicity and impact of SeNPs and selenite on the spheroid's development, models of thyroid carcinoma metastasis (MDA-T120). We found a significant reduction in cell viability with SeNP-chitosan and selenite compared to controls with a decrease in reactive oxygen species production.

Keywords: Selenium, SeNPs, Thyroid Cancer, MDA-T120.

1. Introduction

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for 85-90% of all cases [1]. Current clinical treatments for PTC are primarily surgical resection and radiation therapy. However, these common cancer treatments are usually accompanied by unavoidable side effects, e.g., when tumor tissues are removed, surgical resection may remove some normal tissue [2]. The current challenge for scientists is to develop new therapies that are less invasive and consequential for the well-being of patients with more efficient against the propagation and growth of tumors.

Selenium (Se) is an essential micronutrient compound, it participates in the upkeep and integrity of cellular systems by impacting cellular redox statuses and the ability to detoxify compounds, free radicals, and reactive oxygen species (ROS) [3]. Several *in vitro* studies have shown a beneficial effect of high Se doses against cancer cells [4-5]. The healthy intake range for Se is particularly narrow, and it is between 25 and 70 µg/day for adults, and while it has been considered as a potential cytotoxic/chemotherapeutic agent due to its higher-level toxicity, it cannot be administered systemically in a free form (selenite) [6]. In contrast, selenium nanoparticles (SeNPs) are well tolerated when administered *in vivo* models systemically and show cytotoxic effects on ovarian cancer cells [7-8].

The aim of this pilot study is to test, for the first time, the cytotoxicity of SeNPs on the spheroid developments, models of thyroid carcinoma metastases (MDA-T120).

2. Materials and methods

a. Characteristics and cell culture

We used models MDA-T120 (ATCC, Maryland, US), which represent a papillary thyroid carcinoma cell line of female gender with 72 years old. Cells were cultured in RPMI-1640 medium (Sigma - Aldrich, UK) supplemented by 20% bovine serum albumin (BSA, Sigma-Aldrich), 5 µg/mL and 1% penicillin-streptomycin solution (v/v) (Sigma-Aldrich, UK). Cells were kept at 37°C and 5% CO_2 and regularly passaged using 0.25% trypsin and 0.1% EDTA (v/v). We checked the cell viability and their fluctuations using an optic microscope.

b. Spheroid's growth, treatment, and viability assay

Cell viability was assessed by using the CellTiterGlo assay (BMGLabtech, Fluostar Omega, UK). 5,000 cells/well were loaded into 96-well plates with a round bottom and Ultra Low Attachment coating (Corning, UK). After 24 h, the spheroid was formed, then we added 100

µL of fresh medium containing a 2X concentration of sodium selenite or selenium nanoparticles-chitosan (SeNP-chitpsan). For the viability assay, a range of rising doses (0.01 µg/mL to 20 µg/mL) was applied by simple dilution in the appropriate medium for 24, 48 or 72h. After treatment, we removed the 100 µL of medium from the wells and we added 100 µL of CellTiterGlo. Plates were shaken for 10 min and equilibrated at room temperature for 25 min before luminescence measurements.

c. ROS assay

MDA-T120 cells were plated in monolayers at 20 000 cells per well in dark 96-well plates and grown overnight. After media removal, cells were washed once by PBS and incubated for 1h with cellular ROS detection reagent (Red Fluorescence, Abcam186027). A 6x IC50 concentration of selenite or SeNPs was then added, and the plate was incubated at 37°C for the duration of the assay. Fluorescence was analyzed at different time points from 30 min to 10h (520 nm excitation filter, 605 nm emission filter, BMGLabtech Fluostar Omega, UK).

3. Results

Before the formation of the spheroids, we checked the fluctuation of cells viability, as shown in figure 1. We had a very important fluctuation about 80% of the flask surface.

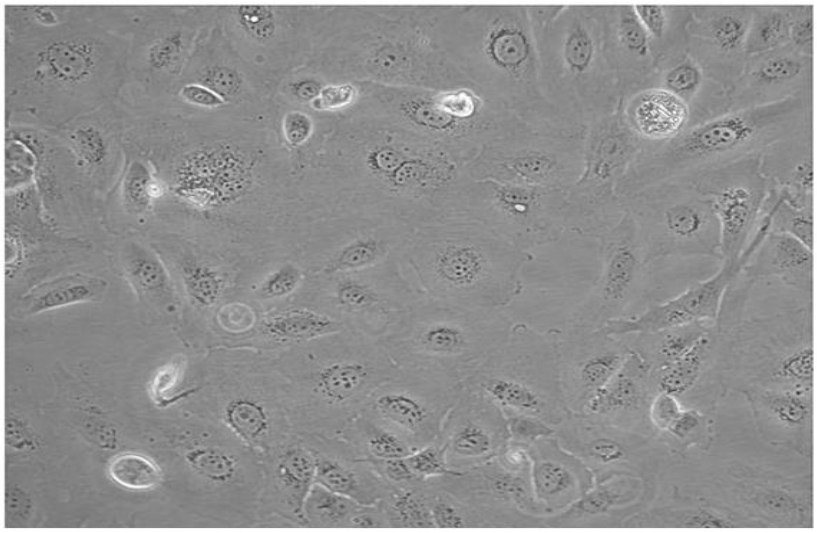

Figure 1. Microscopic aspect of cell culture.

