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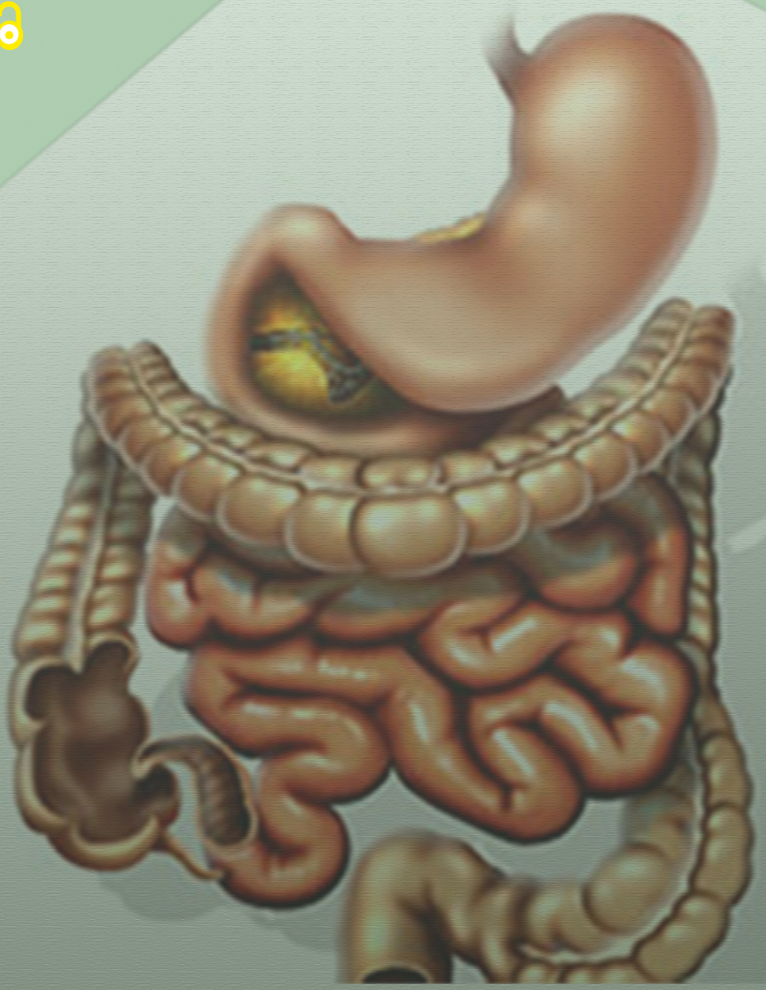

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Mini Review

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Percutaneous Endoscopic Gastrostomy: Use and Abuse in Clinical Practice

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ABSTRACT

Nowadays, Percutaneous Endoscopic Gastrostomy (PEG) is considered the method of choice for long-term enteral feeding, and is spreading all over the world because of its effectiveness and easy carrying out. This review encompasses indications and contraindications of PEG tube placement, and deals with the problem of the growing disconnect between scientific evidence and clinical practice. Despite the evidence shows an advantage in the outcome from PEG placement only in selected subgroups of patients, this technique is also used for questionable indications in clinical practice, such as advanced dementia, permanent vegetative state, and even in end-life patients. Such an overuse is indirectly confirmed by several studies reporting a high 30-day mortality rate after PEG placement in elderly patients. The decision of placing PEG in end-stage patients involves very complex ethical issues, and the authors of this review are not so pretentious as to think themselves capable of dealing with and solving such a dramatic issue. However, patients' interests should be better protected by a case-by-case decision making, based not only on technical competence, but also on sympathetic awareness, avoiding to perform procedures that can be disadvantageous for the patients.

KEYWORDS: Percutaneous Endoscopic Gastrostomy (PEG); Indications; Contraindications; Clinical practice; Ethical Issues.

ABBREVIATIONS: PEG: Percutaneous Endoscopic Gastrostomy; ESPEN: European Society for Clinical Nutrition and Metabolism; LCIG: Levodopa-carbidopa intestinal gel; PEG-J: PEGs with jejunal extension.

INTRODUCTION

Malnutrition is a common problem that affects up to 40% of hospitalized patients, increasing their morbidity and mortality. The problem of malnutrition is often not recognized, and patients can often remain malnourished throughout their hospital stay.[1]

The guidelines of the European Society for Clinical Nutrition and Metabolism (ESPEN) underline that frail and dysphagic patients benefit by adequate nutritional support, that can reduce the complications after bone fractures, prevent and help to heal pressure ulcers, and prolong survival.[2] Nowadays, Percutaneous Endoscopic Gastrostomy (PEG) is considered the method of choice for long term feeding when nutritional intake is likely to be inadequate for more than four to six weeks. Indeed, nutritional support by PEG can stop the decline in quality of life caused by insufficient nutritional intake.[2,3]

The first PEG was performed in a pediatric patient in 1979, and the first paper was published in 1980,[4] generating great interest. Many efforts were done to improve some technical aspects, and the "push" and "introducer" endoscopic techniques were suggested as more effective alternatives to the original "pull" technique.[5,6] Moreover, non-endoscopic, radiologically-controlled techniques were also proposed.[7] In the last two decades, the number of PEG procedures has exponentially increased worldwide.[8]

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INDICATIONS AND CONTRAINDICATIONS

Indications

PEG tube placement has two main classic indications: feeding access and gut decompression.[9] Moreover, an increasing number of PEGs with jejunal extension (PEG-J) have recently been placed in patients with advanced Parkinson's disease, to enable the intra-jejunal infusion of Levodopa-carbidopa intestinal gel (LCIG). The LCIG infusion was developed to overcome the limitations of oral levodopa-carbidopa treatment. The LCIG system (Duodopa®) consists of a suspension of levodopa-carbidopa monohydrate in an aqueous gel that is continuously delivered *via* a portable infusion pump to the proximal small intestine through a PEG-J.[10]

Table 1 reports the main conditions for which adult patients are commonly referred for insertion of a PEG, even if PEG tube placement may also be useful in the setting of severe bowel motility disorders.[11] However, PEG are increasingly requested and inserted for indications with uncertain long term outcomes.

ENTERAL NUTRITION
Neurological dysfunctions
• *Cerebral vascular accident*
• *Motor neurone disease*
• *Multiple sclerosis*
• *Parkinson's disease*
• *Dementia*
Brain trauma
Oncological indications
• *Head and neck cancer*
• *Esophageal tumours*
• *Sequelae after radiation therapy or surgery*
Intensive care patients
Miscellaneous
• *Cachexia*
• *Burns*
• *Fistula*
GUT DECOMPRESSION
Advanced malignancies causing chronic intestinal obstruction/ileus
DRUGS ADMINISTRATION (PEG-J)
Advanced Parkinson's disease

Table 1: Main indications for PEG placement in adult subjects.

Contraindications

Besides the general contraindications to upper gastrointestinal endoscopy, absolute contraindications to PEG tubes placement are few: the most common is the severe coagulophaty, whereas infrequent contraindications are the portal hypertension with gastric varices, peritonitis, sepsis, digestive tract ischemia and gastric cancer. Some authors consider the impossibility of obtaining transillumination an absolute contraindication,[12] but high success rates of PEG placement have been reported even without transillumination.[13]

Relative contraindications include recent gastrointestinal bleeding, severe hepatomegaly or splenomegaly, moderate or severe ascites, presence of prior abdominal surgery (especially procedures involving the stomach) and morbid obesity. Furthermore, recent myocardial infarction, hemodynamic instability and respiratory distress are obvious systemic contraindications to PEG placement.[12,14]

A GROWING DISCONNECT BETWEEN EVIDENCE AND PRACTICE

Since its introduction in the eighteens, PEG tube placement has become the preferred way for those patients requiring long-term nutrition, who have functionally normal gastrointestinal tract but who cannot meet their nutritional needs because of inadequate oral intake. In the last years, the number of patients with PEG is exponentially increasing,[15] but the beneficial effects of PEG feeding on morbidity and mortality have been described only in certain subgroups of patients. For instance, randomized studies in patients after stroke who received PEG feeding have shown improved nutritional outcomes, higher survival, and earlier discharge.[16] However, in 2012 a Cochrane systematic review on the interventions for dysphagia and nutritional support in acute and subacute stroke, did not show any difference between PEG and nasogastric tube feeding for case fatality or composite outcome of death or dependency, even though PEG was associated with fewer treatment failures and gastrointestinal bleeding, and higher feed delivery and albumin concentration.[17]

Several clinical studies have also shown clear benefits of PEG feeding in patients with head and neck cancer, either in terms of improving nutritional status, or less discomfort and lower rates of complications such as bleeding, blockage and dislodgment of the tube.[18,19]

In patients with motor neuron disease, PEG is usually placed to maintain adequate nutrition when the patients have difficult chewing and swallowing. However, a recent Cochrane Database Systematic Review observed that there are no randomized controlled trials to indicate whether enteral tube feeding is beneficial compared to continuation of oral feeding, with regards to survival, maintenance of adequate nutrition, and quality of life, although non-randomized evidence suggested a benefit from enteral feeding.[20]

The most relevant disconnect between literature evidence and clinical practice has been observed in elderly patients with advanced cognitive impairment. Swallowing impairments are known to increase with age. Estimates of the prevalence of dysphagia in older adults range from 15% of those living in the community[21] to 40-60% of those living in a care home.[22] Placement of PEG tube is increasingly being advocated in these patients to provide nutrition, hydration, and to administer medications with the long-term goal of improving quality of life and life span.

