SERUM TOTAL CALCIUM, IONISED CALCIUM AND CORRECTED TOTAL CALCIUM CONCENTRATIONS IN KWASHIORKOR AND NEPHROTIC SYNDROME PATIENTS

MJ Dreyer BSc (Med)(Hons)
Department of Chemical Pathology, School of Pathology and Pre-Clinical Sciences, University of Limpopo, Medunsa Campus
email: Manie.Dreyer@ul.ac.za

Abstract
Calcium in blood plasma circulates in two main states: The non-diffusible protein-bound calcium which constitutes approximately 45% of the total plasma calcium and the diffusible free calcium fraction which can be further subdivided into complexed calcium and ionised calcium. The ionised calcium fraction is the physiologically active form.

Albumin is the protein which binds approximately 80% of the protein-bound calcium fraction in plasma, therefore any changes in plasma albumin concentration will directly influence the plasma total calcium concentration.

To demonstrate this hypothesis, a study was done on patients with decreased plasma albumin concentrations to determine the effect on their total calcium, ionised calcium and corrected total calcium concentrations.

Our study clearly illustrates that ionised calcium and corrected total calcium concentrations are better indicators of calcium homeostasis in patients with hypoproteinaemia, than total calcium concentrations alone. Medical staff therefore, cannot only rely on total calcium concentrations in these patients.

Keywords
Ionised calcium, corrected total calcium.

INTRODUCTION

The calcium fractions in blood
As illustrated in Figure 1, essentially all of the calcium in blood is present in plasma and the concentration of total calcium (tCa) in plasma is approximately 2.50mmol/L. Calcium circulates in the plasma in two main states: the non-diffusible protein-bound calcium which constitutes approximately 45% of the total plasma calcium, and the diffusible free calcium fraction. The latter can be further subdivided into complexed calcium and ionised calcium (Ca^{2+}) which is the physiologically active form.[1]

Protein-bound calcium
The concentration of protein-bound calcium in plasma depends on the protein concentration and is approximately directly proportional to this.[2]

About 0.94mmol/L (37%) of the tCa concentration is bound to plasma proteins. The degree to which calcium is bound to protein varies significantly with the type of protein. In the normal individual about 80% of the non-diffusible fraction is bound to albumin, and the remainder is bound to globulins.[2]

The binding of calcium to globulins is still under investigation. Some studies indicate that the gammaglobulins probably do not bind any calcium but special gammaglobulins, which may be present in high concentrations in patients with multiple myeloma, may exhibit significant calcium binding, resulting in an increase in plasma tCa concentration.[3]

The degree of binding may vary in certain disease states and with changes in pH. The binding is pH dependent because hydrogen (H+) and Ca^{2+} ions compete for binding sites on the albumin molecule. With a high pH (alkalosis) more Ca^{2+} is bound and therefore the plasma concentration of Ca^{2+} decreases. The opposite is the case with a low pH (acidosis).[3,4]

Complex calcium
Of the tCa concentration of 2.50mmol/L, about 0.26mmol/L (10%) is complexed by a number of anions, especially anions with two or more negative charges (EDTA, citrate) but also monovalent anions like bicarbonate and lactate. Of all the anions in plasma that complex calcium, the concentration of bicarbonate is the highest (chloride does not complex calcium),
therefore the bicarbonate bound calcium ($CaHCO_3$) accounts for the major portion of the complex fraction.\cite{5}

**Ionised (free) calcium ions**

Of the tCa concentration of 2.50 mmol/L, about 1.30 mmol/L (52%) accounts for the ionised or free calcium fraction in plasma. The major portion of this fraction is inactivated by electrostatical forces due to other electrolytes in plasma, mainly sodium (Na$^+$) and chloride (Cl$^-$). The remainder, approximately 0.38 mmol/L (15%) is the physiologically active fraction.\cite{1,4}

In view of the effect of pH on the Ca$^{2+}$ fraction, any results obtained must be interpreted in the light of the acid-base status of the patient.\cite{1,2}

**Regulation of the calcium balance**

The different calcium transport processes in the human body are regulated mainly by three different hormones:\cite{6,7}

1. **Parathyroid hormone (PTH)**
   - Retrieving calcium and phosphate from bone
   - Conserving calcium by increasing its reabsorption by the renal tubules
   - Facilitating the excretion of phosphate by inhibiting its reabsorption by the renal tubules
   - Stimulating the kidney to convert 25-(OH)$_2$D$_3$, the inactive vitamin D precursor, to active vitamin D$_3$, 1,25-Dihydroxy vitamin D$_3$ [1,25-(OH)$_2$D$_3$]

2. **1,25-Dihydroxy vitamin D$_3$ [1,25-(OH)$_2$D$_3$]**
   - This hormone increases the intestinal absorption of calcium ingested in the diet

3. **Calcitonin (CT)**
   - This hormone inhibits the activity of osteoclasts and thus suppresses bone resorption