The morphological aspect of the two spheroids is presented in Figure 2, the first one is a control spheroid without any added agent and the second one is a spheroid after treatment with SeNP-chitosan, we noticed a decrease size of the treated spheroid.

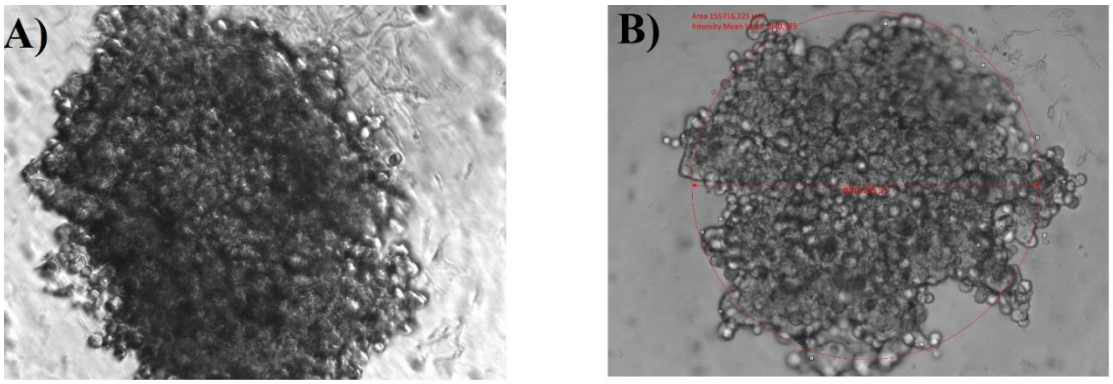

Figure 2. Microscopic aspect of spheroid in A) normal condition, B) treatment with SeNP-chitosan.

Figure 3 illustrated the treatment of MDA-T120 cell spheroids, we found a significant reduction in cell viability with selenite and SeNP-chitosan compared to controls (without any treatment). MDA-T120 cell spheroids were more sensitive to selenite than SeNP-chitosan. We observed a significant decrease in cell viability with both. SeNPs and selenite have similar cytotoxic effects. SeNPs are significantly lower toxic than selenite in *in vivo* models, whilst having a cytotoxic effect.

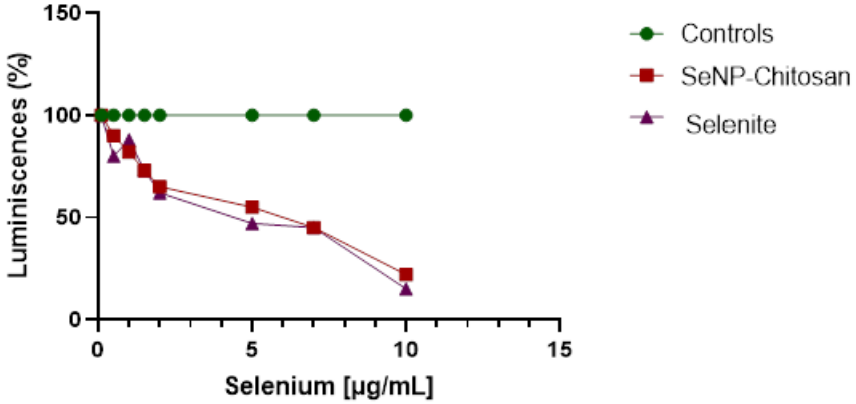

Figure 3. MDA-T120 cytotoxicity in the presence of SeNP-chitosane and selenite. MDA-T120 were grown as 5 x 10^3 cell spheroids for 24 h then treated with an raising range of concentration (0 to 20 µg/mL) of selenite and SeNP-chitosane over 24h and cellular cytotoxicity monitored. Cytotoxicity was evaluated by CellTiterGlo endpoint experiment.

Reactif Species Oxygen production over times shown that SeNPs induced cytotoxicity in MDA-T120 cell lines, with cell-type specific responses, and could therefore offer significant benefits and deliver at cytotoxic doses *in vivo* studies [9-10] (Figure 4).

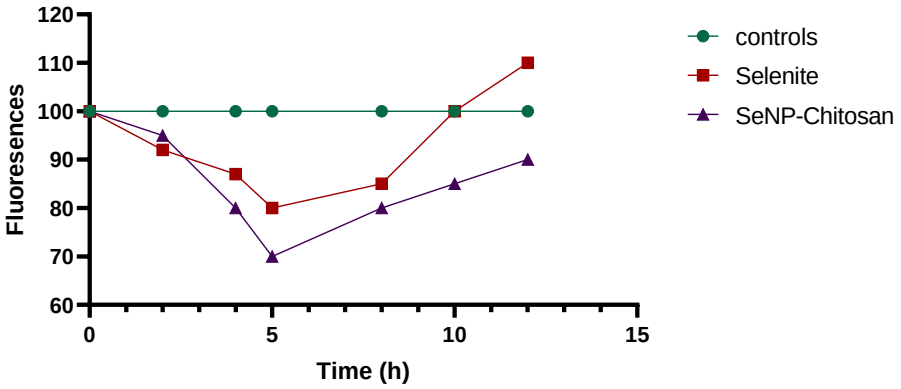

Figure 4. ROS production in MDA-T120. Cells were grown as a 2D at 50 x 10^3 cells per well, incubated with ROS probes, and treated with selenite and, SeNP-chitosan. ROS red fluorescent assay SeNPs showed that selenite, and SeNPs treatments at IC50 decreased the production of ROS.

