ISSN 1948-9366 (online)

World Journal of *Gastrointestinal Surgery*

World J Gastrointest Surg 2015 April 27; 7(4): 52-70

Published by Baishideng Publishing Group Inc

WJGS World Journal of Gastrointestinal Surgery

A peer-reviewed, online, open-access journal of gastrointestinal surgery

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2012-2016

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World Journal of Gastrointestinal Surgery

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World Journal of Gastrointestinal Surgery
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NAME OF JOURNAL
World Journal of Gastrointestinal Surgery

ISSN
ISSN 1948-9366 (online)

LAUNCH DATE
November 30, 2009

FREQUENCY
Monthly

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
April 27, 2015

World Journal of Gastrointestinal Surgery

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4240/wjgs.v7.i4.52

World J Gastrointest Surg 2015 April 27; 7(4): 52-59
ISSN 1948-9366 (online)

ORIGINAL ARTICLE

Clinical Trials Study

Endoscopic ultrasound guided radiofrequency ablation, for pancreatic cystic neoplasms and neuroendocrine tumors

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Author contributions: Habib N developed the device concept and designed the study; Pai M, Senturk H, Reddy N, Kahaleh M and Brugge W materialized the design concept and designed the study; Senturk H, Lakhtakia S, Reddy N, Cicinnati VR, Kaba I and Beckebaum S contributed in patient screening, recruitment and procedures; Pai M, Senturk H, Lakhtakia S, Kaba I and Beckebaum S were responsible for the acquisition of data; Pai M and Habib N done the analysis and the interpretation of data; Pai M and Habib N drafted the manuscript; Habib N, Senturk H, Lakhtakia S, Reddy N, Cicinnati VR, Drymousis P, Kahaleh M and Brugge W did critical revisions of the manuscript and had input of important intellectual content; Pai M and Habib N did the statistical analysis; Pai M and Habib N were responsible for administrative, technical and material support; Habib N had the study supervision.
Conflict-of-interest: None of the other authors have a conflict of interest or a financial disclosure to declare.
Data sharing: Further technical data and device application details are available from Nagy Habib.

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Received: June 19, 2014
Peer-review started: June 24, 2014
First decision: August 17, 2014
Revised: February 11, 2015
Accepted: March 5, 2015
Article in press: March 9, 2015
Published online: April 27, 2015

Abstract

AIM: To outline the feasibility, safety, adverse events and early results of endoscopic ultrasound (EUS)-radiofrequency ablation (RFA) in pancreatic neoplasms using a novel probe.

METHODS: This is a multi-center, pilot safety feasibility study. The intervention described was radiofrequency ablation (RF) which was applied with an innovative monopolar RF probe (1.2 mm Habib EUS-RFA catheter) placed through a 19 or 22 gauge fine needle aspiration (FNA) needle once FNA was performed in patients with a tumor in the head of the pancreas. The Habib™ EUS-RFA is a 1 Fr wire (0.33 mm, 0.013") with a working length of 190 cm, which can be inserted through the biopsy channel of an echoendoscope. RF power is applied to the electrode at the end of the wire to coagulate tissue in the liver and pancreas.

RESULTS: Eight patients [median age of 65 (range 27-82) years; 7 female and 1 male] were recruited in a prospective multicenter trial. Six had a pancreatic cystic

neoplasm (four a mucinous cyst, one had intraductal papillary mucinous neoplasm and one a microcystic adenoma) and two had a neuroendocrine tumors (NET) in the head of pancreas. The mean size of the cystic neoplasm and NET were 36.5 mm (SD ± 17.9 mm) and 27.5 mm (SD ± 17.7 mm) respectively. The EUS-RFA was successfully completed in all cases. Among the 6 patients with a cystic neoplasm, post procedure imaging in 3-6 mo showed complete resolution of the cysts in 2 cases, whilst in three more there was a 48.4% reduction [mean pre RF 38.8 mm (SD ± 21.7 mm) *vs* mean post RF 20 mm (SD ± 17.1 mm)] in size. In regards to the NET patients, there was a change in vascularity and central necrosis after EUS-RFA. No major complications were observed within 48 h of the procedure. Two patients had mild abdominal pain that resolved within 3 d.

CONCLUSION: EUS-RFA of pancreatic neoplasms with a novel monopolar RF probe was well tolerated in all cases. Our preliminary data suggest that the procedure is straightforward and safe. The response ranged from complete resolution to a 50% reduction in size.

Key words: Endoscopic ultrasound; Radiofrequency ablation; Pancreas; Cystic neoplasms; Neuroendocrine tumors

Core tip: This manuscript presents a pilot, safety feasibility study with the results of the first in humans endoscopic ultrasound (EUS) guided radiofrequency ablation (RFA) for cystic neoplasms and neuroendocrine tumors of the pancreas with a novel EUS-RFA catheter. EUS-RFA is feasible and well tolerated. EUS-RFA with this novel catheter provides endoscopic treatment option other than surgical resection for pancreatic lesions.

Pai M, Habib N, Senturk H, Lakhtakia S, Reddy N, Cicinnati VR, Kaba I, Beckebaum S, Drymousis P, Kahaleh M, Brugge W. Endoscopic ultrasound guided radiofrequency ablation, for pancreatic cystic neoplasms and neuroendocrine tumors. *World J Gastrointest Surg* 2015; 7(4): 52-59 Available from: URL: http://www.wjgnet.com/1948-9366/full/v7/i4/52.htm DOI: http://dx.doi.org/10.4240/wjgs.v7.i4.52

INTRODUCTION

Incidental pancreatic solid or cystic lesions are diagnosed with increased frequency due to the widespread use of abdominal cross-sectional imaging to investigate unrelated symptoms. In a large single-centre study, pancreatic cysts were diagnosed in 1.2% of 24000 individuals subjected to abdominal cross-sectional imaging[1]. As a result, the majority of these lesions are diagnosed at an earlier stage, before they become invasive and present with jaundice, pancreatitis or abdominal pain[2]. Lesions such as neuroendocrine tumors (NET), mucinous cystadenomas and intraductal papillary mucinous neoplasms have the potential of malignant transformation. This risk is lower with NET, but significantly higher with mucinous lesions[3].

The standard treatment of solid or cystic pancreatic lesions with malignant potential has been surgical resection, with lesions in the pancreatic head requiring a Whipple resection whereas pancreatic tail lesions are treated with distal pancreatectomy. Both types of resection carry significant morbidity and mortality, resulting in unacceptably high risk/benefit ratios for many elderly patients with co-morbidities[4,5]. Currently, patients deemed unfit for major pancreatic surgery are offered cross-sectional imaging surveillance at regular intervals according to the International Association of Pancreatology Guidelines[6]; these guidelines recommend annual imaging for lesions < 10 mm, 6-monthly imaging for cysts 10-20 mm and 3-monthly imaging for lesions larger than 20 mm. However, controversy exists regarding the optimal follow up of patients with primary pancreatic lesions, underlying the need for minimally invasive ablative techniques as alternative to surgical resection.

Radiofrequency ablation (RFA) has been used percutaneously and intraoperatively to treat primary and secondary liver cancers by achieving localized tumor necrosis[7-10]. Endo-biliary application of radiofrequency (RF) has been developed in our unit and used in patients with inoperable bile duct and pancreatic head adenocarcinomas presenting with biliary obstruction[11]. Many alternative techniques of endoscopic ultrasound (EUS)-guided tumor ablation have been described, including RF ablation, photodynamic therapy, laser ablation, and ethanol injection[12].

EUS-RFA could achieve complete ablation of pancreatic cysts with malignant potential in patients unfit for surgery, thus eliminating the requirement for long-term surveillance in this group of individuals. Gaidhane *et al*[13] showed that EUS-RFA of the pancreatic head using Habib EUS-RFA catheter (Emcision Ltd., United Kingdom) through a 19 gauge needle was well tolerated in 5 Yucatan pigs with minimum amount of pancreatitis. The aim of this study is to outline the safety, feasibility, adverse events and early results of EUS-RFA in patients with pancreatic neoplasms using a novel probe.

MATERIALS AND METHODS

Patients

Eight patients were subjected to EUS-RFA of a neoplastic lesion in the head of the pancreas. A novel monopolar RF catheter [Habib™ EUS-RFA catheter, Emcision Ltd., London (CE Marked)] (Figure 1) was placed through a 19 or 22 gauge fine needle aspiration (FNA) needle.

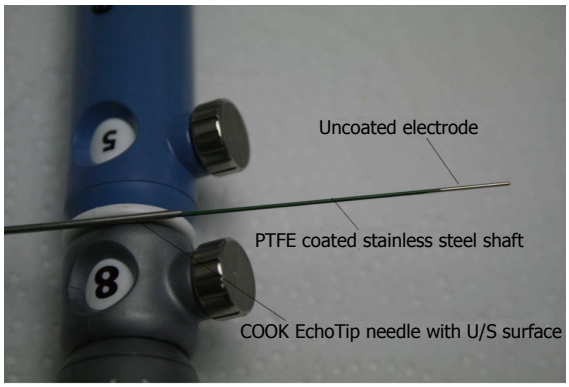

Figure 1 Close up of the Habib™ endoscopic ultrasound-radiofrequency ablation catheter showing uncoated electrode at the tip and the PTFE Coated stainless steel shaft.

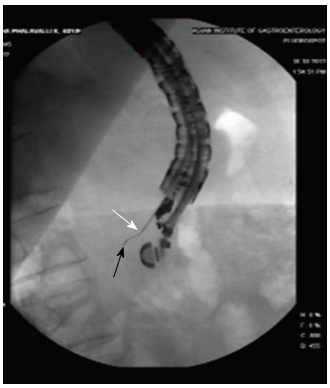

Figure 2 Fluoroscopic view of Habib™ endoscopic ultrasound-radiofrequency ablation catheter (black arrow) protruding out of the endoscopic ultrasound Biopsy needle (white arrow).

Inclusion criteria were age over 18 years, patients with a cystic pancreatic lesions that were not suitable surgical candidates and patients that consented to participate in the study. Exclusion criteria included patients younger than 18 years, patients not consenting to participate in the study, uncorrected coagulopathy and cardiac pacemakers *in situ*.

All patients were investigated with blood tests; haematological, biochemical, tumor markers as well as radiological investigation including computed tomography scan and ultrasound scans. On follow-up, patients had clinical examination, blood tests and cross sectional imaging to assess the pancreatic lesion. The follow-up ranged from 3 to 6 mo. Data are presented as mean plus or minus standard deviations of the mean or median with range. Research was carried out in accordance with the Helsinki Declaration.

Description of device

The Habib™ EUS-RFA is a 1 Fr wire (0.33 mm, 0.013") with a working length of 190 cm, which can be inserted through the biopsy channel of an echoendoscope. RF power is applied to the electrode at the end of the wire to coagulate tissue in the liver and pancreas. This is a monopolar device and is used in conjunction with a patient grounding/diathermy pad.

Intervention

Habib™ EUS-RFA catheter comes in a dispensing sheath. The catheter is removed from the dispensing sheath and connected to the adaptor cable, which is then connected to the generator. Power in the generator is set to the required wattage we used 5-25 Watts in our patient group). A patient grounding/diathermy pad is applied as close to the operating field as possible, since the catheter is monopolar. We applied the pad on the lower back of the patient. The entire area of the grounding pad should be reliably applied to the patient's body to avoid skin burns.

