

# Study of the relationship between calcium ion and thyroid hormones, liver enzymes in some patients of hypocalcaemia and hypercalcaemia

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**Objective** This study focuses on the relationship between calcium ion concentration in human body and thyroid hormones with liver enzymes to determine the effect of some physiological and biochemical parameter of liver [glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), Bilirubin] and thyroid stimulating hormone (TSH; T<sub>3</sub>, T<sub>4</sub>), on the levels of calcium ion in the blood.

**Methods** The current study included 40 patients from two different hospitals in Karbala city. This study has been investigated to find the effect of thyroid hormones (T<sub>3</sub>, T<sub>4</sub>, TSH) and liver enzymes (GOT, GPT, Bilirubin) on calcium ion concentration.

**Results** The results revealed there is no significant difference ( $P > 0.01$ ) between calcium ion concentration and thyroid hormones (T<sub>4</sub>, T<sub>3</sub>, TSH) in each groups of different ages, respectively. Therefore, weak correlation between calcium and hormones pointed to an increase or decrease of calcium ion concentration effect by these hormones.

**Conclusion** There is no significant difference ( $P > 0.01$ ) between liver enzymes (GOT, GPT, Bilirubin) and calcium ion concentration in each groups of different ages respectively, although weak correlation between calcium and hormones that pointed to an increase or decrease of calcium ion concentration is also affected by these enzymes.

**Keywords** calcium ion, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, Bilirubin, T<sub>3</sub>, T<sub>4</sub>, TSH

## Introduction

Calcium is a body's abundant divalent cation and more than 99% of the body calcium is concentrated in the skeletal system and approximately 1% is rapidly exchangeable with blood calcium. Small amounts of calcium located outside the bone circulates in the serum partly bound to protein and partly ionized. Calcium has a major role in the transmission of nerve that include normal heart beat and has vital role in cardiac action potential and is essential for cardiac pacemaker automaticity, and this ion is also involved in blood clotting and hormone secretion.<sup>1,2</sup> Intracellular calcium is a key of intracellular second messenger that play a pivotal role in controlling various cellular processes such secretion, differentiation, proliferation, motility and cell death. In contrast, extracellular calcium is crucial for a spectrum of physiological phenomena including blood coagulation, muscle function and maintenance of skeletal integrity.<sup>3</sup> Calcium balance is tightly regulated by the interplay between gastrointestinal absorption, renal excretion, bone reabsorption, vitamin D and parathyroid hormone (PTH) system.<sup>4</sup> The thyroid gland plays a role in calcium metabolism and regulation, by secreting when needed and the thyroid stimulating hormones (TSHs) are key to regulate the metabolism of thyroid gland to produce both T<sub>4</sub> (thyroxine) and T<sub>3</sub> (tri-iodothyronine). T<sub>4</sub> is the main product and is converted in the periphery via de iodination to T<sub>3</sub> which is the main biological active of thyroid hormones. The production of TSHs is regulated by thyrotrophic; therefore, thyroid stimulating hormone in response is regulated by a negative feedback mechanism related to serum level of free T<sub>3</sub> and T<sub>4</sub>.<sup>5</sup> The liver enzyme has an important role in amino acid metabolism. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes found mainly in the

liver. They are also found in red blood cells, heart muscle tissues and other organs such as the pancreas and kidney.<sup>6</sup> AST and ALT formerly was called serum glutamic oxaloacetate transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), respectively. AST or ALT levels are valuable aid primarily in the diagnosis of liver diseases but not specific for diagnosis of liver disease.<sup>7</sup>

## Materials and Methods

### 1. Collection of sample

The specimens were collected from two hospitals, Al-Hussainy hospital and Al-Hindia hospital in Karbala city. Venous bloods were collected in a laboratory, as specimens, from 40 patients. The average age was 10–45 years.

### 2. Separating serum from blood

First, blood taken from patients by syringe was placed in the plane tubes (not anticoagulant tube). Then, these tubes were centrifuged at 5000 rpm for 5 min. The serum thus separated from blood was transferred to another tube and gave a special number and name to the tube to avoid its loss.

### 3. Measurement of calcium

The first test is the measurement of calcium ion in the body via electrolytes instrument in both hospitals, Al-Hussainy and Al-Hindia.

### 4. Hormonal test procedure (TSH-T<sub>3</sub>-T<sub>4</sub>) by ELISA

- i. Prepare both the samples (serum) and reagents in room temperature.
- ii. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate.
- iii. Pipette 50 µl of the appropriate serum reference, control or specimen into the assigned well.

