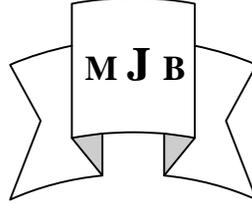# Serum Electrolytes and Minerals Status in Asthmatic Patients on Corticosteroids

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Abstract

Sixty nine asthmatic patients (thirty five males and thirty four females) used corticosteroid therapies as oral tablets and inhaler forms, thirty eight patients used oral tablets and thirty one patients used inhaler form. Thirty five apparently healthy individuals with out any disease were taken as a control group.

Blood samples were drawn from patient and control groups after overnight fasting. The serum obtained from the blood was used for spectrophotometric estimation of the level of total protein, albumin, chloride, total calcium, phosphorus, total magnesium and zinc concentrations and serum alkaline phosphatase activity. Sodium and potassium levels were estimated by flame photometer. Ionized calcium and ionized magnesium levels were calculated by using two mathematical formulae.

The results showed that oral corticosteroid caused significant increase in mean serum sodium level (P < 0.001), and significant decrease in mean serum potassium (P < 0.001), total magnesium (P < 0.001), ionized magnesium (P < 0.001), zinc (P < 0.001), corrected total calcium (P < 0.001), ionized calcium (P < 0.001), phosphorus (P < 0.001) and alkaline phosphatase activity levels (P < 0.001) when compared with those of the control group and those who used inhaled corticosteroid. The significant decrease in the mean serum chloride is (P < 0.01) when compared with the control group and (P < 0.05) when compared with those who used inhaled corticosteroid.

Also the results elucidated that oral corticosteroid had no significant influence on mean serum total protein (P > 0.05) and albumin (P > 0.05) levels when compared with those of the control group and those who used inhaled corticosteroid. The obtained results explained that inhaled corticosteroid had no significant effect on all the measured parameters when compared with those of the control group (P > 0.05).

It was concluded that oral corticosteroids possess more undesirable effects on the measured parameters than inhaled form as appeared by the changes in the measured parameters. Thus, the measurement of serum electrolytes and the correction of mineral status is quite important during corticosteroid therapy.

الخلاصة

تسع وستون من مرضى الربو (خمس وثلاثون رجلا و أربعة وثلاثون امرأة) استخدموا العلاجات الكورتيكوستيرويدية على شكل حبوب فموية واستنشاق ،ثمان وثلاثون مريضا استخدموا الحبوب الفموية وواحد وثلاثون مريضا استخدموا الشكل المستنشق. خمس وثلاثون شخصا ظاهريا انهم اصحاء لا يعانون من أي مرض كانوا قد اخذوا كمجموعة ضبط.

نماذج الدم سحبت من مجاميع المرضى ومن الضبط بعد صوم طوال الليل. المصل الذي استحصل من الدم قد استعمل لتقدير مستوى تراكيز البروتين الكلي والالبومين والكلور و الكالسيوم الكلي والفسفور والمغنيسيوم الكلي والزنك ونشاط خميرة الفوسفاتيز القاعدية باستخدام مقياس الضوء الطيفي . مستويا الصوديوم والبوتاسيوم قدرا بمضواء اللهـب . مـستويا الكالـسيوم المتــأين والمغنيسيوم المتأين حسبا باستخدام معادلتين رياضيتين.

أظهرت النتائج ان الكورتيكوستيرويد الفموي قد سبب ارتفاعا معنويا في معدل مستوى الصوديوم (0.001 > P) وانخفاضا معنويا في معدل مستويات البوتاسيوم (0.001 > P) ، المغنيسيوم الكلي (0.001 > P) ، المغنيسيوم المتــأين (0.001 > P) ، الزنك (0.001 > P) ، الكالسيوم الكلي المصحح (0.001 > P) ، الكالسيوم المتأين (0.001 > P) والفسفور (0.001 > P) في المصل، والانخفاض المعنوي في معدل نشاط خميرة الفوسفاتيز القاعدية (0.001 > P) في المصل عندما قورنت مع أولئك من

مجموعة الضبط وأولئك الذين يستخدمون الكورتيكوستيرويد المستنشق. وكان الانخفاض المعنوي في معدل مستوى الكلور ($P >$ 0.01) عندما قورن مع مجموعة الضبط و ($P < 0.05$) عندما قورن مع أولئك الذين يستخدمون الكورتيكوستيرويد المستنشق.

