

# Performance of chloride/phosphate test in patients with primary hyperparathyroidism

*Is it related to calcium level?*

Ayman A. Mismar, MD, Gabriele Materazzi, MD, Marco Biricotti, MD, Nader M. Albsoul, MD, Nidal A. Younes, MD, Paolo Miccoli, MD.

## ABSTRACT

**الأهداف:** دراسة حساسية فحص نسبة كلوريد/الفوسفات  $>33$  كاختبار لتشخيص مرض فرط الغدة جارة الدرقية الأولي (pHPT) وعلاقته بنسبة الكالسيوم في الدم.

**الطريقة:** تم استعراض بيانات 120 مريضاً تم تشخيصه بمرض فرط الغدة الفوق الدرقية تم علاجهم جراحياً في قسم الجراحة، مستشفى سيزانيلو، بيزا، إيطاليا خلال الفترة من مارس 2010م حتى يونيو 2011م. تم تقسيمهم إلى أربع مجموعات فرعية وفقاً لمستويات الكالسيوم عندهم. وقد تم قياس نسبة الكلوريد/الفوسفات لكل مريض، ومن ثم تم قياس حساسية الاختبار باعتبار أن النتيجة أكثر من 33 مشخصة للمرض. ومن ثم جرى التحقق من وجود علاقة بين مستوى الهرمون جار الدرقية و حساسية الفحص.

**النتائج:** كانت حساسية فحص نسبة كلوريد/الفوسفات للمجموعة ككل (0.883 (0.809-0.932)). للمرضى الذين يعانون من الكالسيوم في الدم فوق المستويات العادية كانت حساسية الفحص (0.9340 (0.857-0.973)). أما المجموعة التي كانت تعاني من ارتفاع كلس الدم ( $>1$  ملغم / دل) فوق مستوى طبيعي فقد كانت حساسية الفحص 93%. وقد شكلت مجموعة المرضى الذين كانت نسبة الكالسيوم عندهم ضمن الحدود الطبيعية حوالي 24%؛ ولهذه المجموعة بلغت حساسية الفحص (0.724 (0.562-0.887))، وخصوصيته (0.763 (0.628-0.898))، وكانت القيمة التنبؤية الإيجابية (0.700 (0.536-0.864)) والقيمة التنبؤية السلبية (0.784 (0.651-0.916)). لم تظهر أي علاقة مباشرة بين أداء الفحص ومستوى الهرمون جار الدرقية في الدم.

**خاتمة:** أن فحص نسبة كلوريد/الفوسفات تشكل أداة جيدة لتوقع مرض فرط الغدة جارة الدرقية الأولي وقد أظهر أداء مرضياً لدى مجموعة المرضى الذين كانت نسبة الكالسيوم عندهم ضمن الحدود الطبيعية.

**Objectives:** To examine the sensitivity of the chloride/phosphate (Cl/PO<sub>4</sub>) ratio with a cut-off point of  $>33$  as a diagnostic test for primary hyperparathyroidism (pHPT) in surgically proven patients, and its performance at different calcium levels.

**Methods:** This is a retrospective medical records based study. Data of 120 patients diagnosed with pHPT, already operated in the Department of Surgery, Cisanello Hospital, Pisa, Italy between March 2010 and June 2011 were reviewed. They were divided into 4 subgroups according to their calcium levels. The Cl/PO<sub>4</sub> ratio was measured for each patient, with a cut-off point of 33, sensitivity of Cl/PO<sub>4</sub> test was measured. Test sensitivity was calculated for each subgroup, and a correlation with the parathyroid hormone (PTH) level was investigated. Performance of the equation was tested for the normocalcemic patients with a suitable control group.

**Results:** The sensitivity of Cl/PO<sub>4</sub> ratio for the whole group was 0.883 (0.809-0.932). The sensitivity was 0.9340 (0.857-0.973) for patients with serum calcium above normal levels. A similar result of 0.933 (0.830-0.978) was demonstrated for the subgroup with hypercalcemia  $<1$  (mg/dL) above normal level. Normocalcemic patients constituted 24%; for this subgroup, the sensitivity test was 0.724 (0.562-0.887), specificity was 0.763 (0.628-0.898), positive predictive value was 0.700 (0.536-0.864), and negative predictive value was 0.784 (0.651-0.916). No correlation was identified between the performance of formula and serum PTH level.

**Conclusion:** The Cl/PO<sub>4</sub> test seems to be a good tool to anticipate pHPT and showed a fair performance in normocalcemic patients.

