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Quantitative analysis of the steroidal calcitriol-mediated regulation of the serological components responsible for IDA and TSH disorders in reproductive and nonproductive women

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Abstract

Anemia and thyroid disorders are global health issues that affect all ages but are more apparent in women. In this case, some serological components responsible for IDA and TSH disorders in women have been found actively regulated through a complex steroidal-calcitriol mediated pathway. This research has been investigated the correlation between Calcitriol and the serological components responsible for IDA and TSH disorders in childbearing and non-child-bearing women of different health conditions. Experimental sampling from 452 women suffering from both IDA and TSH disorders were taken, aged between 0 and 70 years. Serological parameters, such as iron, total iron-binding capacity and ferritin, were assessed for IDA profiling, whereas thyroid-stimulating hormone and free thyroxin were for TSH profiling based on the individual's serum calcitriol status. The resulted serological data were interpreted using sophisticated computer programming language and algorithms for quantitative biochemical analysis. The study resulted in a significant correlation between FT4 and Calcitriol ($P<0.0001$) for all age groups. TSH also showed strong interactions with the fluctuation of calcitriol level ($P<0.0001$), except for the children aged below 10 years ($P<0.063$). The iron, TIBC, TSH, and FT4 showed phenomenal regulation with the steroidal-calcitriol concentration for congenital patients. Unlike the others, ferritin has a substantial connection with Calcitriol ($P<0.0064$) fluctuation in the serum. To ratify, the concentrations of TSH, FT4, iron, TIBC, and ferritin were found to be significantly interconnected in terms of serum calcitriol level in women suffering from IDA and TSH disorders simultaneously. To understand the accuracy and efficacy of the Calcitriol in IDA and TSH disorders, some other inflammatory markers and parathyroid hormone analysis are need in future studies, besides a large number of samples.

Keywords: TSH disorder, IDA, TIBC, ferritin, FT4, Calcitriol mediated regulation, reproductive and non-reproductive women, quantitative serological assessment.

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Introduction

Anemia is a global public health concern nowadays that affects developing countries and developed countries with the major consequences of human health hazards. According to the previous data, it affects one-quarter of the global population, with pregnant women and young children having a higher prevalence rate than men [1]. Turning to the factors, iron deficiency is the major cause of poor nutrition, which correspondingly results in severe Anemia with the consequence of both mother and child's death [2]. Iron is vital to all biological functions, including DNA synthesis, respiration, cell proliferation, energy production, and so on [3]. Over 2 billion people are affected by iron deficiency worldwide [4, 5], and the ubiquity of Anemia among pregnant women and young children due to Iron depletion has been well documented [6, 7]. Age is also significantly correlated with IDA in females of childbearing and pregnancy [8]. Additionally, premenopausal women who stick to a restrictive diet and usually intake a little amount of iron are mostly at risk of iron deficiency since they also lost iron during their menstrual cycle; however, in 2002, WHO reported iron deficiency anemia (IDA) as one of the most important factors to the global burden of disease [9]. Therefore, frequent screening of IDA is very significant. Numerous iron indicators are used to screen for IDA, and a potential example is how serum ferritin can be used as a diagnostic tool in clinical practice [10]; a combination of two serum transferrin markers are used in detecting IDA in regular hemodialysis anemic patients [11].

Thyroid dysfunction is also one of the most prevalent endocrine disorders worldwide [12]. Globally about 1.6 billion people are at risk of developing thyroid disorder due to iodine deficiency [13]. There are two types of thyroid disorder means- hyperthyroidism and hypothyroidism. Iron status in humans is inextricably related to thyroid function. IDA deteriorates thyroid metabolism and retards the physical and mental development of both young and adult individuals. Based on several investigations, both ID and Anemia are interconnected with hypothyroidism which significantly increases serum TSH levels and decreases serum iron, serum ferritin, Free T4, transferrin, RBC count, and so on [14-18]. Ferritin is a universal protein that acts as an iron carrier, and serum ferritin level is negatively correlated with serum TSH levels [19, 20]. In addition to iron and ferritin, Values of TIBC, FT3, and FT4 were significantly lower in hypothyroid patients suffering from IDA [21, 22]. This lower serum ferritin level is also

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associated with reducing sex hormones along with TSH, which exaggerates another endocrine dysfunction [23]. To summarize the inter-connection, on the one hand, deficiency of iron can produce hypothyroidism [24]. On the other hand, alterations in thyroid status change serum iron metabolism and hematological index [16].