كذلك اوضحت النتائج بأن الكورتيكوستيرويد الفموي لم يتسبب بتأثير معنوي على معدل مستويات البروتين الكلي ($P >$ 0.05) والألبومين ($P > 0.05$) في المصل عندما قورنت مع أولئك من مجموعة الضبط وأولئك الذين يستخدمون الكورتيكوستيرويد المستنشق. فسرت النتائج المستحصلة بأن الكورتيكوستيرويد المستنشق لم يتسبب بتأثير معنوي على جميع العوامل المقاسة عندما قورنت مع أولئك من مجموعة الضبط ($P > 0.05$). استنتج بأن شكل الكورتيكوستيرويد الفموي يمتلك تأثيرات غير مرغوب بها على العوامل المقاسة أكثر مقارنة بالشكل المستنشق. لهذا السبب، قياس الالكتروليتات في المصل وتصحيح حالة المعادن مهم فعلا اثناء العلاج الكورتيكوستيرويدي.

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Introduction

Asthma is a chronic inflammatory disease of the airways that is associated with increased response-veness of the airways to environmental stimuli and variable airflow obstruction. It is a result of the interaction between genes and environment, and several genetic and environmental factors [1].

The prevalence of asthma increased steadily over the latter part of the last century in countries with a Western lifestyle and is also increasing in developing countries. In childhood, more common in boys, but following puberty females are more frequently affected. The socio-economic impact of asthma is enormous, particularly when poor control leads to days lost from school or work, hospital admissions and, for some patients, a premature death [2].

The prevalence of asthma has increased dramatically in recent years, with the largest increases and the highest prevalence in youth 18 years old and younger [3]. Large increases in the prevalence of asthma and allergic diseases have been reported in industrialized countries during the last twenty to thirty years [4].There is strong evidence for differences in the prevalence of allergic diseases between urban and rural areas in Europe and in non-industrialized countries, with higher prevalence of allergic diseases reported in urban areas [5].

Asthma treatments include medical treatment. For quick relief most clinically useful beta-2 agonists have a rapid onset of action (5-30 minutes) and provide relief for 4-6 hours as (Epinephrine, pirbuterol, terbutaline) [6].

Corticosteroids have been used to treat asthma since 1950 and are presumed to act by their broad anti-inflammatory efficacy [7].

Inhaled corticosteroids (ICSs) are the most effective agents in this category. Several studies demonstrated that treatment with ICS for one month or more significantly reduces the pathological signs of airway inflammation in asthma and airway hyperresponsiveness continues to improve with prolonged treatment[8]. The ICSs in current use (beclomethasone, budesonide and fluticasone) are characterized by low oral bioavailability because of high first-pass metabolism in the liver. Patients, who can not manage inhaled therapy, can be given oral therapy, although this will be accompanied by more systemic side effects. Oral prednisolone is very effective for severe exacerbations [9].

Minerals are inorganic (non-carbon-containing) elements one needs in relatively small amounts to help regulate body functions, aid in the growth and maintenance of body tissues, and help release energy [10]. Minerals that are required in relatively large amounts in the body are

commonly grouped as major elements [11]. The major minerals, those that the body needs in amounts exceeding 100 milligrams per day, include calcium, sodium, phosphorous, magnesium, potassium and chloride. The essential trace minerals, those that one needs in minute amounts, include copper, iron fluoride, iodide, selenium and zinc. The (electrolytes) term is used to refer to the minerals sodium, potassium and chloride [12]. The alkaline phosphatases (ALPs) are a group of isoenzymes that hydrolyse organic phosphates at high pH. They are present in most tissues but in particularly high concentrations in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta [13].

