World J. Surg. 24, 1319–1322, 2000
DOI: 10.1007/s002680010218

WORLD
Journal of
SURGERY

Intraoperative Parathyroid Aspiration and Parathyroid Hormone Assay as an Alternative to Frozen Section for Tissue Identification

Nancy D. Perrier, M.D.,[1] Philip Ituarte, Ph.D., M.P.H.,[1] Shoichi Kikuchi, M.D.,[1] Allan E. Siperstein, M.D.,[1] Quan Yang Duh, M.D.,[2] Orlo H. Clark, M.D.,[1] Robert Gielow, M.A.,[1] Timothy Hamill, M.D.[3]

[1]Department of Surgery, University of California, San Francisco, UCSF/Mt. Zion Medical Center, 1600 Divisadero Street, San Francisco, California 94115, USA
[2]Surgical Services, Veterans Affairs Medical Center, San Francisco, California 94121, USA
[3]Department of Clinical Pathology, University of California, San Francisco, UCSF/Mt. Zion Medical Center, 1600 Divisadero Street, San Francisco, California 94115, USA

Abstract. Most people would agree that successful parathyroidectomy depends on two important variables: the surgeon's recognition and excision of the abnormal parathyroid gland(s) and the pathologist's confirmation that the removed tissue is parathyroid tissue. Frozen section is usually employed to confirm the identity of parathyroid tissue, but occasionally confirmation cannot be made without a permanent section, as with intrathyroidal glands. This study proposes a new method of expeditious and easy confirmation of parathyroid tissue utilizing the immunoassay for quick measurement of intraoperative parathyroid hormone (IOPTH). By directly aspirating the suspected adenoma, the assay becomes a rapid diagnostic tool that can be used as an alternative to frozen section. In cases where the surgeon is already planning to employ the assay, the elimination of frozen section is cost-effective. Intraoperative aspiration of histologically confirmed parathyroid adenomas was performed on 12 consecutive patients undergoing parathyroid surgery. Parathyroid glands were aspirated with a 22-gauge syringe after gland excision. Aspirates were placed in 1 to 3 ml of buffered saline. A similar process was performed on 12 thyroid controls. Specimens were centrifuged, aliquotted, and stored at −70°C. The parathyroid hormone value was analyzed electively by rapid assay and the values recorded. For all parathyroid aspirates, the rapid assay value was > 1500 pg/ml, exceeding the uppermost limit of the diagnostic chart. Values for thyroid aspirates ranged from 58 to 85 pg/ml (mean 75.7 pg/ml). In all cases tissue confirmation was achieved with permanent section. Values were 100% sensitive and specific. Measurement of PTH from intraoperative aspiration of suspected parathyroid adenomas is clinically useful in patients for whom frozen section would routinely be employed. Values > 1500 pg/ml secure the tissue diagnosis. There is no additional cost in cases where IOPTH monitoring is already being utilized to confirm cure. The elimination of frozen section could be cost-effective and, for some institutions, actually decrease the operating time as the IOPTH assay takes only 15 minutes. PTH assay is an accurate diagnostic technique and to date is 100% sensitive and specific for differentiating between parathyroid tumors and thyroid nodules.

Successful parathyroidectomy depends on the surgeon's recognition and excision of the abnormal parathyroid gland and the pathologist's confirmation that the removed specimen is parathyroid tissue. Frozen section is usually employed to confirm the identity of parathyroid tissue, especially during repeat operations. In these patients normal anatomic relations are disturbed: Landmarks may be absent, and scarring and adhesions distort the field and complicate the surgical technique. Parathyroid identification is also challenging in patients with brown fat because the beige-brown color is the same as that of parathyroid tissue. Occasionally, as in cases of intrathyroidal parathyroid glands, parathyroid glands with a microfollicular pattern, or uncircumscribed oxyphilic cells, the tissue closely resembles microfollicular or Hurthle cell thyroid adenoma tissue and identity cannot be confirmed with frozen section [1]. Polarization microscopy is usually not helpful, and tissue confirmation must be made on permanent sectioning. In these cases, results are not available until the following day. Previous studies have reported the value of aspiration biopsy of parathyroid adenomas but recommended doing so preoperatively, under ultrasonographic guidance [2].