4. Discussion

Selenium is an essential micronutrient for human health. In recent years, several research studies have focused on Se as a potential cancer preventive agent, particularly in populations with markedly Se low intakes [11]. At doses above the nutritional requirement, Se is a toxic element, but clinical trials using selenite as an anti-cancer therapy have demonstrated a protective effect against liver and breast cancer [12-13].

In literature no *in vivo* study has described the effect of SeNPs cytotoxicity on spheroid with thyroid carcinoma models. In this pilot study we found a promising results for the potential therapeutic uses of SeNPs compared to aqueous selenium, mainly due to their increased accumulation and specificity against cancer cells [14]. In addition, SeNPs have been found to trigger cell death mechanisms in hepatocarcinoma and breast cancer [15-16].

In animal study, Sadeghian *et al.*, [17] showed that SeNPs were less toxic and more bioactive with antioxidant activity than sodium selenium. In another animal study with mouse model demonstrated that SeNPs has promising anticancer activity against Ehrlich's ascites carcinoma [18].

In human studies, Kong *et al.*, [19] found that SeNPs decreased the growth of prostate cancer cells by degradation of androgen receptors which are essentiel for the normal functioning of prostate cells. SeNPs activated the Akt/Mdm2 pathway, reducing the transcriptional activity of androgen receptors by regulating its mRNA and protein expression [19]. Ali *et al.*, [20] demonstrated that pretreatment of lung cancer cells with SeNPs decreased the lipid peroxidation, inflammation (TNF-α) and C reactive protein level. Vekariya *et al.*, [21] reported

that SeNPs modulate estrogen receptor-alpha signaling in breast cancer cells and led to rise the expression of cytochrome C, Bax, and P-p38. Furthermore, SeNPs could induce apoptosis and necrosis in breast cancer cells with reduced CD44 expression, caused disorganization and dysregulation of intracellular cytoskeleton F-actin in MCF-7 cells [22]. In *in vivo* study, on fibro-sarcoma cell lines (HT-1080), demonstrated that SeNPs inhibit the matrix metalloprotein 2 expression which is principally involved in tumor invasion [23]. Several studies proved that SeNPs has promising anti-proliferation activity with no toxic effect and inhibit lung cells line during S phase [24-25]. Even though the effects of SeNPs seem attractive in these cells' lines; however, to make a concrete conclusion, *in vivo* studies are mandatory which are hither to not much explored.

5. Conclusion

We found a significant reduction in cell viability with selenite and SeNP-chitosan compared to controls with a decrease in ROS production. We provided valuable preliminary evidence representing the potentiality of SeNPs as a candidate for thyroid cancer treatment. This work supports the concept that SeNPs can be investigated in further as therapeutic option not only for thyroid cancer, but before that we should investigate more the pathway of the cellular mechanism.

Author Contributions: L.C. and L.S.K.T. designed the study. L.S.K.T did measurement. L.S.K.T write the paper. L.C and N. D-M supervise the word and correct the last version. All authors have read and agreed to the published version of the manuscript.

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References

[1] M. E. Cabanillas, D. G. McFadden, et C. Durante, « Thyroid cancer », *The Lancet*, vol. 388, nº 10061, p. 2783-2795, déc. 2016, doi: 10.1016/S0140-6736(16)30172-6.

[2] X. Zou, Z. Jiang, L. Li, et Z. Huang, « Selenium nanoparticles coated with pH responsive silk fibroin complex for fingolimod release and enhanced targeting in thyroid cancer », *Artif. Cells Nanomedicine Biotechnol.*, vol. 49, nº 1, p. 83-95, janv. 2021, doi: 10.1080/21691401.2021.1871620.

[3] D. M. Townsend, K. D. Tew, et H. Tapiero, « Sulfur containing amino acids and human disease », *Biomed. Pharmacother.*, vol. 58, nº 1, p. 47-55, janv. 2004, doi: 10.1016/j.biopha.2003.11.005.

[4] K. Bai, B. Hong, Z. Hong, J. Sun, et C. Wang, « Selenium nanoparticles-loaded chitosan/citrate complex and its protection against oxidative stress in d-galactose-induced aging mice », *J. Nanobiotechnology*, vol. 15, nº 1, Art. nº 1, déc. 2017, doi: 10.1186/s12951-017-0324-z.

[5] C. D. Davis, P. A. Tsuji, et J. A. Milner, « Selenoproteins and cancer prevention », *Annu. Rev. Nutr.*, vol. 32, p. 73-95, août 2012, doi: 10.1146/annurev-nutr-071811-150740.

[6] M. Vinceti *et al.*, « Selenium for preventing cancer », *Cochrane Database Syst. Rev.*, janv. 2018, doi: 10.1002/14651858.CD005195.pub4.