Materials and Methods
Materials
Subjects: include the control and patient groups

A. Group 1: Thirty five (23 males and 12 females) apparently healthy subjects were chosen as healthy people. They were non smokers; don't have any history of chronic diseases. Their age range is from (23) to (48) years with the mean age and standard deviation (34.428 ± 9.555).

B. Group 2: Thirty one asthmatic patients (17 males and 14 females) were included in this study. Their age range is from (22) to (53) years with the mean age and standard deviation (34.096 ± 10.274) years. They were non smokers and on inhaled corticosteroid therapy [beclomethasone dipropionate (BDP)] (Beclosone)[R] with duration of treatment for (1) year. They received short courses of systemic corticosteroids in the past.

C. Group 3: Thirty eight asthmatic patients (18 males and 20 females) were participated in this study. Their

age range is from (26) to (51) with the mean age and standard deviation (33.921 ± 11.075). They were non smokers and on oral corticosteroid therapy [prednisolone tablets (prisolone)[R]] with a duration of treatment for (1) year. The patients in group 2 and 3 were chronic stable asthmatics; they were not recently discharged from the hospital.

Patients selection and collection of specimens

Only asthmatic patients who were treated with corticosteroid drugs were included in this study. Post menopausal asthmatic women were excluded from the study. Patients with diarrhea, vomiting, a history of hepatic, cardiac, renal, bone, diabetes mellitus, thyroid gland disorders, hypertension, epilepsy or other diseases that may interfere with the study along with patients who take other drugs in addition to corticosteroids were also excluded from the study, except those on salbutamol inhaler (vental)[R].

The study was carried from the first of November 2009 to the end of May 2010. The samples of patients were obtained from, Asthma and Allergy Center in Hilla city. The study was performed at the laboratory of Biochemistry Department in College of Medicine, University of Babylon. A questionnaire was taken for each patient and control subjects.

Blood sampling

Venous blood samples were drawn from healthy control individuals and asthmatic patients after fasting by using disposable syringes in the sitting position. Five milliters of blood were obtained from each subject without using tourniquet, and was pushed slowly into plain disposable tubes with out anticoagulant. The blood was allowed to clot for 10-15 minutes and serum was obtained by centrifugation

at 2500 rpm for approximately 10-15 minutes. Then serum samples were placed into new clean disposable plain tubes.

Methods

Serum alkaline phosphatase activity determined by BioMerieux SA,

France kit. Serum total protein concentration determined by Biolabo SA, France kit. Serum albumin concentration determined by Biolabo SA, France kit. Serum total calcium concentration determined by Linear Chemicals, S.L., Spain kit. Serum phosphorous concentration determined by Human, Germany kit. Serum total magnesium concentration determined by Linear Chemicals, S.L., Spain kit.

Serum zinc concentration determined by LTA s.r.l., Italy kit. Serum chloride concentration determined by Biolabo SA, France kit. All the above mentioned parameters were measured by using spectrophotometer. The serum concentrations of sodium and potassium were measured by flame photometer.

Corrected total calcium was calculated according to the formula: [14].

Corrected calcium concentration mmol/L = measured calcium mmol/L + 0.02 × (40 - albumin g/L)

The concentration of ionized calcium in serum was calculated according to the formula: [15].

$$\text{Ionized calcium (mmol/L)} = \frac{60 \times \text{measured calcium (mmol/L)} - K'/12}{K' + 60}$$

K' = 0.19 × total protein (g/L) + albumin (g/L)

The concentration of magnesium ion in serum was calculated from measurement of concentrations of total serum protein and total serum magnesium according to the equation: [16].

$$[100.4\,G\,Z/100\,G-P]^2 + (33.77 + 2.42\,f\,P - f\,Mg)[100.4\,G\,Z/100\,G-P] - 33.77\,f\,Mg = 0$$

P = total protein in gram per 100 ml of serum.