Calcitriol is an activated form of vitamin D, which is semi-synthetic and hormonally active. Vitamin D is a fat-soluble steroid hormone mainly produced in the skin when exposed to sunlight. Vitamin D may also be acquired from the ingested diet to a minor extent [25]. To explain further, vitamin D biosynthesis in the skin becomes initiated by UV rays of sun lights which convert 7-dehydrocholesterol to pre-vitamin D3; then, it is thermally isomerized to vitamin D3. Calcitriol is the main vitamin D metabolite and is the best determiner of vitamin D nutritional status in the flow. However, this metabolite is not the active form of vitamin D, which requires a further activation step, and this happens in the presence of the catabolic enzyme 25OHD-1-α-hydroxylase (CYP27B1) to finally generate Calcitriol (1,25-(OH)2D3) [26, 27]. In recent years, Calcitriol has taken close attention because its deficiency entails the risks of various human diseases such as gestation-associated disorders [28]. According to previous studies, Calcitriol (activated vitamin D) insufficiency affected over one billion people globally [29, 30]. The lower calcitriol levels are associated with higher serum TSH levels [25] and lower hemoglobin and ferritin levels [31]. Studies highly suggested the supplementation of vitamin D in case of hypothyroidism and Anemia, along with the high recommendation for the screening of the vitamin D deficiency in all hypothyroid patients [25, 32]. It is practically required for monitoring iron nutritional status as it exaggerates thyroid disorders in reproductive age and pregnant women [14]. Thyroid dysfunctions must also be considered by physicians treating Anemia to ensure early detection and proper treatment [33]. Based upon various factors like lifestyle, climatic conditions, sun exposure, and diet, vitamin D supplementation should be determined. Literature reveals that there is no study conducted yet on Bangladeshi patients suffering from iron deficiency anemia and thyroid deterioration, especially in women.

Considering all the aforementioned facts, this current study aims to evaluate the relationship between Calcitriol, IDA, and TSH disorders. At the same time, the serological significance of Calcitriol was determined in regulating both the iron contents and thyroid cycle of women of all ages simultaneously. In addition, a new dimension of diagnosing and studying the serological

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status of the anemic and thyroid patients depending on their calcitriol profiles can be achieved, through which it should be clinically suggestive as a steroidal biomarker for IDA and TSH disorders.

Materials and methods

Clinical diagnosis

The current research started with diagnosing serum calcitriol levels [34] of the 452 women suffering from IDA and TSH simultaneously, as suggested by their doctors and 6 healthy women, to be used as control. To analyze the regulatory effects of Calcitriol on the IDA and TSH disorders among the patients, several specific serological parameters were considered, such as serum iron (µg/dl), total iron-binding capacity (TIBC, µg/dl), and ferritin (ng/dl) for IDA confirmation. Whereas, serum thyroid-stimulating hormone (TSH, µIU/ml) and free thyroxine (FT4, ng/dl) were assessed to determine the TSH disorders. The iron (µg/dl) and TIBC (µg/dl) level of the patients' serum were quantitatively analyzed using 'Dimension®IRON Flex® reagent cartridge (DF85)' and 'Dimension®Flex® Reagent IBCT' (Siemens Healthcare Diagnostics Inc., USA) respectively, following the referred methodology [35, 36]. The ferritin level (ng/dl) was tested quantitatively with 'Beckman Coulter Access Ferritin Calibrators (S0-S5)' considering its established protocol [36, 37]. On the other hand, 'ADVIA®Centaur™ TSH-3 Kit' (Siemens, USA) and 'ADVIA®Centaur™ FT4 Kit' (Siemens, USA) were used to diagnose TSH (µIU/ml) and FT4 (ng/dl) respectively. Different patient groups were classified following their age, reproductive status, and past clinical history in all aspects. All of those serum components were tested depending on their respective calcitriol level counts to identify if there were any precise correlation with Calcitriol or not. For transparent analysis, clinical data of the same serological tests and the calcitriol profiling of several normal women were also used as a control to compare with the IDA and TSH patients. The ethical clearance was officially taken from the Committee of Ethical Issues of JMC, Bangladesh Medical and Dental Council (BMDC), with the Principal's authorization and the Pathology Unit's Supervisor.

Post-diagnostic quantitative assessment

To study the quantitative interactivity of the aforementioned serum components based on the individuals' calcitriol level, different bioanalytical parameters were preferred, including- two-way ANOVA test, Brown-Forsythe test, Bartlett's test, and Tukey's multiple comparisons test 'p

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values,' as the primary factors. In addition, least-square mean (LSM), mean difference (MD); standard error of the difference (SED); the difference between predicted means (DBPM), and 95% CI of difference (95% CID) were analyzed as the secondary parameters. In this study, Calcitriol was assigned to be considered as a serological marker only if its quantitative values belong to $P<0.02$ in all the primary and secondary parameters, to each of the selected serum components; so that the analysis can be more authentic as compared to the $P<0.05$ scale [38].

Software tools for data analysis and validation

The biostatistical analysis and computational algorithms were performed using computational 'R Programming Scripts' (version R-4.0.2, for Linux) [39, 40] and 'GraphPad Prism' (version 8.1.2, for Mac OS) [41, 42, 43] premium software packages.