[7] A. R. Shahverdi *et al.*, « Characterization of Folic Acid Surface-Coated Selenium Nanoparticles and Corresponding In Vitro and In Vivo Effects Against Breast Cancer », *Arch. Med. Res.*, vol. 49, nº 1, p. 10-17, janv. 2018, doi: 10.1016/j.arcmed.2018.04.007.

[8] B. Toubhans *et al.*, « Selenium nanoparticles trigger alterations in ovarian cancer cell biomechanics », *Nanomedicine Nanotechnol. Biol. Med.*, vol. 29, p. 102258, oct. 2020, doi: 10.1016/j.nano.2020.102258.

[9] K. Bai, B. Hong, Z. Hong, J. Sun, et C. Wang, « Selenium nanoparticles-loaded chitosan/citrate complex and its protection against oxidative stress in d-galactose-induced aging mice », *J. Nanobiotechnology*, vol. 15, nº 1, p. 92, déc. 2017, doi: 10.1186/s12951-017-0324-z.

[10] G. Huang *et al.*, « Autophagy is an important action mode for functionalized selenium nanoparticles to exhibit anti-colorectal cancer activity », *Biomater. Sci.*, vol. 6, nº 9, p. 2508-2517, 2018, doi: 10.1039/C8BM00670A.

[11] M. P. Rayman, « Selenium in cancer prevention: a review of the evidence and mechanism of action », *Proc. Nutr. Soc.*, vol. 64, nº 4, p. 527-542, nov. 2005, doi: 10.1079/PNS2005467.

[12] T. Chen et Y.-S. Wong, « Selenocystine induces reactive oxygen species–mediated apoptosis in human cancer cells », *Biomed. Pharmacother.*, vol. 63, nº 2, p. 105-113, févr. 2009, doi: 10.1016/j.biopha.2008.03.009.

[13] A. R. Shahverdi *et al.*, « Characterization of Folic Acid Surface-Coated Selenium Nanoparticles and Corresponding In Vitro and In Vivo Effects Against Breast Cancer », *Arch. Med. Res.*, vol. 49, nº 1, p. 10-17, janv. 2018, doi: 10.1016/j.arcmed.2018.04.007.

[14] A. Khurana, S. Tekula, M. A. Saifi, P. Venkatesh, et C. Godugu, « Therapeutic applications of selenium nanoparticles », *Biomed. Pharmacother.*, vol. 111, p. 802-812, mars 2019, doi: 10.1016/j.biopha.2018.12.146.

[15] H. H. Ahmed, W. K. B. Khalil, et A. H. Hamza, « Molecular mechanisms of Nano-selenium in mitigating hepatocellular carcinoma induced by *N* -nitrosodiethylamine (NDEA) in rats », *Toxicol. Mech. Methods*, vol. 24, nº 8, p. 593-602, déc. 2014, doi: 10.3109/15376516.2014.956912.

[16] K. K. Vekariya, J. Kaur, et K. Tikoo, « ERα signaling imparts chemotherapeutic selectivity to selenium nanoparticles in breast cancer », *Nanomedicine Nanotechnol. Biol. Med.*, vol. 8, nº 7, p. 1125-1132, oct. 2012, doi: 10.1016/j.nano.2011.12.003.

[17] S. Sadeghian, G. A. Kojouri, et A. Mohebbi, « Nanoparticles of Selenium as Species with Stronger Physiological Effects in Sheep in Comparison with Sodium Selenite », *Biol. Trace Elem. Res.*, vol. 146, nº 3, p. 302-308, juin 2012, doi: 10.1007/s12011-011-9266-8.

[18] M. Kumari *et al.*, « Curcumin loading potentiates the chemotherapeutic efficacy of selenium nanoparticles in HCT116 cells and Ehrlich's ascites carcinoma bearing mice », *Eur. J. Pharm. Biopharm.*, vol. 117, p. 346-362, août 2017, doi: 10.1016/j.ejpb.2017.05.003.

[19] L. Kong *et al.*, « The suppression of prostate LNCaP cancer cells growth by Selenium nanoparticles through Akt/Mdm2/AR controlled apoptosis », *Biomaterials*, vol. 32, nº 27, p. 6515-6522, sept. 2011, doi: 10.1016/j.biomaterials.2011.05.032.

[20] E. N. Ali, S. EL-sonbaty, et F. Salem, « Evaluation of selenium nanoparticles as a potential chemopreventive agent against lung carcinoma », *Int. J. Pharm. Biol. Chem. Sci.*, vol. 2, p. 38-46, janv. 2013.

[21] K. K. Vekariya, J. Kaur, et K. Tikoo, « ERα signaling imparts chemotherapeutic selectivity to selenium nanoparticles in breast cancer », *Nanomedicine Nanotechnol. Biol. Med.*, vol. 8, nº 7, p. 1125-1132, oct. 2012, doi: 10.1016/j.nano.2011.12.003.

[22] J. Pi *et al.*, « Selenium nanoparticles induced membrane bio-mechanical property changes in MCF-7 cells by disturbing membrane molecules and F-actin », *Bioorg. Med. Chem. Lett.*, vol. 23, nº 23, p. 6296-6303, déc. 2013, doi: 10.1016/j.bmcl.2013.09.078.