G = specific activity of serum = 0.00292 × P + 1.007

f = liters of serum that contain 1 kilo of water =

1000 / G (4225.6 – 3225.6 G).

Mg = total magnesium in milli-equivalent per liter of serum.

Z = ionized magnesium in milli-equivalent per liter of serum.

Results and Discussion

Serum total protein and albumin were measured in the sera of group1, group2 and group3; the results are shown in table 1.

Table 1 Serum total protein and albumin concentrations in the group1, group2 and group3.

Parameters	Groups	Mean ± SD	Range
Total protein (g/dL)	Group1	7.553 ± 0.485	6.325 – 7.993
	Group2	7.470 ± 0.480	6.082 – 8.00
	Group3	7.610 ± 0.376	6.853 – 8.100
Albumin (g/dL)	Group1	4.180 ± 0.263	3.502 – 4.431
	Group2	4.134 ± 0.278	3.351 – 4.486
	Group3	4.191± 0.237	3.393 – 4.488

Table 1 shows that there is no significant difference in the mean serum total protein ($P > 0.05$) and albumin ($P > 0.05$) levels in the studied groups, thus total protein and albumin effects on the measured parameters are excluded.

Compared with systemic steroids, the dose of ICSs is low, and systemic absorption of most preparations via the gastrointestinal tract undergoes extensive first-pass metabolism, hence, their systemic side effects are fewer [17], this may explain the reason in this study in which it was observed no significant effect of inhaled BDP on the measured parameters when compared with that of the controls.

Serum sodium, potassium and chloride were measured in the sera of group1, group2 and group3; the results are shown in table 2.

Table 2 Serum sodium, potassium and chloride concentrations in the group1, group2 and group3.

Parameters	Groups	Mean ± SD	Range
Sodium (mmol/L)	Group1	141.771 ± 2.808	136 –145
	Group2	140.806 ± 2.587	137 –146
	Group3	146.894 ± 0.863 *** ***	146 – 149
Potassium (mmol/L)	Group1	4.117 ± 0.439	3.5 – 4.9
	Group2	3.961 ± 0.432	3.4 –4.8
	Group3	3.305 ± 0.089 *** ***	3.1 – 3.4
Chloride (mmol/L)	Group1	102.135 ± 3.136	98.122–107.685
	Group2	101.611 ± 3.221	97.876–107.135
	Group3	99.955 ± 1.645 ** *	97.658–103.641

Notes: The stars below elucidate the significant difference at p values:
*** Mean that group3 significantly different from group1 at ($p < 0.001$).
*** Mean that group3 significantly different from group2 at ($p < 0.001$).

**   Mean that group3 significantly different from group1 at ($p < 0.01$).
*   Mean that group3 significantly different from group2 at ($p < 0.05$).

Table 2 shows that the mean serum level of sodium is not significantly different between group1 and group2, but shows a significant increase in the mean serum level of sodium in the group3 than the other groups; this is due to oral corticosteroid therapy. Corticosteroid drug may possesses glucocorticoid effects (action on organic metabolism), mineralocorticoid effects (action on inorganic metabolism) or both with different potencies. Mineralocorticoid effects: increase retention of sodium by the renal tubule, and increase potassium excretion in the urine. Silva J., *etal*. showed that synthetic glucocorticoid (prednisolone) has little mineralocorticoid effects [18].

Also table 2 shows that the mean serum level of potassium is not significantly different between group1and group2, but shows a significant decrease in the mean serum level of potassium in the group3 than the other groups, this can be attributed to oral corticosteroid therapy.