In a recent retrospective analysis, Mendiratta, et al. reported that in the USA. PEG tube use in hospitalized elderly patients increased significantly, and PEG placement in patients

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with Alzheimer's dementia doubled (5%-10%) over a 10-year period.[23] These data are in keeping with those reported by other authors, who observed a high prevalence (18%-34%) of PEG tube use among US nursing home residents with advanced cognitive impairment, and about one third of them were patients with dementia.[24-27] Such a habit is not limited to the USA, being observed in many other countries, even though with different percentages.[28]

However, despite the widespread use, benefits associated with PEG placement in patients with dementia or advanced cognitive impairment remain quite questionable. There are few studies examining PEG insertion and outcome, and there is a particular dearth of studies using randomized controlled trials to examine outcomes. PEG placement has been associated with futile procedures and significant mortality and morbidity.[29] Although large prospective studies have examined outcomes of PEG feeding in patients with dementia, a Cochrane review showed no evidence of increased survival, reduced pressure ulcers, or improved quality of life, nutritional status, function, behaviour, or psychiatric symptoms of dementia in patients with advanced dementia who were fed using gastrostomy tubes.[30] Moreover, some studies reported a high 30-day mortality rate (22%) after PEG placement in elderly patients,[31] and a 30% mortality rate during hospital stay in inpatients undergoing PEG.[32] For these reasons, many authors claim that PEG is not indicated in severe dementia,[30] and should be cautiously pondered in patients older than eighty years with moderate dementia.[31]

ETHICAL ISSUES

The use of artificial nutrition and hydration, especially by PEG, resulted in media attention also as a consequence of some emblematic cases that modified the way of thinking of the public opinion.

Nutrition and hydration are intuitively and instinctively linked to the concept of the life itself. It follows that physicians run the risk of deciding to place a PEG on the basis of their own opinion about the significance of the end-life time.[33] Patients are usually considered terminal by many societies of palliative care when their life expectancy is below six months. However, the decision of placing a PEG involves different ethical considerations in presence of different morbidities, such as cancer, amyotrophic lateral sclerosis, dementias, Alzheimer's disease and so on.[34] For instance, artificial nutrition does not prolong survival in patient with advanced cancer. However, PEG is a relatively simple and safe way to administer artificial nutrition at home, and could not be considered as a therapeutic obstinacy but, on the contrary, as an opportunity to offer a more acceptable quality of life to patients with short life expectancy.

The ethical issue of PEG in the last six months of life must be distinguished from the ethical issue of the long-term artificial nutrition in patients with neurological disorders that can strongly compromise either the quality or the dignity of life. However, different cultures may have a different concept of dignity of life, which may also vary from person to person.

Before seventies, most patients with advanced dementia refusing nutrition died of starvation. Afterwards, enteral nutrition became increasingly used, and to date most patients with end-stage dementia undergo artificial nutrition by nasogastric tube or PEG.

Indeed, religious beliefs play a key role in such an increasing request of enteral nutrition,[35] and the presence of a home care nursing can extend the request of PEG placement, to make easier the management of enteral nutrition. When PEG is not placed, patients usually undergo parenteral support or long-term enteral nutrition *via* nasogastric tube. Therefore, both medical and ethical issues are not represented by the dichotomy PEG or no nutrition, but by the comparison between PEG and parenteral nutrition, or between PEG and enteral nutrition *via* nasogastric tube, which is uncomfortable and less safe than PEG, but has some advantage such as the easier and scarcely invasive positioning, usually not requiring a written informed consent.[36,37]

Finally, PEG placement in patients with permanent vegetative state involves ethical issues so complex and deep that overcome the mere technical aspects of PEG placement, and the authors of this paper are not so pretentious as to think themselves capable of dealing with and solving such a dramatic issue.

CONCLUSIONS

Placement of a percutaneous endoscopic gastrostomy feeding tube has become a common medical intervention instituted to maintain or improve a patients' nutritional status. After its introduction in clinical practice in 1980, the use of PEG has exponentially increased because of its easy carrying out, low complication rate, and long-term cheapness. However, these characteristics have also prompted to its overutilization, according to what claimed by Gauderer, one of the two inventors of PEG.[38] In fact, improved nutritional status and survival have been demonstrated only in selected subgroups of patients, whereas the use of PEG tubes in advanced dementia did not show any benefit with regards to outcomes and survival. Since the advanced cognitive impairment and permanent vegetative state are growing indications to PEG placement, this technique is often used inappropriately, because of unrealistic and inaccurate expectations of what it can achieve.

Based on this evidence it could be worthwhile to explain to the family that it is inappropriate and useless place the PEG in terminally ill patients.

We agree with Brody, et al. who stated "we seem to have forgotten the difference between people who die because they stop taking in food and water, and people who stop taking in food and water because of the natural dying process".[39]

In conclusion, we believe that patients' interests should be better protected by a case-by-case decision making, based not only on technical competence, but also on sympathetic awareness.

CONFLICTS OF INTEREST

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Case Report

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Massive Gastric Variceal Bleeding in a Patient with Chronic Pancreatitis

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SUMMARY

Left-sided portal hypertension (LSPH) is a rare form of portal hypertension that usually occurs as a result of isolated obstruction and thrombosis of the splenic vein.

We present a case of a 45-year-old male patient with history of repetitive chronic pancreatitis exacerbations, who was hospitalized because of the severe gastric variceal bleeding and hemorrhagic shock, developed as a complication of the splenic vein thrombosis and LSPH.

Splenectomy is the treatment of choice, but in this case, because of the patient`s severe general condition and significant risk of new surgical procedure (extensive fibrous adhesions in abdomen), transcatheter embolization of the splenic artery was undertaken as the method of "nonsurgical splenectomy".

Upper endoscopy was performed two weeks after embolization and it showed significantly reduced gastric varices, with no signs of bleeding, what brings us indirectly to a conclusion that the embolization significantly decreased pressure in the portal circulation.

KEYWORDS: Left-sided portal hypertension (LSPH); Pancreatitis; Gastric varices; Bleeding; Embolization.

ABBREVIATIONS: LSPH: Left-sided portal hypertension; MSCT: Multi Slice Computer Tomography; SVT: Splenic Vein Thrombosis.

INTRODUCTION

Variceal bleeding is the final outcome of many events that start with increase of portal blood pressure, development of varices and their progressive dilatation up to their rupture and bleeding.

Variceal bleeding mostly occurs as a result of portal hypertension within liver cirrhosis, and it can occur from oesophageal varices, gastric varices or ectopic varices along digestive tract.

Left-sided portal hypertension (LSPH) is a rare form of portal hypertension that usually occurs as a result of isolated obstruction and thrombosis of the splenic vein, and it can cause bleeding from isolated gastric varices.

LSPH should be considered in all cases of gastrointestinal bleeding accompanied by

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normal liver function, splenomegaly and passable portal vein.

In this paper we present a case of a male patient with history of chronic pancreatitis that was presented by abundant bleeding from upper gastrointestinal tract caused by LSPH due to splenic vein thrombosis.

CASE REPORT

In October 2007, a 45-years-old male patient was admitted to gastrointestinal department because of the bleeding from upper GI tract which presented with abundant hematemesis and clinical signs of hemorrhagic shock.

In patient's history it is important to highlight alcohol consumption for many years. First time he was hospitalized in 1993 because of acute pancreatitis caused by alcohol, and several next year's he was hospitalized because of repetitive chronic pancreatitis exacerbations.

Six years after the first hospitalization complete diagnostic work up was done and abdominal ultrasound showed enlarged spleen with many perisplenic collaterals and suspicion on splenic vein thrombosis was set. During the same hospitalization patient develops clinical signs of acute abdomen and small intestinal obstruction and was transferred to surgical department.

Explorative laparotomy revealed numerous, tight small bowel adhesions so adhesiolysis was performed. During repeated hospitalization in March 2003 upper endoscopy showed emphasised and voluminous folds of gastric large curve that were suspected to be isolated varices.