We propose intraoperative needle aspiration of suspected parathyroid tissue for a quick measurement of intraoperative parathyroid hormone (IOPTH). This novel application of the assay can by used in cases in which the IOPTH measurement is already being employed. Direct aspiration of the suspected adenoma is quick, easy, cost-effective, and reliable for confirming it is tissue of parathyroid origin. This technique is an ideal alternative to frozen section for parathyroid gland identification and is especially helpful when the pathologist has difficulty distinguishing between thyroid adenoma and parathyroid adenoma. It is a complementary modality in situations where the surgeon is already planning to employ the assay [3].

Patients and Methods

Patients

During the months of October 1998 through May 1999 a total of 65 parathyroidectomies were performed by a single surgeon at the

This International Association of Endocrine Surgeons (IAES) article was presented at the 38th World Congress of Surgery International Surgical Week (ISW99), Vienna, Austria, August 15–20, 1999.
Correspondence to: O.H. Clark, M.D.

University of California at San Francisco/Mount Zion Medical Center. There were 41 female and 25 male patients. Fifty-six operations were performed for primary, sporadic hyperparathyroidism, three for tertiary hyperparathyroidism, and two each for parathyroid cancer, secondary hyperparathyroidism, or multiple endocrine neoplasia (MEN) type I disease. Five operations were for persistent disease, and one was for recurrent disease. IOPTH assays were utilized in all cases to assess hormone decline as an indication of complete removal of hyperfunctioning tissue. Aspiration of parathyroid or other tissue possibly of parathyroid origin was performed on 41 patients in whom the surgeon desired frozen section for tissue confirmation.

Procedure

Under direct vision, a 5 cc syringe with a 1.5 inch (3.8 cm) 23-gauge needle was used to aspirate suspected in situ or ex vivo parathyroid tissue. Two or three passes were used to obtain a sufficient sample. Although the assay has not been designated for use with tissue aspirates, samples were collected by aspiration of the tissue in question with a syringe containing 2 ml of saline solution and then injecting the saline into ethylenediaminetetraacetic acid (EDTA) tubes for analysis. The vials were placed on ice and transferred to the chemistry laboratory.

Biochemical Assay

The Quick-IntraOperative Intact PTH assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) is a chemiluminescence immunometric assay. The assay utilizes chemiluminescence acridinium esters as a label in the presence of a hydrogen peroxide and a sodium hydroxide trigger. In their presence, the acridinium esters are oxidized to an excited state. The subsequent return to the ground state causes an emission of light that is quantified. A certified clinical laboratory scientist with training in conducting high complexity tests performed each assay. Aliquots (1 ml) of EDTA tissue aspirates were transferred to 1.5 ml tubes and centrifuged at 6400 rpm for 1.5 minutes. After centrifugation the supernatant was ready for immediate use. Calibrated, quality control EDTA tissue aspirate (200 μl) was added to each of two 12 $\times$ 75 mm borosilicate glass tubes. Acridium ester-labeled antibody (100 μl) was then added to the bottom of each tube. An antibody-coated styrene bead was added to each tube, and then all tubes were incubated in the QuiCk-Pak Kinetic Enhancer at 45°C while rotating at 400 rpm for 7 minutes. The styrene beads were saline-washed three times, utilizing 2 ml of working saline solution, in the QuiCk-Pak Automated Washer. The styrene beads were then transferred into clean, dry 12 $\times$ 75 mm borosilicate glass tubes. Each tube/bead was then placed in the QuiCk-Pak Quantifier where trigger solutions were added to the tube/bead and the resulting chemiluminescence was measured in relative light units (RLU) [4].