**Hypercalcaemia and hypocalcaemia**

Hypercalcaemia and hypocalcaemia may best be defined in terms of plasma concentrations of ionised calcium, rather than plasma concentrations of total calcium, because it is the ionised calcium fraction that accounts for most of the biological activity of calcium in neuromuscular and cellular functions as well as regulating the secretion of certain hormones.\cite{8,9}

**Hypercalcaemia**

Pathologically, three general mechanisms may lead to the development of hypercalcaemia:

1. Increased mobilisation of calcium from bone, by far the most common mechanism
2. Increased absorption of calcium from the gastrointestinal tract
3. Decreased urinary excretion of calcium

The manifestations of hypercalcaemia differ among patients. Mild hypercalcaemia may be totally asymptomatic and may only be detected during a routine blood chemistry determination; however, hypercalcaemia may be so severe as to produce lethargy, disorientation, coma and death.\cite{8,9}

**Hypocalcaemia**

Six general mechanisms may be responsible for the development of hypocalcaemia:

1. Absence of parathyroid hormone
2. Abnormalities in vitamin D metabolism or decreased magnesium levels that make bone resistant to the action of parathyroid hormone
3. A genetic disorder – pseudohypoparathyroidism, where the target organs do not respond to the action of parathyroid hormone
4. A decreased absorption of calcium from the gastrointestinal tract
5. Translocation of calcium between different compartments of the body
6. Increased urinary excretion of calcium

The clinical manifestations of hypocalcaemia vary greatly among patients. Patients adjust well to low levels of serum calcium and seldom become symptomatic. However, patients who suddenly become hypocalcaemic, such as those with postsurgical hypoparathyroidism, may develop tetany, even after a moderate decrease in serum calcium.\cite{8,9}

**Calcium homeostasis in patients with hypoproteinaemia**

There are several conditions which can be associated with a decrease in albumin concentration of which nephrotic syndrome, hepatic cirrhosis, glomerulonephritis, oedema, kwashiorkor and extensive burns are the most important.\cite{10}

**Kwashiorkor patients**

A deficiency in dietary protein among young children, especially in economically poor areas, is the single most important nutritional problem in the world today and leads to the clinical condition known as kwashiorkor. This disease is characterised by growth retardation, anaemia, hypoproteinaemia, frequently with oedema and fatty infiltration of the liver with ensuing fibrosis. The mortality rate in untreated children has been reported to be as high as 30% to 90%.\cite{11}

**Nephrotic syndrome patients**

Normally, traces of protein present in urine are not readily detected. The most common cause of frank proteinuria is renal disease, such as glomerulonephritis and nephrotic syndrome.
The latter is a clinical syndrome characterised by increased glomerular permeability with massive proteinuria, lipuria, hypoalbuminaemia, oedema and hyperlipidaemia. The major protein excreted is albumin, although globulins are sometimes also present in urine.[12]

MATERIALS AND METHODS

Ethical approval to perform this study was obtained from the Medical Research and Ethics Committee of the Medical University of Southern Africa (University of Limpopo, Medunsa campus). Blood samples for serum total protein (TP), albumin, total calcium and plasma ionised calcium concentrations were taken from seventeen kwashiorkor patients and three patients with nephrotic syndrome. All patients were admitted at the Ga-Rankuwa Hospital (Dr George Mukhari Hospital), North West province.

For serum TP, albumin and tCa determinations, venous blood samples were collected in the morning (+/- 08:00 - 10:00) from patients in a recumbent position. Samples were collected without stasis into red capped BD Vacutainer tubes (BD Diagnostics, Franklin Lakes, USA). After clotting, the blood was centrifuged and the serum drawn off and stored in tightly capped plastic tubes at 4°C, until analysed the same day. Serum total protein and albumin levels were determined utilizing a Beckman CX7. Clinical System (Beckman Instruments, Inc Brea, USA) which employs the following methodologies: Total protein levels were determined by using the Biuret reaction, and albumin levels were determined by using the Bromcresol Green (BCG) dye binding technique.

All serum tCa values referred to in this study were determined by Atomic Absorption Spectroscopy (AAS) utilising a Varian 1475 series atomic absorption spectrophotometer.[13,14] All the ionised calcium values referred to in this study were determined with an ICA1 ionised calcium analyser (Radiometer AS, Copenhagen, Denmark). It employs two measuring electrodes, one for the determination of the Ca²⁺ concentration and one for measuring the pH. The instrument can also convert the Ca²⁺ value at the existing pH of the specimen to a Ca²⁺ value at normal body pH (7.40). All measurements were performed at 37°C. The Ca²⁺ specimens taken for this study were heparinised venous whole blood specimens, collected in a syringe which was heparinised with the special heparin (S 4500 Heparin) supplied with the instrument. The specimen should be properly stoppered, at all times, to prevent CO₂ loss. This type of specimen is easy to collect and to preserve and it is unnecessary to transport the syringe on ice.[15,16]

The following formula was used in this study for the calculation of corrected total calcium concentrations in patients with albumin concentrations of < 40g/L.[17]

Corrected total calcium (mmol/L) = [tCa] + 0.02 x (40 - Alb).

