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Article

Determination of vitamin D3 in biological fluids by Sophonix Device that operates on chemiluminescent immunoassay (cLIA)

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Abstract: Vitamin D deficiency, affecting over a billion people globally, is now recognized as a steroid hormone crucial for immunity and bone health. Hashimoto's thyroiditis, an autoimmune thyroid condition, is linked to low vitamin D. This study compared vitamin D and calcium levels in 105 individuals from Baghdad (2023): healthy controls, hypothyroidism, and hyperthyroidism patients. Results revealed a significant decrease in vitamin D levels in the hyperthyroidism group compared to the healthy group (p-value = 0.008), while the hypothyroidism group showed no statistically significant difference (p-value = 0.056). Calcium levels were significantly reduced in hypothyroidism patients (p-value = 0.000) and moderately reduced in hyperthyroidism (p-value = 0.000).Hyperthyroidism was characterized by low serum TSH levels alongside elevated T4 and T3, whereas hypothyroidism showed the highest TSH levels and reduced T4. These findings highlight a clear association between thyroid disorders and imbalances in vitamin D and calcium, emphasizing the need to monitor these parameters in diagnosis and management.

Keywords: Vitamin D3, calcium, TSH, T3, T4.

Introduction

Vitamin D

Vitamin D refers to a group of fat-soluble secosteroids that play a key role in enhancing the intestinal absorption of calcium, magnesium, and phosphate, along with numerous other biological effects (Holick, 2006). There are several forms of vitamin D (D1 to D5), but the most important for humans are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) (Grundmann, von Versen-Höynck, & endocrinology, 2011).

Vitamin D is synthesized in the skin upon exposure to sunlight (as vitamin D3), while only about 5–10% of it is obtained through dietary sources (vitamin D2 and D3))Amithabh, Gireesh Kumar, Kumar, Selvapandian, & Baskar, 2025). Ultraviolet Blight (290-320 nm) is the primary source of vitamin D. When the skin is exposed to sunlight, 7-dehydrocholesterol is converted into vitamin D3 (Matsuoka et al., 1992). This vitamin D3 is then processed in the liver by the enzyme vitamin D 25-hydroxylase, transforming it into 25-

hydroxyvitamin D (25(OH)D), also known as calcidiol or calcifediol. In the kidneys, the active form of vitamin D3, 1,25-dihydroxyvitamin D (1,25(OH)2D), also referred to as calcitriol, is produced from 25(OH)D through the action of the enzyme α-hydroxylase, which is encoded by the CYP27B1 gene (Bikle & biology, 2014).

Vitamin D status is primarily assessed by measuring serum levels of 25(OH)D. The key function of calcitriol (the active form of vitamin D3) is to regulate calcium and phosphate concentrations. Calcitriol enhances the absorption of calcium and phosphate in both the intestines and kidneys and is essential for bone mineralization (van Driel, van Leeuwen, & endocrinology, 2017).

In addition to this, calcitriol plays a role in the regulation of cell growth, as well as immune and neuromuscular functions (Chakraborti, 2011). Low vitamin D levels have been linked to autoimmune thyroid diseases (AITD) such as Hashimoto's thyroiditis (HT) and Graves' disease (GD). Moreover, impaired vitamin D signaling has been suggested to contribute to thyroid tumorigenesis (Kim, 2017).

Thyroid

The thyroid gland produces thyroid hormones essential for the proper functioning of physiological systems. The hypothalamus-pituitary-thyroid (HPT) axis regulates the synthesis of these hormones through feedback mechanisms. When thyroid hormone levels drop, the hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary to increase the secretion of thyroid-stimulating hormone (TSH). TSH then acts on thyrocytes, promoting the production of thyroid hormones (Jonklaas, Tefera, & Shara, 2018).

The synthesis of thyroid hormones requires the active uptake of iodide via the sodium/iodide symporter (NIS), the production of thyroglobulin (Tg), and the iodination of Tg by the enzyme thyroid peroxidase (TPO) (Fekete & Lechan, 2014). When thyroglobulin is broken down, the thyroid hormones triiodothyronine (T3) and thyroxine (T4) are released. Although the thyroid produces more T4 than T3, the ratio is approximately 14:1 (Dayan & Panicker, 2009).