Transabdominal ultrasound with Doppler could not show vascular structures due to meteorism. Endoscopic ultrasound with Doppler of gastric fundus confirmed existence of isolated gastric varices. Multi Slice Computer Tomography (MSCT) with contrast visualized extremly enlarged spleen (8 cm x 15 cm x 19 cm) and numerous convolution of wide veins with diameter up to 2 cm which led from spleen to stomach, up to oesophagus and peripancreatically. Splenic vein did not fill in with contrast so it was concluded that splenic vein thrombosis occurred. Patient missed his control follow up for few years, and then in April 2007 he was again admitted in emergency room because of obstructive symptoms- recurrent vomiting and inability to eat. Endoscopically and radiologically fixed bulbostenosis was confirmed, next to early known gastric varices. Emergency surgical treatment was indicated so antecolic gastroenetroanastomosis with entero-enteroanastomosis section Braun was done. Surgical procedure was complicated due to numerous fibrous adhesions and that is the reason why splenectomy did not take place. (Figure 1)

At admission in October 2007 the patient was extremly pallor, with cold periphery, tachycardia and very low blood pressure. Laboratory findings showed significant posthemorrhagic anaemia: erythrocytes 2.19x10^{12}/L (4,34-5,72x10^{12}/L), haemoglobin 55 g/L (138-175 g/L), haematocrit 0.173 L/L (0,415-0.530 L/L), MCV 89.2 fL (83.0-97.2 fL), Prothrombic time (PV) 0.15 (0.70-1.30), thrombocytes 205x109/L (158-424x109/L). After patient was stabilized receiving crystalloid fluids, transfusion of concentrated erythrocytes and fresh frozed plasma emergency oesophagogastroduodenoscopy was performed. In oesophagus and stomach there was fresh blood and in stomach big coagulum that was not able to be removed completely but huge gastric varices were seen beneath. Due to significant varices and acute bleeding endoscopic haemostasis could not be performed. Control gastroscopy after emptying blood from stomach showed enlarged varices on major gastric curve.

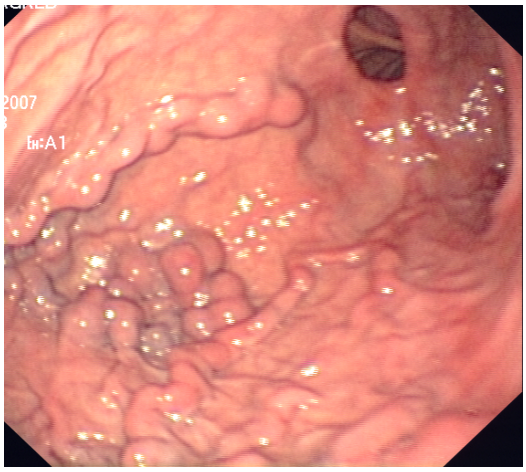

Figure 1: Upper gastrointestinal endoscopy. Isolated varices on the greater curvature of the stomach.

As endoscopic haemostasis was not able and risk of repetitive bleeding very high, consultation between gastroenterologist, surgeon, and intervent radiologist concluded that therapeutic method of choice was transcatheter embolization of splenic artery due to high risk of operation.

Three days after admission to hospital digital subtraction angiography of abdominal aorta was performed to demonstrate beginning of coeliac truncus and splenic artery. During procedure coeliac truncus was selectively catheterized and catheter was placed in main tree of splenic artery all the way to its distal segment. Within splenic hilus significant curve of splenic artery was visualized. (Figure 2). The main tree of splenic artery was embolized by serial of spirals whose diameter was from 5 to 8 mm, and the goal was devascularization and development of collateral blood path to stop necrosis upgrowth. Control angiography did not show flow within main tree of splenic artery, so the goal of intervention was achieved. (Figure 3)

Procedure finished without complications, and during follow up there were no signs of sepsis or splenic abscess. Few days after procedure patient's condition improved. Fifteen days after transcatheter embolization control oesophagogastroduo-

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denoscopy showed significant reduction of varices on major gastric curve (Figure 4). Control MSCT showed ischaemic splenic upper half and reduced gastric varices. During 3 years of follow up there was not recurrent bleeding from gastric varices.

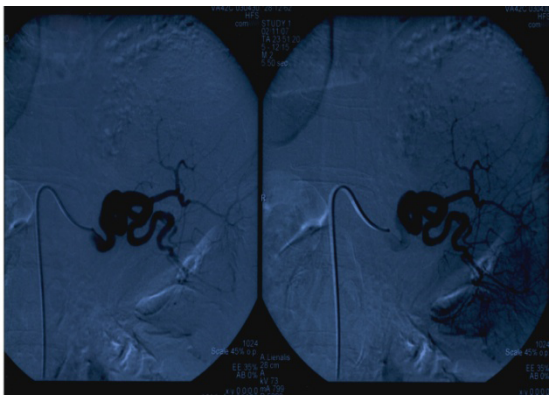

Figure 2: DSA of abdominal aorta. Tortuotic lienal artery filled with contrast before embolization procedure.

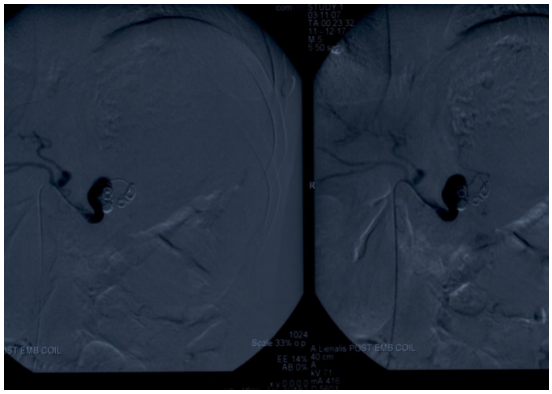

Figure 3: DSA of abdominal aorta. Lienal artery after DSA embolization with a series of coils 5-8 mm. There is no sign of flow inside lienal artery.

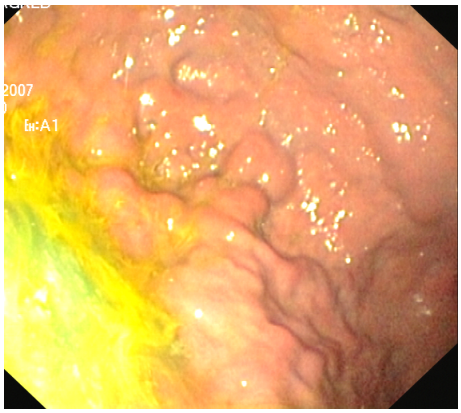

Figure 4: Upper gastrointestinal endoscopy. Significantly reduced gastric varices after embolization of the lienal artery.

DISCUSSION

We presented a case of a 45-years-old male patient with recurrent bleeding from gastric varices caused by a rare form of portal hypertension.

Left-sided portal hypertension (LSPH) represents localized form of portal hypertension which originates as a result of isolated splenic vein obstruction, mostly connected with thrombosis (Splenic Vein Thrombosis-SVT).[1] Because of their anatomical relation every pancreatic condition can effect splenic vein.[2,3] Acute and chronic pancreatitis and pancreatic tumors are main causes of SVT.[2,4] Exact mechanism of thrombosis development is obviously multifactorial and includes intrinsic injury of endotel with inflammatory and neoplastic process and extrinsic damage connected with venous compression from outside due to fibrosis, pseudocyst that lies next to vein or adjacent oedema.

LSPH is a rare cause of upper GI tract bleeding. Obstruction of splenic vein results with vein hypertension in collateral flows of *vv.gastricas breves*, *vv.coronariae ventriculi*, *vv.gastroepiploice* and veins of upper part of stomach. Usually after obstruction splenic blood goes through *vv.gastricae breves* to gastric vein plexus. As pressure and flow in those structures grow, dilatation of submucous veins occurs and gastric varices develop. As most of the patients have no symptoms it is very hard to predict accurate incidence of this disorder. But percentage of LSPH in all patients with portal hypertension is less than 5.[3] First clinical manifestation of splenic vein thrombosis and left-sided portal hypertension is mostly acute or chronic bleeding from oesophageal or gastric varices.[5] Splenomegaly is one of the major characteristics of long lasting portal hypertension, and in LSPH splenomegaly is more expressed than in liver cirrhosis.[6]

In our paper we presented a case of a male patient with history of repetitive chronic pancreatitis exacerbations due to aethylic etiology. With continuous, long lasting alcohol consumption he developed chronic pancreatitis and consequently by repetitive exacerbations he developed fixed bulbostenosis and gastric varices due to splenic vein thrombosis. The diagnosis of gastric varices was established endoscopically four years after first, clinically proven bleeding. At that time he presented with abundant bleeding from big, isolated gastric varices and clinical signs of hemorrhagic shock.