Results

In this research, the concentrations of TSH, FT4, iron, TIBC, and ferritin level were found regulating with the concentrations of the serum calcitriol level of the corresponding women suffering from TSH and IDA disorders. Individuals patients belonging between 11 to 70 years possess a very strong correlation of both the TSH and FT4 level ($P<0.0001$) with their respective calcitriol counts (in the scale of significance $P<0.02$) **(Figure 1)**. Though there is an insignificant relationship between the TSH and calcitriol level ($P<0.063$) among the patients ranged between 0 to 10 years **(Figure 1A)**, the correlation between FT4 and Calcitriol is highly significant ($P<0.0001$) for the same age group **(Figure 1B)**. The upper and lower values of TSH and FT4 are- 3.1 (µIU/ml), 1.2 (µIU/ml), and 1.25 (ng/dl), 1.01 (ng/dl) respectively, for the women aged below 10 years. In both aspects, the calcitriol upper and lower values are- 25.3 (ng/ml) and 8.19 (ng/ml).

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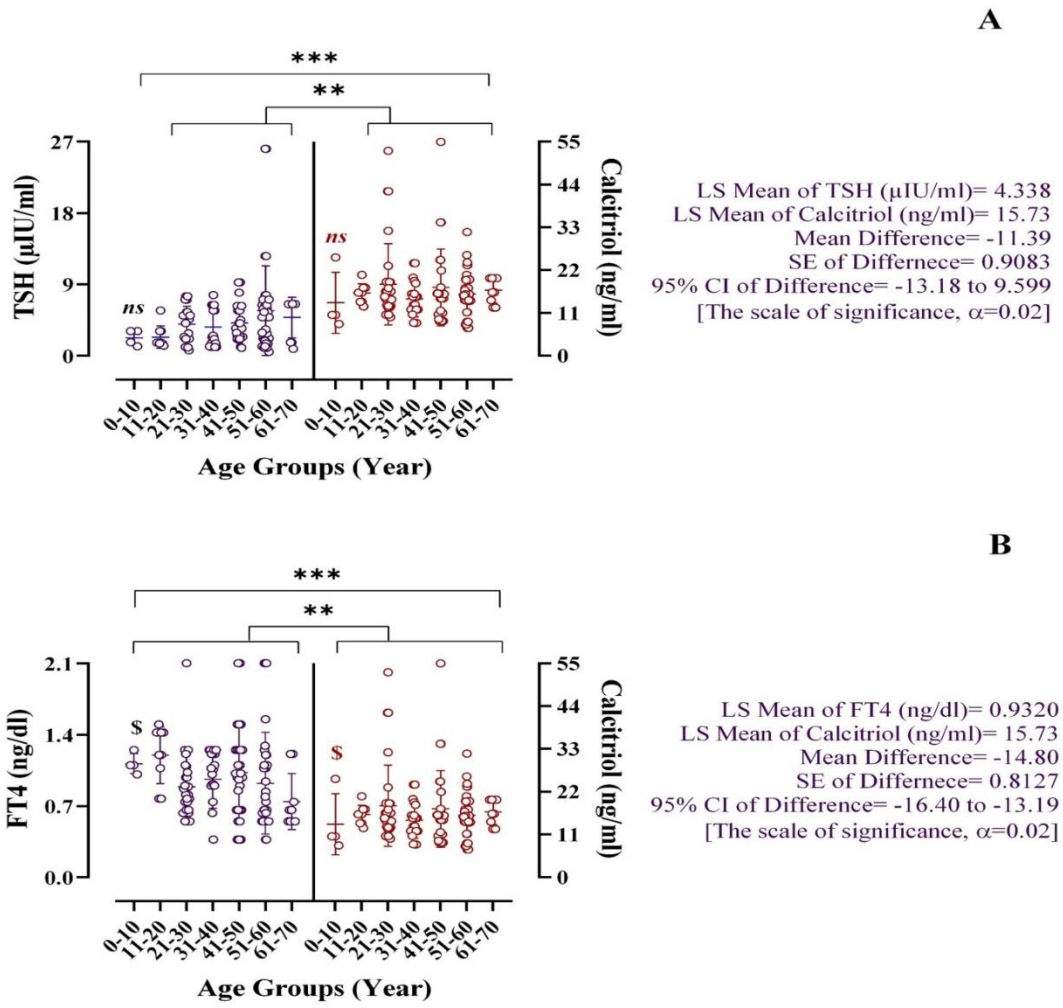

Figure 1: (A)-The correlation between the thyroid-stimulating hormone (TSH) and Calcitriol; **(B)**-The correlation between free thyroxin (FT4) and calcitriol status in thyroid disorder women patients. µIU/ml (micrograms international units per millilitre), ng/mL (nanograms per millilitre), LS mean (the mean of Longitudinal Section), SE (standard error), CI (confidence interval).