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- iv. Add 100 µl of working reagent A (T3, T4, TSH), enzyme conjugated solution to all the wells.
- v. Swirl the microplate gently for 20–30 sec to mix and cover it.
- vi. Incubate 60 min at room temperature.
- vii. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- viii. We used automatic plate washer and washed plates for 3 times.
- ix. Add 100 µl of working signal reagent solution to all wells. Don't shake the plate after substrate addition.
- x. Incubate at room temperature for 15 min.
- xi. Add 50 µl of stop solution to each well and gently mix for 15–20 sec.
- xii. Read the absorbance in each well at specific wave length compatible with hormones that we tested (T3, T4, TSH).
- xiii. The result should be read within 30 min of adding the stop solution.

5. Biochemical test procedure (include GOT, GPT, bilirubin)

We used Reflatrone device to determine this parameter. This procedure includes:

- i. Working the device (Reflatrone).
- ii. Take specific strip for test that you need (GOT, GPT, Bilirubin) and add in the dedicated space one drop or 32 µl from the patient serum.
- iii. Put the strip in the device and wait for some minutes and read the result.

6. Statistical analysis

We used the correlation test to deduce correlation factor between calcium ion concentration and thyroid hormones and liver enzymes at probability level 0.01 and degree freedom, n-2.<sup>8</sup>

## Results and Discussion

### The Relationship between Calcium Ion Concentration and Thyroid Hormones

The present study included 40 patients and tested the relationship between thyroid hormones (T3, T4), TSH and calcium ion concentration; in other words, this experiment studied the effect of thyroid hormones on calcium ion concentration. Through this study we

observed the relationship between calcium ion concentration and thyroid hormones (T4, T3), TSH. There is no significant difference when  $P > 0.01$  in all groups of different ages. Weak correlation between calcium ion concentration and thyroid hormones (T4, T3, TSH) pointed to an increase of calcium ion concentration depending on an increase of these hormones, but it was weak. This result is shown in Table 1 and Fig. 1. The calcium sensing receptor (CaSR) represents the molecular mechanism by which parathyroid cell detect change in blood ionized calcium concentration and modulate parathyroid hormone (PTH) secretion to maintain serum calcium levels within a narrow physiological range.<sup>9</sup> Depending on some studies that observed the blood ionized calcium concentration are remarkably stable in healthy individual because of the hemostatic system involving the action of the three calcitropic hormone on the organ of bone. Therefore, the secretion of PTH is highly dependent on the ionized calcium concentration and represent a simple negative feed-back loop. The serum PTH concentration decreases as the serum PTH secretion is not entirely suppressible; however, there is relatively narrow range of regulation of PTH secretion by

extracellular calcium.<sup>10</sup> The major effect of PTH is to maintain normal ionized serum calcium concentration. PTH stimulates the bone reabsorbing releasing calcium into extracellular fluid. The rise in calcium concentration is caused principally by two effects, first is the effect of PTH to increase calcium and phosphate absorption from the bone and second is a rapid effect of PTH to decrease the excretion of calcium by the kidneys.<sup>11</sup> Effect of exercises on thyroid hormone T3 demonstrated that this effect can lead to increase T3 hormone.<sup>12</sup> Renal calcium influenced by thyroid hormone was decrease in hyperthyroidism and increase in hypothyroidism.<sup>3</sup> Other studies showed the elevated level of total and free T4 hormone also have been reported in patient with acute psychiatric illness, iodinated contrast agent also elevate T4 hormone level by inhibiting peripheral conversion of T4 to T3. The decrease of T4 hormone occurs in patient with most severe monothyroid illness.<sup>13</sup> The serum calcium level decreased significantly in patient with high TSH concentration in contrast with normal TSH. Also, renal calcium influenced by thyroid hormones have been decreased in hypothyroidism and increased in hypothyroidism.<sup>9</sup> The inhibitory action of calcium on the

Table 1. Relationship between calcium and TSH, T3 and T4

Parameters	Ca <sup>+2</sup> – TSH	Ca <sup>+2</sup> – T4	Ca <sup>+2</sup> – T3
Age			
10–20 years (n = 12)	0.4117	0.4105	0.1566
20–30 years (n = 10)	0.10502	0.00604	0.20412
30–40 years (n = 18)	0.08956	0.17519	0.00602

\*The number refers to correlation factor (r value).

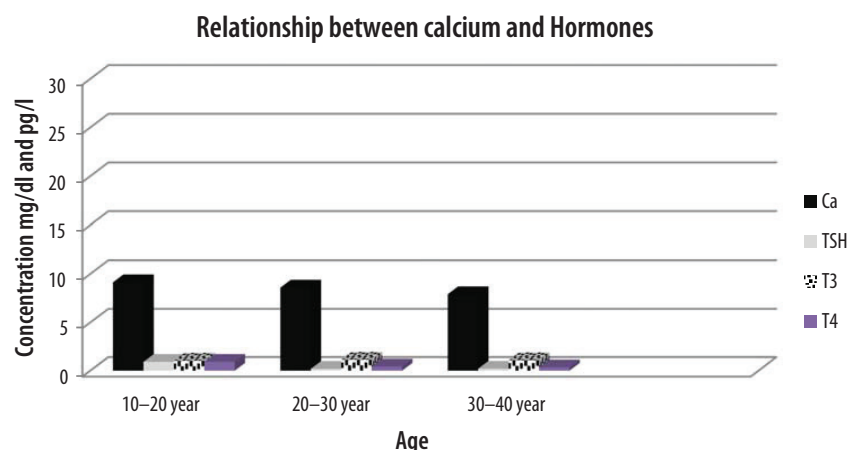


Fig. 1 Mean value of calcium and thyroid hormones.