The echoendoscope is manoeuvred to obtain proper sonographic visualization of the target lesion. Under EUS control, a 19 gauge biopsy needle (with stylet) is introduced into the target lesion. In pancreatic cystic lesions, effort was made to completely aspirate the cyst before applying RFA. The tip of the needle was positioned near the far end of the lesion. In case of pancreatic NET also, the FNA needle was positioned at the deepest part of the tumor. The stylet is removed from the biopsy needle and Habib™ EUS RFA catheter is gently pushed inside the hollow of the biopsy needle until it cannot be pushed any further. Carefully maintaining this position of the Habib™ EUS RFA probe, the FNA needle is gradually withdrawn by 3 cm in order to disengage contact between the active part of the RF catheter located at the tip and the metallic FNA needle. Fluoroscopy assists in visualization of the RFA probe protruding beyond the tip of the needle (Figure 2). The tip of the probe is floppy, and may take a curved shape in emptied cystic lesons.

RF energy is applied for 90-120 s at the set wattage. In larger lesions, the Habib™ EUS RFA probe and needle is pulled back as one unit and repositioned to ablate near end of the lesion (Figures 3-5). This process can be repeated as many times, as needed to ensure complete ablation of the lesion. In larger pancreatic lesions, repeat puncture with the FNA needle is done in a different axis (after withdrawing the RFA probe, with or without replacing with stylet). The patients were managed post procedure as per standard hospital practice for EUS interventional procedures.

RESULTS

Eight patients [median age of 65 (range 27-82) years; 7 female and 1 male] were recruited in a prospective multicentre trial. Six had a pancreatic cystic neoplasm (four a mucinous cyst, one had IPMN and one a microcystic adenoma). In all six cases, diagnosis was based on imaging reviewed by an expert radiologist. The remaining two cases, had a NET in the head of pancreas (previously documented with diagnostic FNA cytology and not suitable for surgical intervention). The mean size of the cystic neoplasms and NETs were

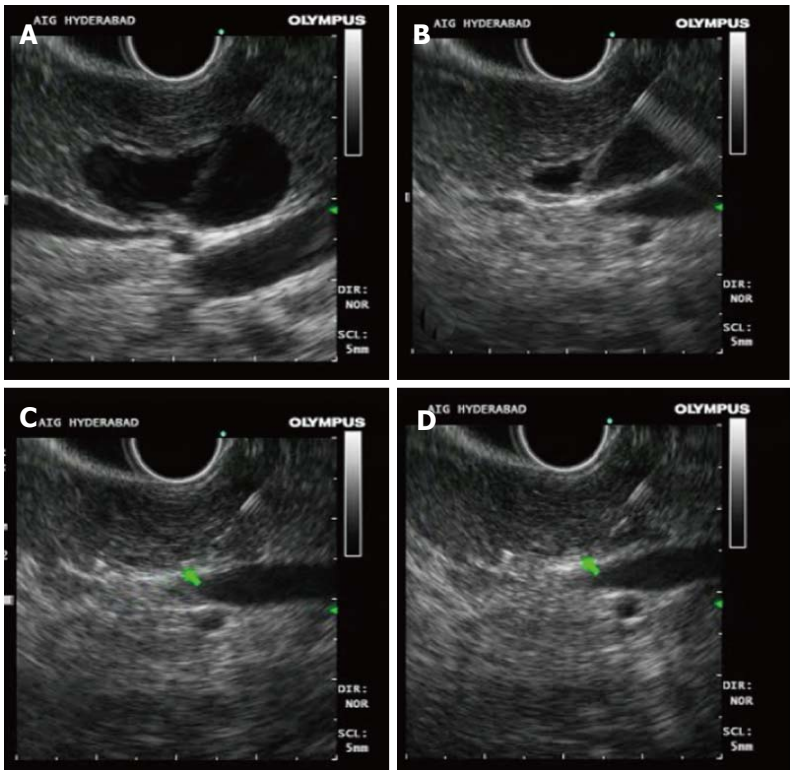

Figure 3 Endoscopic ultrasound pictures of radiofrequency ablation of pancreatic cyst. A: Pancreatic cyst with the biopsy needle in position; B: Aspiration of the pancreatic cyst; C and D: Complete aspiration of the cyst followed by radiofrequency ablation using the endoscopic ultrasound radiofrequency ablation catheter.

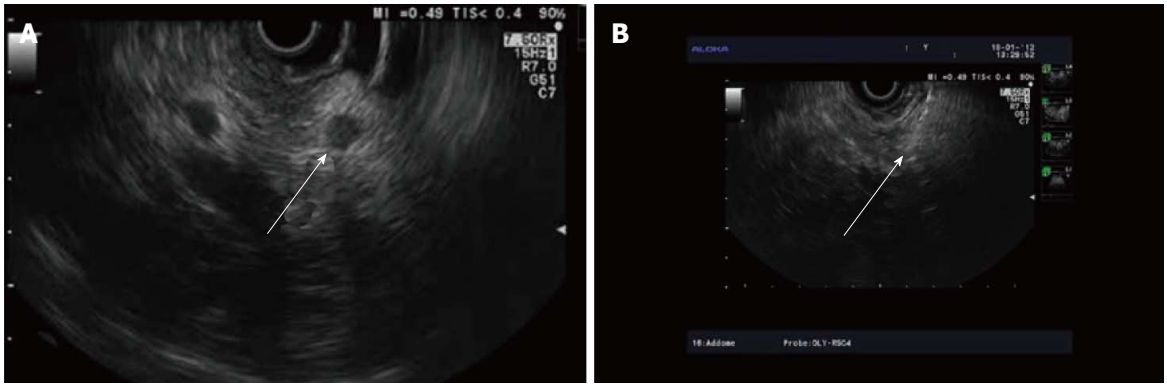

Figure 4 Endoscopic ultrasound Pictures of radiofrequency ablation of pancreatic cyst. A: Pancreatic cyst Pre ablation (arrow); B: Pancreatic cyst aspirated completed and the radiofrequency ablation with in process using the endoscopic ultrasound radiofrequency ablation catheter (arrow).

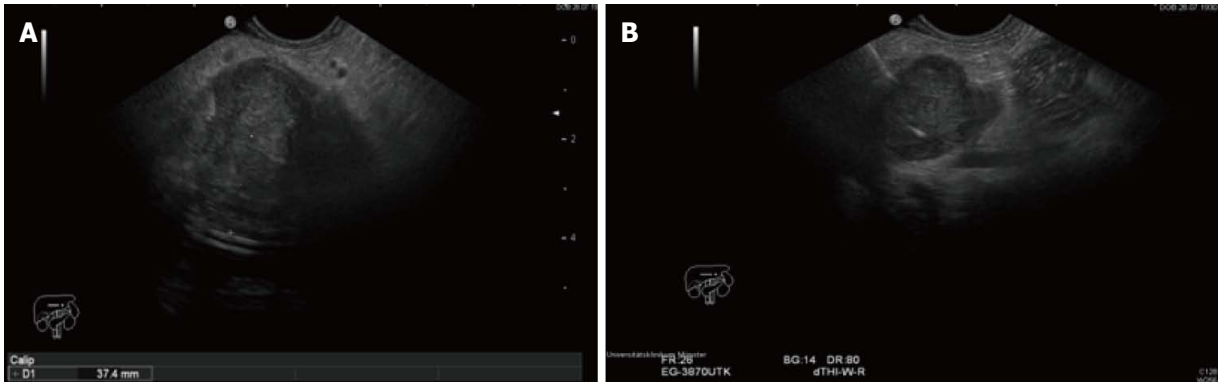

Figure 5 Endoscopic ultrasound radiofrequency ablation of pancreatic neuroendocrine tumors. A and B: Endoscopic ultrasound pictures of the pancreatic neuroendocrine tumors pre and during ablation.

36.5 mm (SD ± 17.9 mm) and 27.5 mm (SD ± 17.7 mm) respectively. RF [Rita (Model 1500X) or ERBE

Table 1 Patient characteristics and procedure specifications

Age	Sex	Diagnosis	No. of RF applications/session	No of sessions	Dead/ alive
5 Watts					
82	F	Mucinous cyst	3	1	Alive
73	F	Mucinous cyst	5	1	Alive
46	F	Microcystic adenoma	5	1	Alive
15 Watts					
40	F	Mucinous cyst	3	1	Alive
27	F	Mucinous cyst	2	1	Alive
20 Watts					
57	F	NET	6	1	Alive
82	F	NET	4	2	Alive
25 Watts					
78	M	IPMN	7	1	Alive

IPMN: Intraductal papillary mucinous neoplasm; NET: Neuroendocrine tumors; RF: Radiofrequency; F: Female; M: Male.

Table 2 Outcome after endoscopic ultrasound radiofrequency ablation of pancreatic cystic neoplasm and neuroendocrine tumors

No.	Diagnosis	Pre ablation size (mm)	Post ablation size (mm)	Adverse events
1	Mucinous cyst	30	10	No
2	Mucinous cyst	40	Cyst not seen	No
3	Microcystic adenoma	20	8	No
4	Mucinous cyst	70	45	Mild pain
5	Mucinous cyst	24	Cyst not seen	Mild pain
6	IPMN	35	17	No
7	NET	15	Change in vascularity	No
8	NET	40	Central area of necrosis 15 mm	No

IPMN: Intraductal papillary mucinous neoplasm; NET: Neuroendocrine tumors.

(Model ICC 200) was applied at 5 watts, 15 watts, 20 watts and finally 25 watts in 3, 2, 2 and one patients respectively over 90 s for each watt setting (Table 1). The median number of applications was 4.5 (range 2-7). Patients with cystic neoplasm and one patient with NET had one session of RFA each, whilst a second patient with NET had two sessions of RFA.

The EUS-RFA was completed in all cases. Amongst the 6 patients with pancreatic cystic neoplasm, the post procedure imaging in 3-6 mo showed complete resolution of the cysts in 2 patients, whilst in 3 patients there was 48.4% reduction [mean pre RF 38.8 mm (SD ± 21.7 mm) *vs* mean post RF 20 mm (SD ± 17.1 mm)] in size (Table 2). Using cross sectional imaging in 2 patients with NET, a change in vascularity and central necrosis after EUS-RFA was demonstrated. There were no episodes of post-procedural pancreatitis, perforation or bleeding within 48 h. Two patients had mild abdominal pain that resolved in 3 d.

DISCUSSION

RFA is a well-recognized, safe and effective modality for the treatment of focal malignant diseases[14,15]. RFA uses high-frequency alternating current to generate thermal energy and thus coagulative necrosis to the tissue[16]. The technique is minimally invasive and has very good tolerability which are the major advantages[17]. RFA is increasingly applied in pancreatic lesions[18], including unresectable pancreatic carcinoma where RFA has an acceptable mortality but high morbidity[16,17,19-21]. In general, adverse events are associated with the duration of ablation. Pancreas is very thermo-sensitive, and when heat is applied on normal pancreas it produces an inflammatory response causing edema and later fibrosis and occasionally cystic transformation[18]. Massive necrosis of the pancreas following RFA have been reported, probably due to sequential ablations done in close proximity at the same session[17,20].