[23] M. Shakibaie, M. R. Khorramizadeh, M. A. Faramarzi, O. Sabzevari, et A. R. Shahverdi, « Biosynthesis and recovery of selenium nanoparticles and the effects on matrix metalloproteinase-2 expression », *Biotechnol. Appl. Biochem.*, vol. 56, nº 1, p. 7-15, mai 2010, doi: 10.1042/BA20100042.

[24] H. Luo, F. Wang, Y. Bai, T. Chen, et W. Zheng, « Selenium nanoparticles inhibit the growth of HeLa and MDA-MB-231 cells through induction of S phase arrest », *Colloids Surf. B Biointerfaces*, vol. 94, p. 304-308, juin 2012, doi: 10.1016/j.colsurfb.2012.02.006.

[25] S. Ramya, T. Shanmugasundaram, et R. Balagurunathan, « Biomedical potential of actinobacterially synthesized selenium nanoparticles with special reference to anti-biofilm, anti-oxidant, wound healing, cytotoxic and anti-viral activities », *J. Trace Elem. Med. Biol.*, vol. 32, p. 30-39, oct. 2015, doi: 10.1016/j.jtemb.2015.05.005.

Conclusion and Perspectives

This research is in line with the objectives of promoting health and consequently, sustainable development in Algeria. The present initiative is a contribution to a better understanding of the factors favoring the incidence of cancer, including a diet deficient in Se and other elements.

In fact, the incidence of papillary thyroid cancer (PTC) has grown significantly in recent years, and Algeria is not excluded. The relationship between nutritional status and thyroid cancer has been debated for a long time and the results of studies are inconclusive.

In this investigation, we observed a significant variation of Se status in patients with PTC: low concentrations of plasma Se and GPx3 activity are observed in patients compared to healthy controls, while in contrast, GPx1 activity is increased. This fluctuation could be a consequence of lack of Se in soil due to poor atmospheric import from the ocean (and consequent draught) in the last 10 years and change of cereal import from Se naturally rich US wheat to European wheat, and this could be the cause of various pathologies including cancer. It can also be explained by divers' polymorphisms changing the protein structure of selenoproteins, or oxidative stress present in the thyroid cell and not eliminated.

Our predictive model showed a significant association between low plasma Se level. However, the relationship between Se deficiency and thyroid cancer remains ambiguous and further studies are needed to clarify the exact metabolism.

Regarding Fe and Ca, their concentrations seem to be disturbed in thyroid cancer patients compared to healthy controls.

The assessment of Cu and Zn plasma levels showed higher Cu and lower Zn concentrations in PTC patients compared to healthy controls, yielding a highly significant difference in plasma Cu/Zn ratio. In addition, Cu isotopic composition was strongly different in thyroid cancer patients as compared to healthy controls. Notably, the $\delta^{65}Cu$ values differed in opposite direction for plasma vs. the thyroid tumor tissue samples. Our study supports the hypothesis that Cu/Zn and isotopic composition have a high potential to be used as additional biomarkers in the detection of thyroid cancer.

We have also tested different concentrations of selenite and SeNPs on PTC spheroids, a model of human papillary carcinoma (MDA-T120). We observed a significant reduction in cell viability compared to controls with a decrease in ROS production. We support the concept that SeNPs can be further investigated as a non-invasive and therapeutic option for the treatments in thyroid cancer, but before that we should understand its action mechanism.

The results of this thesis are conclusive and innovative. They show that the homeostasis of micro and macro elements is disturbed in cancer patients compared to controls. It is necessary to recognize the role of trace elements in the protection of the human being against the diseases of this century and to give an overview on the impact of a deficit, or an excess, of these elements on public health. This research will enrich epidemiological studies aiming at establishing meta-analyses which alone have the statistical power to provide recommendations.

Among future lines of research, we would recommend the following ones

1. **Soil mapping**

The most abundant source of micro and macro nutrients is generally obtained in part from local plant sources. It is important to map soil types according to Food and Agriculture Organization (FAO) classification and their trace element content, especially the Algerian soils for which few data are available, and to standardize this type of test in the future (like in the FOREG maps in Northern Europe) in order to evaluate the nutritional constitution of the soils and to be able to follow international recommendation.

2. **Wheat import**

The most important Se intake is from cereals. Algeria is importing wheat to cover a large part of dietary needs. Depending on where they grow, Se content in wheat differs. In the US Great plains, wheat is naturally rich in Se due to black shale it grows on. However, Algeria is now importing EU and Ukraina or Russia wheat. It would be important to quantify the Se provided by these different sources (and the volume imported in each case). Wheat import is already recognized as a “Virtual Water Flow” and such a study could allow to deverlop the “Virtual Selenium Flow” concept.

3. **Dietary intake**

It is necessary to determine the nutritional habits of patients, possibly with a follow-up and questionnaire of recall 24h, 72h or weekly. This allows to know not only the nutritional habits of the patients and in general population at risk, but also to evaluate if a sufficient intake of micronutrient according to Recommended Dietary Allowances "RDA" is observed. We can also compare with healthy people. Indeed, several studies had shown a significant association between obesity, BMI, and thyroid cancer.