thyroid gland has been suspected since the last century but more recent studies have confirmed its effect. It is known that calcium decreases thyroid activity and that its absorption increases thyroid insufficiency.<sup>14</sup> The thyroid gland normally enlarge in response to an increase demand for thyroid hormone that occur in puberty, pregnancy, iodine deficiency and immunologic viral or genetic disorder in this increase in thyroid follicles and thyroid hormone synthesis. Therefore, elevation of thyroid hormone leads to graves' disease or other thyroid abnormalities. The primary characteristics of graves' disease are diffuse thyroid enlargement (as much as two to three times the normal size, 40–60 grams), then the tumor release TSH that induce

elevated thyroid synthesis and release and are not responsive to normal hormonal feedback control. Such patients present with many symptoms of hyperthyroidism.<sup>15</sup> However, peripheral metabolism of thyroid hormone can be changed significantly by condition, which can alter the deionization pathway and lead to change in the circulating level of thyroid hormones. The biological effect of short-term change in the thyroid hormone level are not currently completely understood but are potentially important in the body adjustment to stressful or catabolic state. Low dietary calcium intake and hypocalcaemia are major stimuli for PTH secretion leading to parathyroid gland hyperplasia.<sup>16</sup>

### Relationship between Calcium Ion Concentration and the Liver Enzyme GOT, GPT and Bilirubin

In the present study, we tested the relationship between calcium ion concentration and liver enzymes (GOT, GPT and Bilirubin). We observed there is no significant difference ( $P \geq 0.01$ ) between calcium ion concentration and liver enzymes (GOT, GPT and Bilirubin) in all groups of different ages respectively. Weak correlation between calcium and liver enzyme pointed to the increase or decrease parameter effects on calcium ion concentration. This result is shown in Fig. 2 and Table 2.

A significant decrease in total and ionized calcium phosphorus and chloride and an increase in sodium were also observed. Significant increase was found in most enzymes such as AST, GK and GGT.<sup>17</sup> A diet supplement by calcium leads to an increase in serum glutamate oxaloacetate transaminase and glutamate pyruvate transaminase. Addition of calcium ion significantly reduced liver GST and elevated liver GPX activities.<sup>18</sup> Human erythrocytes that incubated with different concentration of calcium chloride (0.17–1.67 mm) showed hemolytic after addition of bilirubin. Hemolysis was observed only when cell were incubated first with calcium followed by bilirubin and not vice versa. This hemolytic was found to be dependent upon several factors such as concentration of bilirubin, time of incubation of RBCs with calcium and time of incubation of bilirubin with the calcium-loaded RBC<sup>19</sup> and difference in the calcium-induced bilirubin dependent hemolytic phenomenon have been found among different mammalian erythrocytes.<sup>20</sup>

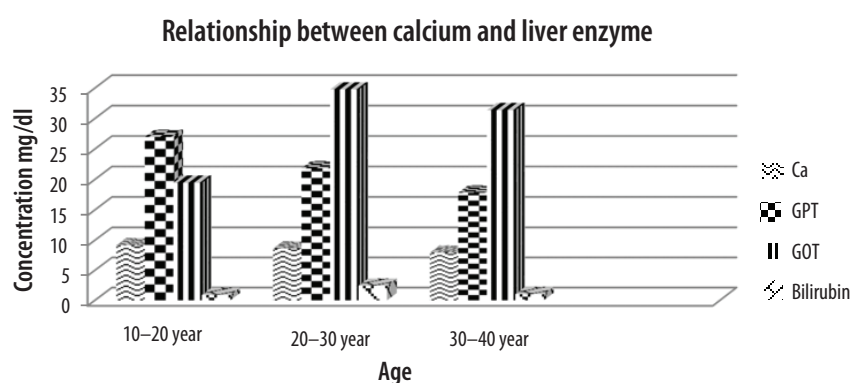


Fig. 2 Mean value of calcium and liver enzymes.

Table 2. Relationship between calcium and (GOT, GPT and Bilirubin)

Age	Parameters	Ca <sup>+2</sup> – GPT	Ca <sup>+2</sup> – GOT	Ca <sup>+2</sup> – Bilirubin
10–20 years (n = 12)		0.2273	0.5427	0.0660
20–30 years (n = 10)		0.2424	0.03136	0.02636
30–40 years (n = 18)		0.01288	0.0263	0.0942

\*The number refers to correlation factor (r value).

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