Vitamin D in Autoimmune Thyroid Diseases

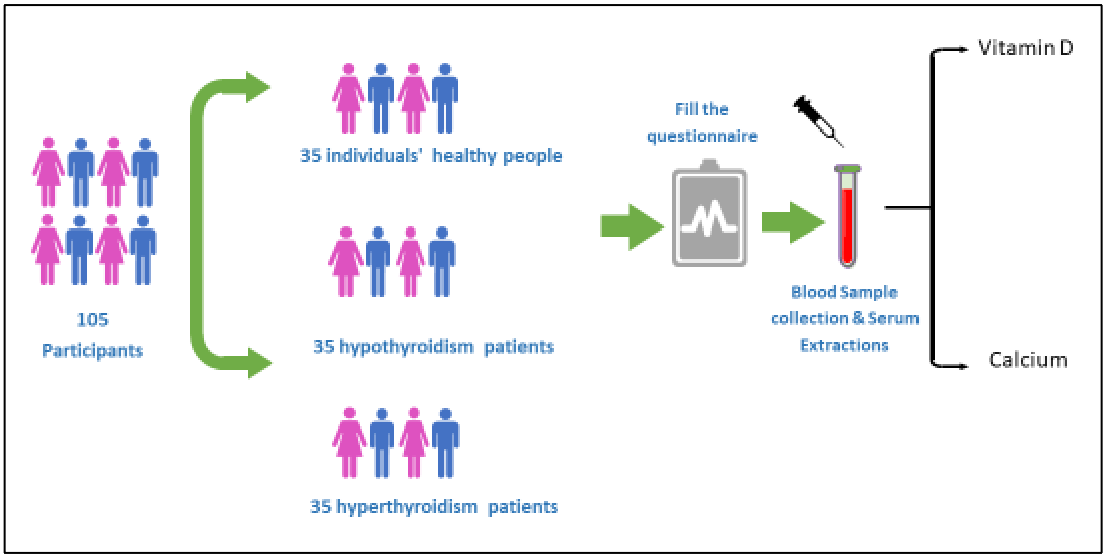
Autoimmune thyroid diseases are characterized by an immune system attack on the thyroid gland (Klecha, Barreiro Arcos, Frick, Genaro, & Cremaschi, 2008). These conditions are among the most common autoimmune disorders, with an approximate prevalence of 5%. The two primary types of autoimmune thyroid diseases are Hashimoto's thyroiditis, which leads to hypothyroidism, and Graves' disease, which results in hyperthyroidism. Both are T-cell-mediated autoimmune disorders marked by lymphocytic infiltration of the thyroid (Fountoulakis & Tsatsoulis, 2004). Vitamin D supplementation has shown beneficial effects in animal models of Graves' disease and thyroiditis. Numerous human studies have also been conducted to explore the role of vitamin D in autoimmune thyroid diseases (Zhao et al., 2025). Genetic research has identified that polymorphisms in the vitamin D receptor (VDR) and other genes involved in vitamin D signaling are linked to an increased risk of autoimmune thyroid diseases (Kurylowicz et al., 2006).

The incidence of thyroid cancer is on the rise, affecting both follicular thyroid cells and neuroendocrine cells. Studies, both in vitro and in vivo, have demonstrated a beneficial effect of vitamin D in the treatment of thyroid cancer. In vitro, research has shown that calcitriol and its analogue, MART-10, can inhibit cell proliferation (Feldman, Krishnan, Swami, Giovannucci, & Feldman, 2014).

The Study Plan

Five milliliters of blood from thirty samples from both genders were divided into three groups: healthy people, hyperthyroidism patients, and hypothyroidism patients. The samples were collected after questionnaires to correspond with the samples obtained for the study. The samples were placed in a centrifuge to separate the blood and obtain serum; the last step started measuring the Vitamin D as shown in Figure 1-1.

Figure (1-1) The scheme explains the study plan with the steps collected and the parameters



The Study's Aim

In this study, approximately 105 cases were studied, which were distributed among healthy people, hypothyroidism patients, and hyperthyroidism patients.

This study assesses the effect of thyroid disorders on the levels of vitamin D and calcium in the blood of healthy people, hypothyroidism patients, and hyperthyroidism patients.