RESULTS

The established normal reference ranges for the relevant param-

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Total protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>Total calcium (mmol/L)</th>
<th>Ionized calcium corrected for pH 7.4 (mmol/L)</th>
<th>Corrected total calcium (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>35</td>
<td>18</td>
<td>2.09</td>
<td>1.25</td>
<td>2.53</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>32</td>
<td>10</td>
<td>1.79</td>
<td>1.20</td>
<td>2.39</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>33</td>
<td>16</td>
<td>1.84</td>
<td>1.22</td>
<td>2.32</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>46</td>
<td>23</td>
<td>2.18</td>
<td>1.27</td>
<td>2.52</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>31</td>
<td>10</td>
<td>1.90</td>
<td>1.26</td>
<td>2.50</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>36</td>
<td>12</td>
<td>1.78</td>
<td>1.20</td>
<td>2.34</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>38</td>
<td>13</td>
<td>1.86</td>
<td>1.25</td>
<td>2.40</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>37</td>
<td>13</td>
<td>1.79</td>
<td>1.26</td>
<td>2.33</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>38</td>
<td>16</td>
<td>1.97</td>
<td>1.19</td>
<td>2.45</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>50</td>
<td>23</td>
<td>2.00</td>
<td>1.23</td>
<td>2.34</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>37</td>
<td>20</td>
<td>2.06</td>
<td>1.25</td>
<td>2.46</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>43</td>
<td>22</td>
<td>2.07</td>
<td>1.27</td>
<td>2.43</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>36</td>
<td>18</td>
<td>1.91</td>
<td>1.20</td>
<td>2.35</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>35</td>
<td>18</td>
<td>1.84</td>
<td>1.24</td>
<td>2.28</td>
</tr>
</tbody>
</table>

Table II: Hypocalcaemic male (M) and female (F) kwashiorkor patients.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Total protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>Total calcium (mmol/L)</th>
<th>Ionized calcium corrected for pH 7.4 (mmol/L)</th>
<th>Corrected total calcium (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>42</td>
<td>22</td>
<td>1.70</td>
<td>1.05</td>
<td>2.06</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>35</td>
<td>18</td>
<td>1.80</td>
<td>1.11</td>
<td>2.24</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>28</td>
<td>13</td>
<td>1.04</td>
<td>0.66</td>
<td>1.58</td>
</tr>
</tbody>
</table>

Table III: Normocalcaemic male (M) and female (F) nephrotic syndrome patients.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Total protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>Total calcium (mmol/L)</th>
<th>Ionized calcium corrected for pH 7.4 (mmol/L)</th>
<th>Corrected total calcium (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>44</td>
<td>19</td>
<td>2.07</td>
<td>1.27</td>
<td>2.49</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>39</td>
<td>10</td>
<td>1.83</td>
<td>1.21</td>
<td>2.43</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>37</td>
<td>14</td>
<td>2.02</td>
<td>1.23</td>
<td>2.54</td>
</tr>
</tbody>
</table>
In this study, the serum tCa concentrations were all well below the lower limit of the normal reference range, whereas the whole blood Ca²⁺ and corrected total calcium levels were within the normal reference ranges. Furthermore, none of these patients showed any clinical symptoms of hypocalcaemia and were therefore classified as being normocalcaemic.

As can be seen from Table II, the serum tCa, whole blood Ca²⁺, and corrected total calcium concentrations were all decreased. Also, these patients showed neuromuscular excitability, a distinctive feature of severe hypocalcaemia. The relevant values of three patients with nephrotic syndrome are listed in Table III.

It is evident from Table III that these patients were actually normocalcaemic due to the normal whole blood Ca²⁺ and corrected total calcium concentrations, but the serum tCa levels were decreased due to the loss of calcium binding proteins.

**DISCUSSION**

The aim of this study was to demonstrate the relationship between total calcium, ionised calcium and corrected total calcium concentrations in patients with abnormally low TP and albumin concentrations.

It is evident from the results that, if serum tCa concentrations were the only available parameter for assessment of the calcium status of the normocalcaemic patients, all of them would have been diagnosed as hypocalcaemic. This clearly illustrates that ionised calcium estimations and corrected total calcium concentrations are superior to that of total calcium concentrations, especially with abnormal total protein and albumin levels.

It is also evident that there is a good relationship between Ca²⁺ and corrected total calcium concentrations in the three hypocalcaemic patients.

**CONCLUSION**

The initial step in the diagnostic approach to hypocalcaemia is to make sure that there is a true reduction in the concentration of ionised calcium, but due to the limited availability of ionised calcium analysers, it is recommended that laboratories automatically (routinely) include in the results report a calculated corrected total calcium concentration, if possible, for evaluation of the calcium homeostasis, especially in patients with hypoproteinaemia.

It was also proven in this study that total calcium concentrations alone, can be misleading in patients with abnormal protein concentrations.

**ACKNOWLEDGEMENTS**

I am indebted to the late Prof. J Dauth for his support and constructive advice during this study and to Ms. K van Staden for her outstanding secretarial services in the preparation of this manuscript.