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*From the Department of General Surgery (Mismar, Albsoul, Younes), Faculty of Medicine, University of Jordan, Amman, Jordan, and the Department of General Surgery (Materazzi, Biricotti, Miccoli), Faculty of Medicine, University of Pisa, Pisa, Italy.*

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*Address correspondence and reprint request to: Dr. Ayman A. Mismar, Department of General Surgery, Faculty of Medicine, University of Jordan, Amman 11942, Jordan. Tel. +962 799060822. Fax. +962 (6) 5353388. E-mail: aymanmismar@yahoo.com*

Primary hyperparathyroidism (pHPT), which is characterized by the autonomous overproduction of parathyroid hormone, is diagnosed in approximately 100,000 patients every year in the United States.<sup>1</sup> The classical clinical presentation of pHPT is described by the pentad painful bones, kidney stones, abdominal groans, psychic moans, and fatigue overtones.<sup>2</sup> With the introduction of calcium screening programs and its implementation worldwide, pHPT was started to be diagnosed early. In a well-defined population in Rochester, Minnesota, the proportion of patients representing the classical symptoms or complications of pHPT decreased from 22% in the pre-screening era (1965-1974) to 8%; following the introduction of automated serum calcium screening (1974-1982) to 1.6% in the post-screening era (1983-1992).<sup>3</sup> Due to the early detection, severe forms of pHPT with bone diseases and renal stones are rarely diagnosed these days.<sup>4,5</sup> High serum calcium level is the usual clue for pHPT diagnosis.<sup>6</sup> Normal calcium levels, however, might be seen in 10-20% of patients.<sup>7</sup> Even those patients who are thought to be asymptomatic will often show symptoms or metabolic complications when carefully evaluated with standardized health questionnaires.<sup>8,9</sup> Truly asymptomatic pHPT is rare, occurring in only 2-5% of patients.<sup>2,10</sup> Diagnostic lab tests include serum phosphate, chloride and parathyroid hormone (PTH) assay. The chloride/phosphate (Cl/PO<sub>4</sub>) ratio with a cut-off point of >33 was reported to be highly efficient in pHPT anticipation.<sup>11,12</sup> Its importance is augmented in those patients who have serum calcium within normal levels or just above normal level. In this subgroup of patients, a high index of suspicion is necessary to draw attention to the pHPT possibility. There are no studies in literature on the performance of Cl/PO<sub>4</sub> test in relation to the serum calcium level.

The present study aims to examine the sensitivity of this formula in an Italian population depending on results obtained from surgically proved primary hyperparathyroid patients' records, to check its sensitivity at different calcium levels, to check a relation with parathyroid hormone concentrations and to examine its performance in the group of normocalcemic primary hyperparathyroidism.

**Methods.** A literature review using a PubMed search looking for chloride in primary hyperparathyroidism, phosphate in primary hyperparathyroidism, chloride/phosphate ratio in primary hyperparathyroidism and Reeves equation was carried out. A pre-hand approval by the ethical authority of the Department of Surgery, University of Pisa, Pisa, Italy according to the principles

of Helsinki Declaration was achieved. Medical records of 120 patients diagnosed with pHPT, who were operated in Cisanello Hospital between March 2010 and June 2011, were reviewed. All of them had normal kidney function, normal vitamin D level in blood and a post-operative histopathological report of either parathyroid adenoma or hyperplasia. Collected data included age, gender, calcium, phosphate, chloride, parathyroid hormone (PTH) level, and pathological diagnosis; the patients who did not fulfill the previous criteria and those who had incomplete biochemical data were excluded. Whenever there was more than one reading, the highest was recorded for calcium, chloride and PTH, while the lowest was recorded for phosphate. The Cl/PO<sub>4</sub> ratio was measured for each patient. With a cut-off point of 33, the sensitivity of Cl/PO<sub>4</sub> test was measured. The patients were then divided into 4 groups depending on their calcium levels (<10.5, 10.5-11.5, 11.5-12.5, >12.5). Test sensitivity was first measured for each subgroup and then compared. Then, the relation between PTH level and Cl/PO<sub>4</sub> test performance was studied. Six patients were excluded as the PTH level was reported by the endocrinologist and no official lab record was available. Based on the pre-operative PTH level, 114 patients were stratified and divided into 4 groups with a cut-off point of 25%, 50% and 75%. Test sensitivity was measured for each group.

Subgroup with calcium level <10.5 was studied separately. Data for 38 patients were collected as a control group by reviewing the hospital database with inclusion criteria: serum calcium level of 10-10.5 and a serum PTH level within the normal range, gender distribution was controlled to meet the study group. The sample size determination was based on statistical analysis of the maximum error of estimates. The analysis showed that a sample of 26 elements is statistically adequate to perform the analysis. A hypothesis test was carried out based on the comparison of the mean of 2 samples with different variances and samples size. All items of the first sample are healthy people while all the items of the second sample have pHPT. The Cl/PO<sub>4</sub> ratio was calculated and the average of this ratio for both samples were obtained, and the variance was calculated for both samples. The null hypothesis was Cl/PO<sub>4</sub> ratios were equal for both samples; Cl/PO<sub>4</sub> was not a good indicator of the presence of pHPT. Whereas, the alternative hypothesis was Cl/PO<sub>4</sub> ratio for the first sample was higher than that for the second sample. The 5% level of confidence ( $\alpha=0.05$ ) was used to carry out the hypothesis test.