METHODOLOGY:

Materials and Method:

In this study, samples were collected over two months, starting in November. Seventy samples from both genders of thyroid disorder patients (hypothyroidism and hyperthyroidism) with a range of ages (40 to 80) years were collected from Al-Amal National Hospital (specialized in treating cancerous tumors), depending on them (T3, T4, TSH), and thirty-five other samples of healthy people with a mean age (31 ± 9) years were collected from individuals from different areas in Baghdad randomly after obtaining people's approval.

Regarding gender: In all groups (normal, hyperthyroidism, and hypothyroidism), it indicates that the vast majority of people in these groups are female.

A 5 ml sample was taken in a gel tube after separating the sample in the Fusion Center. The serum was isolated using Eppendorf and placed in the refrigerator until work was done. Note that the sample was sufficient to perform all of the T3, T4, TSH, vitamin D3, and calcium analyses. 10 µL was consumed to perform the T3 analysis, 100 µL to perform the TSH analysis, 10 µL to perform the T4 analysis, 50 µL to perform the vitamin D3 analysis, and 10 µL to perform the calcium analysis.

ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of human T3, T4, and TSH in serum, plasma, and cell culture media.

The Sophonix device was used to determine vitamin D, which depends on the principle of its operation on chemiluminescent immunoassay (cLIA). The serum was inserted into the device for 17 minutes, and then the results were given.

The cobas c 311 analyzer device was used to determine calcium, which depends on the principle of its operation that measures the amount of photons (the intensity of light) absorbed after it passes through the sample solution.

The samples of people using nutritional supplements were excluded to ensure more accurate vitamin D3 and calcium values.

Corrected total calcium is adjusted based on the levels of albumin in the blood using the following formula:

Corrected Calcium = Measured Calcium + 0.8 × (4.0 − Albumin)

Where Measured Calcium: The calcium level measured in the test. Albumin: The albumin level in g/dL. The value "4.0" represents the normal albumin level (Lubna A.A. Al-Assaf, 2024).

The equation for ionized calcium to account for total calcium is adjusted based on the levels of albumin in the blood using the following formula:

Ionized Calcium = (0.55 * total calcium) - (0.3 * Albumin)]

Where 0.55 = empirically derived correction factor, valid for a pH range of 7.2 to 7.6.

The ionized calcium correction equation based on albumin changes is not as common as the total calcium correction, because ionized calcium represents free calcium that is not bound to proteins. However, it is used in most studies because although the ionized calcium correction equation for pH effects is used to estimate the normal level based on changes in pH, the common equation is based on the principle: if the pH is less than 7.4 (acidity), the corrected ionized calcium is higher than measured. If the pH is higher than 7.4 (alkalinity), the corrected ionized calcium is lower than measured.

This equation is useful in cases where the pH changes significantly, such as acidosis or alkalosis. The values should be interpreted with caution based on the clinical situation. Therefore, an estimation equation inspired by the correction of total calcium from the theoretical effect of albumin on it was used:

Corrected Ionized Calcium =Measured Ionized Calcium + [0.02 × (4.0 − Albumin)]

4.0: Normal value for albumin level.

Finally, it should be noted that this equation is used less commonly since ionized calcium is not directly affected by albumin like total calcium.

It is always preferable to rely on the results of directly measured ionized calcium, because corrections may be less accurate. Kits from Thermo Fisher Scientific were used.

Statistical Analysis:

IBM SPSS version 27 was used for the statistical analysis. The independent t-test was used to analyze the difference between thyroid disorder patients (hypothyroidism and hyperthyroidism) in addition to healthy individuals. A statistically significant value was defined as $p<0.05$, and a highly significant value was defined as $p<0.01$.

THE RESULTS AND DISCUSSIONS

The Results

Table (3-1) displays the results of the sophonix device's emission of the serum samples for vitamin D. The normal range for vitamin D concentration in blood serum is between 30 and 50 ng/ml. It contains data for several vital signs in three groups: the normal group and the groups related to hypothyroidism and hypothyroidism.

Regarding TSH (thyroid hormone): In the normal group, the average TSH level is 1.64 (within the normal range of 0.27-4.20). In the hyperthyroidism group, the TSH value is very low (0.09). In the hypothyroidism group, the TSH value is significantly high (9.27).

In the T3 (triiodothyronine) analysis: In the normal group, the average T3 level is 2.05. In the hyperthyroidism group, it is 2.03. In the hypothyroidism group, it is 1.88 (within the normal range of 1.2-3.10) for the samples taken.