In recent years there have been reports of prospective studies using RFA in locally advanced pancreatic adenocarcinoma. In 2010, Girelli *et al*[22] reported ultrasound-guided RFA during laparotomy in fifty patients with locally advanced pancreatic cancer. In this prospective study the main outcome measures were short-term morbidity and mortality. In thirty four patients the tumor was located in the pancreatic head or the uncinate process and in 16 in the body or tail; median diameter was 40 (inter-quartile range 30-50) mm. Abdominal adverse events occurred in 24% of patients. Half of those were directly associated with RFA (two pancreatic fistulas and four cases of portal vein thrombosis) and were managed conservatively. When the applied heat was reduced from 105 degrees C to 90 degrees C there was a significant reduction in adverse events (ten *vs* two of 25 patients; $P = 0.028$). Median postoperative hospital stay was 10 (range 7-31) d. The authors concluded that RFA of locally advanced pancreatic cancer is feasible and relatively well tolerated. In another observational study, the same group compared patients with locally advanced pancreatic carcinoma treated with either primary RFA (group 1) or RFA following any other primary treatment (group 2)[23]. In total, 107 consecutive patients were treated with RFA of which 47 patients in group 1 and 60 in group 2. Median overall survival was 25.6 mo and it was significantly shorter in group 1 than in group 2 (14.7 mo *vs* 25.6 mo; $P = 0.004$). In this study the authors reported that RFA after alternative primary treatment was associated with prolonged survival.

RFA has been proposed by many groups as a strong adjuvant for antitumor response as it induces an immune response targeting tumor antigens[24-26]. *In situ* tumor destruction by RFA provides the immune system with an antigen for the induction of antitumor immunity. Antigen-presenting cells take up antigens in the periphery after which they induce specific

immune responses[25]. Wissniowski *et al*[24] reported that RFA can induce a tumor-specific T-cell reaction in the non-reactive neoplasm-bearing host, probably by overcoming immune tolerance and leading to the presentation of otherwise cryptic neoplastic antigens. In another study, ablation of hepatocellular carcinoma (HCC) was found to induce a functional transient activation of myeloid dendritic cells associated with increased serum levels of TNF-α and IL-1β with a sustained antitumoral immune response[26]. Moreover, animals treated with subtotal RF ablation showed significant increases in tumor-specific class Ⅰ and Ⅱ responses to male minor histocompatibility (HY) antigens and tumor regression[27]. Subtotal RF ablation produces an enhanced systemic antitumor immune response and tumor regression which is related to increased dendritic cell infiltration. RFA can also induce a tumor-specific proliferative T cell response and even transplantable protective immunity[28].

Intraoperative RFA uses a larger device with higher energy and is associated with significant morbidity and mortality. However, EUS guided RFA is a more conservative approach and avoids surgical intervention. Goldberg *et al*[29] applied EUS guided RFA to the pancreas of 13 Yorkshire pigs at 285 ± 120 mA for 6 min resulting in discrete zones of coagulation necrosis in the porcine pancreas. Only one of the 13 animals had increased lipase levels and mild focal pancreatitis. No other significant adverse events were observed. A more recent study in 2008 demonstrated the feasibility and efficacy of EUS RFA using a newly developed bipolar ablation probe combining RFA and cryotechnology in 14 pigs. The size of the ablation achieved was related to the duration of ablation; when applied for 900 s there was a high complication rate in the healthy pancreas. Adverse events were less common compared to conventional RFA needles[18]. In a recent study by Kim *et al*[30], EUS-RFA of the pancreas was applied on 10 adult mini pigs. An 18 gauge endoscopic RFA probe was used to ablate the body and tail of the pancreas, with an output power of 50 W for 5 min. On histology, there was a spherical necrotic lesion surrounded by fibrous tissue localized in the pancreatic parenchyma. The mean diameter of the ablated tissue was 23.0 ± 6.9 mm. No major procedure-related adverse events were observed, and all pigs survived without any distressed behavioural pattern for 7 d until autopsy. Another minimally invasive technique for treatment of pancreatic cystic lesions with moderate success is the EUS-guided injection of ethanol into the cyst, with reported efficacy of 33.5%-62% in achieving cyst resolution[31,32]. The adverse events associated with this technique are significant, with a reported risk of severe post-procedural pain and pancreatitis of 4%-20%. Also, the presence of multiple septations within the cyst reduces the efficacy of ethanol injection. Another limitation of ethanol ablation is that this method would not be suitable for treatment of solid pancreatic lesions. A major potential advantage of EUS-RFA of cystic tumors is that it could be done in a minimally invasive way, with the likelihood of fewer adverse events than the alcohol injection because the area of ablation can be assessed and monitored in real-time by EUS.

EUS-RFA using Habib EUS-RFA catheter (Emcision Ltd., United Kingdom) through a 19 gauge needle for ablation of lymphatic and pancreatic tissue, was reported in two animal studies. In the former study[33], EUS-guided RFA ablation of mediastinal lymph nodes was successfully attempted in six pigs. RFA was performed with the ERBE Vaio generator (ERBE, Tuttlingen, Germany) with bipolar settings of 10 watts, effect 2 for 2 min. During the procedure, the probe was visible in all cases. No evidence of ablation effect in the surrounding tissue or at the needle puncture site was seen on gross examination. There was a direct correlation between the probe length and the size of necrosis. In the pancreatic study using the same catheter, five Yucatan pigs underwent EUS-guided RFA of the head of the pancreas[13]. RFA was applied with 6 mm of the probe exposed at 4 watts for 5 min, 5 watts for 0.9 min, and 6 watts for 0.2 min. Then, with 10 mm of the probe exposed in the pancreas, RFA was performed at 4 watts for 4.3 min, 5 watts for 1.4 min, and 6 watts for 0.8 min. Autopsy showed moderate level of pancreatitis, with involvement of 20% of the proximal pancreatic tissue in only one pig. There was minimal tissue damage in the other animals. In this study EUS-guided RFA of the pancreatic head with the monopolar probe through a 19 gauge needle was well tolerated with a minimal amount of pancreatitis.

We have reported in this prospective study the application of RFA *via* the novel Habib EUS-RFA catheter (Emcision Ltd., United Kingdom) for pancreatic cystic neoplasms and NET. The concept of treating pre-malignant asymptomatic pancreatic lesions by means other than surgical resection is appealing, as the latter is associated with major morbidity and some mortality. This study shows that such an approach is feasible and safe. Our patients were discharged hours without any major adverse events. However, it is conceivable that the application of RF energy in the pancreatic parenchyma may be associated with some adverse events. Such adverse events may include (but not necessarily limited to) acute pancreatitis, pancreatic leaks, infection of necrotic pancreatic tissue post treatment and bleeding. Using lower energy also allows for repeating the ablation with low morbidity as per clinical indication. EUS-RFA of pancreatic neoplasms with a novel monopolar RF probe was well tolerated in all patients. These preliminary data results suggest that the procedure is technically easy and safe. The response ranged from complete resolution to a 50% reduction in diameter. Further multicenter experience is required before widespread use of this novel procedure.

ACKNOWLEDGMENTS

Nagy Habib is shareholder and director of EMcision

which designed and developed the device. None of the other authors have a conflict of interest or a financial disclosure to declare.

COMMENTS

Background

The aim of this report is to outline the feasibility, safety, adverse events and early results of endoscopic ultrasound guided radiofrequency ablation (EUS-RFA) in pancreatic neoplasms using a novel RF probe. The Habib™ EUS-RFA is monopolar catheter with a 1 Fr wire (0.33 mm, 0.013") with a working length of 190 cm, which can be inserted through the biopsy channel of an echoendoscope. RF power is applied to the electrode at the end of the wire to coagulate tissue in the liver and pancreas.

Research frontiers

This first in human study shows that EUS-RFA with this novel catheter provides an endoscopic treatment option other than surgical resection for pancreatic lesions.

Innovations and breakthroughs

The standard treatment of solid or cystic pancreatic lesions with malignant potential has been surgical resection. Pancreatic resections carry significant morbidity and mortality, resulting in unacceptably high risk/benefit ratios for many elderly patients with co-morbidities. There is an unmet need for minimally invasive ablative techniques as alternative to surgical resection.

Applications

Our results show that the procedure is technically easy and safe. The response in this series ranged from complete resolution to a 50% reduction in diameter. Therefore it might be an excellent alternative for patients that are not suitable surgical candidates.

Terminology

Radiofrequency ablation is the procedure of destructing tissue with the use of heat generated from high frequency alternating current (in the range of 350-500 kHz). It is a widely accepted method of tissue destruction for primary solid organ tumors. It has been used in the management of primary liver and lung tumors in patients that are not suitable surgical candidates and in secondary malignancies as part of the treatment algorithm.

Peer-review

This author congratulated demonstrating wonderful study.

REFERENCES

1 **Spinelli KS**, Fromwiller TE, Daniel RA, Kiely JM, Nakeeb A, Komorowski RA, Wilson SD, Pitt HA. Cystic pancreatic neoplasms: observe or operate. *Ann Surg* 2004; **239**: 651-657; discussion 657-659 [PMID: 15082969 DOI: 10.1097/01.sla.0000124299.57430.ce]

2 **Le Borgne J**, de Calan L, Partensky C. Cystadenomas and cystadenocarcinomas of the pancreas: a multiinstitutional retrospective study of 398 cases. French Surgical Association. *Ann Surg* 1999; **230**: 152-161 [PMID: 10450728 DOI: 10.1097/00000658-199908000-00004]

3 **Kitagawa Y**, Unger TA, Taylor S, Kozarek RA, Traverso LW. Mucus is a predictor of better prognosis and survival in patients with intraductal papillary mucinous tumor of the pancreas. *J Gastrointest Surg* 2003; **7**: 12-18; discussion 18-19 [PMID: 12559180 DOI: 10.1016/S1091-255X(02)00152-X]

4 **Cameron JL**, Riall TS, Coleman J, Belcher KA. One thousand consecutive pancreaticoduodenectomies. *Ann Surg* 2006; **244**: 10-15 [PMID: 16794383 DOI: 10.1097/01.sla.0000217673.04165.ea]

5 **Birkmeyer JD**, Sun Y, Wong SL, Stukel TA. Hospital volume and late survival after cancer surgery. *Ann Surg* 2007; **245**: 777-783 [PMID: 17457171 DOI: 10.1097/01.sla.0000252402.33814.dd]

6 **Tanaka M**, Chari S, Adsay V, Fernandez-del Castillo C, Falconi M, Shimizu M, Yamaguchi K, Yamao K, Matsuno S. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. *Pancreatology* 2006; **6**: 17-32 [PMID: 16327281 DOI: 10.1159/000090023]

7 **Jiao LR**, Hansen PD, Havlik R, Mitry RR, Pignatelli M, Habib N. Clinical short-term results of radiofrequency ablation in primary and secondary liver tumors. *Am J Surg* 1999; **177**: 303-306 [PMID: 10326848 DOI: 10.1016/S0002-9610(99)00043-4]

8 **Weber JC**, Navarra G, Jiao LR, Nicholls JP, Jensen SL, Habib NA. New technique for liver resection using heat coagulative necrosis. *Ann Surg* 2002; **236**: 560-563 [PMID: 12409660 DOI: 10.1097/00000658-200211000-00004]

9 **Pai M**, Frampton AE, Mikhail S, Resende V, Kornasiewicz O, Spalding DR, Jiao LR, Habib NA. Radiofrequency assisted liver resection: analysis of 604 consecutive cases. *Eur J Surg Oncol* 2012; **38**: 274-280 [PMID: 22209064 DOI: 10.1016/j.ejso.2011.12.006]

10 **Pai M**, Spalding D, Jiao L, Habib N. Use of bipolar radiofrequency in parenchymal transection of the liver, pancreas and kidney. *Dig Surg* 2012; **29**: 43-47 [PMID: 22441619 DOI: 10.1159/000335732]