The serum iron, TIBC, and ferritin level fluctuate dramatically with the concentration of calcitriol present for all the selected patients from different age groups, resulted in the current serological quantitative analysis **(Figure 2)**. The iron level increases (150µg/dl) and decreases (9µg/dl) with the increase and decrease of calcitriol level as 54.98 ng/ml and 8 ng/ml, respectively. The correlation between iron and Calcitriol is found significant (P<0.0001) among

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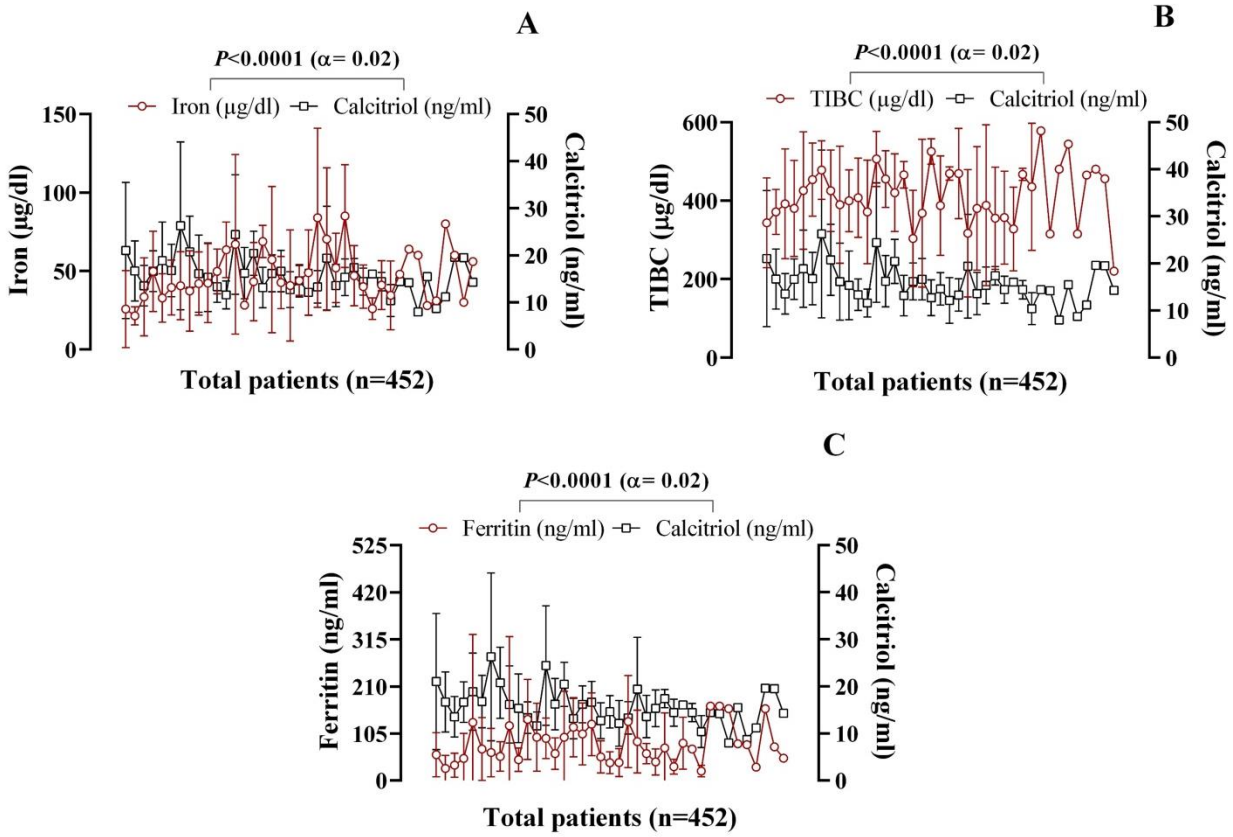

Figure 2: (A)-The correlation between iron and Calcitriol; (**B)**-The correlation between TIBC and Calcitriol; (**C)**-The correlation between ferritin and calcitriol status in anemic women of reproductive ages. TIBC (total iron binding capacity), Calcitriol (activated vitamin D3), µg/dL (micrograms per deciliter), ng/mL (nanograms per milliliter).

The reproductive patients **(Figure 2A)**. On the other hand, TIBC increased (580µg/dl) with the reduction of Calcitriol (8ng/ml) and decreased (156µg/dl) with the progress of calcitriol concentration (52.7ng/ml), means their serological profiles are reversible to each other **(Figure 2B)**. Similarly, ferritin downregulates (5ng/ml) as the calcitriol level promotes (52.7ng/ml), resembling the findings of TIBC as well **(Figure 2C)** among the reproductive women. In both the cases of TIBC and ferritin, the values are equally significant (P<0.0001) when correlated with their calcitriol limits **(Figure 2B and C)**. The correlation of iron, TIBC, and ferritin in respect to the calcitriol level has also been found significant to the nonproductive women (P<0.0001 for each in the scale of significance P<0.02), except the women aged below fourteen years.