As for the T4 (thyroxine) analysis: It was found that in the normal group, the average T4 level is 84.2. In the hyperthyroid group, the T4 level is significantly elevated to 163.67 (within the normal range of 66-181) for the samples obtained. While in the hypothyroid group, the T4 level decreases to 26.4.

Regarding the analysis of total calcium (Ca): In the normal group, the average calcium level is 9.22 (within the normal range of 8.5-10.5) while in the hyperthyroid group, it increases to 10.82 and in the hypothyroid group, it decreases to 7.51.

Vitamin D3 (VitD3): In the normal group, the vitamin D3 level is 24.49 (less than the normal range of 30-50) Although the Iraqi lands are sunny now, people are not exposed to the sun due to the hot weather and the nature of the clothing in the country, making people less exposed to the sun, noting that to get your sufficient needs of vitamin "D", it is recommended to be exposed directly to the sun at the right times.

During the summer, exposure to sunlight can be done from 8:30 am to 10:30 am, and from 2 pm to 4 pm. In the winter season, it can be done from 10 am to 2 pm because the wavelength of ultraviolet B (UVB) rays is 290-220 nm during this period, which is necessary for the skin for 20 minutes, four times a week (Papadopoulou et al., 2025). In the hyperthyroid group, it decreases further to 13.07. Compared to the hypothyroid group, the average vitamin D3 is 16.54.

Albumin (Alb.): For the three groups, and therefore to obtain the corrected and ionized calcium values, the level was observed in the normal group, the average albumin is 4.49. In the hyperthyroid group, it is 4.23. In the hypothyroid group, it is 4.53. (Within the normal range 3.4-5.4).

(Correct total Ca): In the normal group, it is 8.83. (Within the normal range 8.5-10.5) In the hyperthyroid group, it increases very slightly to 10.63. While in the hypothyroid group, it decreases significantly to 7.09.

Ionized calcium: In the normal group, it is 3.73. (Below the normal range 4.5-5.3) In the hyperthyroid group, it is 4.68, which is within the normal range while it decreases to 2.77 Hypothyroid group.

Corrected ionized calcium: In the normal group, it is 3.51. (Below the normal range 4.5-5.3) In the hyperthyroid group, it is 4.58. In the hypothyroid group, it decreases to 2.54.

Table (3-1) The emissions of serum samples in sophonix for Vitamin D and calcium in cobas c311

Dependent Variable	Group	Normal range	Mean	Std. Deviation	Std. Error
Age	Normal	-	31	9	-
	Hyperthyroidism		61	14	-
	Hypothyroidism		45	13	-
TSH	Normal	0.27-4.20	1.64	0.80	0.25
	Hyperthyroidism		0.09	0.06	0.02
	Hypothyroidism		9.27	3.38	0.95
T3	Normal	1.2-3.10	2.05	0.48	0.15
	Hyperthyroidism		2.03	0.58	0.18
	Hypothyroidism		1.88	0.54	0.17
T4	Normal	66-181	84.20	15.44	4.88
	Hyperthyroidism		163.67	26.62	8.87
	Hypothyroidism		26.40	8.22	2.60
Ca	Normal		9.22	0.48	0.15

	Hyperthyroidism	8.5-10.5	10.82	0.34	0.11
	Hypothyroidism		7.51	0.74	0.23
VitD3	Normal	30-50	24.49	6.42	2.03
	Hyperthyroidism		13.07	5.32	1.68
	Hypothyroidism		16.54	12.98	4.11
Alb.	Normal	3.4-5.4	4.49	0.54	0.17
	Hyperthyroidism		4.23	0.49	0.15
	Hypothyroidism		4.53	0.70	0.22
Correct total Ca	Normal	8.5-10.5	8.83	0.63	0.20
	Hyperthyroidism		10.63	0.41	0.13
	Hypothyroidism		7.09	0.75	0.24
Ionized calcium	Normal	4.5-5.3	3.73	0.30	0.10
	Hyperthyroidism		4.68	0.19	0.06
	Hypothyroidism		2.77	0.39	0.12
Corrected ionized calcium	Normal	4.5-5.3	3.51	0.47	0.15
	Hyperthyroidism		4.58	0.34	0.11
	Hypothyroidism		2.54	0.53	0.17

∗ The range for vitamin D if <5 ng/ml is a very severe deficiency, between 5-10 ng/ml severe deficiency, between 10-20 ng/ml deficiency, while suboptimal between 20-30 ng/ml, optimal between 30-50 ng/ml, in addition between 50-70 ng/ml is upper normal, but >150 ng/ml is overdose (Yousif & Muhsin, 2019).