11 **Steel AW**, Postgate AJ, Khorsandi S, Nicholls J, Jiao L, Vlavianos P, Habib N, Westaby D. Endoscopically applied radiofrequency ablation appears to be safe in the treatment of malignant biliary obstruction. *Gastrointest Endosc* 2011; **73**: 149-153 [PMID: 21184881 DOI: 10.1016/j.gie.2010.09.031]

12 **Yoon WJ**, Brugge WR. Endoscopic ultrasonography-guided tumor ablation. *Gastrointest Endosc Clin N Am* 2012; **22**: 359-369, xi [PMID: 22632957 DOI: 10.1016/j.giec.2012.04.017]

13 **Gaidhane M**, Smith I, Ellen K, Gatesman J, Habib N, Foley P, Moskaluk C, Kahaleh M. Endoscopic Ultrasound-Guided Radiofrequency Ablation (EUS-RFA) of the Pancreas in a Porcine Model. *Gastroenterol Res Pract* 2012; **2012**: 431451 [PMID: 23049547 DOI: 10.1155/2012/431451]

14 **Chen MH**, Yang W, Yan K, Gao W, Dai Y, Wang YB, Zhang XP, Yin SS. Treatment efficacy of radiofrequency ablation of 338 patients with hepatic malignant tumor and the relevant complications. *World J Gastroenterol* 2005; **11**: 6395-6401 [PMID: 16419172]

15 **Toyoda M**, Kakizaki S, Horiuchi K, Katakai K, Sohara N, Sato K, Takagi H, Mori M, Nakajima T. Computed tomography-guided transpulmonary radiofrequency ablation for hepatocellular carcinoma located in hepatic dome. *World J Gastroenterol* 2006; **12**: 608-611 [PMID: 16489676]

16 **Matsui Y**, Nakagawa A, Kamiyama Y, Yamamoto K, Kubo N, Nakase Y. Selective thermocoagulation of unresectable pancreatic cancers by using radiofrequency capacitive heating. *Pancreas* 2000; **20**: 14-20 [PMID: 10630378 DOI: 10.1097/00006676-200001000-00002]

17 **Elias D**, Baton O, Sideris L, Lasser P, Pocard M. Necrotizing pancreatitis after radiofrequency destruction of pancreatic tumours. *Eur J Surg Oncol* 2004; **30**: 85-87 [PMID: 14736529 DOI: 10.1016/j.ejso.2003.10.013]

18 **Carrara S**, Arcidiacono PG, Albarello L, Addis A, Enderle MD, Boemo C, Campagnol M, Ambrosi A, Doglioni C, Testoni PA. Endoscopic ultrasound-guided application of a new hybrid cryotherm probe in porcine pancreas: a preliminary study. *Endoscopy* 2008; **40**: 321-326 [PMID: 18389449 DOI: 10.1055/s-2007-995595]

19 **Siriwardena AK**. Radiofrequency ablation for locally advanced cancer of the pancreas. *JOP* 2006; **7**: 1-4 [PMID: 16407612]

20 **Wu Y**, Tang Z, Fang H, Gao S, Chen J, Wang Y, Yan H. High operative risk of cool-tip radiofrequency ablation for unresectable pancreatic head cancer. *J Surg Oncol* 2006; **94**: 392-395 [PMID: 16967436 DOI: 10.1002/jso.20580]

21 **Spiliotis JD**, Datsis AC, Michalopoulos NV, Kekelos SP, Vaxevanidou A, Rogdakis AG, Christopoulou AN. High operative risk of cool-tip radiofrequency ablation for unresectable pancreatic head cancer. *J Surg Oncol* 2007; **96**: 89-90 [PMID: 17345594 DOI: 10.1002/jso.20764]

22 **Girelli R**, Frigerio I, Salvia R, Barbi E, Tinazzi Martini P, Bassi C. Feasibility and safety of radiofrequency ablation for locally advanced pancreatic cancer. *Br J Surg* 2010; **97**: 220-225 [PMID: 20069610 DOI: 10.1002/bjs.6800]

23 **Cantore M**, Girelli R, Mambrini A, Frigerio I, Boz G, Salvia R, Giardino A, Orlandi M, Auriemma A, Bassi C. Combined modality treatment for patients with locally advanced pancreatic adenocarcinoma. *Br J Surg* 2012; **99**: 1083-1088 [PMID: 22648697

DOI: 10.1002/bjs.8789]
24 **Wissniowski TT**, Hänsler J, Neureiter D, Frieser M, Schaber S, Esslinger B, Voll R, Strobel D, Hahn EG, Schuppan D. Activation of tumor-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits. *Cancer Res* 2003; **63**: 6496-6500 [PMID: 14559842]
25 **den Brok MH**, Sutmuller RP, van der Voort R, Bennink EJ, Figdor CG, Ruers TJ, Adema GJ. In situ tumor ablation creates an antigen source for the generation of antitumor immunity. *Cancer Res* 2004; **64**: 4024-4029 [PMID: 15173017 DOI: 10.1158/0008-5472.CAN-03-3949]
26 **Ali MY**, Grimm CF, Ritter M, Mohr L, Allgaier HP, Weth R, Bocher WO, Endrulat K, Blum HE, Geissler M. Activation of dendritic cells by local ablation of hepatocellular carcinoma. *J Hepatol* 2005; **43**: 817-822 [PMID: 16087270 DOI: 10.1016/j.jhep.2005.04.016]
27 **Dromi SA**, Walsh MP, Herby S, Traughber B, Xie J, Sharma KV, Sekhar KP, Luk A, Liewehr DJ, Dreher MR, Fry TJ, Wood BJ. Radiofrequency ablation induces antigen-presenting cell infiltration and amplification of weak tumor-induced immunity. *Radiology* 2009; **251**: 58-66 [PMID: 19251937 DOI: 10.1148/radiol.2511072175]
28 **Zerbini A**, Pilli M, Penna A, Pelosi G, Schianchi C, Molinari A, Schivazappa S, Zibera C, Fagnoni FF, Ferrari C, Missale G. Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. *Cancer Res* 2006; **66**: 1139-1146 [PMID: 16424051 DOI: 10.1158/0008-5472.CAN-05-2244]
29 **Goldberg SN**, Mallery S, Gazelle GS, Brugge WR. EUS-guided radiofrequency ablation in the pancreas: results in a porcine model. *Gastrointest Endosc* 1999; **50**: 392-401 [PMID: 10462663 DOI: 10.1053/ge.1999.v50.98847]
30 **Kim HJ**, Seo DW, Hassanuddin A, Kim SH, Chae HJ, Jang JW, Park do H, Lee SS, Lee SK, Kim MH. EUS-guided radiofrequency ablation of the porcine pancreas. *Gastrointest Endosc* 2012; **76**: 1039-1043 [PMID: 23078928 DOI: 10.1016/j.gie.2012.07.015]
31 **DeWitt J**, DiMaio CJ, Brugge WR. Long-term follow-up of pancreatic cysts that resolve radiologically after EUS-guided ethanol ablation. *Gastrointest Endosc* 2010; **72**: 862-866 [PMID: 20883866 DOI: 10.1016/j.gie.2010.02.039]
32 **DiMaio CJ**, DeWitt JM, Brugge WR. Ablation of pancreatic cystic lesions: the use of multiple endoscopic ultrasound-guided ethanol lavage sessions. *Pancreas* 2011; **40**: 664-668 [PMID: 21562447 DOI: 10.1097/MPA.0b013e3182128d06]
33 **Sethi A**, Ellrichmann M, Dhar S, Hadeler K-GERD, Kahle E, Seehusen F, Klapper W, Habib N, Fritscher-Ravens A. 503 EUS-Guided Lymph Node Ablation With Novel Radiofrequency Ablation Probe: A Feasibility Study. *Gastrointest Endosc* 2012; **75** (Suppl 4): AB147 [DOI: 10.1016/j.gie.2012.04.079]

P- Reviewer: Cidon EU, Chiu KW, Ogura T, Tepes B, Watanabe M
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

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DOI: 10.4240/wjgs.v7.i4.60

World J Gastrointest Surg 2015 April 27; 7(4): 60-66
ISSN 1948-9366 (online)

CASE REPORT

Glucagon receptor gene mutations with hyperglucagonemia but without the glucagonoma syndrome

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Author contributions: Miller HC wrote the paper and analysed genetic data; Kidd M and Modlin IM performed genetic studies; Drymousis P, Vlavianos P and Frilling A contributed to the clinical work up; Cohen P and Dina R conducted the histopathology, Klöppel G reviewed the histology; Frilling A designed the research, analysed data and reviewed and edited the manuscript.
Ethics approval: This study is part of our project R12025: Genetic signature, metabolic phenotyping and integrative biology of neuroendocrine tumors. Ethics approval REC number: 07/MRE09/54.
Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrolment.
Conflict-of-interest: None of the authors have any relevant affiliations or financial involvements to disclose.

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Received: July 17, 2014
Peer-review started: July 18, 2014
First decision: December 17, 2014
Revised: January 21, 2015
Accepted: February 10, 2015
Article in press: February 12, 2015
Published online: April 27, 2015

Abstract

Pancreatic neoplasms producing exclusively glucagon associated with glucagon cell hyperplasia of the islets and not related to hereditary endocrine syndromes have been recently described. They represent a novel entity within the panel of non-syndromic disorders associated with hyperglucagonemia. This case report describes a 36-year-old female with a 10 years history of non-specific abdominal pain. No underlying cause was evident despite extensive diagnostic work-up. More recently she was diagnosed with gall bladder stones. Abdominal ultrasound, computerised tomography and magnetic resonance imaging revealed no pathologic findings apart from cholelithiasis. Endoscopic ultrasound revealed a 5.5 mm pancreatic lesion. Fine needle aspiration showed cells focally expressing chromogranin, suggestive but not diagnostic of a low grade neuroendocrine tumor. OctreoScan® was negative. Serum glucagon was elevated to 66 pmol/L (normal: 0-50 pmol/L). Other gut hormones, chromogranin A and chromogranin B were normal. Cholecystectomy and enucleation of the pancreatic lesion were undertaken. Postoperatively, abdominal symptoms resolved and serum glucagon dropped to 7 pmol/L. Although H and E staining confirmed normal pancreatic tissue, immunohistochemistry was initially thought to be suggestive of alpha cell hyperplasia. A count of glucagon positive cells from 5 islets, compared to 5 islets from 5 normal pancreata indicated that islet size and glucagon cell ratios were increased, however still within the wide range of normal physiological findings. Glucagon receptor gene (GCGR) sequencing revealed a heterozygous deletion,

K349_G359del and 4 missense mutations. This case may potentially represent a progenitor stage of glucagon cell adenomatosis with hyperglucagonemia in the absence of glucagonoma syndrome. The identification of novel *GCGR* mutations suggests that these may represent the underlying cause of this condition.

Key words: Hyperglucagonemia; Glucagon receptor gene; Mutation; Adenomatosis; Pancreas

Core tip: We identify novel mutations in the glucagon receptor gene in a patient with hyperglucagonemia but no glucagonoma syndrome. Physicians dealing with pancreatic disorders should be aware of this unusual condition.

Miller HC, Kidd M, Modlin IM, Cohen P, Dina R, Drymousis P, Vlavianos P, Klöppel G, Frilling A. Glucagon receptor gene mutations with hyperglucagonemia but without the glucagonoma syndrome. *World J Gastrointest Surg* 2015; 7(4): 60-66 Available from: URL: http://www.wjgnet.com/1948-9366/full/v7/i4/60.htm DOI: http://dx.doi.org/10.4240/wjgs.v7.i4.60

INTRODUCTION

Glucagon cell adenomatosis has been reported by Henopp *et al*[1] as an independent previously unrecognised disease of the endocrine pancreas. Multiple pancreatic neoplasms exclusively producing glucagon, associated with glucagon cell hyperplasia of the islets and unrelated to multiple endocrine neoplasia (MEN) type 1 (MEN 1), p27 MEN or von Hippel-Lindau (VHL) syndromes, are the hallmarks of the condition[2]. To date very few such patients have been reported[1,3,4].