The amount of bound labeled antibody was directly proportional to the concentration of parathormone (PTH) in the sample. A six-point standard curve was generated and plotted using RLU versus the PTH concentration for each standard. The concentration of the quality control and patient samples were generated relative to the standard curve. Patient sample results were anticipated to fall within the standard curve range of 0 to 1500 pg/ml. Aspirates from parathyroid tissue were expected to be substantially higher than the upper limit of the standard curve. Dilutions of 1:10 were made for the first five specimens. No other aspirates were diluted. Thyroid tissue, lymph node, and other nonparathyroid tissue were expected to generate results in the lower standard curve range.

In 35 cases (53%) the laboratory had personnel available to perform the assay immediately, while the patient was in the operating room. Because blood was not involved there was no need to centrifuge the specimens, and results were available in less than 7 minutes. In 31 cases (47%) personnel were not immediately available, and specimens were frozen at −72°C and analyzed within 30 days.

Data Analysis

All specimens underwent tissue confirmation by permanent histologic evaluation. The final pathologic diagnoses were compared with the paired IOPTH diagnoses for all patients. All data were entered as received into an Excel spreadsheet. The Pearson correlation coefficient was used to measure the strength of association between PTH aspirate values and PTH serum levels. Sensitivities and specificities were calculated.

Results

Among the 65 patients for whom IOPTH was employed, 65 aspirates were obtained from 41 patients. There were no complications. In all 44 aspirates from documented parathyroid tissue, parathyroid hormone rapid assay values were higher than the upper limit of normal for the assay (1500 pg/ml). The results from aspirates diluted 1:10 were also higher than the upper limit of normal. There was no quantitative way of measuring the value when it was off the standard curve. The values for aspirates from two parathyroid carcinoma specimens were also higher than the upper limit of normal for the assay. All aspirates from nonparathyroid tissue had IOPTH values that were impressively lower than those for aspirates of parathyroid origin. Fifteen aspirates from thyroid nodules had a mean value of 157.5 pg/ml (range 16–562 pg/ml). Three aspirates from thymic tissue had a mean value of 60 pg/ml (range 49–71 pg/ml), and one fat aspirate had a value of 47 pg/ml (Fig. 1).

The Pearson correlation coefficient for the association between nonparathyroid aspirate PTH and preexcision PTH was $r = -0.24$ ($p = 0.54$). This suggested no correlation between the preexcision parathyroid hormone level in serum with the tissue concentration of nonparathyroid tissue. Aspirations successfully defined either parathyroid or nonparathyroid tissue in all 65 aspirates. Laboratory technicians were blinded to the frozen section results. Values were 100% sensitive and specific.

Discussion

Intraoperative parathyroid hormone monitoring as an adjunct to parathyroidectomy is a means to confirm removal of hyperplastic parathyroid tissue by monitoring glandular hyperfunction and secretion [5–7]. The intraoperative immunochemiluminescence assay (ICMA) uses two antibodies to measure intact PTH, which has a half-life of 3 to 4 minutes [4]. In cases where immunochemiluminescent technology is already being used, another innovative utilization of IOPTH is for tissue diagnosis. Values higher than

