Value of Measuring C-Terminal Parathyrin in Differential Diagnosis of Hypercalcemia

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We measured calcium, phosphate, chloride, albumin, C-terminal parathyrin, and \( \beta_2 \)-microglobulin in serum from 102 hypercalcaemic patients: 42 with primary hyperparathyroidism and 60 with neoplasia. The calcium concentrations and the discriminant function index of Johnson et al. (Clin Chem 28, 333–338, 1982) were higher in malignant hypercalcemia than in primary hyperparathyroidism. The diagnostic efficiency of the index and of parathyrin concentration was 82% and 78%, respectively. Using the ratio of parathyrin to \( \beta_2 \)-microglobulin increased the diagnostic efficiency to 98%; the ratio of the discriminant index to parathyrin concentration had a diagnostic efficiency of 100%. We conclude that C-terminal assay by itself is no better than the discriminant function index.

Additional Keyphrases: diagnostic sensitivity and specificity of various tests and ratios · hyperparathyroidism · cancer · discriminant function index · \( \beta_2 \)-microglobulin

Differential diagnosis of the etiology of hypercalcemia is a common clinical problem that can be difficult. Among its common causes are malignancy and primary hyperparathyroidism (1, 2), for which several diagnostic tests and indices have been described, including measurements of serum phosphate (3), plasma chloride (4), urinary phosphate excretion, acid/base status (5), and parathyroid hormone (6–9). Because diagnostic groups could not be distinguished on the basis of these tests alone, discriminant function analysis has been tried in an attempt to increase diagnostic efficiency. Palmer et al. (10) used the ratio of phosphate to chloride in serum to differentiate parathyroid and nonparathyroid causes of hypercalcemia. Others have described multivariate analyses designed to distinguish the cause of hypercalcemia (11, 12). Using tests available in a general hospital laboratory, Johnson et al. (13) described a mathematical equation, which they termed the “HJHM chloride index,” for use in differentiating hypercalcemic patients.

Theoretically, measurement of parathyrin (parathyroid hormone) should be the best diagnostic test. However, it circulates in the form of several fragments. The 84-amino-acid polypeptide is cleaved to form C-terminal and N-terminal fragments. The biologically active forms are the N-terminal fragment, which has a short biological half-life, and the intact molecule. Because the C-terminal fragment has a much longer half-life, antibodies developed against parathyrin have until recently been directed toward this fragment (6–9).

The cleaved fragments of parathyrin are metabolized and excreted by the kidney, the concentrations of C-terminal parathyrin activity being increased in the circulation in renal failure. Hypercalcemia itself can lead to a decrease in the glomerular filtration rate and therefore can cause a secondary increase in C-terminal parathyrin.

In 1982, when a commercial parathyrin assay became available we decided to investigate its usefulness in establishing a diagnosis of hypercalcemia and to compare it with the discriminant function index of Johnson et al. (13). We also measured serum \( \beta_2 \)-microglobulin, a protein that is filtered and metabolized by the kidney, postulating that values for \( \beta_2 \)-microglobulin in serum could help us assess the contribution of renal function to increases in C-terminal parathyrin activity.

Materials and Methods

Retrospective Study

To establish whether the discriminant function index described by Johnson et al. (13) was applicable to our laboratory, and to re-define the cutoff points for this index, we retrospectively analyzed data from 134 patients for whom the reported calcium concentration in serum was 2.70 mmol/L or greater (normal reference interval, 2.20–2.60 mmol/L). We also noted the results for serum chloride, albumin, and inorganic phosphate, and determined the final diagnosis as arrived at from the surgical, histological, or radiological evidence recorded in the case notes.

Prospective Study

In the prospective study, we examined results for serum samples being analyzed with the SMAC multichannel continuous-flow analyzer (Technicon Instruments Corp., Basingstoke, U.K.) and collected and froze those samples having a calcium concentration of 2.70 mmol/L or greater. We also recorded the results for albumin, inorganic phosphate, and chloride from the SMAC profile, and re-analyzed aliquots of these stored samples for C-terminal parathyrin and \( \beta_2 \)-microglobulin.

To determine parathyrin concentration, we used a commercial kit (Immuno Nuclear Corp., Stillwater, MN 55082), following the manufacturer’s protocol except that we added the tracer 4 h after the antibody, to enhance sensitivity. We measured \( \beta_2 \)-microglobulin by a modified radial immunodiffusion technique involving agarose and antiserum to \( \beta_2 \)-microglobulin from Kallestad Laboratories, Austin, TX 78701 (also available from the Immunology Department, Royal Hallamshire Hospital, Sheffield, U.K.).

Preliminary experiments showed that delaying the separation of serum from blood cells for as long as 2 h did not affect the values obtained for either parathyrin or \( \beta_2 \)-microglobulin.

The final classification of these patients was also based on clinical, surgical, histological, and radiological findings recorded in the patients’ case notes.