Most patients present with abdominal pain and increased serum glucagon levels but fail to exhibit the characteristics of the glucagonoma syndrome (necrolytic migratory erythema, diabetes mellitus, stomatitis and weight loss)[5]. While macroscopic tumors are evident on imaging in some, numerous microadenomas scattered throughout the pancreas and enlarged islets are the findings in others[1,3,4]. Malignancy has not been identified in any cases reported to date. The underlying cause of glucagon cell hyperplasia and consequent development of glucagon cell neoplasia without the glucagonoma syndrome remains unknown. Yu *et al*[3], Zhou *et al*[6] have proposed that malfunction of the glucagon receptor (GCGR) and/or glucagon may be responsible for the disease after detection of a homozygous missense mutation, c.256C>T (P86S) in the *GCGR* of a patient.

We present another example of hyperglucagonemia without morphological evidence of neoplasia or the glucagonoma syndrome in which we identified *GCGR* mutations which may represent the underlying pathogenic cause of the condition.

CASE REPORT

A 36 years old Caucasian female with no previous medical or known family history was referred to us in 2011 with a 10 year history of non-specific diffuse abdominal pain. She repeatedly underwent complete gastrointestinal diagnostic work-up over a period of 8 years which revealed no pathologic results. In 2009, she had been diagnosed with cholelithiasis on abdominal ultrasound. Upon referral to our centre in 2011, extensive investigations including upper and lower intestinal endoscopy, computerised tomography and magnetic resonance imaging (MRI) were carried out. Apart from the previously diagnosed cholelithiasis, no other pathology was evident. Endoscopic ultrasound (EUS) confirmed calculi in the gallbladder and a mild dilatation of the distal common bile duct. In addition, a 5.5 mm hypoechoic lesion with irregular margins was detected in the pancreatic tail. Fine needle aspiration (FNA) revealed cells focally expressing chromogranin A. The features were suggestive but not diagnostic of a low grade neuroendocrine tumor. Somatostatin receptor scintigraphy showed no foci of increased uptake. While serum gastrin, vasoactive intestinal polypeptide, somatostatin, and pancreatic polypeptide were within the normal range, glucagon was elevated to 66 pmol/L (normal: 0-50 pmol/L). Serum fasting and postprandial glucose was normal. Neuroendocrine tumor markers chromogranin A and chromogranin B were not elevated. At laparotomy, a sub-centimeter lobulated lesion was found at the inferior margin of the pancreatic tail corresponding with the lesion identified on EUS. No further lesions were identified in the remaining pancreas after meticulous bimanual exploration and intraoperative ultrasound. There were no enlarged peripancreatic lymph nodes. The pancreatic tail lesion was enucleated and cholecystectomy performed. A grade 1 pancreatic fistula developed postoperatively and resolved within 2 wk. The further course was uneventful and the patient was entirely asymptomatic. Moreover, she reported that the abdominal pain she experienced over the last decade had completely disappeared. Serum glucagon was assessed 1 mo postoperatively after the pancreatic morphology returned to normal on imaging. It was found to have decreased to 7 pmol/L. Serum glucagon was monitored at regular intervals (see Table 1). At the last follow-up, 31 mo after surgery, the patient remained asymptomatic with a normal MRI result, serum glucagon was 10 pmol/L and insulin was within the normal range.

Histology (H and E) showed features of normal pancreatic tissue. Immunohistochemical examination for glucagon and insulin was undertaken using the technique of Henopp *et al*[1]. Approximately 20% of the islet cells were glucagon positive and 80%

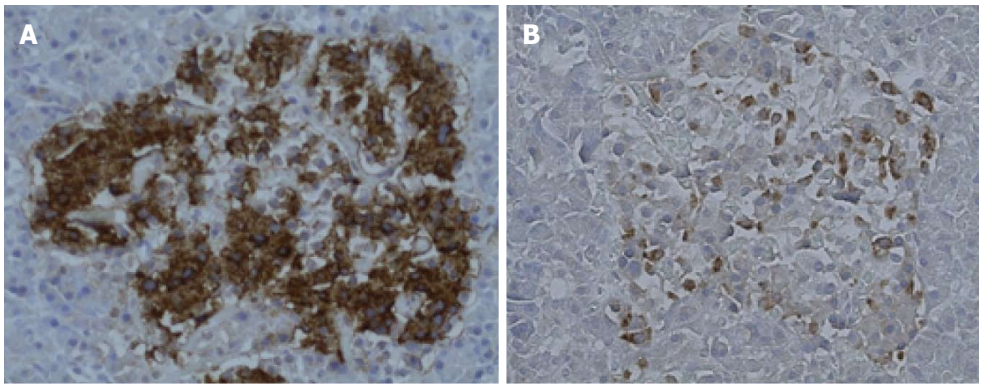

Figure 1 Islet from patient. Immunohistochemistry for A: Insulin; B: Glucagon, x 200 magnification (600 μm at maximum diameter).

Table 1 Serum glucagon levels

Time	Serum glucagon (pmol/L) (normal range: 0-50 pmol/L)
Pre-surgery	
2 mo	66
Post-surgery	
1 mo	7
5 mo	28
6 mo	6
17 mo	15
20 mo	29
31 mo	10

Table 2 Islet size and number of glucagon positive cells in the current case compared to 5 normal pancreata

	Average[1] islet size (μm)	Average[1] number glucagon positive cells	
		Count 1	Count 2
Patient	192	29.2	28.8
Control 1	256	59.8	61.8
Control 2	186	25.6	25.2
Control 3	255	32.2	31.6
Control 4	260	52.4	53.4
Control 5	190	44.6	42.6

[1]Average of 5 pancreatic islets.

insulin positive. Glucagon cell hyperplasia was initially considered (Figure 1). In order to investigate this further, glucagon cell counts were done with 5 islets from 5 normal pancreatic controls and compared to 5 islets from the patient (Table 2). The counts showed that the average islet size and the average number of glucagon positive cells per islet were increased in the patient, however still within the wide range observed in normal pancreatic tissue.

Methods of genetic investigation

A peripheral blood sample was obtained from the patient, her daughter and a healthy individual as a normal control (informed consent obtained). Genomic DNA (gDNA) was extracted using the DNeasy Blood and Tissue Kit according to the protocol (Qiagen, catalogue number: 9506). Polymerase chain reaction (PCR) amplification of exons 2-13 (and most of exon 14) of *GCGR* and the intron:exon borders was carried out using previously described primers[6]. Purified PCR products were sequenced by the W.M. Keck Biotechnology Resource Laboratory at Yale University, New Haven, United States using an automated Applied Biosystems 373A Stretch DNA sequencer (Perkin-Elmer, Norwalk, United States). PCR products were sequenced using forward primers. If ambiguous peaks were evident, the sequence was confirmed with the reverse primers[7]. Bioedit software was used to analyse the sequencing results[8]. Sequencing products were compared to the control sample and the national centre for biotechnology information (NCBI) reference sequences for the human *GCGR*, DNA (NG_016409.1), mRNA (NM_000160.3) and protein (NP_000151.1).

MEN1 sequencing was carried out on gDNA from the peripheral blood. PCR amplification of exons 2-10 of *MEN1* was undertaken using previously described primers[9,10]. The DNA extraction, sequencing and analysis were carried out using the same technique as for *GCGR*. The reference sequence used was NCBI GenBank: U93237.1.

VHL sequencing was carried out on gDNA from the peripheral blood. PCR amplification of exons 1-3 of *VHL* was undertaken using primers previously described[11]. The DNA extraction, sequencing and analysis were carried out using the same technique as for *GCGR*. The reference sequence used was NCBI GenBank: NM_000551.3.

Results of genetic analysis

A heterozygous deletion of 33 nucleotides in exon 11 of the *GCGR* was detected. This corresponded to a K349_G359del in the GCGR with the loss of the following 11 amino acids, KSTLTLIPLLG. There were also 5 heterozygous point mutations including 4 missense mutations, E362K, V368L, K381E, S389N and 1 synonymous mutation (Figure 2). There were no mutations in *MEN1* or *VHL*. No mutations were

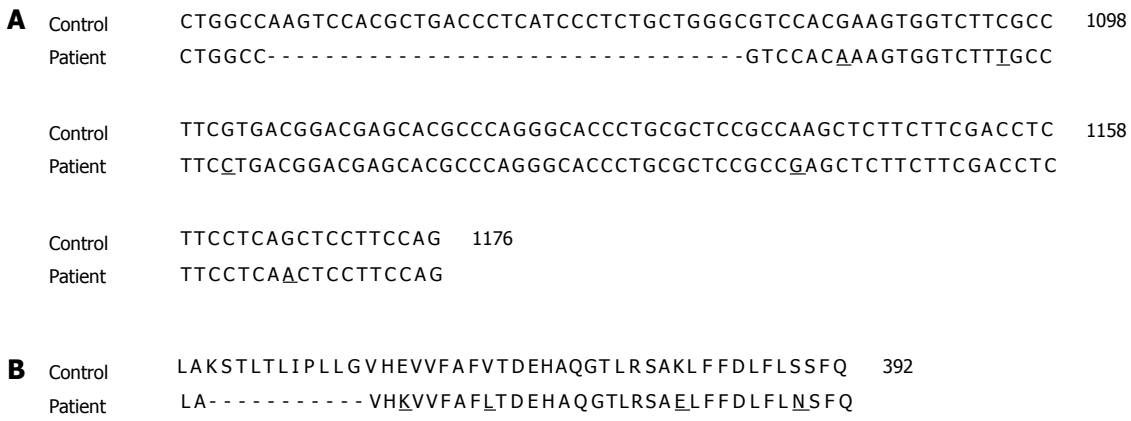

Figure 2 Genetic findings. A: Sequencing results showing a heterozygous 33 nucleotide deletion and 5 point mutations in exon 11 of the *GCGR*; B: Amino acid sequence showing K349_G359del, E362K, V368L, K381E, S389N in the GCGR. Alignments done using Clustal W multiple sequence alignments software[25]. Numbers indicate the position of the last residue shown along the GCGR cDNA/protein.

detectable in the daughter.

DISCUSSION

This case report represents the second case of hyperglucagonemia which has been associated with a specific genetic lesion in the *GCGR.* The case could potentially represent a progenitor stage of an entity leading to glucagon cell adenomatosis.

To date 8 individuals exhibiting characteristics of glucagon cell adenomatosis with hyperglucagonemia but without glucagonoma syndrome have been reported in the literature[1,3,4,6,12,13]. It is a matter of debate whether all cases cited completely fulfil the criteria of glucagon cell adenomatosis as defined by Henopp *et al*[1]. For example, the individual described by Yu *et al*[3] had not only raised serum glucagon levels but also pathologic values of pancreatic polypeptide. The patient reported by Balas *et al*[13] in 1988 had normal serum glucagon levels; however immunohistological findings in the resected pancreas were consistent with glucagon cell adenomatosis. In our patient, although the morphology of the resected pancreatic islets was within the broad range of findings reported in unaffected pancreata, we speculate that a cluster of hyperfunctioning cells might potentially be responsible for the development of hyperglucagonemia. Functional studies would be needed to confirm this theory.