The use of inhaled beta-2 agonists as salbutamol in patients with chronic asthma may have no effect on serum potassium level [19].
Patients on prolonged therapy with, corticosteroids tend to become hypokalemic due to the mineralocorticoid effect on the distal renal tubules [13].

Finally table 2 shows that the mean serum level of chloride is not significantly different between group1 and group2, but shows a significant decrease in the mean serum level of chloride in the group3 than the other groups. There may be some acid - base disturbance in the patients who received oral corticosteroid therapy.

Corticosteroid treatment may cause raised venous serum bicarbonate and decreased serum chloride [20]. Chloride is the major anion that counterbalances the major cation, sodium [21]. A change in plasma sodium concentration must be matched by a change in anion concentration. The major anions of the ECF are chloride and bicarbonate [22]. In the kidney, chloride reabsorption changes in a reciprocal fashion to bicarbonate reabsorption. When plasma chloride concentration changes independently of plasma sodium concentration, it is thus usually due to an acid-base disorder [23]. In the presence of hypokalemia, the kidneys conserve potassium and thus increase hydrogen ion excretion (recall that these ions compete for renal excretion). The serum chloride is relatively lower than sodium, as an elevation in the serum bicarbonate level causes the chloride level to drop. Norma M. said that as one anion increases, another tends to decrease to maintain electroneutrality [24].

Serum phosphorous, total magnesium, and zinc concentrations and alkaline phosphatase activity were measured also corrected total calcium, ionized calcium, ionized magnesium were calculated in group1, group 2 and in group3. The results are showed in table 3.

Table3 Serum corrected total calcium, ionized calcium, phosphorous, total magnesium, ionized magnesium, and zinc concentrations and alkaline phosphatase activity for group1, group2 and group3.

Parameters	Groups	Mean ± SD	Range
Corrected total calcium (mmol/L)	Group1	2.264 ± 0.113	2.105 – 2.491
	Group2	2.225 ± 0.170	1.799 – 2.471
	Group3	1.864 ± 0.154 *** ***	1.475 – 2.120
Ionized calcium (mmol/L)	Group1	1.129 ± 0.050	1.036 – 1.216
	Group2	1.117 ± 0.069	0.927 – 1.241
	Group3	0.919 ± 0.926 *** ***	0.697 –1.111
Phosphorous (mmol/L)	Group1	1.439 ± 0.090	1.252 –1.615
	Group2	1.428 ± 0.138	1.165 – 1.611
	Group3	1.100 ± 0.077 *** ***	0.897 – 1.266
Total magnesium (mmol/L)	Group1	0.752 ± 0.081	0.660 –1.013
	Group2	0.719 ± 0.064	0.598 –0.861
	Group3	0.565 ± 0.058 *** ***	0.442 – 0.684
Ionized magnesium (mmol/L)	Group1	0.473 ± 0.048	0.408 – 0.624
	Group2	0.454 ± 0.039	0.369 – 0.533
	Group3	0.353 ± 0.032 *** ***	0.288 – 0.420
Parameters	**Groups**	**Mean ± SD**	**Range**
Zinc (mol/L)	Group1	11.037±1.213	8.736 –14.200
	Group2	11.395 ± 1.646	9.101–16.830
	Group3	8.704 ± 1.007 *** ***	6.228 – 10.175
Alkaline phosphatase activity (U/L)	Group1	71.070 ± 15.335	38.950–103.680
	Group2	67.872 ± 19.276	23.853 –93.407
	Group3	50.213 ± 13.107*******	25.989–78.491

Note: The stars in table 3 give the same indications as in table 1.

Table 3 shows that the mean serum level of calcium is not significantly different between group1 and group2, but shows a significant decrease in the mean serum level of calcium in the group3 than the other groups, this effect can be attributed to oral corticosteroid therapy.

Direct effects of corticosteroids include the suppression of intestinal calcium absorption, decreased renal tubular calcium reabsorption with increased urinary calcium excretion [25]. Hypercalciuria is due to increased bone resorption and decreased renal tubular reabsorption of calcium, which occurs despite elevated serum levels of PTH [26].