The listed results indicate statistically significant differences between groups (Normal, Hyperthyroidism, Hypothyroidism) in some variables. The following is a brief explanation of the results based on the statistical values:

1. Gender: Although most of the samples obtained were from women, we were able to find the following results: There are no statistically significant differences between groups in terms of gender (Sig. = 0.673), as it was proven that gender does not affect the change in values.

2. Age: We regret that there is an age difference between the groups, as healthy people were younger than those with thyroid disorders due to its widespread spread in Iraq recently, which added to the difficulty of obtaining healthy samples from elderly people, in addition to the difficulty of the approvals we need to obtain the sample and the unwillingness of the people themselves to conduct the analysis. In general, a large statistically significant difference was observed between Normal and Hyperthyroidism (Sig. = 0.000). While there was a statistically significant difference between Normal and Hypothyroidism (Sig. = 0.018).

3. TSH: No statistically significant difference between Normal and Hyperthyroidism (Sig. = 0.073). However, the difference was statistically significant between Normal and Hypothyroidism (Sig. = 0.000).

4. T3: No statistically significant difference between Normal and Hyperthyroidism (Sig. = 0.941). The same was the case between Normal and Hypothyroidism (Sig. = 0.489).

5. T4: A statistically significant difference between Normal and Hyperthyroidism (Sig. = 0.000). The same statistically significant difference was given between Normal and Hypothyroidism (Sig. = 0.000).

6. Calcium (Ca): A statistically significant difference between Normal and Hyperthyroidism (Sig. = 0.000). A statistically significant difference between Normal and Hypothyroidism (Sig. = 0.000).

7. Vitamin D3: Statistically significant difference between Normal and Hyperthyroidism (Sig. = 0.008). While no statistically significant difference between Normal and Hypothyroidism (Sig. = 0.056).

8. Albumin (Alb.): No statistically significant difference between Normal and Hyperthyroidism (Sig. = 0.34). No statistically significant difference between Normal and Hypothyroidism (Sig. = 0.89). As expected, albumin was studied to measure other parameters related to calcium as mentioned in the practical part.

9. Corrected, ionized, and corrected ionized calcium: These three parameters gave statistically significant differences between Normal and Hyperthyroidism as well as Normal and Hypothyroidism with values (Sig. = 0.00 for both), as shown in table (3-2).

Table (3-2): Comparisons between the studied vitamin D and calcium of thyroid disorders patients' group and the healthy group.

Dependent Variable	Group		Sig.
Gender	Normal	Hyperthyroidism	0.673
		Hypothyroidism	0.673
Age	Normal	Hyperthyroidism	0.000
		Hypothyroidism	0.018
TSH	Normal	Hyperthyroidism	0.073
		Hypothyroidism	0.000
T3	Normal	Hyperthyroidism	0.941
		Hypothyroidism	0.489
T4	Normal	Hyperthyroidism	0.000
		Hypothyroidism	0.000
Ca	Normal	Hyperthyroidism	0.000
		Hypothyroidism	0.000
VitD3	Normal	Hyperthyroidism	0.008
		Hypothyroidism	0.056
Alb.	Normal	Hyperthyroidism	0.34
		Hypothyroidism	0.89
Correct total Ca	Normal	Hyperthyroidism	0.00
		Hypothyroidism	0.00
Ionized calcium	Normal	Hyperthyroidism	0.00
		Hypothyroidism	0.00
Corrected ionized calcium	Normal	Hyperthyroidism	0.00
		Hypothyroidism	0.00
*. The mean difference is significant at the 0.05 level.			

The Dissuasion

During the review of the results, it was noted that there were statistically significant age differences (described in detail above), =, thyroid hormone T4, calcium, corrected calcium, ionized calcium, and corrected ionized calcium between the groups.

While variables such as gender, thyroid hormone T3, and albumin (the reasons for measuring this parameter were explained above) did not show statistically significant differences.