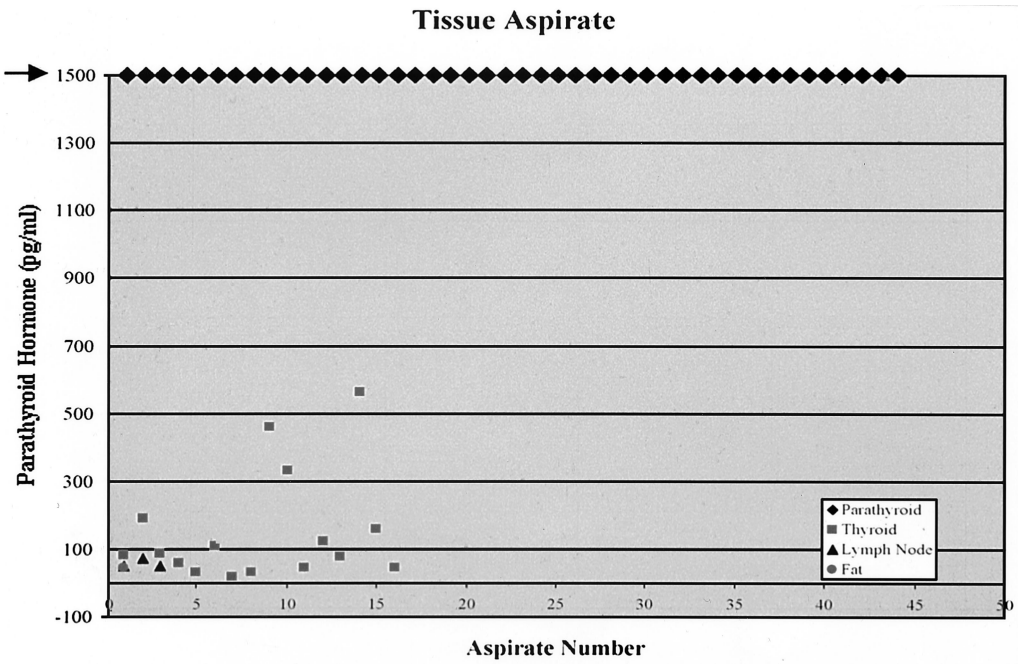

Fig. 1. Intraoperative parathyroid aspiration and parathyroid hormone assay as an alternative to frozen section for tissue identification. Hormone values for aspirations of pathologically confirmed parathyroid (diamonds), thyroid (squares), lymph nodes (triangles), and fatty tissues (circles).

1500 pg/ml secured the diagnosis that the tissue was parathyroid in all cases. It was not, however, able to differentiate normal from hyperplastic, adenomatous, or carcinomatous tissue. In retrospect, we attempted to dilute the parathyroid samples to bring the aspirate value into the upper limit of the standard curve. Four parathyroid aspirates were diluted 1:10 with normal saline. Values still remained outside the upper limit of the normal curve. Higher dilutions (1:100 or 1:1000) should be evaluated in the future to determine if there is any other diagnostic capability of the quick PTH assay. Although an experienced surgeon can successfully identify abnormal and normal parathyroid glands in 95% of patients, in difficult cases or during reoperations with an abundance of scar tissue tissue confirmation is necessary. Frozen section can provide a definitive diagnosis 99% of the time, with incorrect diagnosis occurring in only 1.3% of cases [2]. Inaccurate diagnoses are caused by section artifact, sampling error, and errors in judgment. This is more likely when there is coexistence of nodular thyroid disease, intrathyroidal parathyroid glands with conspicuous follicle formations or abundant oncocytic cells, and thyroid nodules with fatty stroma. As mentioned, in some patients it is impossible to distinguish between a parathyroid and a thyroid adenoma on frozen section and rarely even on permanent section. Immunohistochemical staining may be necessary in these patients [8].

Measuring PTH in the tissues in questions using the quick PTH assay can eliminate the need for frozen section and an on-site pathologist. This can decrease patient cost by abolishing the fee for slide preparation ($200–300) and for the pathologists' interpretation ($200–300). In some institutions it can decrease the operating time and patient cost, as assay values can be obtained in 7 minutes or less, which is generally quicker than frozen section results. Although not directly charted, in our experience rapid confirmation of tissue expedited the exploration in more than half of the cases when IOPTH and frozen section were both utilized intraoperatively.

The cost of an IOPTH kit varies from $1000 to $1200. Each kit includes the materials to run nine aliquots in double. Once the seal is broken, the kit is not reusable and must be discarded at the end of the day. To assess PTH decline, each patient requires, at minimum, a before and after sample. In these situations, seven extra aliquots are available. When using these "extra" materials for tissue identification there is no additional charge to the patient, as the fee for the kit is a one-time fee. High-volume centers can use one kit for two or three patients on the same day if samples are accurately coordinated.