We calculated the discriminant function of Johnson et al.—the HJHM chloride index (13)—as follows: HJHM chloride index = 191.25 – (0.65 \times chloride, mmol/L) – (2.14 \times albumin, g/L) + (14.44 \times inorganic phosphate, mmol/L).
Results

Retrospective Study

Of the 134 patients in this study, 65 had hypercalcemia resulting from malignancy and 61 because of primary hyperparathyroidism. In eight patients the cause was not identified, and they were excluded from further study. The age of the patients with malignancy ranged from 37 to 96 (mean 72) years; for those with primary hyperparathyroidism the range was 30–66 (mean 43) years. After inspecting the distribution of the values for the index in the two groups of patients, we decided to change the lower cutoff point from 43 (the value used by Johnson et al.) to 55. This increased the diagnostic efficiency from 80% to 89% and the predictive value of a positive test result from 61% to 84%.

Prospective Study

Of the 102 hypercalcemic patients in this study, 42 had primary hyperparathyroidism and 60 had neoplasia. Table 1 shows the results for the index and for calcium, parathyrin, and the concentrations of \( \beta_2 \)-microglobulin in serum for both groups. The patients with malignant hypercalcemia had lower concentrations of parathyrin than the other patients, but values for the other three variables were all higher. None of the patients with primary hyperparathyroidism had a serum calcium concentration as high as 3.20 mmol/L (range 2.70–3.12); therefore, a calcium concentration of 3.20 mmol/L or more in serum was strongly indicative of non-parathyroid hypercalcemia. As shown in Table 2, the diagnostic efficiency of results for serum calcium only was 76%. The index, as expected, was higher in cases of non-parathyroid hypercalcemia (Table 1); its diagnostic efficiency was 82% (Table 2). Applied to the hyperparathyroid group, the index correctly predicted the diagnosis in 39 of the patients (94%).

Mean concentrations of parathyrin were higher in patients with primary hyperparathyroidism, but there was wide overlap between the two groups. None of the patients with malignancy had a parathyrin value exceeding 1.3 \( \mu g/L \); at this cutoff value, the predictive efficiency of parathyrin alone was 78%, slightly less than that of the index.

Measurement of parathyrin alone misclassified almost half (45%) of the patients with primary hyperparathyroidism. Because of the feedback between calcium and parathyrin, we investigated the diagnostic efficiency of the ratio of parathyrin to calcium, the ratio being 0.28–1.08 in patients with primary hyperparathyroidism and 0.01–0.45 in patients with non-parathyroid disease. Use of a ratio of 0.4 as the cutoff point gave an overall diagnostic efficiency of 84%.

The HJHM chloride index was sensitive in detecting patients with primary hyperparathyroidism (94%) whereas parathyrin, on the other hand, was normal for patients with neoplasms but variable for those with primary hyperparathyroidism. Using the combination of these two results—i.e., the ratio of the index to parathyrin—increased the diagnostic efficiency to 100% (Table 2). Similarly, the ratio of parathyrin to \( \beta_2 \)-microglobulin had a diagnostic efficiency of 98%, only one case of non-parathyroid hypercalcemia being misclassified.

Discussion

The higher incidence of primary hyperparathyroidism in the present series (40%) as compared with that reported in other series (e.g., 30% in the series reported by Fiskcn et al. (1)) could be due to selective referral to a surgeon with special interest in parathyroid surgery in this hospital (Hull Royal Infirmary), which serves a large population. Although the value for serum calcium was higher in neoplasia, its diagnostic usefulness is limited (1, 14). The HJHM chloride index, arrived at by use of commonly available laboratory tests (13), had an efficiency of 80% in the retrospective study, improved to 88% by changing to the lower cutoff point. However, when this was applied to the prospective study the efficiency was still only 82%.

Although values for parathyrin were significantly higher in primary hyperparathyroidism than in neoplastic disease, the diagnostic efficiency was only 78%, not much different from that obtained by using the index or serum calcium determinations alone. A similar diagnostic efficiency of parathyrin (75%) can be calculated from the data of Wong and Frier (14). In an attempt to improve the diagnostic efficiency, we examined the ratios of parathyrin to calcium and the index to parathyrin in the prospective study. The diagnostic efficiency was not improved for the former but it did improve (to 100%) for the latter.

An increased \( \beta_2 \)-microglobulin concentration in serum can be caused by a decrease in glomerular or tubular function, because this protein is freely filtered and reabsorbed by the tubules. Because parathyrin is metabolized by the renal tubules, we measured serum \( \beta_2 \)-microglobulin, which is also metabolized by the renal tubules. Values for \( \beta_2 \)-microglobulin were higher in malignant hypercalcemia and correlated significantly with results of C-terminal parathyrin assay. The diagnostic efficiency of the ratio of parathyrin to \( \beta_2 \)-microglobulin was 98% (Table 2).

We conclude that C-terminal parathyrin assay by itself is no better than the discriminant function analysis of Johnson et al. or serum calcium alone, and that evaluations
parathyrin/Ca ratios do not improve diagnostic efficiency. In difficult cases, and to make the diagnostic test more efficient, we suggest C-terminal parathyrin assay together with β2-microglobulin or in conjunction with a discriminant function such as that of Johnson et al. (13).

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References