The majority of individuals had glucagon cell adenomatosis, but were asymptomatic with respect to evidence of the glucagonoma syndrome. The results of imaging ranged from no pathologic findings to diffuse pancreatic enlargement associated with multiple tumors of various sizes. Abdominal pain is present in most individuals as was the case in our patient (Table 3). While the case we present exhibited normal uptake on somatostatin receptor scintigraphy, diffusely increased uptake was reported on OctreoScan® in a patient with diffuse pancreatic enlargement and multiple tumors by Henopp *et al*[1]. In our patient the positive staining for chromogranin on FNA was thought to be suggestive of a neuroendocrine tumor. This might reflect the small number of cells obtained from the FNA, with a sampling error leading to a higher proportion of chromogranin positive cells (*e.g.,* if FNA sampling comprised an islet). In comparison to two reported cases which had highly elevated serum glucagon levels, our patient had only slightly increased serum glucagon (Table 3). The lack of standardised serum glucagon reporting in the majority of cases and the small number of patients means it is difficult to tell if the levels in our patient were truly lower than average.

The majority of previously reported patients demonstrated numerous microadenomas expressing almost exclusively glucagon and/or glucagon cell hyperplasia. This observation prompted Henopp *et al*[1,14] to postulate that diffuse glucagon cell hyperplasia might represent a precursor from of glucagon cell neoplasia. In the case described by Yu *et al*[3], 60%-80% of the hyperplastic islet cells stained positive for glucagon but negative for insulin. A similar trend was noted by Henopp *et al*[1]. In our patient, the pancreatic morphology was unusual, nevertheless still within the wide range of physiological findings. Approximately 20% of the islet cells expressed glucagon while 80% expressed insulin. Based on this observation and only mildly increased serum glucagon, we hypothesize that the disease might have been diagnosed at a very early stage prior to evidence of hyperplasic transformation and development of overt morphological evidence of neoplasia/s. While a subcentimeter nodule at the pancreatic tail was evident on EUS and confirmed intraoperatively, standard histology showed regular findings. This scenario resembles a report by Martignoni *et al*[4] of hyperglucagonemia but no microadenomas.

Both of the two previously reported patients for whom follow-up data was available showed increased serum glucagon levels after pancreatic resection in the presence of negative imaging results[1,3]. These findings underline the presumption of disease persistence. Our patient however, had normal serum glucagon levels at 31 mo after surgery (10 pmol/L) (Table 1). Due to the

Table 3 Hyperglucagonemia without the glucagonoma syndrome-review of the literature

	Martignoni *et al*[4]	Henopp *et al*[1] (patient 2)	Yu *et al*[3], Zhou *et al*[6]	Present case
Patient	54, M	43, F	60, F	36, F
Origin	-	-	Persian	Caucasian
Clinical symptoms	Abdominal pain Diarrhea[1]	Abdominal pain	Abdominal pain Constipation	Abdominal pain
Serum Glucagon (pmol/L)	Elevated	Elevated (25-fold)[2]	17011[3]	66
Imaging	Negative	Positive	Positive	Negative (positive on EUS)
OctreoScan®	Negative	-	Negative	Negative
Localization	No focal abnormality	Tail	Uncinate	Tail
Pancreatic pathology	α-cell hyperplasia nesidioblastosis	α-cell hyperplasia, large cystic tumor and small solid tumors, multiple microadenomas	α-cell hyperplasia non-functioning pancreatic NET microglucagonoma microadenoma	Normal pancreatic morphology on standard H and E staining
GCGR	-	-	Homozygous gDNA point mutation	Heterozygous gDNA deletion 5 point mutations
Other Genes	-	Negative for *MEN1*/*VHL* gDNA mutations	-	Negative for *MEN1*/*VHL* gDNA mutations
Relatives *GCGR*	-	-	Brother Negative	Daughter Negative

[1]Mild diabetes was initially suspected but then found to be unlikely; [2]The glucagon levels were only measured postoperatively; [3]Glucagon levels converted to pmol/L from pg/mL. M: Male; F: Female; EUS: Endoscopic ultrasound; GCGR: Glucagon receptor.

genetic predisposition of the disease we cannot exclude the possibility that at some point in the future the disease may recur therefore our patient requires life-long follow up. Any future increases in serum glucagon levels could potentially represent the emergence of alpha cell hyperfunction consistent with the concept of a residual genomic lesion representing a diffuse alpha cell abnormality in the remaining pancreatic islets.

The GCGR is a member of the class B G protein-coupled receptor family, glucagon binding triggers downstream signalling, allowing glucagon to regulate blood glucose levels by stimulating glycogenolysis[15,16]. The knockout mouse for *GCGR* expresses high glucagon levels associated with pancreatic enlargement, glucagon cell adenomatosis and microglucagonomas or glucagonomas at 10-12 mo when compared to their heterozygous littermates[17,18]. Based on these observations, Yu *et al*[3,6] sequenced *GCGR* and the glucagon gene in their patient with hyperglucagonemia, alpha cell hyperplasia and microglucagonoma. They detected a homozygous c.256C>T (P86S) mutation in *GCGR* resulting in lower binding affinity of GCGR P86S to glucagon and hypothesized that this mutation was responsible for the alpha cell hyperplasia and hyperglucagonemia. They showed *in vitro* that the GCGR P86S localized to the plasma membrane but bound glucagon with less avidity than wild type GCGR; a greater glucagon concentration was thus needed to trigger downstream signalling *via* adenylate cyclase activation[6]. Neuroendocrine cells undergoing hyperplastic changes is particularly relevant for MEN1 conditions however they probably also occur in sporadic cases. Very recently Klöppel *et al*[14] identified 3 further patients with germline *GCGR* mutations and glucagon cell adenomatosis unrelated to MEN1 or VHL syndromes. The genetic lesions present in the *GCGR* were not described, however a further 3 patients had glucagon cell adenomatosis in the absence of any *GCGR* mutation[14].

Our case represents the second case with genetic lesions described in the *GCGR* associated with hyperglucagonemia in the absence of the glucagonoma syndrome. The heterozygous K349_G359del and E362K, V368L, K381E, S389N mutations could potentially represent a loss of function mutation in the *GCGR*. Functional studies would be needed to show if these mutations might be the cause of the hyperglucagonemia observed in our patient. All mutations were in exon 11 towards the C terminal end of GCGR. The point mutations appear to represent rather conservative amino acid changes in terms of hydrophobicity. Lysine and glutamate have a positively and a negatively charged R group respectively and the serine to asparagine change represents an alteration from a hydroxyl R group to a carboxamide R group. Site directed mutagenesis studies have noted that D385 is relevant to the specificity of glucagon/GCGR binding[19]. Since this residue is close to the K381E mutation site and adjacent to the glucagon binding site, the alteration in R group may affect glucagon binding. However in the absence of high resolution crystal structure data for the human glucagon receptor (except for the extracellular N terminal domain) and site directed mutagenesis studies for these sites, the effects of these genetic changes cannot be directly inferred[15].

The K349_G359del falls within the 6th transmembrane domain of GCGR, therefore the 11 amino acid deletion could prevent GCGR from inserting into the plasma membrane. This would prevent GCGR binding to glucagon[20]. In structural studies where COS-1 cells were transfected with the rat glucagon receptor gene, truncation mutants lacking any of the different

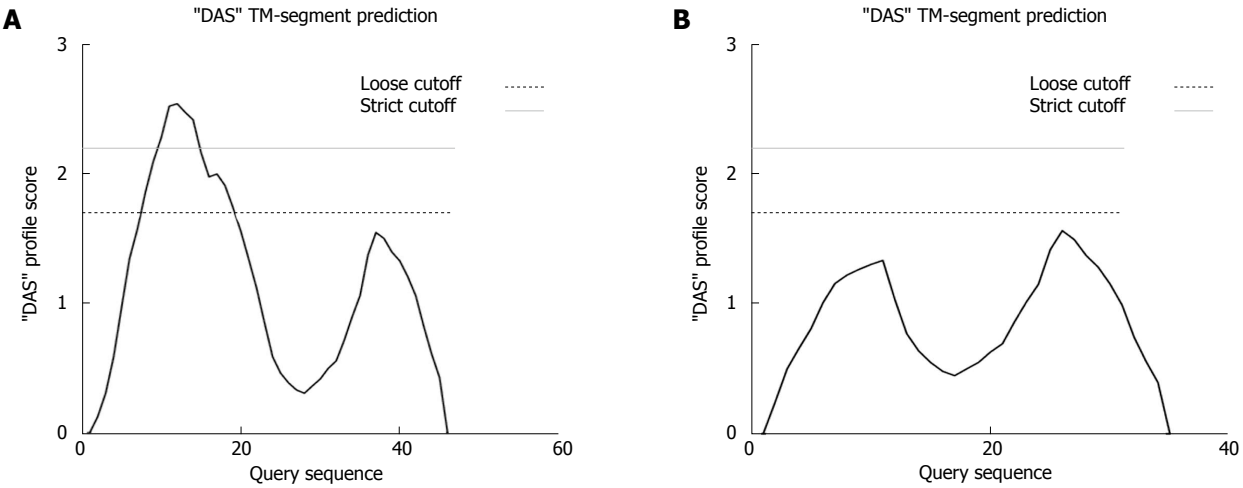

Figure 3 Membrane topology prediction. The loss of 11 amino acids from the glucagon receptor (GCGR) 6th transmembrane domain is predicted to prevent the insertion of GCGR into the plasma membrane. A: Predicted normal GCGR membrane topology; B: Predicted K349_G359del GCGR membrane topology. Software used: Meta^{TM}[22]; input amino acid sequence shown in Figure 2B. DAS: Distributed Annotation System.

transmembrane domains, were not localized to the plasma membrane suggesting that all 7 transmembrane domains are needed for correct membrane insertion[21]. The K349_G359del mutation is predicted by membrane topology prediction software to prevent the GCGR from properly inserting into the plasma membrane[22] (Figure 3). If this was the case, then the GCGR would be miss-localized preventing glucagon binding. This would however need to be confirmed by *in vitro* protein localization studies and assays to check glucagon binding efficiency in the presence of the deletion. In addition, since the mutation in the *GCGR* is heterozygous, there would still be a normal gene copy present which might allow sufficient glucagon signalling via the remaining receptors to give normal function. However, the clinical pathology evident in the presence of hyperglucagonemia seems to suggest that this may not be the case.

The phenotype could potentially represent incomplete dominance leading to the modest elevation of serum glucagon in our patient. Alternatively, it is possible that this individual might have a second mutation in the other copy of the *GCGR* within some of the pancreatic alpha cells which could potentially be causing them to become hyperfunctional.

It has been previously suggested that incretin treatment is associated with the development of alpha cell hyperplasia since pancreata from autopsies of incretin treated persons exhibit alpha cell hyperplasia (and beta cell hyperplasia) and some had glucagon expressing microadenomas[23,24]. A possibility exists that as incretin usage increases, alpha cell hyperplasia may become more prevalent.

In conclusion, we have identified a novel heterozygous K349_G359 deletion and 4 missense mutations in the *GCGR* which appear to be associated with hyperglucagonemia without the glucagonoma syndrome. Physicians dealing with pancreatic disorders should be aware of this very unusual condition. Further study leading to a better understanding of this disease entity would be of benefit to patients. The further usage of *GCGR* sequencing in such individuals should be undertaken to provide additional information on the breadth of the spectrum of mutational abnormalities associated with alpha cell transformation and excess glucagon production.