Conclusions

For patients in whom the IOPTH assay is already being used, we recommend IOPTH aspiration biopsy of selected parathyroid tissue for rapid biochemical confirmation. Among our 66 patients, IOPTH levels were noticeably elevated in parathyroid tissue but not in nonparathyroid tissue. Utilization of IOPTH has the potential to decrease operating time and patient cost. Results are easy to interpret and highly sensitive and specific.

Résumé

Fond du problème: La plupart des médecins s'accorderait pour dire que le succès d'une parathyroïdectomie dépend de deux variables: l'identification et l'excision de toutes les glandes anormales et la confirmation par l'anatomopathologiste que le tissu enlevé est effectivement du tissu parathyroïde. Habituellement, on s'appuie sur l'examen anatomopathologique extemporané pour confirmer l'identité du tissu parathyroïdien mais, de temps à autre, la confirmation ne peut être faite sans ablation et examen définitif de la pièce, comme dans le cas de glandes parathyroïdes intrathyroïdiennes. Cette étude propose une nouvelle méthode de confirmation de tissu parathyroïde, rapide et facile, utilisant un examen immunologique pour mesurer

le taux de parathormone peropératoire (IOPTH). Par ponction/aspiration directe de l'adénome suspect, cet examen devient un outil diagnostique rapide qui peut être utilisé comme alternative à l'examen extemporané. Dans le cas où le chirurgien projetterait d'utiliser cet examen au préalable, l'élimination de l'examen extemporané devient cout-efficace. Méthodes: La ponction/aspiration d'un adénome parathyroïdien, confirmé histologiquement en post-opératoire, a été réalisée chez 12 opérés consécutifs. Après l'excision, la glande parathyroïde a été ponctionnée avec une aiguille calibre 22. Les échantillons ont été placés dans 1-3 cc de sérum physiologique, tamponné. On a effectué le même procédé chez 12 témoins. Les échantillons ont été centrifugés, divisés en lots égaux et ensuite, stockés à −70°C. Le taux de parathormone a été analysé de manière sélective par un dosage rapide et les résultats enregistrés. Résultats: Le taux de parathormone a été supérieur à 1500 pg/ml, au-dessus de la limite supérieure de la normale, dans tous les échantillons. Les valeurs des échantillons de ponction/aspiration de la thyroïde ont été comprises entre 58-85 pg/ml, avec une moyenne de 75.7 pg/ml. Dans tous les cas, on a vérifié le diagnostic par un examen histologique définitif. La sensibilité et la spécificité ont été de 100%. Conclusion: Doser la PTH à partir de la ponction/aspiration d'adénome de la parathyroïde en peropératoire est une alternative à l'examen extemporané systématique. Une valeur supérieure à 1500 pg/ml est diagnostique. Il n'y a aucun coût supplémentaire au cas où le taux de PTH aurait été monitoré en peropératoire de toute manière pour confirmer l'ablation. L'élimination de l'examen extemporané pourrait être coût-efficace et pour certaines institutions, représenter une diminution du temps opératoire puisque le dosage ne dure que 15 minutes. Le dosage de PTH proposé est une technique diagnostique précise pour distinguer une tumeur de la parathyroïde et des nodules de la thyroïde, et jusqu'à présent, est 100% sensible et spécifique.