365

Packe G. *et al*. found no significant difference in serum calcium between asthmatics on inhaled BDP and those who had never taken inhaled or systemic corticosteroids [27].

Also table 3 shows that the there is no significant difference in the mean serum level of phosphorous between group1 and group2, but shows a significant decline in the mean serum level of phosphorous in the group3 than other groups, thus the cause can be attributed to oral corticosteroid therapy.

Corticosteroids increase urinary phosphorous excretion [19]. The hyperphosphaturia observed in patients taking glucocorticoids is due in part to secondary hyperparathyroidism. The other causative factor is a glucocorticoid-induced change in Na^+- H^+ exchange activity, which causes a decrease in sodium-gradient-dependent phosphate uptake in the proximal tubule [26].

Hypophosphatemia can cause respiratory muscle fatigue, and reduction of tissue oxygen extraction in patients with acute asthma [19]. Bootsma *et al.* [28] observed that serum phosphate was not significantly changed by BDP.

Additionally table 3 shows that there is no significant difference in the mean serum level of magnesium between group1 and group2, but shows a significant decline in the mean serum level of magnesium in the group3 than other groups, thus the cause can be attributed to oral corticosteroid therapy.

There was no association of hypomagnesemia with inhaled beta-agonists and inhaled steroids [29], thus low mean serum magnesium level in the group3 compared to other groups may be the result of oral corticosteroid treatment. Khosrow A.,and Hamid R. said that receiving glucocorticoid drugs for long times especially its over use by the patients in acute states may cause depletion of magnesium in human through urinary excretion [30].

Low serum concentrations of magnesium have been associated with diminished respiratory power that improves with administration of magnesium [31]. Magnesium appears to be important in bone cell activity. It is shown to be mitogenic for osteoblasts and its depletion causes cellular growth inhibition, in vitro [32]. Fatouh *et al*. found that serum magnesium was significantly lower in asthmatics receiving short courses of oral corticosteroid compared to controls and to those not receiving steroid [33]. Our results are consistent with those obtained by Khalid S. in that no relation was found between the regular uses of ICSs and serum magnesium [34].

Further table 3 shows that there is no significant difference in the mean serum level of zinc between group1 and group2, but shows a significant decrease in the mean serum level of zinc in the group3 than the other groups, this effect may be due to oral corticosteroid. Steroidal anti-inflammatory drugs have been found to increase urinary loss of zinc [35].

Zinc is needed for osteoblastic activity, and ALP activity [36]. Our results are in agreement with the findings of Flynn *et al*. in that all the patients receiving oral corticosteroid therapy had lower circulating zinc level [37].

ALP is produced by osteoblasts during bone formation, and in the absence of liver dysfunction the total serum levels reflects mineralization rates [38]. Glucocorticoids decrease the number and function of osteoblasts [39]. Glucocorticoids have a biphasic effect on bone. Physiologic concentrations and brief exposure enhance the function of differentiated osteoblasts, whereas prolonged periods

inhibit synthetic processes [26]. Ionized magnesium is one of the ALP activators, and ionized zinc is a constituent metal ion [40]. In this study the significant decline in the mean serum ALP level in the group3 than the other groups as shown in table 3 may be due to the inhibitory effect of oral corticosteroid on osteoblasts which are source of the enzyme, also may be due to its effect on the levels of zinc and magnesium which are important for the activity of the enzyme. Jin S. and Wei F. [41] found substantially reduced ALP levels in asthmatic patients who had received glucocorticoid therapy. Bootsma *et al.* [28] observed that serum ALP was not significantly changed by BDP.

Finally it was concluded that oral corticosteroids have more undesirable effects on electrolytes, minerals and bone than ICSs; therefore, it is better to use ICSs whenever possible.

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