4. **Mapped the trace elements present in the thyroid gland**

For decades, thyroid pathologies, such as cancer, have been linked to iodine. However, iodine is not the only micronutrient present in the gland, other elements are present and have a

crucial role in the proper functioning of the gland. With the new technologies and instruments such as Laser Induce Breakdown Spectroscopy (LIBS) one can detect even low concentrations of the order to 1 ppm. Therefore, one can consider mapping the different micronutrients in the gland and follow the concentration distributions. This new tool can, after further studies, provide an early clinical diagnosis and new sources of information for clinicians to understand the origin and development of pathologies.

5. **Genetic study**

A linkage study between polymorphisms of selenoproteins involved in the gland e.g., Thr92Ala (rs225014 T/C) and ORFa-Gly3Asp (rs12885300 C/T) polymorphisms in the DIO2 gene and proto-oncogenes (BRAF) by using sophisticate methods like Next Generation Sequencing (NGS), would be a major contribution to this field, as it could explain the relationship between Se and thyroid cancer.

Moreover, Se also can influence histone methylation, as already studied in ovarian cancer, and this can also be exploited in thyroid cancer.

6. **Se-NPs Se-Fe NPs and treatments**

We have found that Se-NPs reduced the viability of spheroid model of human papillary carcinoma and decreased the concentration of ROS, however other studies have suggested that the combined use of selenium and Iron Oxide Nanocomposites gave better effect on spheroid cells. We need to further investigate these effects at the molecular level in order to better understand the antitumor functions of SeNPs.

7. **Fe and ferroptosis in cancer development/treatment**

Activation of regulated cell death is a potential cancer treatment strategy. Ferroptosis induced by ROS contributes to tumor growth suppression. With ferroptosis activation, widely recognized as a novel target for drug discovery, an increasing number of small molecules can be identified to induce ferroptosis directly - or indirectly - by targeting Fe metabolism and lipid peroxidation. A better understanding of the mechanism of ferroptosis and the role of ferroptosis in cancer will create new opportunities for diagnosis and therapeutic intervention.

8. **Use of Isotope as early diagnosis tool**

In our work, we have demonstrated that there is a disturbance of copper isotope in patients, the concept is to follow the evolution of cancer and measure to the isotope at different times in the same patients (a cheap and non-invasive tool, to be used before radiotherapy, during and after). Since the isotopes have a small concentration in the human body, they reflect the cell

state and can be used as a biomarker for thyroid cancer and could be useful in the present type of treatment. We also need to increase the number of cases and biopsies in order to obtain strong correlations between the Se status and thyroid cancer. We also need to investigate new isotopes such as Se isotopes.

Annexes

Annex 1. Questionnaire

Code :

- Nom :
- Prénom :
- Date et lieu de naissance :
- Age :
- Adresse, ville :
- Numéro de téléphone :
- Etat civil :
- Nombre de frères et sœurs :

☐ Célibataire ☐ Marié ☐ Veuf ☐ Divorcé

- Nombre d'enfants :

1. Profession :
2. Salarié : Assuré :
3. Niveau d'instruction :

☐ P ☐ M ☐ S ☐ U ☐ Formation :

4. Habitation :

☐ Maison individuelle ☐ Collective ☐ Appartement

☐ Haouch : ☐ autre :

5. Consanguinité : ☐ 1 degré ☐ 2degré ☐ 3 degré autre
6. Groupe sanguin :
7. Motif d'admission :

ATCD familiaux : de Pathologie

1. Père :

2. Mère :
3. Frères / sœurs :
4. Grands parents :
5. Oncles /tantes :

ATCD Personnels de maladie :

2 Type de pathologie associée :

☐ HTA ☐ Diabète ☐ autres

5. L'âge de début :
6. Traitements entrepris :
7. Posologie :
8. Chirurgicaux :
9. Toxique :

☐ Alcool ☐ Drogue ☐ Cigarette

Histoire de la maladie :

1. Type d'affection de la thyroïde diagnostiqué :
   - ☐ Hypothyroïdie
   - ☐ Hyperthyroïdie
   - ☐ Thyroïde de Hashimoto
   - ☐ Goitre, hypertrophie de la glande thyroïde, nodule thyroïdien
   - ☐ Maladie de Basedow
   - ☐ Cancer de la glande thyroïde
   - ☐ Autre
   - ☐ Je ne sais pas
2. Date du diagnostic :
3. Traitements ou examens réalisés :
   - ☐ Iode radioactif
   - ☐ Ablation
   - ☐ Biopsie par aspiration
   - ☐ Chirurgie de la thyroïde

☐ Thyroïdectomie

☐ Autre

4. Date de chaque traitement ou examen :
5. Symptômes :
6. Médicaments pour traiter cette affection :
7. Information pertinente ou commentaire supplémentaire :
8. Complément alimentaire :

. Examen :

1. Poids :
2. Taille :
3. Tour de taille : Tour de hanche:
4. Bilan lipidique : Cholestérol Total : c-HDL : c-LDL : Triglycérides :
5. Bilan glucidique : glycémie : hémoglobine glyquée :
6. Bilan Hématologique (FNS) :
7. Bilan ionique :
8. Bilan hépatique :
9. Fonction rénale : urée : créatinine :

Annex 2. Consent Request Form

République Algérienne Démocratique et Populaire

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE

Université Abou-Bekr-Belkaid Faculté des sciences Département de biologie

Madame, Monsieur,

Vous participez à un projet de recherche portant sur le statut nutritionnel des patients atteints de cancer de la thyroïde. Dans le cadre de ce projet, un prélèvement d'échantillons de sang est proposé. Le volume de ce prélèvement est de 5 ml de sang.