COMMENTS

Case characteristics

36 years old patient with a 10 year history of non-specific diffuse abdominal pain.

Clinical diagnosis

A sub-centimeter lobulated lesion was found at the inferior margin of the pancreatic tail, no further lesions were identified in the remaining pancreas after meticulous bimanual exploration and intraoperative ultrasound.

Differential diagnosis

Fine needle aspiration revealed cells focally expressing CgA. The features were suggestive but not diagnostic of a low grade neuroendocrine tumor.

Laboratory diagnosis

Serum glucagon was elevated to 66 pmol/L (normal: 0-50 pmol/L). Other gut hormones were within the normal range.

Imaging diagnosis

Endoscopic ultrasound identified a 5.5 mm hypoechoic lesion with irregular margins in the pancreatic tail.

Pathological diagnosis

Histology (H and E) showed features of normal pancreatic tissue. Glucagon cell hyperplasia was initially considered based on glucagon immunohistochemistry. Further investigation revealed that the average islet size and the average number of glucagon positive cells per islet were increased in the patient, however still within the wide range observed in normal pancreatic tissue.

Treatment

At laparotomy, a sub-centimeter lobulated lesion was found at the inferior margin of the pancreatic tail and was enucleated.

Related reports

This is a very rare disease entity. Genetic lesions in the glucagon receptor (*GCGR*) have only been described in one individual in the literature in the context of glucagon cell adenomatosis with hyperglucagonemia but without glucagonoma syndrome. Several additional cases exhibiting the characteristics of glucagon cell adenomatosis with hyperglucagonemia but without glucagonoma syndrome have been published however their *GCGR* mutation status remains unknown.

Experiences and lessons

The authors have identified novel *GCGR* mutations which appear to be associated with hyperglucagonemia without the glucagonoma syndrome. Physicians dealing

with pancreatic disorders should be aware of this very unusual condition.

Peer-review

This is an interesting case of an entity not described before.

REFERENCES

1 **Henopp T**, Anlauf M, Schmitt A, Schlenger R, Zalatnai A, Couvelard A, Ruszniewski P, Schaps KP, Jonkers YM, Speel EJ, Pellegata NS, Heitz PU, Komminoth P, Perren A, Klöppel G. Glucagon cell adenomatosis: a newly recognized disease of the endocrine pancreas. *J Clin Endocrinol Metab* 2009; **94**: 213-217 [PMID: 18957496 DOI: 10.1210/jc.2008-1300]

2 **Pellegata NS**, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Höfler H, Fend F, Graw J, Atkinson MJ. Germline mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proc Natl Acad Sci USA* 2006; **103**: 15558-15563 [PMID: 17030811 DOI: 10.1073/pnas.0603877103]

3 **Yu R**, Nissen NN, Dhall D, Heaney AP. Nesidioblastosis and hyperplasia of alpha cells, microglucagonoma, and nonfunctioning islet cell tumor of the pancreas: review of the literature. *Pancreas* 2008; **36**: 428-431 [PMID: 18437091 DOI: 10.1097/MPA.0b013e31815ceb23]

4 **Martignoni ME**, Kated H, Stiegler M, Büchler MW, Friess H, Zimmermann A, Schirp U, Nitzsche EU. Nesidioblastosis with glucagon-reactive islet cell hyperplasia: a case report. *Pancreas* 2003; **26**: 402-405 [PMID: 12717275 DOI: 10.1097/00006676-200305000-00016]

5 **Eldor R**, Glaser B, Fraenkel M, Doviner V, Salmon A, Gross DJ. Glucagonoma and the glucagonoma syndrome - cumulative experience with an elusive endocrine tumour. *Clin Endocrinol* (Oxf) 2011; **74**: 593-598 [PMID: 21470282 DOI: 10.1111/j.1365-2265.2011.03967.x]

6 **Zhou C**, Dhall D, Nissen NN, Chen CR, Yu R. Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, alpha cell hyperplasia, and islet cell tumor. *Pancreas* 2009; **38**: 941-946 [PMID: 19657311 DOI: 10.1097/MPA.0b013e3181b2bb03.A]

7 **Kidd M**, Eick G, Shapiro MD, Camp RL, Mane SM, Modlin IM. Microsatellite instability and gene mutations in transforming growth factor-beta type II receptor are absent in small bowel carcinoid tumors. *Cancer* 2005; **103**: 229-236 [PMID: 15599934 DOI: 10.1002/cncr.20750]

8 **Hall T.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999; **41**: 95-98

9 **Mayr B**, Apenberg S, Rothämel T, von zur Mühlen A, Brabant G. Menin mutations in patients with multiple endocrine neoplasia type 1. *Eur J Endocrinol* 1997; **137**: 684-687 [PMID: 9437237 DOI: 10.1530/eje.0.1370684]

10 **Wang EH**, Ebrahimi SA, Wu AY, Kashefi C, Passaro E, Sawicki MP. Mutation of the MENIN gene in sporadic pancreatic endocrine tumors. *Cancer Res* 1998; **58**: 4417-4420 [PMID: 9766672]

11 **Huang Y**, Zhou D, Liu J, Zhou P, Li X, Wang Z. Germline mutations of the VHL gene in seven Chinese families with von Hippel-Lindau disease. *Int J Mol Med* 2012; **29**: 47-52 [PMID: 21972040 DOI: 10.3892/ijmm.2011.808]

12 **Anlauf M**, Schlenger R, Perren A, Bauersfeld J, Koch CA, Dralle H, Raffel A, Knoefel WT, Weihe E, Ruszniewski P, Couvelard A, Komminoth P, Heitz PU, Klöppel G. Microadenomatosis of the endocrine pancreas in patients with and without the multiple endocrine neoplasia type 1 syndrome. *Am J Surg Pathol* 2006; **30**: 560-574 [PMID: 16699310 DOI: 10.1097/01.pas.0000194044.01104.25]

13 **Balas D**, Senegas-Balas F, Delvaux M, Bertrand C, Fagot-Revurat P, Rumeau JL, Ribet A. Silent human pancreatic glucagonoma and "A" nesidioblastosis. *Pancreas* 1988; **3**: 734-739 [PMID: 2851784 DOI: 10.1097/00006676-198812000-00015]

14 **Klöppel G**, Anlauf M, Perren A, Sipos B. Hyperplasia to neoplasia sequence of duodenal and pancreatic neuroendocrine diseases and pseudohyperplasia of the PP-cells in the pancreas. *Endocr Pathol* 2014; **25**: 181-185 [PMID: 24718881 DOI: 10.1007/s12022-014-9317-8]

15 **Koth CM**, Murray JM, Mukund S, Madjidi A, Minn A, Clarke HJ, Wong T, Chiang V, Luis E, Estevez A, Rondon J, Zhang Y, Hötzel I, Allan BB. Molecular basis for negative regulation of the glucagon receptor. *Proc Natl Acad Sci USA* 2012; **109**: 14393-14398 [PMID: 22908259 DOI: 10.1073/pnas.1206734109]

16 **Mayo KE**, Miller LJ, Bataille D, Dalle S, Göke B, Thorens B, Drucker DJ. International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol Rev* 2003; **55**: 167-194 [PMID: 12615957 DOI: 10.1124/pr.55.1.6.167]

17 **Gelling RW**, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, Charron MJ. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci USA* 2003; **100**: 1438-1443 [PMID: 12552113 DOI: 10.1073/pnas.0237106100]

18 **Yu R**, Dhall D, Nissen NN, Zhou C, Ren SG. Pancreatic neuroendocrine tumors in glucagon receptor-deficient mice. *PLoS One* 2011; **6**: e23397 [PMID: 21853126 DOI: 10.1371/journal.pone.0023397]

19 **Runge S**, Gram C, Brauner-Osborne H, Madsen K, Knudsen LB, Wulff BS. Three distinct epitopes on the extracellular face of the glucagon receptor determine specificity for the glucagon amino terminus. *J Biol Chem* 2003; **278**: 28005-28010 [PMID: 12724331 DOI: 10.1074/jbc.M301085200]

20 **Authier F**, Desbuquois B. Glucagon receptors. *Cell Mol Life Sci* 2008; **65**: 1880-1899 [PMID: 18292967 DOI: 10.1007/s00018-008-7479-6]

21 **Unson CG**, Cypess AM, Kim HN, Goldsmith PK, Carruthers CJ, Merrifield RB, Sakmar TP. Characterization of deletion and truncation mutants of the rat glucagon receptor. Seven transmembrane segments are necessary for receptor transport to the plasma membrane and glucagon binding. *J Biol Chem* 1995; **270**: 27720-27727 [PMID: 7499239 DOI: 10.1074/jbc.270.46.27720]

22 **Klammer M**, Messina DN, Schmitt T, Sonnhammer EL. MetaTM - a consensus method for transmembrane protein topology prediction. *BMC Bioinformatics* 2009; **10**: 314 [PMID: 19785723 DOI: 10.1186/1471-2105-10-314]

23 **Yu R**. Pancreatic α-cell hyperplasia: facts and myths. *J Clin Endocrinol Metab* 2014; **99**: 748-756 [PMID: 24285676 DOI: 10.1210/jc.2013-2952]

24 **Butler AE**, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes* 2013; **62**: 2595-2604 [PMID: 23524641 DOI: 10.2337/db12-1686]

25 **Larkin MA**, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007; **23**: 2947-2948 [PMID: 17846036 DOI: 10.1093/bioinformatics/btm404]

P- Reviewer: Guan YS **S- Editor**: Tian YL **L- Editor**: A
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DOI: 10.4240/wjgs.v7.i4.67

World J Gastrointest Surg 2015 April 27; 7(4): 67-70
ISSN 1948-9366 (online)

CASE REPORT

Appendiceal tie syndrome: A very rare complication of a common disease

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Ethics approval: The study was reviewed and approved by the BP Koirala Institute of Health Sciences Institutional Review Committee.
Informed consent: The study participant provided informed written consent.
Conflict-of-interest: None declared.

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Received: December 11, 2014
Peer-review started: December 13, 2014
First decision: December 26, 2014
Revised: January 25, 2015
Accepted: February 9, 2015
Article in press: February 11, 2015
Published online: April 27, 2015

Abstract

Acute appendicitis is the most common surgical emergency that we encounter. Adynamic Intestinal obstruction due to appendicitis or its complication may be seen time and often. Mechanical obstruction because of appendicitis is uncommon and even rarer for a closed loop obstruction to occur. Although it was described as early as 1901, very few cases have been reported. We report the case of a 20 years male who presented with generalized colicky pain abdomen, abdominal distension, vomiting and obstipation for three to four days. Vital signs were stable. His abdomen was distended and peritonitic, especially in the right iliac fossa. Rest of the physical examination was unremarkable. Blood tests were normal except for leucocytosis with neutrophilia. An abdominal X-ray finding was indicating a small bowel obstruction. A midline laparotomy was performed. On intraoperative examination, distended loops of small bowel from the jejunum to the distal ileum was observed, and a constricting ring around the terminal ileum created by a phlegmonous appendicitis with its tip adherent to the root of mesentery was found, obstructing an edematous loop of small bowel without signs of ischemia. As the bowel was viable simple appendectomy was done. Postoperatively, he had an uneventful recovery and was discharged after 3 d.

Key words: Appendicitis; Appendicular band; Intestinal obstruction; Mechanical small bowel obstruction; Closed loop obstruction

Core tip: It is safe to say, almost no-one will become a surgeon without seeing or doing appendectomy. It is so common, yet time and often we are deceived by it. As we know, abdomen is a "Pandora's box", we never know what come up sometimes and this is a perfect example. We report a case of mechanical small bowel obstruction due to acute appendicitis that was timely and successfully managed surgically.

