Utility of Endoscopic Ultrasound Multimodal Examination with Fine Needle Aspiration for the Diagnosis of Pancreatic Insulinoma – A Case Report

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ABSTRACT Insulinomas are benign insulin-secreting neuroendocrine tumors originating in the pancreatic beta cells. Symptoms are caused by hypoglycemia and clinical diagnosis is based on establishing their relationship to fasting, usually via a fasting test. The most conclusive imaging tests are endoscopic ultrasound (EUS) and CT. Surgery is the treatment of choice. A 33 year old male presented with a 2-year history of hunger which had intensified in the previous 6 months with added accompanying symptoms, culminating with an acute episode - loss of consciousness and seizures - which resolved after administering i.v. glucose. A fasting test was performed, with results suggestive for an insulinoma. Dual-phase CT showed a mass in the tail of the pancreas but no contrast enhancement. EUS was used for further assessment: B-mode showed a hypoechoic focal mass with a cystic component, on contrast enhancement the pattern was hypovascular, and elastography showed soft tissue. EUS fine needle aspiration (FNA) was performed and the immunohistochemistry (IHC) assay was conclusive for a neuroendocrine tumor of the pancreas. Treatment consisted of caudal pancreatectomy, with no recurrence after 1-year follow-up. Although this case started with a classic clinical presentation of an insulinoma, imaging studies related to tumor vascularization raised doubts about the actual diagnosis. Nevertheless, multimodal EUS assessment with FNA was considered to be the most appropriate diagnostic technique for detection, characterization and staging of the mass. EUS findings together with the IHC assay were able to offer the definite diagnosis of a benign neuroendocrine tumor and allowed us to refer the patient for appropriate treatment.

KEY WORDS insulinoma, multimodal EUS, EUS-guided FNA, immunohistochemistry assay

Introduction Insulinomas are insulin-secreting neuroendocrine tumors that originate in the beta cells of the pancreatic islets and they are the most common cause of hypoglycemia resulting from endogenous hyperinsulinism. They are usually solitary, generally well encapsulated, firmer than normal pancreas, and highly vascular. Only a small percentage of insulinomas are malignant (5 – 16%). The majority are diagnosed between the ages of 40 to 45 years. Symptoms are caused by hypoglycemia, characteristically associated with fasting and may be neuroglycopenic or adrenergic, and there may be an interval of several years between the onset of the symptoms and the diagnosis. Diagnosis of insulinoma is based on clinical history and establishing the relationship between symptoms and fasting, but an extended fasting test is usually preferred. Several imaging methods have been put into use, mainly for localizing the suspected lesion, with the best approach being dual-phase helical CT together with EUS. Treatment usually consists of surgical resection and the prognosis is very good.

The aim of this article is to present a case of a pancreatic insulinoma in which the diagnosis was made difficult by some atypical test results. In this case EUS with FNA and subsequent IHC assay was the method of choice for obtaining a definite diagnosis.

Case report A 33 year old male presented with a 2-year history of intense hunger which had caused a constant weight gain. In the last 6 months, the sensation had intensified and accompanying symptoms such as malaise, vision troubles and dizziness had developed. The symptoms were most frequent in the morning and after prolonged fasting. There was an acute episode one month before: the patient was found unconscious and exhibiting tonic-clonic seizures. The emergency medical team measured a 29 mg/dl capillary glycemia and promptly administered i.v. glucose, which led to the rapid resolution of symptoms. Afterwards, the patient self-monitored his glycemia and made diet changes (several meals...
per day with slow and fast carbohydrates), resulting in a temporary increase of glucose levels after overnight fasting to 65 – 75 mg/dl. This improvement lasted less than a month, the values then dropping to 45 mg/dl. Hormone workup had excluded an endocrine disease.