La conservation de vos échantillons de sang s'effectuera dans des conditions strictes de confidentialité des données médicales sans que votre nom, votre prénom ou d'autres données directement nominatives ne soient transmises avec le prélèvement.

Conformément à la loi du 6juillet 1992 portant code de la déontologie médicale. Le protocole est lu est approuvé par le conseil régionale de déontologie médicale.

Après avoir lu cette information et pu poser toutes les questions que je jugeais utiles au médecin qui me suit.

J'accepte qu'un prélèvement de sang soit effectué conformément aux objectifs et aux méthodes décrits ci-dessus.
Prénom et nom du patient …….. : Date : .....

Signature :

Un exemplaire original signé de ce formulaire de consentement doit être conservé par le patient.

Abstract

The link between mineral elements and cancer has not yet been proven. Therefore, thanks to a biogeochemical approach, we undertook to enrich, by data not yet recorded the impact of certain elements and their various isotopes on thyroid cancer in Algerian population.

The first objective of this work is to study the profile of the selenium (Se) status in 47 patients of the west Algerian region, affected by thyroid carcinoma and 55 healthy controls. This status includes plasma Se content, the activity of glutathione peroxidases (GPx3 and GPx1) and the plasma content of selenoprotein P (SePP). The second objective is the use of copper isotope fractionation (δ^{65}Cu) as a new diagnostic tool for papillary thyroid carcinoma (PTC). Plasma levels of copper (Cu), zinc (Zn), iron (Fe), and calcium (Ca) were also studied. The last objective is the study of the impact of Se nanoparticles (Se-NPs) and selenite ions on the development of spheroids thyroid carcinoma metastasis (MDA-T120) models.

Plasma Cu concentrations were higher (1.35 ± 0.33 vs. 1.06 ± 0.22 mg/L, $p < 0.0001$), and Zn concentrations were lower (0.94 ± 0.20 vs. 1.03 ± 0.15 mg/L, $p < 0.05$) in patients compared with controls. Accordingly, the Cu/Zn ratio was significantly higher in PTC compared with controls ($p < 0.0001$). Plasma δ^{65}Cu levels in patients were significantly lower in PTC patients. Thyroid tumor tissues had high δ^{65}Cu values.The predictive model showed a significant association between low plasma Se level and the risk for PTC (OR = 5.02; 95% CI: 1.51 - 16.73; P = 0.009). Regarding plasma Fe and Ca concentrations, our results show significant differences in patients compared with controls. We found a significant reduction in cell viability in the presence of selenite and SeNP-chitosan, compared to controls with a decrease in reactive oxygen species production. The area under the ROC curve (AUC) obtained for Cu/Zn and δ^{65}Cu was 0.81 and 0.78, respectively ($p < 0.001$), indicating exceptional discrimination in favor of using Cu/Zn and δ^{65}Cu ratio as biomarkers of PTC.

The results of this thesis show that the homeostasis of minerals elements is altered in cancer PTC patients. This study suggests novel biomarkers for thyroid carcinoma diagnostic and the use of Se nanoparticles in the treatment of PTC.

Keywords: Selenium, Copper, Zinc, Iron, Calcium, δ^{65}Cu, Thyroid cancer, Selenium nanoparticles.

Résumé

Le lien entre les éléments minéraux et le cancer n'a pas encore été prouvé. Ainsi, grâce à une approche biogéochimique, nous avons entrepris d'enrichir, par des données non encore répertoriées, l'impact du statut de certains éléments et de leurs différents isotopes sur le cancer de la thyroïde dans la population algérienne.

Le premier objectif de ce travail est d'étudier le profil du statut en sélénium (Se) chez 47 patients de la région ouest algérienne, atteints de carcinome thyroïdien et 55 témoins sains. Ce statut comprend la teneur en Se du plasma, l'activité des glutathion peroxydases (GPx3 et GPx1) et la teneur plasmatique en sélénoprotéine P (SePP). Le deuxième objectif est l'utilisation du fractionnement isotopique du cuivre (δ^{65}Cu) comme nouvel outil de diagnostic du carcinome papillaire de la thyroïde (CPT). Les niveaux plasmatiques de cuivre (Cu), de zinc (Zn), de fer (Fe) et de calcium (Ca) ont également été étudiés. Le dernier objectif est l'étude de l'impact des nanoparticules de Se (SeNPs) et des ions sélénites sur le développement de modèles sphéroïdes de métastases de carcinome thyroïdien (MDA-T120).