Resumen

Antecedentes: Se acepta que el éxito de la paratiroidectomía depende de que el cirujano reconozca y extirpe la/s glándula/s patológica/s y de que el anatomopatólogo confirme que el especimen extirpado corresponde a tejido paratiroideo. Habitualmente, se utilizan los cortes por congelación para confirmar la identidad del tejido paratiroideo, pero en ocasiones, como ocurre con las glándulas intratiroideas, esta comprobación no puede realizarse sin una resección previa definitiva. El objetivo de este estudio es proponer un método nuevo, rápido y fácil que confirme la existencia de tejido paratiroideo mediante la determinación intraoperatoria de la hormona paratiroidea, utilizando un inmunoensayo rápido (IOPTH). Tras la aspiración directa del pretendido adenoma podemos llegar a un rápido diagnóstico que puede incluso, sustituir al estudio histológico de los cortes por congelación. En los casos en los que el cirujano emplee el IOPTH puede prescindir del estudio microscópico, lo que abarata los costos hospitalarios. Métods: En 12 pacientes intervenidos por adenoma paratiroideo, confirmado mediante estudio microscópico, se procedió a realizar aspiración de la glándula, una vez extirpada, mediante una jeringa del calibre 22. El aspirado se mezcló con 1-3 cc de suero salino tamponado. El mismo procedimiento se efectuó con 12 nódulos tiroideos que sirvieron de control. Los especímenes se centrifugaron en partes alícuotas y se almacenaron a −70°C. Se analizaron selectivamente los valores de paratohormona mediante un test rápido de inmunoensayo, registrándose los valores obtenidos. Resultados: En todos los aspirados de paratiroides los valores detectados con el test rápido excedieron los 1500 pg/ml es decir, el límite más alto de la escala del test. Por el contrario, en el material aspirado de tejido tiroideo los valores detectados oscilaron entre 58-85 pg/ml con una media de 75.7 pg/ml. El diagnóstico se confirmó en todos los casos mediante estudio microscópico definitivo. La sensibilidad y especificidad de las determinaciones fue del 100%. Conclusiones: La determinación de la PTH en el aspirado intraoperatorio de posibles adenomas paratiroideos es útil desde el punto de vista clínico, sobre todo en pacientes en los que se efectúa de forma rutinaria estudio microscópico mediante cortes por congelación. Valores superiores a los 1500 pg/ml permiten una total certeza diagnóstica. Este método no origina costes adicionales en los casos en que se monitoriza la IOPTH con objeto de confirmar la curación del paciente. En algunos hospitales se prescinde ya del estudio anatomopatológico, reduciéndose así los costos. El test de la IOPTH precisa tan solo 15 minutos, por lo que disminuye el tiempo operatorio. La determinación de la PTH es una técnica diagnóstica segura, cuya especificidad y sensibilidad alcanza el 100% permitiendo diferenciar los tumores paratiroideos de los nódulos tiroideos.

Acknowledgments

This work was supported in part by Mt. Zion Health System–Department of Surgery, The Edwin H. Zeller Fund, and Friends of Endocrine Surgical Oncology.

References

1. Ranchod, M.: Parathyroid gland. Pathology *3*:405, 1996
2. Clark, O.H., Gooding, G.A., Ljung, B.M.: Locating a parathyroid adenoma by ultrasonography and aspiration biopsy cytology. West. J. Med. *135*:154, 1981
3. Westra, W.H., Pritchett, D.D., Udelsman, R.: Intraoperative confirmation of parathyroid tissue during parathyroid exploration: a retrospective evaluation of the frozen section. Am. J. Surg. Pathol. *22*:538, 1998
4. Eisenberg, H., Pallotta, J., Sherwood, L.M.: Selective arteriography, venography and venous hormone assay in diagnosis and localization of parathyroid lesions. Am. J. Med. *56*:810, 1974
5. Ramaley, A.T., Woods, J.J., Glickman, J.W.: Point of care testing of parathyroid hormone during the surgical treatment of hyperparathyroidism. Med. Lab. Observer *31*:20, 1999
6. Irvin, G.L., Deriso, G.T.: A new, practical intraoperative parathyroid hormone assay. Am. J. Surg. *168*:466, 1994
7. Boggs, J.E., Irvin, G.L., Molinari, A.S., Deriso, G.T.: Intraoperative parathyroid hormone monitoring as an adjunct to parathyroidectomy. Surgery *120*:954, 1996
8. Winkler, B., Gooding, G.A., Montgomery, C.K., Clark, O.H., Arnaud, C.: Immunoperoxidase confirmation of parathyroid origin of ultrasound guided fine needle aspirates of the parathyroid glands. Acta. Cytol. *31*:40, 1987