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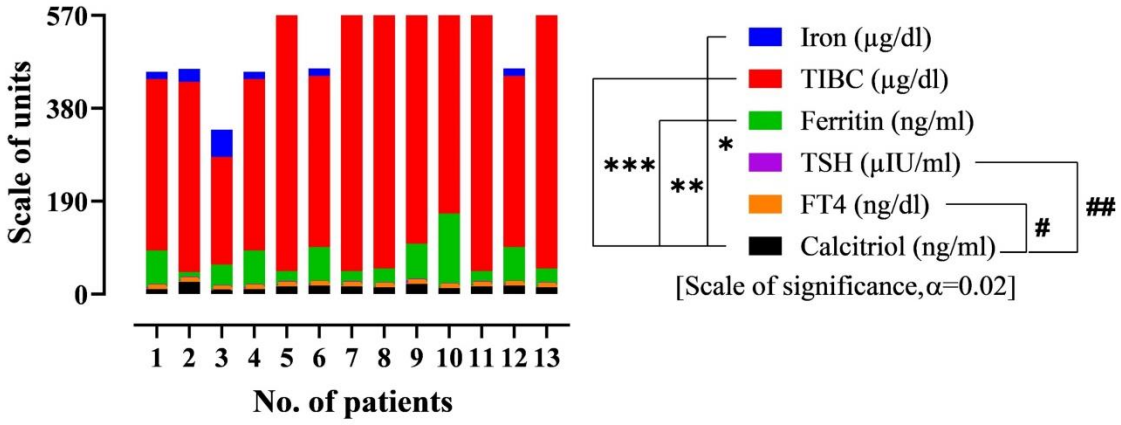
This study experienced 13 congenital cases of IDA and TSH disorders among all women. Surprisingly, according to their calcitriol profiles, all serological parameters have been found highly significant considering their two-way ANOVA and 'Tukey's multiple t-tests' of variables **(Figure 3)**. For the congenital patients, individual correlations of TIBC, TSH, and FT4 with their calcitriol concentration is exactly P<0.0001 **(Figure 3A)**.

A



B

Level of divergence			
Interactives	**DBPM**	**SED**	**95% CID**
Calcitriol - TSH	-13.00	1.215	-15.64 to -10.35
Calcitriol – FT4	-14.16	1.250	-16.88 to -11.44
Calcitriol – Iron	18.13	6.690	3.552 to 32.71
Calcitriol – TIBC	440.1	33.62	366.9 to 513.4
Calcitriol – Ferritin	42.21	10.38	19.60 to 64.81

Figure 3: (A)-Two-way ANOVA and 'Tukey's multiple t-tests' showing the correlation of the variables with Calcitriol among 13 congenital cases of IDA and TSH disorder. The stacked columns demonstrate the relationship of Iron, TIBC, Ferritin, TSH, and FT4 against Calcitriol in the case of 13 congenital patients who had Iron Deficiency Anemia and Thyroid Deficiency. **(B)**- Level of divergence of the obtained values from Fig 3A has 95% confidence considering the DBPM, SED, CID parameters. TIBC (Total iron-binding capacity), TSH (Thyroid-stimulating hormone), FT4 (Free thyroxine), Calcitriol (Activated vitamin D3), * and # (Level of significance).

In contrast, ferritin shows a strong correlation but unlike the others with Calcitriol, means (P<0.0064). The values obtained from the interactive components means iron, TIBC, ferritin, TSH, and FT4, with the Calcitriol, have been found very authentic considering their DBPM, SEM, and CID parameters, which established the findings as 95% confident **(Figure 3B)**. It's

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resulted that there is no null ('0') values in their 95% CID, which means the quantitative outputs are highly authentic and statistically significant. The 'Z value' for 95% confidence is 1.96 (Z=1.96) as calculated from the statistical parameters **(Figure 3)**.