The statistical analysis based on t distribution was carried out and the t value of the test and the degree of

freedom were calculated. The results of the hypothesis test was based on a comparison between the critical and the calculated t values. Sensitivity, specificity, and negative and positive predictive values of Cl/PO<sub>4</sub> test with a cut-off point of 33 were calculated with 95% confidence intervals.

**Results.** The study group consisted of 120 patients (100 females; 20 males). The mean age was 57.7 years, according to which the 4 subgroups were comparable. The sensitivity of Cl/PO<sub>4</sub> ratio for the whole group was 0.883 (0.809-0.932). Twenty-nine patients had the maximum measured calcium levels in the normal range; average level for this subgroup was 10.2 mg/dL, and the test sensitivity for this group was 0.724 (0.562-0.887). For all patients who had calcium higher than the normal level, the sensitivity increased to 0.9340 (0.857-0.973). For the subgroup >12.5 mg/dL, the sensitivity was 1 (0.598-1). The results are shown in Table 1.

The patients with pHPT and normal calcium levels had a higher Cl/PO<sub>4</sub> ratio in comparison to the control group, which was statistically significant. The statistical analysis based on t distribution was carried out and the t value of the test and the degree of freedom which were found to be 6.41 and 53 (round off 52.7) respectively.

So, based on the previous values the null hypothesis can be rejected at a very high level of significance ( $p < 0.0005$ ). A 95 % confidence interval would give an estimation of the difference between the 2 means to be higher than 8.7 and <16.6 (Table 2).

The sensitivity, specificity, and positive and negative predictive value for the Cl/PO<sub>4</sub> test with a cut-off point of 33 in patients with pHPT and normal calcium levels are shown in Table 3.

Regarding the relation between PTH level and Cl/PO<sub>4</sub> ratio, the test sensitivity was 0.897 (0.715-0.973) at the cut-off point of 25% of the study sample, 0.821 (0.624-0.932) at 25-50%, 0.897 (0.715-0.973)

**Table 3 -** The sensitivity, specificity, and predictive values for the test Cl/PO<sub>4</sub> in patients with normocalcemic primary hyperparathyroidism.

Group	Disease	Not diseased	Predictive values
Study	True positive 21	False Positive 9	PPV 0.700 (0.536-0.864)
Control	False Negative 8	True Negative 29	NPV 0.784 (0.651-0.916)
	Sensitivity	Specificity	
	0.724 (0.562-0.887)	0.763 (0.628-0.898)	
PPV - positive predictive value, NPV - negative predictive value			

**Table 1 -** The sensitivity of CL/PO<sub>4</sub> ratio test at different calcium levels.

Calcium (mg/dL)	Age in years (SD)	F/M	Number	CL/PO <sub>4</sub> (SD)	>33	Sensitivity*	Sensitivity†
<10.5	56.27 (2.19)	26/3	29	41.06 (8.7)	21	0.724 (0.525-0.866)	0.724 (0.525-0.866)
10.5-11.5	56.36 (10.2)	51/9	60	42.73 (8.5)	56	0.933 (0.830- 0.978)	0.9340 (0.857- 0.973)
>11.5-12.5	59.9 (10.9)	17/6	23	44.66 (11.5)	21	0.913 (0.705- 0.985)	
>12.5	58.15 (15.1)	6/2	8	45.9 (8.23)	8	1 (0.598- 1)	
Total			120		106	0.883 (0.809-0.932)	
PO <sub>4</sub> - Phosphate, Cl - Chloride, *includes 4 subgroups (<10.5, 10.5-11.5, 11.5-12.5, and >12.5)							
†Group with >10.5 calcium levels and it includes 3 sub-groups (10.5-11.5, 11.5-12.5, >12.5)							

**Table 2 -** A comparison between the study subgroup of normocalcemic hyperparathyroid patients and a control group with normal parathyroid hormone level and a comparable calcium level.