Awale L, Joshi BR, Rajbanshi S, Adhikary S. Appendiceal tie syndrome: A very rare complication of a common disease. *World J Gastrointest Surg* 2015; 7(4): 67-70 Available from: URL: http://www.wjgnet.com/1948-9366/full/v7/i4/67.htm DOI: http://dx.doi.org/10.4240/wjgs.v7.i4.67

INTRODUCTION

Acute appendicitis is one of the most common surgical problems that we encounter. Diagnosis is not always so straightforward and can impose a dilemma. Indeed appendicitis is well known to cause mechanical small bowel obstruction because of adhesion. On the contrary, very few cases of mechanical small bowel obstruction developed as a direct result of acute appendicitis have been reported in literature[1,2]. In 1901, Hotchkiss *et al*[3] first reported it but till date only a handful of cases has been reported. The clinical feature of small bowel obstruction may obscure the clinical picture of appendicitis, making its diagnosis further challenging if not impossible. Hence, the preoperative diagnosis is very difficult and is made during laparotomy. Its paucity makes this case interesting.

CASE REPORT

A 20 years young male presented with the four days history of worsening generalized colicky pain abdomen, three days history of abdominal distension and bilious vomiting and three days history of obstipation. There was no history of previous abdominal surgery. On examination he was afebrile and vital signs were stable. His abdomen was distended, with visible bowel loops remarkably in the center abdomen and peritonitic, especially in the right iliac fossa, with exaggerated bowel sound. The rectal examination was normal. Rest of the physical examination was unremarkable. Laboratory parameters were within normal limits, except for the leukocytosis (16800/μL) with neutrophilia. A plain abdominal skiagram (Figure 1) showed dilated jejunal and ileal loops with multiple air-fluid levels indicating a small bowel obstruction.

The patient was kept nil per oral with active nasogastric aspiration. Intravenous fluid, prophylactic intravenous antibiotics and analgesics were started. Meanwhile the patient was planned for emergency laparotomy with a diagnosis of mechanical small bowel obstruction of unknown etiology.

A midline laparotomy was performed. On intra-operative examination, distended loops of small bowel from the jejunum to the distal ileum were observed. These loops were followed distally to reveal a constricting ring around the terminal ileum (Figure 2) created by a phlegmonous appendicitis (as represented in Figure 3) with its tip adherent to the root of mesentery (Figure 4), obstructing an edematous loop of terminal ileum without signs of ischemia (Figure 2). As the bowel was viable simple appendectomy was done and the abdomen was closed with a drain in the pelvis.

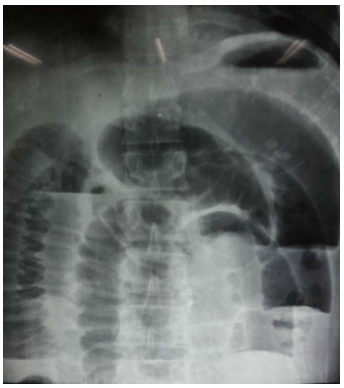

Figure 1 Abdominal radiograph showing multiple distended loops of small bowel with fluid levels.

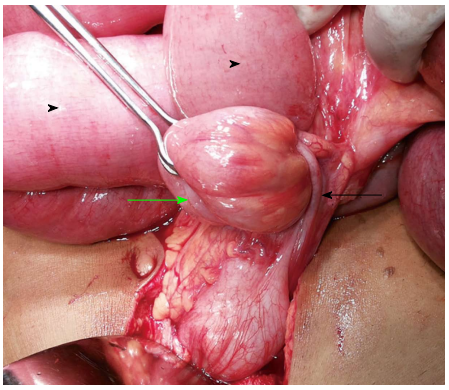

Figure 2 The Appendix (black arrow) encircling the loop of terminal Ileum (green arrow) with dilatation of proximal small bowel (black arrowheads).

Postoperatively, he had an uneventful recovery. The drain was removed on the 2nd operative day, and was orally started after around 48 h with the evidence of bowel movement. Subsequently, he was discharged on the 4th postoperative day. Histopathology report of excised appendix revealed acute appendicitis. He was doing well till 2 mo.

DISCUSSION

The first case of intestinal obstruction due to acute appendicitis was described by Hotchkiss[3]. In 1909, Hawks[4] divided the causes into mechanical and septic appendicitis or a combination of both. Appendix is a mobile organ and has variable position. Hence, during appendicitis it has tendency to get adhere to surrounding structures resulting in mechanical small bowel obstruction, and an increased length seems to facilitate the phenomenon[5].

In 2009, Bhandari *et al*[6] classified intestinal obstruction because of appendicitis into four types: adynamic, mechanical, strangulation, and caused by mesenteric ischemia. Adynamic obstruction or paralytic

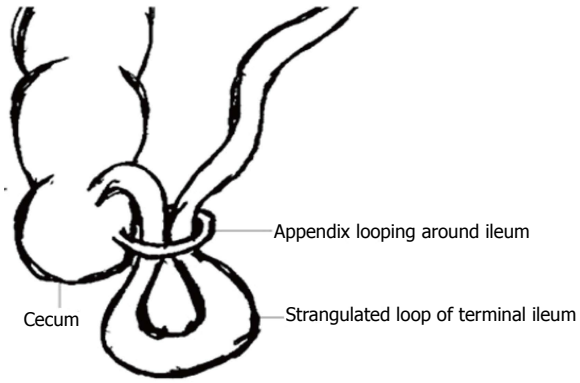

Figure 3 Depiction of Appendix wrapping around loop of Ileum. (Reproduced with permision from Menon *et al*[7]).

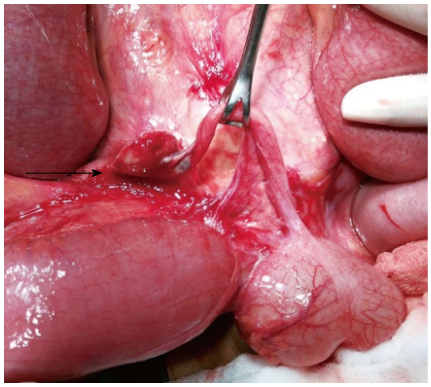

Figure 4 Inflamed and odematous tip of Appendix adherent to the root of mesentry (black arrow).

ileus is undoubtedly the most common type and is due to the appendicular inflammation spreading to the surrounding structures (caecum, small bowel or posterior peritoneum). Strangulation may result from a long standing closed loop obstruction, which can be due to the long appendix constricting around a loop of small bowel, or when it is adhere to surrounding structures and a part of bowel herniates through the gap. In 2005, Assenza *et al*[5] reported only six such cases in the review. Mesenteric ischemia due to appendicitis causing intestinal obstruction is the rarest one.

Among the mechanical causes, the vast majority are due to the formation of appendicular abscess that compresses the loops of small bowel, and postoperative adhesions that occur years after treatment[6]. There are two basic situations where the appendix may also cause a mechanical obstruction[5]; appendicular tip attached to the mesentery surrounding an ileal loop, producing compression of its lumen and the appendicular tip attached to the intestinal serosa, producing the obstruction by direct compression or torsion of a loop. There are only ten cases reported in literature reviewed by O'Donnell *et al*[2], *i.e.,* a loop obstruction caused by the loop of the appendix attached to the mesentery, in the context of acute appendicitis, which is similar to the one in our case.

The paucity of this condition makes it very challenging in making its preoperative diagnosis. In the early inflammation phase, CT (Computerized Tomography) may help to clinch the diagnosis. After resolution of appendicitis, its role is very limited[5-7]. Thorough history and clinical examination, imaging findings and high index of suspicion may help in diagnosis. Diagnostic laparoscopy may be a valuable option.

Treatment is straightforward and depends on intraoperative findings. Appendectomy is sufficient if intervened early, as in our case. It may require small bowel or ileocaecal resection when there is strangulation.

Closed loop and strangulating obstruction of the small bowel are serious lesions that require emergency surgery. An accurate and early diagnosis of intestinal strangulation is essential in patients with small bowel obstruction to minimize the risks of morbidity and mortality. Delayed operation potentially results in high mortality. Preoperative, diagnosis of Appendiceal tie syndrome[7] is always difficult. Early surgical intervention in case of small bowel obstruction can reduce the postoperative risk.

COMMENTS

Case characteristics

A 20 years young male presented with generalized colicky pain abdomen, abdominal distension, bilious vomiting and obstipation.

Clinical diagnosis

Acute abdomen, Mechanical small bowel Intestinal obstruction.

Differential Diagnosis

Congenital anomalous bands, Intestinal malrotation.

Laboratory Diagnosis

Laboratory tests showed a leukocytosis (16800/μL; 4000-11000) rest within normal range (including haemoglobin, haematocrit, creatinine, ABG analysis).

Imaging diagnosis

An abdominal X-ray radiography indicated remarkably multiple air-fluid levels.

Pathological diagnosis

Pathology findings indicated acute appendicitis.

Treatment

Appendectomy.

Related reports

Acute appendicitis, as a cause of mechanical small bowel obstruction is very rare. Long inflamed appendix may lead to this problem. Close loop obstruction by loop of the appendix, in the context of appendicitis, is even rarer. A literature review in 2005 identified only six such cases leading to strangulation.

Term explanation

Appendiceal tie syndrome also called as appendicular band or knot syndrome is an extremely rare surgical entity, in which there is entrapment of bowel loop by the appendix, acting as constricting ring, and may lead to its strangulation.

Experiences and Lessons

Sometimes a very common disease like appendicitis can surprise you with its very rare presentation. But the key thing is the early intervention before it really does the damage, that is, to prevent strangulation.

Peer-review

This manuscript is a well designed with visual materials and will contribute to the literature.

REFERENCES

1 **Harris S**, Rudolf LE. Mechanical small bowel obstruction due

to acute appendicitis: review of 10 cases. *Ann Surg* 1966; **164**: 157-161 [PMID: 5947287]

2 **O'Donnell ME**, Sharif MA, O'Kane A, Spence RA. Small bowel obstruction secondary to an appendiceal tourniquet. *Ir J Med Sci* 2009; **178**: 101-105 [PMID: 18256872 DOI: 10.1007/s11845-008-0125-3]

3 **Hotchkiss LW**. V. Acute Intestinal Obstruction following Appendicitis. A Report of Three Cases Successfully Operated upon. *Ann Surg* 1901; **34**: 660-677 [PMID: 17861052]

4 **Hawkes F**. III. The Prevention of Intestinal Obstruction Following Operation for Appendicitis. *Ann Surg* 1909; **49**: 192-207 [PMID: 17862302]

5 **Assenza M**, Ricci G, Bartolucci P, Modini C. Mechanical small bowel obstruction due to an inflamed appendix wrapping around the last loop of ileum. *G Chir* 2005; **26**: 261-266 [PMID: 16329768]

6 **Bhandari L**, Mohandas P. Appendicitis as a cause of intestinal strangulation: a case report and review. *World J Emerg Surg* 2009; **4**: 34 [PMID: 19818149 DOI: 10.1186/1749-7922-4-34]

7 **Menon T**, Martin RJ, Cameron D, Rao S. Appendiceal tie syndrome. *Australas Radiol* 2007; **51** Spec No.: B133-B136 [PMID: 17875135]

P- Reviewer: Bandyopadhyay SK, Ince V, Petronella P, Steele SR
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