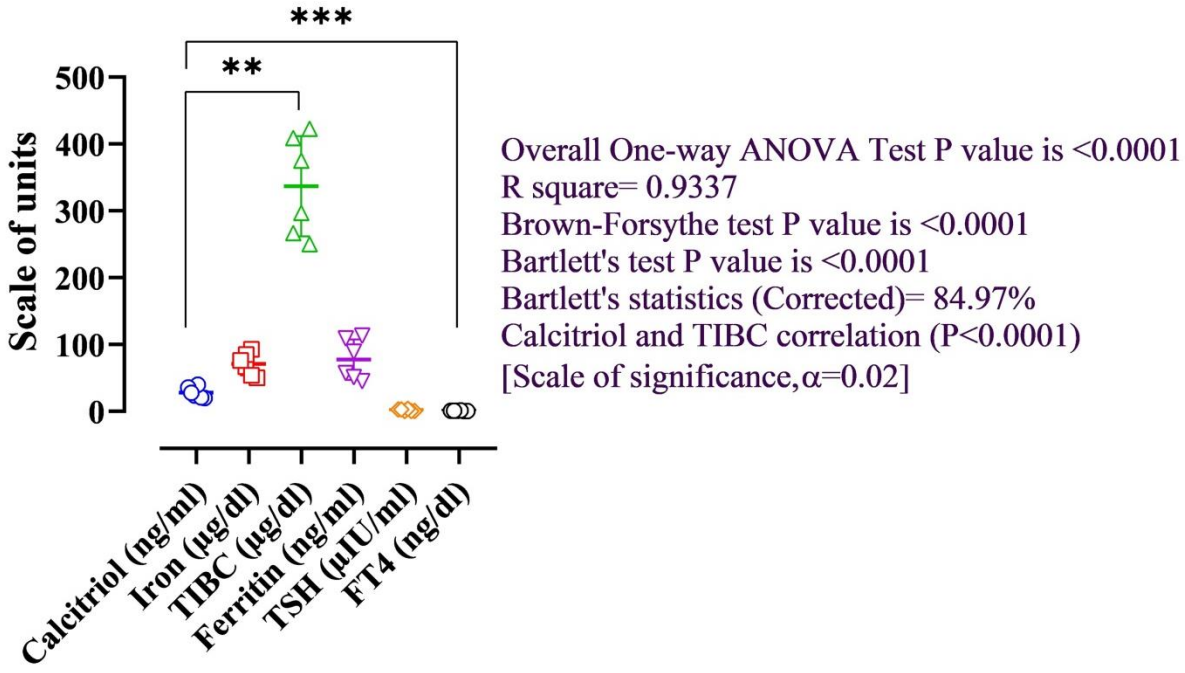

Figure 4: Figure: Level of Calcitriol, Iron, TIBC, Ferritin, TSH, and FT4 of six normal women and the potential relationship between calcitriol level and value of the other five serological parameters Graph. TIBC-Total Iron-binding capacity, TSH-Thyroid stimulating hormone, FT4-Free thyroxine.

The overall results of each parameter were assessed based on the standard data generated by sampling the same serological parameters from six normal women as a control to compare their calcitriol status with the diseased women. The calcitriol level of the normal women ranged between 20ng/ml to 40ng/ml. In response to which, their iron, TIBC, ferritin, TSH and FT4 ranges found as 50-93 µg/dl, 250-423 µg/dl, 45-113 ng/ml, 0.5-4.0 µIU/ml, and 0.70-1.67 ng/dl respectively **(Figure 4)**.

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Discussion

The term "vitamin D" refers to a group of secosteroid compounds, two of which, cholecalciferol (known as vitamin D3) and ergocalciferol (also known as vitamin D2), are most commonly associated with the term [44]. The former is composed of a reduction of 7 dehydrocholesterol in the skin following the exposure to ultraviolet B (UVB) radiation from few dietary sources (mainly fish fat). At the same time, the latter is compounded by plants and fungi, which can form a dietary basis of vitamin D2 for people [45]. Both D2 and D3 are hydrolyzed with 25-hydroxyvitamin D (25(OH)D, calcidiol), the principal form of vitamin D circulation and storing in the liver [46]. The active hormone is formed when 25(OH)D is hydroxylated to 1,25-dihydroxyvitamin D (1,25(OH)2D, Calcitriol). This transformation usually occurs in the kidney and is governed by negative responses due to high Calcitriol and fibroblast growth factor 23 (FGF23) [46]. Calcitriol attaches to the intracellular vitamin D receptor (VDR) that influences the response components of objective genes [47].

Calcitriol has long been recognized as an important hormone in regulating the musculoskeletal system, and it continues to be so today. The extra-skeletal effects of 1,25(OH)2D were also highly researched over the last decade after the presence of vitamin D receptors in almost all tissue types was established [48]. In the context of thyroid disorders, the antiproliferative and differentiating effects of Calcitriol are significant, and its function in modulating the immune system has been demonstrated in autoimmune thyroid disease (AITD) [27, 44]. Several research studies have shown that vitamin D3 has vital roles in maintaining bone health, immunity, and muscles. Several studies showed a link between vitamin D deficiency and thyroid disorders [49]. IDA is a common problem and highly prevalent among Bangladeshi women, especially among pregnant women and females living in low iron water supplies [50]. Many IDA patients remain undiagnosed worldwide as the early stages show minor symptoms.

Furthermore, people with chronic diseases like CVD (cardiovascular diseases) and CKD (chronic kidney diseases) have more significant risks of suffering from IDA [51]. The function of several proteins, metabolic activity, including imbalance of thyroid hormones, may occur due to iron deficiency. Changes in ferritin levels affect thyroid functions. Low levels of TSH and high levels of FT4 occur due to an imbalance in TSI (thyroid-stimulating immunoglobulin), leading to

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hyperthyroidism. FT4 does not bind to proteins, which is good for diagnosing thyroid problems [52].

The potential relationship of activated vitamin D3 (Calcitriol) with the regulation of thyroid-stimulating hormone (TSH) has been identified **(Figure 1A)**. We have collected the TSH in µIU unit and Calcitriol in ng/ml unit of serum concentration from 452 women blood who possessed the TSH and IDA disorders according to the age limit of 0 to 70. According to the current study, the age limit was conducted among the 10 years intervals for the higher frequency of random sample collection. After the statistical analysis of the relationship of TSH and Calcitriol, it showed ($P<0.063$) among the patients ranged between 0 to 10 years. The values of the serological components for the women beneath 10 years can act randomly because their hormonal and metabolic profile remained developmental like the neonatal, which can fluctuate insignificantly under any circumstances [53]. According to the result of **figure 1 (A)**, the LS mean of TSH and Calcitriol is 4.338 and 15.73, in which the Mean Difference and SE of Difference are -11.39 and 0.9083.