A prolonged fasting test was performed under supervision in the hospital of Tg. Mures, after 12 hours of overnight fasting. Initial capillary glycemia was 53 mg/dl and the patient was not exhibiting any symptoms. Fasting was continued, with capillary glycemia measured every 90 minutes or in the case of symptom occurrence. After a total of 18 hours of fasting, the patient exhibited slurred speech, and capillary glycemia showed a value of 42 mg/dl. Three venous blood samples were taken at 5 minute intervals before administering i.v. glucose and a carbohydrate-rich meal. The blood levels for glucose and insulin and the insulin/glucose ratio are presented in table 1.

| Sample | Glycemia (mg/dl) | Insulinemia (µU/ml) | Insulin/glucose ratio |
|--------|------------------|---------------------|----------------------|
| Sample 1 | 29               | 19.9                | 0.68                 |
| Sample 2 | 27               | 25.0                | 0.92                 |
| Sample 3 | 28               | 25.9                | 0.93                 |

Table 1: Fasting test results – glucose and insulin levels with insulin/glucose ratio – 3 measurements at 5 minute intervals

Standard CT scan showed a hypodense focal mass of 20mm in diameter with clear delimitation, located in the tail of the pancreas, and no contrast enhancement was observed on dual-phase examination [Figure 1].

EUS was performed, with several studies during the same examination. B-mode ultrasound showed a hypoechoic focal mass of approximately 20mm in diameter with a cystic component, while elastography showed a predominantly green pattern, consistent with soft tissue, suggestive of a benign mass [Figure 2].

Contrast enhancement with 1 ml of Sono-Vue showed a hypovascular pattern with only a slight increase in the vascularity index, from 2.72% pre-contrast to 13.6% post-contrast, and peri-tumoral power-Doppler signal enhancement [Figure 3].

The liquid component was drained via FNA and then three consecutive punctures of the solid mass were performed. One of the two HE stain slides showed hypercellularity with frequent placards of round-oval cells, some pseudo-rosette-like, others with papillar distribution and moderate nuclear dyskariosis, atypical mitoses and frequent denudated dyskariotic nuclei [Figure 4].
IHC assay of the FNA material obtained the following results: chromogranin with intensely positive cytoplasm marking diffusely throughout the tumor [Figure 5], synaptophysin with positive diffuse cytoplasmatic marking [Figure 6], cytokeratin 7 absent in the tumor cells, cytokeratines AE1/AE3 positive in the paranuclear granule diffusely throughout the tumor cells, cytokeratine 20 and CEA were absent, protein Ki67 was absent (although the fragment was considered too small for an accurate assessment of this marker), protein p53 absent, CA 19-9 antigen weak positive focally inside the cells. No specific insulin markers were used as they were not available at the time of the assay. The pathologist had concluded that the HE stain together with the IHC assay were conclusive for a neuroendocrine tumor of the pancreas.

![Figure 5. FNA sample, IHC staining for chromogranin - intensely positive cytoplasm marking diffusely throughout the tumor](image)

![Figure 6. FNA sample, IHC staining for synaptophysin – diffusely positive immune marking of tumor cells](image)

The patient was referred to a surgical center for a caudal pancreatectomy, which was successfully performed. No recurrence was observed after a follow-up of one year.

**Discussions**

Insulinomas usually occur in patients between 20 to 75 years, age group where our patient fit, although the large majority of tumors are diagnosed between the ages of 40 to 45 years [1, 4]. Symptoms are caused by hypoglycemia, characteristically associated with fasting and thus more frequently occur when a meal is delayed or missed, before the first meal of the day or during exercise [3]. Symptoms may be neuroglycopenic - caused by the lack of glucose in the brain (82 – 92% of patients), or adrenergic - caused by catecholamine release. Neuroglycopenic symptoms include visual disturbances (diplopia, blurred vision), confusion, altered consciousness, weakness, transient motor defects, hemiplegia, dizziness, fatigue, inappropriate behavior, while adrenergic symptoms include anxiety, palpitations, weakness, fatigue, headache, tremor, and sweating [5–7]. During an initial attack patients most commonly exhibit both types of symptoms (49% of cases), as opposed to neuroglycopenic symptoms alone (38%) or just adrenergic symptoms (12%). Up to half of the patients may even enter a coma, while one in ten exhibits convulsions. Patients tend to avoid symptoms by increasing the frequency of meals, and obesity may result [3]. The average duration of neuroglycopenic symptoms prior to diagnosis is often prolonged, ranging between one and five years in 53% of patients, but in rare cases it is possible to extend up to 30 years [6].