The diagnosis of gastric varices is made from clinical status, endocopically and radiologically. Upper endoscopy is a procedure of choice for visualization of gastric varices, especially when performed by experienced doctor. New studies that compare splenoportography and endoscopy reveal that even 90% of gastric varices are able to be diagnosed endoscopically.[7]

Finding of isolated gastric varices during upper endoscopy always require work-up to find if possibly splenic vein thrombosis exists. Generally ultrasound with Doppler is the first method to be done in patients with portal hypertension.[8] According to limitations of this method (interpretation due to operator, bad acustic window), when SVT is suspected it is often necessary to do an extra view angiographically. As splenoportography and digital subtractial angiography are invasive methods, to show porto-lienal vascular system today we use non invasive methods as MSCT and MR angiography. Between MSCT and MR angiography, the last one is more reliable for visualization

of porto-lienal vascular system in relation to Doppler ultrasound and MSCT.[9] Endoscopic ultrasound is sensitive method to show small pancreatic lesions, chronic pancreatitis, changes in vascular structure and oesophagus and gastric varices.[10]

LSPH is one of the therapy accessible disorders caused by portal hypertension.[11] As it is localised form of portal hypertension, therapy needs to be directed to splenic side of portal circulation as hypertension is expressed in that pool. The method of choice in treating patients with LSPH and bleeding is splenectomy due to high mortality and risk of re-bleeding.[12] Removal of spleen decreases vein flow through collateral circulation, shrinks varices and prevents re-bleeding. In patients who did not manifest with bleeding, routine splenectomy is not method of choice because of low risk of bleeding. In patients who are not candidates for surgery, transcatheter embolization of splenic vein can be performed.[13]

During this procedure spirals or other agents (antilogous thromb or gelatinous mass) are transcatheterically put into splenic artery which will lead to artery embolization. In 25% of patients who underwent embolization of splenic artery development of septic complications around spleen is possible, especially development of splenic abscess. In those cases splenectomy needs to be performed.[14] Recurrent bleeding after transarterial catheterization is not common, and prognosis of patients mostly depends on aethyiology of disorder.

Embolization of the splenic artery has been shown as an alternative method which has the same effects as a splenectomy, moreover, it can avoid some severe complications such as post-splenectomy sepsis, or uncontrolled increase of platelet count because of the residual noninfarcted spleen. Koconis, et al.[15] reported that partial splenic embolization made efficacious improvements on the bleeding and hematologic parameters for partial hypertension patients, and the associated morbidity and mortality were acceptable.[16]

Because of extensive fibrous adhesions in abdomen, especially around spleen and pancreas, gastroenterologist, surgeon and radiologist concluded that in case of our patient there is significant risk of surgical procedure. So transcatheter embolization of the splenic artery was undertaken as the method of "non-surgical splenectomy". The procedure underwent without complications, and during follow up there was no recurrent bleeding from gastric varices.

The case we presented indicates a rare cause of segmental portal hypertension. Splenic vein thrombosis, which is usually connected with pancreatic disorders (tumor, inflammation) is cause of Left-sided portal hypertension (LSPH). It should always be considered in patients with normal liver function and obscure splenomegaly, and in patients with isolated gastric varices.[17] Therapy of choice in patients with bleeding is splenectomy, and in patients with high surgical risk transcatheter embolization of the splenic vein. In asymptomatic patients there is no consensus about therapeutic approach.

DISCLOSURES

All authors listed have contributed sufficiently to the project to be included as authors, and all those who are qualified to be authors are listed in the author byline. To the best of our knowledge, no conflict of interest, financial or other, exists.

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Case Report

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Endoscopic Ultrasound Diagnosis of Perianal Rhabdomyosarcoma

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BACKGROUND

Adult primary perianal rhabdomyosarcomas are extremely rare. Therefore, diagnosis can become a challenge. Here we report our experience in diagnosing primary perianal rhabdomyosarcoma by Endoscopic Ultrasound Scan (EUS) and Fine Needle Aspiration (FNA).

KEYWORDS: Endoscopic ultrasound; Rhabdomyosarcomas; Perianal; Diagnosis.

ABBREVIATIONS: EUS: Endoscopic Ultrasound Scan; FNA: Fine Needle Aspiration; CT: Computed Tomography; FISH: Fluorescent *in situ* hybridization; CEA: Carcinoembryonic antigen; HIV: Human Immunodeficiency Virus; HPV: Human papillomavirus.

CASE REPORT

A 27-year-old male with no significant past medical history, presented to the emergency room with symptoms of perianal pain. Additionally, the patient complained of cramps in his legs and buttocks for approximately one month. The cramps were initially minor but progressively worsened and then became constant. His vitals and physical examination were normal except for his digital rectal examination, which was painful and tender. However, no obvious mass was felt in the rectum. His initial blood tests were unremarkable which included hemoglobin-14 g/dl (Nv-13.5-16.5), white cell count-8.9 (Nv-4.5-11), platelets-324 (Nv-150-400), blood urea nitrogen-12 (Nv-7-25), creatinine 1.2 (Nv-0.4-1.24), sodium-140 (Nv-137-145) and potassium of 3.7 (Nv-3.5-5.1). Pelvic Computed Tomography (CT) (Figure 1) showed a mass in the perianal region measuring 12 cm with metastasis to the left iliac and obturator lymph nodes. In view of the CT findings, other lab tests like Carcinoembryonic antigen (CEA)-0.5 (Nv-<3 ng/ml), CA 19-9-10 (Nv-<35 u/ml), Human Immunodeficiency Virus (HIV), Human papillomavirus (HPV) and Chlamydia were checked and were all within normal range. The patient then underwent lower EUS, which showed a 5 cm hypoechoic mass (Figure 2) just outside the anal canal. An FNA was obtained which showed neoplastic cells (Figure 3A). The cells were largely mononuclear, with a few binucleate and multinucleate cells. These cells had a high nuclear cytoplasmic ratio with a relatively round nucleus. Immunohistochemical stain was positive for muscle (desmin) and more precisely for striated muscle (myogenin, Figure 3B), supporting the diagnosis of Rhabdomyosarcoma. Subsequent Fluorescent *in situ* hybridization (FISH) performed using DNA probe specific for the FOXO1 (FKHR) gene on 13q14 showed a signal pattern suggestive of rearrangement of FOXO1 gene. Rearrangement of FOXO1 is associated with a diagnosis of Alveolar Rhabdomyosarcoma. Unfortunately, the patient was a poor candidate for curative treatment due to the presence of distal metastasis. However, he underwent adjuvant chemotherapy and radiation therapy for treatment of his cancer.

DISCUSSION

Adult perianal Rhabdomyosarcomas are extremely rare and the true incidence is unknown.[1] It is estimated to account for less than 1% of all adult solid tumor malignancies.[2]

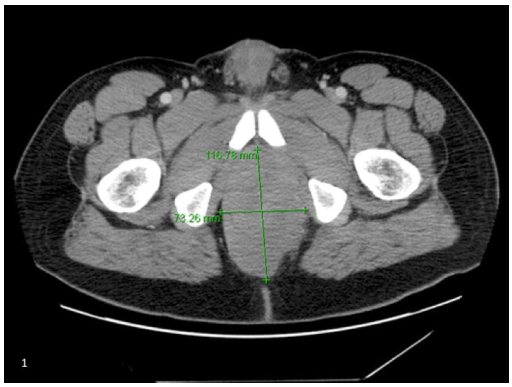


Figure 1: Mass in the perianal region.

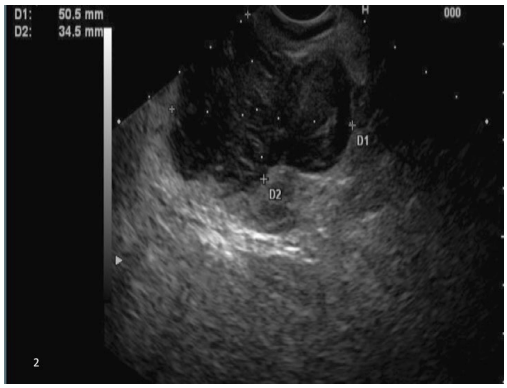
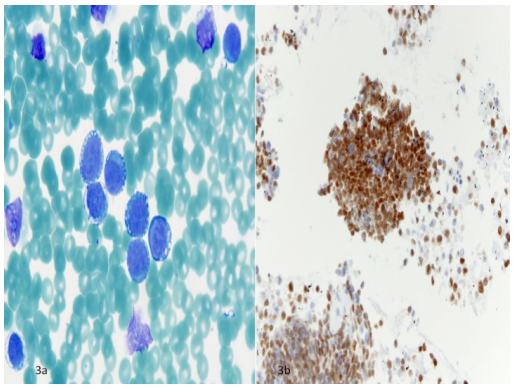


Figure 2: Hypoechoic perianal mass as seen in lower EUS.