Groups	Age in years (SD)	F/M	Ca (mg/dL) mean ± SD	PO <sub>4</sub> (mg/dL) mean ± SD	CL (mmol/L) mean ± SD	CL/PO <sub>4</sub> mean ± SD	Cut-off 33
Control	52.6 (14.32)	32/5	10.16 ± 0.17	3.77 ± 0.94	101 ± 3.6	28.4 ± 7.0	9/38
Study	56.27 (12.19)	26/3	10.17 ± 0.25	2.66 ± 0.57	105.7 ± 2.99	41.06 ± 8.7 $p < 0.0005$	21/29
Ca - Calcium, PO <sub>4</sub> - Phosphate, Cl - Chloride							

**Table 4** - The sensitivity of CL/PO<sub>4</sub> ratio test at different parathyroid hormone levels.

Cut off (%)	PTH range pmol/L	Mean PTH pmol/L (SD)	N	Cl/PO <sub>4</sub> Mean±SD	Cl/PO <sub>4</sub> >33	Sensitivity mean (range)
25	60-135	100.9 (21.7)	29	44.1±8.7	26/29	0.897 (0.715-0.973)
>25-50	140-203	166.3 (17.4)	28	43.1±8.74	23/28	0.821 (0.624-0.932)
>50-75	210-285	240.85 (24.1)	29	46.6±8.84	26/29	0.897 (0.715-0.973)
>75-100	297-1554	618.4 (386.4)	28	52.39±10.12	25/28	0.893 (0.706-0.972)

PTH - Parathyroid hormone, PO<sub>4</sub> - Phosphate, Cl - Chloride

at 50-75%, and 0.893 (0.706-0.972) at 75-100% as shown in Table 4.

**Discussion.** Hyperchloremia (ChlorideN107 mEq/l) occurs in approximately 40% of patients with pHPT, and when present in a hypercalcemic patient it suggests that the patient has pHPT. Another 40% of patients with pHPT have serum chloride level in the high-normal range.<sup>13</sup> Approximately 50% of patients with pHPT have low serum phosphorus level (<2.5 mg/dl) and another 30% have their serum phosphorus in the low-normal range.<sup>14</sup> Even though, PTH assay is the most informative pre-operative diagnostic test, its cost limits its role in screening programs. Reeve et al<sup>12</sup> introduced the concept of CL/PO<sub>4</sub> ratio as a very accurate and inexpensive diagnostic tool for pHPT; besides, with a cut-off point of >33, he reported a sensitivity of 0.940 and specificity of 0.960.<sup>12</sup> Similar figures were also reported by other researchers.<sup>11,15</sup>

Importance of the test is augmented in patients with normocalcemic hyperparathyroidism with minor manifestations. In these patients, a high index of suspicion is important to reach the diagnosis; and the presence of a simple reliable test can aid in this regard. In this study, the Cl/PO<sub>4</sub> ratio showed a direct relation with the serum calcium level. Actually, increase in the ratio is more dependent on the decrease in PO<sub>4</sub>, which is inversely related to the calcium. Whereas, the serum chloride level showed a tendency to be in the upper-normal range, without any direct relation with the serum calcium level.

The Cl/PO<sub>4</sub> test with a sensitivity of 0.9340 (0.857-0.973) for patients with pHPT and hypercalcemia was comparable to literature. It also showed similar performance in patients with mild hypercalcemia <11.5 of 0.933 (0.830-0.978), which makes it a very good diagnostic tool in this subgroup. Twenty-four percent of patients had pHPT with normocalcemia, which is a little bit more than what

is reported in the literature. This could be due to the increased awareness of the disease and the implication of screening programs adopted by the national health system. In this subgroup, the performance of formula declined with a sensitivity of 0.724 (0.562-0.887), 0.763 (0.628-0.898), positive predictive value of 0.700 (0.536-0.864) and negative predictive value of 0.784 (0.651-0.916). But, as the importance of calcium as a screening test is eliminated in these patients, the Cl/PO<sub>4</sub> ratio seems to be a good complementary tool for pHPT anticipation. This research has the advantages of studying the Cl/PO<sub>4</sub> ratio test in a homogeneous Italian population; a community in which it was not studied before. It studied for the first time the performance of the test in relation to serum calcium level and demonstrated a high sensitivity in patients with marginal hypercalcemia and a fair performance in patients who have normocalcemic primary hyperparathyroidism.

A major limitation of the test is the low prevalence of pHPT in the community, which decreases significantly the test positive predictive yield when dealing with the general population. Therefore, a cost benefit study is important before implementation of Cl/PO<sub>4</sub> ratio test in pHPT screening programs. Still the Cl/PO<sub>4</sub> test can have a special importance in primary care clinics where some patients may have calcium levels within the upper-normal range or just above the normal range. In these patients, this simple test can help the physician to predict with a fair accuracy the presence or absence of pHPT. Consequently, modify his investigation and management plan accordingly.

In conclusion, depending on the results and in view of its low cost, availability, and high negative predictive value in normocalcemic patients, The Cl/PO<sub>4</sub> test seems to be a good tool to anticipate pHPT. This study could not demonstrate any direct relation between the sensitivity of Cl/PO<sub>4</sub> test and serum PTH level.

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