Les concentrations plasmatiques de Cu étaient plus élevées (1,35 ± 0,33 vs. 1,06 ± 0,22 mg/L, $p < 0,0001$), et les concentrations de Zn étaient plus faibles (0,94 ± 0,20 vs. 1,03 ± 0,15 mg/L, $p < 0,05$) chez les patients par rapport aux témoins. En conséquence, le rapport Cu/Zn était significativement plus élevé chez les CTP par rapport aux contrôles ($p < 0,0001$). Les taux plasmatiques de δ^{65}Cu étaient significativement plus faibles chez les CTP patients. Les tissus tumoraux thyroïdiens présentaient des valeurs élevées de δ^{65}Cu. Le modèle prédictif a montré une association significative entre un faible taux plasmatique de Se et le risque de CPT (OR = 5,02 ; IC 95 % : 1,51 - 16,73 ; P = 0,009). En ce qui concerne les concentrations plasmatiques de Fe et de Ca, nos résultats montrent des différences significatives chez les patients par rapport aux témoins. Nous avons constaté une réduction significative de la viabilité cellulaire en présence de sélénite et de SeNP-chitosan, par rapport aux contrôles, avec une diminution de la production d'espèces réactives de l'oxygène. L'aire sous la courbe ROC (AUC) obtenue pour Cu/Zn et δ^{65}Cu était de 0,81 et 0,78, respectivement ($p < 0,001$), indiquant une discrimination exceptionnelle en faveur de l'utilisation du rapport Cu/Zn et δ^{65}Cu comme biomarqueurs du CTP.

Les résultats de cette thèse montrent que l'homéostasie des éléments minéraux est altérée chez les patients atteints de CTP, Cette étude propose de nouveaux biomarqueurs pour le diagnostic du carcinome thyroïdien et l'utilisation de nanoparticules de Se dans le traitement du CTP

Mots clés: Sélénium, Cuivre, Zinc, Fer, Calcium, δ^{65}Cu, Cancer de la thyroïde, Nanoparticules de sélénium.

الملخص

لم يتم بعد إثبات الصلة بين العناصر المعدنية والسرطان. لذلك، وبفضل المقاربة البيوجيوكيميائية، تعهدنا بإثراء تأثير بعض العناصر ونظائرها المختلفة على سرطان الغدة الدرقية لدى المجتمع الجزائري من خلال معطيات جديدة.

الهدف الأول من هذا العمل هو دراسة حالة السيلينيوم (Se) في 47 مريضا من منطقة غرب الجزائر، مصابين بسرطان الغدة الدرقية و 55 من الأصحاء. تشمل هذه الحالة محتوى البلازما Se ونشاط الجلوتاثيون بيروكسيداز (GPx3 و GPx1) ومحتوى البلازما من بروتين سيلينوبروتين P (SePP). الهدف الثاني هو استخدام تجزئة نظائر النحاس (δ^{65}Cu) كأداة تشخيصية جديدة لسرطان الغدة الدرقية الحليمي (CPT). كما تمت دراسة مستويات البلازما من النحاس (Cu) والزنك (Zn) والحديد (Fe) والكالسيوم (Ca). الهدف الأخير هو دراسة تأثير الجسيمات النانوية للسيلينيوم (Se-NPs) وأيونات السيلينيت على تطور ورم خبيث لسرطان الغدة الدرقية من الأجسام الشبه الكروية (MDA-T120).

كانت تركيزات النحاس في البلازما أعلى (1,35 ± 0,33 مقابل 1,06 ± 0,22 مغ/ ل، $p < 0,0001$)، وكانت تركيزات الزنك أقل (0,94 ± 0,20 مقابل 1,03 ± 0,15 مغ/ ل، $p < 0,05$) في المرضى مقارنة مع الضوابط . وفقًا لذلك، كانت نسبة Zn/Cu أعلى بشكل ملحوظ في CPT مقارنةً بالضوابط ($p < 0,0001$). كانت مستويات البلازما δ^{65}Cu أقل بكثير لدى المرضى. أنسجة ورم الغدة الدرقية كانت لها قيم δ^{65}Cu عالية. أظهر النموذج التنبئي ارتباطًا كبيرًا بين انخفاض مستوى البلازما Se وخطر الإصابة بـ CPT (OR = 5,02 ؛ IC 95 ٪ : 1,51 - 16,73 ؛ P =0,009). فيما يتعلق بتركيزات الحديد والكالسيوم في البلازما، تظهر نتائجنا اختلافات معنوية في المرضى مقارنة مع الضوابط. وجدنا انخفاضًا كبيرًا في قابلية بقاء الخلية في وجود السيلينيت و SeNP-chitosan، مقارنةً بالضوابط مع انخفاض في إنتاج أنواع الأكسجين التفاعلية. كانت المساحة الواقعة تحت منحنى ROC (AUC) التي تم الحصول عليها من أجل Zn/Cu و δ^{65}Cu 0,81 و 0,78 على التوالي ($p < 0,001$) ، مما يشير إلى تمييز استثنائي لاستخدام نسبة Zn/Cu وδ^{65}Cu كمؤشرات حيوية لـ CPT.

تظهر نتائج هذه الأطروحة أن توازن العناصر المعدنية قد تغير لدى مرضى السرطان. تقترح هذه الدراسة مؤشرات حيوية جديدة لتشخيص سرطان الغدة الدرقية واستخدام الجسيمات النانوية ل Se في علاج CPT.

الكلمات المفتاحية: السيلينيوم ، النحاس ، الزنك ، الحديد ، الكالسيوم ، δ^{65}Cu ، سرطان الغدة الدرقية ، جسيمات السيلينيوم النانوية