Figures 3: Malignant mononuclear cells with high nuclear cytoplasmic ratios and fairly round nuclei (100X; modified Romanowsky stain) and the tumor nuclei stain with myogenin (20X; myogenin immunohistochemical stain).

The prognosis is poor with a 5-year overall survival rate of only 27% in adults without metastasis after curative treatment. The prognosis can be even more dismal with the presence of distal metastasis.[3,4] The most common origin of primary tumors is the head and neck region (35%), followed by the genitourinary and extremities.[5] However, primary tumors can also rarely occur at other anatomic sites like the rectum. Differential diagnoses of perianal tumors are neuroendocrine tumors, hematopoietic/lymphoid malignancies, rectal carcinoma, melanoma, Ewing's sarcoma and desmoplastic small round cell tumor.[6] Tissue diagnosis with immunohistochemical stains is required for a definitive diagnosis. Due to the rarity of the disease, there is no standard way of obtaining the tissue diagnosis. To date, there is only one other case report mentioning EUS as a tool for diagnosing perianal Rhabdomyosarcoma.[7] Open biopsy has been done in the past for diagnosis of Rhabdomyosarcoma. However, EUS is minimally invasive, more accurate, less expensive and safe compared to open biopsy.[8]

CONFLICTS OF INTEREST

None of the authors have conflicts of interest.

CONSENT

No recognizable patient material is used and therefore no patient consent was obtained.

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Case Report

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Brunner Gland Cyst: Two Cases of a Rare Entity and Review of the Literature

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ABSTRACT

Objective: Brunner gland cysts are rare with only 14 cases reported in the literature. The term has been used to consolidate the following entities: Brunner's gland cyst, cystic Brunner's gland hamartoma, Brunner's gland cystadenoma and mucocele of the Brunner gland.
Methods: We present the clinico-pathological features of two cases of Brunner gland cyst along with a review of the literature.
Results: Brunner gland cyst affects slightly more men than women. The age range is 30 to 72 years with an average of 55 years. The lesions range from 1 to 5 cm in size, the average being 2.3 cm in greatest dimension. The majority of lesions are found incidentally in patients with symptoms not specifically related to the lesion. They affect predominantly the first and second portion of duodenum. The majority of the lesions are single and pedunculated filling the submucosa. They can be unilocular or contain multiple cysts divided by fine septae. The epithelial lining is of columnar and clear cells with basally located nuclei.
Conclusion: Brunner gland cysts are benign lesions that are usually not diagnosed pre-operatively. The cases described here add awareness of this entity among pathologists and gastroenterologists allowing for a better recognition of this rare entity.

KEY WORDS: Duodenum; Brunner gland; Cystadenoma; Cyst; Hamartoma.

INTRODUCTION

Cystic lesions of the Brunner gland are rare. They have been referred to as Brunner's gland cyst, cystic Brunner's gland hamartoma, Brunner's gland cystadenoma and mucocele of the Brunner gland. Recently these entities have been grouped under the term Brunner gland cyst. Even with these combined entities, there are only fourteen Brunner gland cysts reported in the English literature. We present two new cases of Brunner gland cyst and compared them to those previously reported. Their clinical presentation and similarities are summarized.

CASE PRESENTATIONS

A 69-year-old Hispanic male was referred by his primary care physician to our institution for further studies of a duodenal lesion that was discovered after a workup for acid reflux. Endoscopic ultrasound showed a 17x6 mm septated cyst that appeared to arise from the submucosa of the second portion of the duodenum (Figures 1 and 2). No other abnormalities were noted. The cyst was resected after lifting the lesion with saline and methylene blue.

The specimen consisted of an ovoid lesion with a 0.5 cm cystic cavity. Microscopic examination revealed a submucosal multicystic lesion lined by tall, columnar cells with round basally located nuclei (Figures 3 and 4). Most of the cells had abundant clear cytoplasm similar

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to those seen in the adjacent normal Brunner glands. In other areas, the lining cells were smaller with pink cytoplasm. The cysts were filled with a serous-like fluid. Necrosis, mitotic activity, and nuclear atypia were absent. The cells contained neutral, Periodic Acid Schiff (PAS) positive mucin, similar to the adjacent Brunner's glands

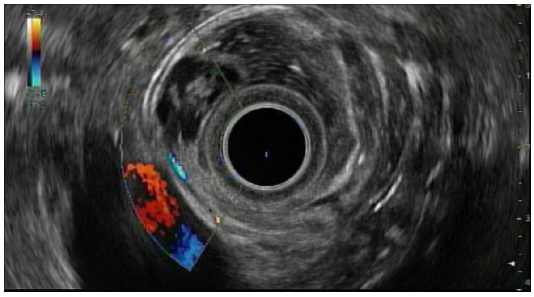

Figure 1: Radial EUS (7.5 MHz) of cystic duodenal Brunner gland cyst. Anechoic lesion in the deep mucosa/submucosa.

Figure 2: Endoscopic image of lobulated lesion in the second portion of the duodenum.

The second patient is a 52-year-old Caucasian man referred to our institution because of epigastric pain and a duodenal mass found at endoscopy. Another endoscopy was performed along with endosonography. An intramural (subepithelial) lesion was found in the second portion of the duodenum. The lesion appeared to originate from within the submucosa (layer 3). The possibilities of pancreatic rest or Brunner's gland hyperplasia were considered. The lesion was hypoechoic, heterogeneous and multicystic that measured 14.5 mm x 10.9 mm. The outer margins were well defined. An intact interface was seen between the mass and the adjacent structures suggesting a lack of invasion. An endomucosal resection of the mass was performed.

Histologically, the multicystic lesion was located in the submucosa of the duodenum and was composed of tall cells with basally located round nuclei. The cytoplasm was clear and abundant. In areas, there were aggregates of glands by the single row of epithelial cells lining the cysts creating a nodular configuration. No mitosis, necrosis or atypia was observed.

DISCUSSION

Rankin, et al. reported the first cystadenoma of the duodenum in 1933.[1] Varnholt, et al. were the first to group Brunner gland cyst, Brunner cyst, mucocele of Brunner gland, and cystic Brunner's gland hamartoma as one entity.[2] Later, Powers, et al. added Brunner gland cystadenoma to this group.[3] There are fourteen cases reported in the English literature.[1-12] Information from these cases and the current two is summarized in Table 1.

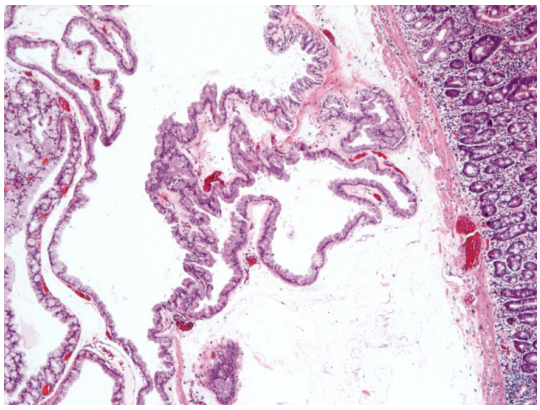

Figure 3: Brunner gland cyst of the duodenum demonstrating multiple small cysts lined by fibrous septa (H&E, 100x).

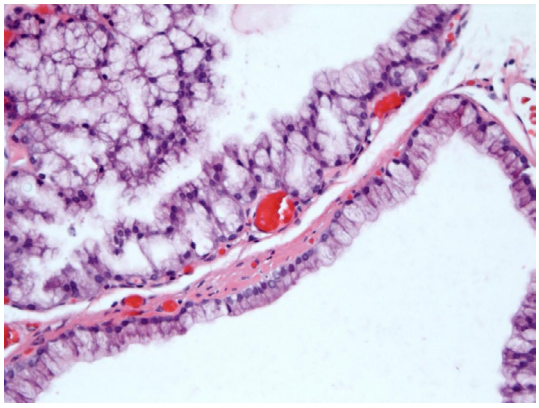

Figure 4: Brunner gland cyst of the duodenum lined by tall, columnar cells with round basally located nuclei (H&E, 400x).