This study analyzed Free T4 (FT4) measurements that are not bound and can freely enter and affect the body tissues. FT4 normal values are 0.7 to 1.9ng/dL **(Figure 4)**. Calcitriol can exert biological effects by binding with VDR (vitamin D receptor). Enzyme 1-alpha-hydroxylase (CYP27B1) catalyzes the calcidiol to Calcitriol and 24-hydroxylase (CYP24A1), Calcitriol inactivating enzyme, are crucial in governing the availability of active vitamin D. The effects of Calcitriol depend on VDR. Their polymorphic variants have been studied to a limited extent in the case of thyroid cancer [54]. In another study, there is an evaluation of the relationship between hypothyroidism and vitamin D, and their results indicated that patients with hypothyroidism suffered from hypovitaminosis D with hypocalcemia [32]. A potential relationship has been obtained between activated vitamin D3 (Calcitriol) and FT4 **(Figure 1B)**. The statistical analysis significant relationship of Calcitriol mediated FT4 regulation ($P<0.0001$) among the patients ranged between 0 to 10 years. The upper and lower values of FT4 and Calcitriol are 1.25 (ng/dl), 1.01 (ng/dl) and 25.3 (ng/ml), 8.19 (ng/ml), respectively; where the LS means of FT4 and Calcitriol are 0.9320 and 15.73, in which the Mean Difference and SE of Difference are -14.80 and 0.8127.

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Iron-deficiency anemia is defined when blood levels of iron will be low, or less than 10 micromoles per liter (mmol/L) for both men and women (normally 10-30 mmol/L) **(Figure 4)**. Iron deficiency anemia increases susceptibility to infectious disease, increased child mortality, slowed child development, and reduced scholastic performance [55]. There is an association of calcitriol deficiency with the regulation of greater risk of Anemia, lower mean hemoglobin (LMH), and higher usage of erythrocyte-stimulating agents [56]. The research results have been depicted a strong correlation between iron and Calcitriol ($P<0.0001$) among the patients **(Figure 2A)**. The calcitriol level of the normal women ranged between 20ng/ml to 40ng/ml, and iron level is 50-93 µg/dl **(Figure 4)**. Here Iron level is increased (150µg/dl) and decreased (9µg/dl) with the increase and decrease of calcitriol level as 54.98 ng/ml and 8 ng/ml, respectively. Total iron-binding capacity (TIBC) plays a pivotal role in indirectly measuring the percentage of transferrin situation involved in positive correlation with vitamin D [57]. Transferrin and vitamin D levels decreased, whereas TIBC levels increased during iron-deficient anemic patients [58]. According to this study, the serum TIBC range for all the patients was between 220 µg/dL to 578 µg/dL with some fluctuations.

In contrast, the activated Vitamin D (Calcitriol) value was between 8 ng/mL and 26.29 ng/mL with a little bit of oscillation. In this research, it was found that serum TIBC result was increased. In contrast, serum calcitriol level was decreased from all the individual patients, consequently indicating iron deficiency anemia [59], as compared to the normal range **(Figure 4)**. The ferritin level in the human body indicates the iron status and iron storage. Ferritin levels are lower in people who have iron deficiency anemia, but they may be higher in people with inflammation and chronic disease-related Anemia [60]. However, in the present analysis, the range of the ferritin level was from 21 ng/mL to 166 ng/mL with some fluctuations, and calcitriol level was between the ranges of 8 to 26.29 ng/mL respected to the normal control. It was noticed that serum calcitriol value was slightly higher, whereas serum ferritin values were significantly lower following the calcitriol scores for all the patients **(Figure 2C)**. The current investigation found a positive association between serum calcitriol and ferritin levels, consistent with previous findings [61, 62].

It is mainly due to maternal risk factors such as inadequate dietary intake of vitamin D, insufficient exposure to sunlight, and pregnancy that occurs close to the people who suffer from

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congenital calcitriol deficiency [63]. Low maternal vitamin D levels may raise the risk of a newborn's deficiency [64]. Besides, spontaneous hypothyroidism affects between 1% and 2% of the population and is more prevalent in older women [65]. Therefore, a poor pregnancy outcome is associated with vitamin D deficiency or insufficiency, which leads to several disorders such as low birth weight of newborns [66]. According to the current serological assessment, the value of Calcitriol (8.19-25.3ng/ml) and iron (15-75 µg/dl) reduced significantly **(Figures 3A and 3B)** than the control group (20-40 ng/ml) and (50-93 µg/dl), respectively **(Figure 4)**. Moreover, the FT4 level decreased slightly from the control 0.7-1.67 ng/dl to congenital 0.77-1.5 ng/dl. On the other hand, TIBC, Ferritin and, TSH indicate an upper range of 220-566 µg/dl, 17-150 ng/ml, and, 1.2-5.7 µIU/ml than the healthy people 250-423 µg/dl, 45-113 ng/ml, and, 0.5-4 µIU/ml, respectively. The results possessed a higher percentage of TIBC and a lower percentage of TSH. The TIBC, TSH, and FT4 have a significant relationship with their calcitriol concentration which is exactly $P<0.0001$, whereas the ferritin shows a strong correlation with Calcitriol ($P<0.0064$) **(Figure 3A and 3B)**.