As for TSH, it showed a significant difference with hypothyroidism as expected, while vitamin D3 showed a significant difference only between healthy people and hyperthyroidism, while it was not given with hypothyroidism.

Two mechanisms may explain why hypothyroid patients often have low serum levels of vitamin D. One possibility is that low vitamin D levels result from poor absorption in the intestines, while the other is that the bodies of these patients may not properly activate vitamin D (Talaei, Ghorbani, Asemi, & metabolism, 2018).

Low vitamin D levels have also been linked to autoimmune thyroid diseases (AITD), such as Hashimoto's thyroiditis (HT) and Graves' disease (GD). Furthermore, impaired vitamin D signalling has been associated with an increased risk of thyroid tumorigenesis (Jara et al., 2025). Both previous studies confirmed the validity of the current study, as it built the reasons for the decrease in vitamin D3.

This is because vitamin D deficiency impairs various functions of the immune system, which in turn increases the likelihood of developing serious autoimmune diseases that target the thyroid gland, leading to its dysfunction (Mackawy, Al-Ayed, & Al-Rashidi, 2013).

The results of this study in TSH analysis between hypothyroidism and healthy groups. Furthermore, T4 and Ca analysis showed significant change among the three groups, while T3 showed no change at all. This result is consistent with the result of Vos, X. G. (2016) (Vos et al., 2016).

The symptoms of hypothyroidism can be different from person to person, the result showed; the highest range of TSH in the hypothyroidism group, more than in normal and hyperthyroidism. Our result agreed with the result of Chen MD (2016) (Chen, Sheu, & biology, 2001).

Several experimental and clinical studies suggest that calcium (Ca) depletion can lead to elevated blood pressure (Rajasree et al., 2001). While changes in various electrolytes, including magnesium (Mg) and calcium, may be modest in some thyroid disorders and might not pose an immediate problem for the patient, these imbalances could be significant in the long term (Baig, Alghalayini, Gazzaz, & Murad, 2024). A potential link between elevated serum levels of substances like calcium and the development of cardiovascular disease has also been observed (SEGAL, 1990).

CONCLUSIONS

Low vitamin D levels in hypothyroid patients are often due to poor intestinal absorption or poor activation of vitamin D. Autoimmune thyroid disorders such as Hashimoto's disease and Graves' disease may be associated with low vitamin D levels.

Poor vitamin D signaling may increase the risk of thyroid tumours. Vitamin D deficiency causes immune system dysfunction, which increases the risk of autoimmune thyroid diseases.

Data show that thyroid disorders (hyperthyroidism or hypothyroidism) affect several biological measures. In hyperthyroidism, TSH levels are typically low while T3 and T4 levels are elevated. In hypothyroidism, TSH levels are elevated while T3 and T4 levels are low.

The study found significantly higher TSH levels in the hypothyroid group compared to the normal thyroid and hyperthyroid groups, which is consistent with previous research. Significant differences were observed in T4 and Ca levels of all types among the three groups. No significant change in T3 levels was observed between groups, which is consistent with a previous study.

Calcium deficiency can lead to high blood pressure. While electrolyte imbalances, including calcium and magnesium, can have long-term health consequences, albeit minor in some cases. Calcium and vitamin D3 are significantly affected in both conditions. But the gist of the matter, it highlights the complex relationship between hypothyroidism, vitamin D levels, and other factors such as calcium and immune function. It emphasizes the potential for significant health effects, including an increased risk of autoimmune diseases and cardiovascular problems.

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ETHICAL CLEARANCE: This study was conducted in compliance with ethical standards and guidelines for research involving human subjects. Ethical approval for the study was obtained from [the ethics committee or institution], ensuring that all procedures adhered to the Declaration of Helsinki principles. Informed consent was obtained from all participants before sample collection. Participants were provided with detailed information regarding the study's objectives, the procedures involved, and their right to withdraw at any point without any consequences. Confidentiality of the participants' data was strictly maintained throughout the study, with all personal information anonymized during analysis and reporting. Additionally, all samples were collected and processed in a manner that prioritized the safety and comfort of participants, ensuring that no undue harm or discomfort occurred during or after the study procedures.

CONFLICT OF INTEREST: There is no conflict of interest associated with this article's publication.

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