Brunner gland cysts are benign, as evidenced by the fact that the lesion described by Golan, et al. was present for 15 years without any long-term consequences.[9] They are widely believed to be retention cysts that develop after obstruction of a larger duct of the Brunner gland outflow tract.[2]

Based on this case and the well-described fourteen previously reported cases, the following conclusions can be made. The lesion can occur throughout the duodenum, specifically any place that contains Brunner glands. The large majority are single lesions with one patient reported to have two.[3] Most of the lesions are pedunculated and located in the submucosa, but sessile lesions have been reported and one was transmural.[8] While some are unilocular, others contain multiple cysts divided by fine septae. The cysts are lined by mucinous, columnar cells with basally located nuclei, resembling normal Brunner gland cells. Eosinophilic cells lining the cyst wall are also common. Only the case described by Chatelain, et al. showed ciliated columnar cells.[7] Atypia is rare, and mitosis is only reported in one case.[10]

Year	Authors	Diagnosis	Age, Sex	Clinical presentation	Location	Size (cm)	Architecture	Depth	# of cysts	Histology characteristics
2015	Moul, et al. (current case)	Brunner gland cyst	69, M	Incidental finding in a patient with acid reflux	Second portion of duodenum	1.7 x 0.7 x 0.3	Sessile	Sub-mucosa	Single	Focal eosinophilic cells
2015	Moul, et al. (current case)	Brunner gland cyst	52, M	Epigastric pain	Second portion of duodenum	1.4 X 1.0	Polypoid	Sub-mucosa	Single	Focal eosinophilic cells
2011	Galiatsatos[4]	Brunner gland cyst	72, M	Incidental	Distal end of the second part of the duodenum	2	Polypoid	Not specified	Not specified	Normal appearing duodenal mucosa and dilated lymphatic channels and capillaries (pictures not available for review)
2009	Park, et al.[5]	Cystic Brunner's gland hamartoma	30, M	Three-day history of nausea, vomiting, and epigastric pain.	Third portion of the duodenum	4 x 3	Pedunculated	Sub-mucosa	Multiple	Lobular collection of mature Brunner's glands, multifocal cystic dilation.
2008	Powers, et al.[3]	Brunner gland cyst	46, F	Dyspepsia and odynophagia	Two lesions located in the second part of the duodenum	1.8 and 2.2	Sessile	Sub-mucosa	Single	Cystic spaces lined by hyperplastic cells similar to Brunner glands cells.
2008	Powers, et al.[3]	Brunner gland cyst	67, F	Incidental in a patient with unexplained iron-deficiency anemia	Second part of the duodenum	1.5	Sessile	Sub-mucosa	Single	Cystic spaces lined by hyperplastic cells similar to Brunner glands cells.
2008	Powers, et al.[3]	Brunner gland cyst	59, F	Incidental in a patient with abdominal pain, heartburn, steatorrhea	Duodenal bulb (first portion)	1.0	Nodule	Sub-mucosa	Single	Cystic spaces lined by hyperplastic cells similar to Brunner glands cells.
2007	Varnholt, et al.[2]	Brunner gland cyst	41, F	Incidental finding in a women being treated for H. pylori-associated gastritis.	Not specified	1.1 x 0.9 x 0.6	Sessile	Sub-mucosa	Single	Cyst lined by a simple cuboidal-to-columnar epithelium.
2003	Yamakawa, et al.[6]	Cystic Brunner's gland hamartoma	64, F	Epigastric pain	Descending duodenum (second portion)	2.4 x 1.1 x .5	Pedunculated	Sub-mucosa	Multiple	Multilocular cysts lined by columnar epithelium ; dilated ductal structures.
2002	Chatelain, et al.[7]	Brunner gland hamartoma with predominant adipose tissue and ciliated cysts	43, M	Two-day history of regurgitation.	Duodenal bulb (first portion)	3.5	Pedunculated	Sub-mucosa	Multiple, ciliated	Prominent mature adipose tissue, hyperplastic lobules of Brunner glands
1980	Fisher[8]	Mucocele of Brunner gland	45, F	Right upper quadrant pain intermittently for two months	Not specified	Not specified	Not specified	Sub-mucosa	Single	Intact duodenal mucosa with prominently dilated glandular spaces, one being large and cystic.
1978	Golan J, et al.[9]	Cystic Brunner's gland hamartoma	64, M	Acute gastrointestinal bleeding; had a known duodenal polyp for 15 years	First part of the duodenum.	5 x 4 x 2	Pedunculated	Sub-mucosa	Multiple	Cysts were lined by columnar and cuboidal epithelium. The surface epithelium was partly pyloric and partly duodenal.

	Taura M, et al.[10]	Brunner's cyst	54, F	Nausea, vomiting, and epigastric pain for several days	Duodenal bulb (first portion)	1.5	Not specified	Sub-mucosa	Single	Lined by tall columnar cells with basal nuclei. Multinucleated cells were intermingled with the epithelial lining cells.
	Wolk DP, et al.[11]	Brunner's gland cystadenoma	68, M	Presented with 20 lb weight loss and eructation.	Third portion of the duodenum, located on the posterior wall	3 x 3	Not specified	Sub-mucosa	Multiple	Multiple fluid-filled cystic spaces confined to the submucosa, lined by Brunner glands
	Hately[12]	Brunner's gland cyst	54, M	Two year history of intermittent vomiting, now after every meal.	First part of the duodenum.	1.5	Sessile	Sub-mucosa	Single	Cyst lined by columnar epithelium and had a direct origin from one of the Brunner glands.
	Rankin and Newell[1]	Simple, multilocular cystadenoma	54, M	18 m history of pernicious anemia and ulcer-like dyspepsia.	-	2	Not specified	Sub-mucosa	Multiple	Cyst lined by cuboidal epithelial cells.

Table 1: Brunner gland cysts reported in the English literature: Patient characteristics, clinical presentation, diagnostic modality and lesion location, and histological characteristics.

Ultrastructural studies performed by Taura, et al. in 1977 demonstrated epithelial cells containing membrane-bound secretory granules in the cytoplasm, mainly in the apical region, and a well-developed Golgi apparatus. The luminal surfaces contained microvilli. These features suggested that the cells of Brunner gland cysts were functionally more active than normal Brunner gland cells. Another distinct histological feature was the lack of neuroendocrine cells within the Brunner gland cysts, which is not true of normal Brunner's glands.[10]

There is no sex predilection; fifty-three percent were male. The age range was 30 to 72 years of age, with the average being 55 years. The lesions ranged from 1 to 5 cm in size, the average being 2.3 cm in greatest dimension. While increased size correlated with more clinical symptoms, the patient with the largest reported lesion was not symptomatic from the lesion but from an adjacent ulcer.[9] Nonetheless, most clinical symptoms are regurgitation, vomiting, and epigastric pain. However, it may be discovered incidentally for unrelated symptoms. Most often, the lesions are located in either the first or second portion of the duodenum. This corresponds to the most common locations of the Brunner's gland, which are mostly concentrated in the first portion and gradually decrease in number throughout the length of the duodenum.[13]

The differential diagnosis on endoscopic imaging includes duplication cysts, lipomas, neuroendocrine tumors and Brunner gland hamartomas. Using endoscopic ultrasound it is possible to differentiate the echostructure (cystic, solid, hypo- or hyperechoic) and wall layer of involvement. The differential diagnosis of hypoechoic/anechoic duodenal lesions would be mainly duplication cysts, stromal cell tumors and neuroendocrine tumors. Our first patient underwent resection to ensure his was not a cystic neuroendocrine lesion. Histologically the main differential diagnosis is Brunner's gland hamartoma, formerly known as Brunner's gland adenoma. These lesions are admixtures of Brunner's glands, ducts, adipose tissue and lymphoid tissue. While their ducts may be dilated, cystic lesions are not characteristic of this entity.[14]

Brunner gland cysts are rare benign lesions of the duodenum. The treatment is surgical excision or polypectomy. Increased awareness of these lesions helps for a better recognition of this entity.

CONFLICTS OF INTEREST: None.

DISCLOSURES

No consent is required to our article publication referenced above.

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Review

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Biphasic Roles of a Small G-Protein, RAC1 in Pancreatic B-Cell

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ABSTRACT

Glucose-stimulated insulin secretion (GSIS) involves cross talk between small G-proteins and their regulating factors. These interactions results in translocation of insulin-laden granules to the plasma membrane for fusion and insulin release. Vesicular transport and fusion events are tightly regulated by signals which coordinate between vesicle- and membrane-associated docking proteins. It is now being accepted that small G-protein, Rac1-mediated Reactive Oxygen Species (ROS) functions as a second messenger in islet β-cell function. Further, evidence from multiple laboratories suggests a tonic increase in ROS generation is necessary for GSIS and fatty acid-induced insulin secretion. On the other hand, Rac1-mediated NADPH oxidase-activation and subsequent generation of excessive ROS under glucolipotoxic conditions and cytokines exposure has proven to be detrimental for islet β-cell function. In this review we overview the normal physiological effects (positive role) and adverse effects (negative role) of activated small G-protein, Rac1 in pancreatic β-cells.

KEYWORDS: Small G-protein; Rac; Insulin secretion; NADPH oxidase; Oxidative stress; Islets.

ABBREVIATIONS: GSIS: Glucose-stimulated insulin secretion; ROS: Reactive Oxygen Species; GEFs: Guanine exchange nucleotide factors; FPR: N-formyl peptide receptor; GDIs: GDP-dissociation inhibitors; GAPs: GTPase-activating proteins; DPI: Diphenyleneiodonium; ZDF: Zucker Diabetic Fatty.