The research has described the values and regulatory relationships of the same serological parameters like iron, TIBC, ferritin, TSH, and FT4, with the Calcitriol of six normal women as a control group, where the range calcitriol level from the control group was found 20ng/ml – 40 ng/ml, which was within the normal limit **(Figure 4)**. On the other hand, the range of iron, TIBC, Ferritin, TSH, and FT4 were found 50-93mcg/dl, 250-423mcg/dl, 45-113n/ml, 0.5-4.0 mcgIUml, respectively, which were also within the normal ranges **(Figure 4)**. The potential calcitriol-based regulation of the other serological parameters has been found in normal six women, used as the standard. Considering the biostatistical analysis, the overall one-way ANOVA test, Brown-Forsythe test, Barlett's test, and Calcitriol, a TIBC correlation P-value were all exactly <0.0001 (in the scale of significance $P<0.02$). The R square (R^2) value was 0.9337, and Barrett's statistics was 84.97%.

There are a lot of variables that were selected based on the inclusion and exclusion criteria. First of all, one of the study's advantages is that the samples were collected from about 452 individual patients and analyzed with automation equipment by skilled persons. The second is that volunteers with other diseases that could induce Anemia, such as Anemia due to inflammation or cause by genetic factors (i.e., thalassemia or sickle cell disease), were all eliminated. Therefore, activated vitamin D insufficiency is connected to Anemia and iron deficiency. Calcitriol

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promotes erythropoiesis, which may help to prevent Anemia. As a result, all patients diagnosed with vitamin D deficiency in the outpatient hospital should be tested for iron deficiency and Anemia. Those who require replacement medication should be provided it. However, Future studies are needed to determine the accuracy and efficacy of Calcitriol in IDA and TSH disorders, in addition to larger samples, biochemicals, inflammation markers, and parathyroid hormone analysis.

Conclusion

It is reasonable to expect that significant calcitriol (activated form of vitamin D) deficiency will occur in the majority of the women suffering from various forms of thyroid autoimmunity and iron deficiency anemia (IDA), based on our previous experience the findings of our research study. The question remains on how to respond in such a circumstance. Based on the results of our serological research study, it can be concluded that the concentrations of TSH, FT4, iron, TIBC, and ferritin were correlated with the levels of serum calcitriol or activated vitamin D3 in women suffering from TSH and IDA abnormalities. Moreover, depending on a woman's reproductive status and age, Calcitriol has been validated as a biomarker for tracking the status of IDA and TSH irregularities. However, more research with larger sample size is required to understand better the serological profiles of patients with IDA and TSH disorders.

Abbreviations

AITD-autoimmune thyroid disease, ANOVA-Analysis of Variance, BMDC-Bangladesh Medical and Dental Council, CID-Confidence Interval of difference, CKD -Chronic Kidney Diseases, CRP-C-Reactive Protein, CVD -Cardiovascular diseases, DNA- Deoxyribonucleic Acid, DBPM-Difference between predicted means, FT3- Free Triiodothyronine, FT4- Free Thyroxine, IDA-Iron Deficiency Anemia, LSM-least-square mean, LMH-Lower Mean Hemoglobin, RBC- Red Blood Cell, MD- mean difference, SED-standard error of the difference, SEM-Standard Error of Measurement, TIBC- Total Iron Binding Capacity, TSH- Thyroid Stimulating Hormone, TSI -Thyroid-stimulating immunoglobulin, UV-B-Ultraviolet B, VDR-vitamin D receptor, WHO-World Health Organization.

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Conceptualization, Methodology, Supervision: Sharmin Ahmed; **Project administration and Co-supervision**: Salauddin Al Azad; **Resources:** Md. Abdul Rashid Mia, **Data curation:** Partha Biswas, Mithila Farjana; **Writing original draft**: All the authors participated equally; **Visualization:** Farzana Alam Arshe, **Investigation:** Sabrina Jahan Mily, Ananya Baidya Ankhi, Mahdi Mubin Shaikat; **Validation and software:** Salauddin Al Azad, Sabeeha Sultana, Kashfia Mawa, Zannatul Naim. **Revision:** Md. Ataur Rahman. **Funding:** Bonglee Kim.

Research ethics

The total research work was conducted under the Ethical Guidelines and Monitoring of Jashore Medical College (JMC), Bangladesh Medical and Dental Council (BMDC) in collaboration with the RPG Authority (Govt. Registration ID: 05-060-06021) under the Project Category C2 (#Project EA No- 10/2021-2022). The Ethical Clearance have been approved by **Prof. Dr. Md. Mohidur Rahman** (Principal, JMC, Bangladesh), **Dr. Md. Azam Saklain** (Associate Prof. and Head of the Pathology Department, JMC, Bangladesh) and **Dr. Sharmin Ahmed Shawon** (Senior Lecturer, Pharmacology Department, JMC, Bangladesh; & Researcher of 'College of Public Health Sciences, Chulalongkorn University', Bangkok, Thailand). **Dr. Sharmin Ahmed Shawon** (BCS 33rd) is the Supervisor of this research.

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Conflict of interest

The authors' have no competing interest at all with the others.

Consent for publication

The authors are very cordial in publishing the manuscript and their consent is clear.

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