Diagnosis of insulinoma is usually based on clinical history and establishing the relationship between symptoms and fasting [1, 4, 8]. Around 75% of insulinomas are clinically characterized by the Whipple triad: presence of symptoms of hypoglycemia, documented low blood sugar at the time symptoms are present, reversal of symptoms by glucose administration [9]. Our patient fit the description, with a 2-year history of symptoms and weight gain due to frequent meals. Neuroglycopenic symptoms were the norm in our case, and there even was an episode of loss of consciousness with convulsions, at which time Whipple’s triad was clearly documented.

Unfortunately, as the symptoms are not specific for insulinomas, and the blood levels of glucose and insulin are not always significantly altered after an overnight fasting, an extended 72 or 48-hour fasting test is preferred, with blood glucose, plasma insulin, and C-peptide levels measured at three- to six-hour intervals. If at any point during the fast the patient becomes symptomatic, plasma insulin and glucose values should be determined before intravenous glucose
is given and the test stopped. Between 65% and 67% of patients with an insulinoma will have symptoms and a blood sugar level lower than 40 mg/dL within 24h of starting the fast, and 93% to 94.5% within 48h [10, 11]. The test is considered positive for insulinoma when plasma glucose levels drop below 40mg/dl insulin levels rise above 10µg/dl and, more importantly, insulin-to-glucose ratio is above 0.3. Our prolonged fasting test was consistent with an insulinoma, showing insulin levels up to 2.5 times the threshold and insulin/glucose ratio more than 3 times the required value for the diagnosis. Several imaging methods have been put into use, mainly for localizing the suspected lesion: CT scan has 82-94% sensitivity, with dual-phase helical CT being more sensitive than single-phase [12]; MRI with gadolinium can be helpful in detecting a tumor in 85% of cases, especially for those with no hypervascular pattern [13]; selective arteriography has an accuracy of 82%, although affected by a false-positive rate of 5%; the sensitivity of somatostatin receptor scintigraphy is 60%, although many insulinomas lack somatostatin receptor subtype 2 for successful identification [14]; endoscopic ultrasonography detects 77 - 94% of insulinomas in the pancreas [14, 15], with an almost 100% yield if it is done in combination with CT scan [16]. In our case, standard and dual-phase CT were first performed, mainly due to the better availability of the method. Although the lesion was found – in the tail of the pancreas – this investigation raised concerns about the diagnosis, as the tumor was hypovascular, which is uncommon for an insulinoma. Some lesions, particularly if they have a cystic component, exhibit delayed enhancement and are best seen or only apparent in the portal venous phase, if at all, and larger lesions often show heterogeneous enhancement in a ringlike pattern or with central areas of necrosis or cystic degeneration [17]. At this point, EUS was considered to be the logical next step and the most appropriate diagnostic technique, since it has a very good resolution for pancreatic masses, multimodal assessment capabilities and tumor samples can be taken via FNA. EUS confirmed the CT findings and provided extra data, showing a semi-cystic, hypoechoic, hypovascular, soft (i.e. suggestive of a benign tumor) mass in the tail of the pancreas, consistent with some of the more uncommon aspects of insulinomas [17]. EUS-FNA with IHC assay was able to offer the definite diagnosis of a benign neuroendocrine tumor. This data, together with the clinical presentation and the results of the fasting test allowed us to conclude, with a very high degree of certainty, that this is the case of a benign insulinoma, and to refer the patient for appropriate treatment.

Because insulinoma resection achieves cure in 90% of patients, it is currently the therapy of choice [1, 18]. The overall prognosis is very good and long-term cure with complete resolution of preoperative symptoms is expected after complete resection. A recurrence rate of 5.4% over a period of 4-17 years was observed in patients with benign insulinomas [19]. In our case, a caudal pancreatectomy was successfully performed with no recurrence after a follow-up of one year.

Conclusions

A seemingly classic clinical case of insulinoma was complicated by unusual findings on imaging methods. EUS was used as a means to shed light over an uncertain diagnosis, because of its high resolution, multimodal capabilities and the possibility to perform FNA with subsequent IHC assay. EUS with FNA and IHC assay was the definitive test that, when faced with a diagnostic dilemma, could offer a diagnosis with a very high degree of certainty – in our case, an insulinoma. Although this is a single case report, it shows the value of this method for the assessment of pancreatic focal masses and recommends it for routine use in similar cases.

Acknowledgement

This work was supported by research grant Partnership – PN II of the ANCS, CNDI – UEFISCDI, project number 2011-3,2-0503.

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