INTRODUCTION

Diabetes is a metabolic disorder with multiple etiologies characterized by chronic hyperglycemia. This results from dysregulated insulin secretion and/or from the resistance to insulin action in peripheral tissues. In the settings of the metabolic disorder, disturbances in carbohydrate, fat and protein metabolism results in a diverse set of complications associated with pancreas, liver, kidney, heart and other vital organs. As per the National Diabetes Statistics Report, 2014, in 2012, 29.1 million Americans (2.9% of the population) have diabetes, which include 1.25 million type 1 diabetic children and adults. Further, 86 million people of age 20 and above are pre-diabetic, and are at increased risk for developing type 2 diabetes. Over decades of research, a greater understanding of the pancreatic β-cells in physiological insulin release has been made to therapeutically target and treat the metabolic disorder. Insulin secretion from islet β-cells is majorly regulated by glucose and other insulin secretagogues. This is mediated through fluctuations in the intracellular calcium, and interplay of soluble secondary messengers like reactive oxygen species (ROS), cyclic nucleotides and hydrolytic products generated from the phospholipases A2, C and D.[1-13] In addition, adenine nucleotides [e.g., ATP]

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and guanine nucleotides [e.g., GTP;[14-17] regulate physiological insulin secretion. Even though many studies have shown the underlying mechanism[s] involved in stimulus-secretion coupling of glucose stimulated insulin secretion (GSIS), the precise molecular and cellular mechanism still remains unknown. However, role of guanine nucleotide-binding protein (G-protein) has been highly researched for their role in insulin release. The signal-transduction system (Adenylylcyclases, ion channels, and phospholipases) involved in insulin release are linked to the receptors for hormones or stimulatory agents *via* G-proteins.

CLASSIFICATION OF G-PROTEINS IN β-CELL

Till date three major classes of G-proteins have been identified in pancreatic β-cells.[18-22] The first class of G-proteins, heterotrimeric G-proteins assists in coupling membrane-associated receptors to their intracellular effectors adenylyl cyclases, ion channels, and phosphodiesterases.[23-25] The second class of G-proteins, small monomeric G-proteins [17-30 kDa] play a vital role in protein organization and trafficking of secretory vesicle.[26] These small G-proteins undergo posttranslational modifications [isoprenylation and methylation] at their C-terminal residues (CAAX motif)[26-30] for their active confirmation. The third class of G-proteins, the elongation factors and Tau proteins are implicated in protein synthesis.

SMALL G-PROTEINS

Based on the substantial evidences on the regulation of pancreatic islet β-cell function, small G-proteins are categorized into three major groups. Rho, Rac1, Cdc42 and ADP-ribosylation factor-6 [Arf6] fall under the first category of small G-proteins and these play an important role in cytoskeletal remodeling and vesicular fusion.[31-48] The second category of small G-proteins comprises of Rap1 and RabGTPases (Rab3A and Rab27).[49] These Rab GTPases assists in priming and docking of insulin-laden secretory granules on the plasma membrane. Unlike first category of small G-proteins, requisite for posttranslational modifications and mechanism[s] involved in the activation of Rab GTPases under the physiological insulin secretagogues remains elusive. However, Rap1 is activated transiently by glucose[50] and undergoes carboxymethylation.[18,51] The third group of small G-proteins consists of Rab2, Rhes and Rem2 which are under-studied,[52-55] whereas, RalA appears to draw direct regulatory effects in exocytosis.[56] Do you have data about small G-proteins expression in pancreas?

ACTIVATION AND DEACTIVATION CYCLE OF SMALL G-PROTEINS

Like heterotrimeric G-proteins, small G-proteins also shuttle between their inactive (GDP-bound) and active (GTP-bound) conformations, and are tightly regulated by various G-protein regulatory factors/proteins. Till date, three regulatory factors have been identified for small G-proteins, viz., Guanine exchange nucleotide factors [GEFs], GDP-dissociation inhibitors [GDIs] and GTPase-activating proteins [GAPs]. GEFs facilitate the translation of the inactive GDP-bound to their active GTP-bound forms, while, the GDIs avert the dissociation of GDP from the G-proteins, thereby keeping them in the inactive conformation (Figure 1). However, GAPs, convert the active GTP-bound to their inactive GDP-bound form by inactivating the intrinsic GTPase activity of the candidate G-proteins. The efficiency of the G-protein activation cascade depends on the relative amounts of active to inactive GTPase. The activity of GTPase can be altered either by accelerating GDP dissociation by GEFs or by inhibiting GDP dissociation by GDIs, or by accelerating GTP hydrolysis by GAPs. Any imbalance in either of the regulatory factors alters the hydrolytic cycle and physiological functions in pancreatic β-cells.[49,57,58]

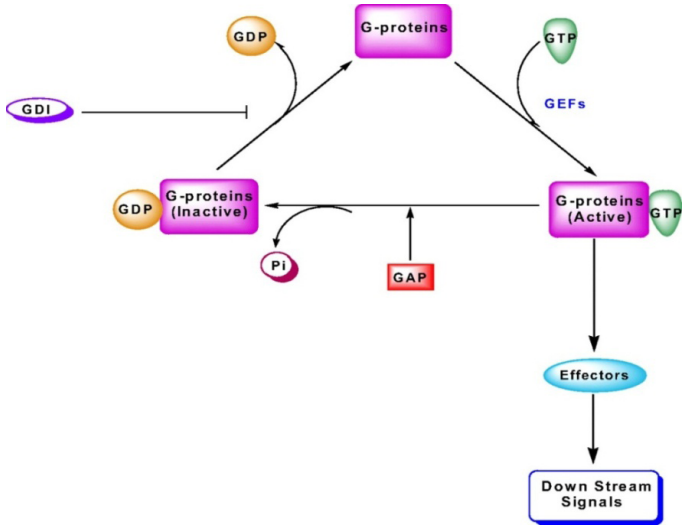

Figure 1: Activation and deactivation cascade of small G-proteins.[49]

SMALL G-PROTEIN-RAC

Rac was first identified and implicated in cellular function with two cDNAs encoding proteins, Rac1 and Rac2.[59] So far three isoforms of Rac proteins, Rac1, Rac2, and Rac3 have been identified in mammals. Both Rac1 and Rac2 share over 90% homology. Rac1 and Rac3 are extensively expressed in diverse tissues, whereas as, Rac2 is restricted to hematopoietic cells. Rac1 and other small G-proteins, Cdc42 and Arf6 have been recognized as key regulatory molecules in vesicle trafficking and organelle dynamics coupled with proliferation and survival of a cell.[19,49] In addition, Rac1 has also been shown to play a vital role in various diseased states including cancer and neurological disorders,[60-62] liver fibrosis[63] and diabetes.[57,58] Furthermore, Rac1 protein has shown to be associated with GLUT4 translocation in the muscle of diabetic patients.[64,65] Furthermore, Rac1 has shown to play an important role in wound healing, bacterial clearance and cell adhesion/migration by regulating actin dynamics in the gut.[66] Rac1 together with cdc42 induces intestinal wound closure, mediated by N-formyl peptide receptor (FPR) stimulation leading to enhanced intestinal epithelial cell restitution.[67] In addition, many pathogenic bacteria secrete factors that

trigger the posttranslational modification and activation of Rho proteins like Rac1 and cdc42 leading to gut epithelial cell death *via* apoptosis.[68] Citalán-Madrid and group have clearly depicted the roles of small G proteins as important signaling molecules in the regulation of epithelial junctions.[69] Herein this review, we describe both the positive and negative roles for Rac1 small G-protein in islet β-cell pathophysiology.

A. Positive Role of Rac1 in Insulin Secretion: Like other small G-proteins, Rac also shuttles between inactive GDP and active GTP conformations to facilitate cellular function. These proteins undergo ADP ribosylation by C3 component of botulinum toxin prior to their association with membrane. However, potential roles for Rac1 in glucose stimulated insulin secretion (GSIS) was first demonstrated by using Clostridium difficile toxins A and B, which irreversibly monoglucosylate and inactivate specific G-proteins (Cdc42 and Rac1).[32] Like Cdc42, Rac1 also undergoes posttranslational carboxymethylation and membrane translocation in the presence of stimulatory glucose concentrations.[32] Expression of an inactive mutant of Rac1 (N17Rac1) in INS-1 cells resulted in significant morphological changes leading to inhibition of GSIS. These findings also confirmed the involvement of small G-protein Rac1 in cytoskeletal remodeling and reorganization.[41] As stated above, Rac1 also requires prenylation for its function. Experiments involving pharmacological and molecular biological inhibition of Rac1 prenylation indicated marked reduction in GSIS in a variety of insulin-secreting β-cells. For an instance, GGTI-2147, a specific inhibitor for geranylgeranylation, one of the post translational modifications, significantly augmented accumulation of Rac1 in cytosol and inhibited GSIS in insulin-producing β-Cell line INS 832/13. Over expression of the regulatory α-subunit of protein prenyltransferase also attenuated glucose-induced insulin secretion in clonal pancreatic β-cells.[31] In addition, a recent study has shown that siRNA-mediated knock down of small G-protein Rac1 attenuated GSIS significantly having no effect on the basal insulin secretion, suggesting a positive modulatory roles for Rac1 in insulin secretion.[70] The importance of these small G-proteins in insulin secretion has been extensively studied *in vitro*; however, studies concentrating on *in vivo* Rac1 knock out models are limited. As Rac1 small G-protein is involved in many physiological processes, knocking out Rac1 might have deleterious effects. In this context, epithelial-specific Rac1-Knockout mice showed epithelial hyperplasia and a reduced basal cell layer.[71] Recent study has shown that, Rac1 specific knockout in pancreatic β-cells has no difference in either β-cell mass or pancreatic islet density explaining the possible compensatory mechanisms by other Rho-GTPases.[72] However, glucose stimulated insulin secretion was attenuated in these mice lacking Rac1 in β-cells both *in vivo* and in isolated islets. Furthermore, Rac1-null mice [βRac1−/−] exhibited impaired glucose tolerance and hypoinsulinemia, suggesting key regulatory roles for Rac1 in normal insulin function.[43] Taken together, these evidences suggest a positive role for Rac1 protein in islet function.

RAC1-NOX SIGNALING IN INSULIN SECRETION

Recent evidence suggests that NADPH oxidase derived tonic increase in reactive oxygen species (ROS) is required for glucose stimulated insulin secretion.[50,73-76] NADPH oxidase (Nox) represent a group of superoxide-generating enzymes which transport electrons through membranes and catalyze the cytosolic NADPH-dependent reduction of molecular oxygen to O_2 •-.[77] Till date, seven Nox family members have been identified i.e., Nox1, Nox2, Nox3, Nox4, Nox5, DUOX1 and DUOX2.[78] The Phagocytic Nox is a multicomponent enzyme complex, composed of membrane components [catalytic glycosylated $gp91^{phox}$ and the regulatory non-glycosylated $p22^{phox}$], cytosolic proteins [$p47^{phox}$, $p67^{phox}$, $p40^{phox}$] and a small GTPase, Rac ½.[78] Activation of Nox requires translocation of cytosolic components to the membrane and association with $gp91^{phox}$/$p22^{phox}$ complex (Figure 2).[79] Furthermore, Nox1 is the first homologue of $gp91^{phox}$ to be described and requires small GTPase Rac for activation.[80-83] In contrast to Nox1, 2 and 3, Nox4 is a constitutively active enzyme and is activated without the necessity for GTPase Rac or the cytosolic components.[84]

In this setting, the functional activation of Rac1 has shown to be critical in holoenzyme assembly and activation of Nox.[78,85-88] In support of this, Gorzalczany and associates have

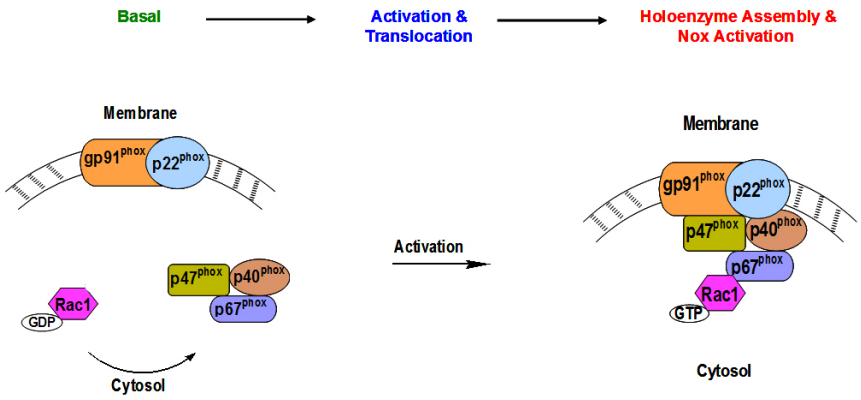

Figure 2: Activation of NADPH oxidase holoenzyme.

shown the activation of Nox and subsequent generation of ROS by targeting Rac1 to the membrane fraction.[89] They also demonstrated that prenylated Rac1 but not the unprenylated form binds to the phagocyte membrane more efficiently to facilitate the superoxide generation. Along these lines, Pi and Collins have overviewed the existing evidence in supporting "secondary messenger" roles of ROS in physiological insulin secretion.[50] In addition, studies have also emphasized roles for Nox in physiological insulin secretion. For example, Diphenyleneiodonium [DPI], a selective inhibitor of Nox, inhibited glucose-induced Nox activity and GSIS.[75] These observations were further confirmed by Morgan and associates suggesting that DPI or $p47^{phox}$ antisense-induced inhibition of Nox attenuated GSIS under static or perifusion conditions.[90] Graciano and co-workers demonstrated regulatory roles for Nox in palmitate-induced superoxide generation and insulin secretion in rat islets.[50] Furthermore, recent findings suggests that prenylation and activation of Rac1 are critical for glucose- and mitochondrial fuel-induced Nox-dependent ROS generation in clonal pancreatic β-cells and rodent islets.[91] In summary, a tonic increase in intracellular ROS is necessary for normal physiological insulin secretion and Rac1 initiates subsequent signaling steps including Nox activation and insulin release.[92]

B. RAC1-Nox Signaling and Metabolic Dysfunction: In addition to the above described beneficial roles for Rac1 in Nox-mediated ROS signaling in islet function, recent evidence also confirmed negative roles for ROS in islet β-cell dysfunction.[91] Excessive ROS generation is considered central to the development of diabetes and its associated complications. Under normal physiological conditions, generation of free radicals is relatively low; however increased levels of circulating glucose promote intracellular accumulation of superoxides leading to metabolic dysfunction. Although, mitochondria remain the primary source for free radicals, emerging evidence implicates Nox as one of the major sources of extra-mitochondrial ROS. Immunological localization and functional regulation of Nox have been described in clonal β-cells, rat and human islets.[50,75,90,92] Studies by Shen and associates in cardiac myocytes have also suggested regulatory roles for Rac1 in the activation of Nox and associated generation of ROS in animal models of diabetes.[93] In addition, significant increase in Nox-mediated oxidative stress and subsequent metabolic dysfunction has been clearly reviewed in a recent article by Kowluru.[70] However, very little is known with regard to regulatory roles of Rac1 in the holoenzyme assembly and activation of Nox in islet β-cells following chronic exposure to glucose, saturated fatty acids or cytokines.

In this context, recent findings demonstrated that prenylation of Rac1 is necessary for glucose-induced Nox activation and ROS generation in isolated β-cells.[91] In addition, studies have also implicated Nox in metabolic dysfunction of the islet β-cell under conditions of glucolipotoxicity and exposure to cytokines.[57,94] Generation of ROS under these conditions appears to be largely due to the activation of Nox, since inhibition of Nox [e.g., DPI, apocynin or siRNA-$p47^{phox}$] or Rac1 activation [e.g., GGTI-2147, NSC23766] markedly attenuated deleterious effects on pancreatic β-cells. In addition, the activation status of Rac1 was shown to be under precise control of Tiam1, a known guanine nucleotide exchange factor for Rac1, but not Cdc42 and Rho G-proteins in isolated β-cells.[95] In further support of this, a marked reduction in high glucose-, high palmitate- cytokine-induced Rac1 and Nox activation and ROS generation in isolated β-cells was observed following treatment with NSC23766, a selective inhibitor of Tiam1/Rac1 signaling axis.[57,94] Using selective inhibitors of protein prenylation, Subasinghe, et al. demonstrated a critical requirement of prenylation of Rac1 for Nox-mediated β-cell dysfunction.[94]

Taken together, these *in vitro* findings clearly implicate participatory roles of Nox in exerting effects at the mitochondrial level including loss in membrane potential, cytochrome C release and activation of caspase-3 culminating in islet β-cell dysfunction.[94,96] In addition, recent studies from Sidarala and colleagues present the evidence that the Rac1-Nox2 signaling is vital in high glucose induced activation of stress activated kinases and loss in GSIS causing islet β-cell dysfunction.[97] Despite these *in vitro* evidences, potential roles for Nox in islet dysfunction in animal models of type 2 diabetes are minimal. However, a recent study systematically examined the functional status of Nox in islets from Zucker Diabetic Fatty [ZDF] rat, which develops obesity, hyperinsulinemia, hyperglycemia and a decline in β-cell function.[58] These *in vitro* observations supported by findings in islets derived from the diabetic rodents [the ZDF rat] and diabetic human islets, form basis for the development of small molecule inhibitors for Rac1 and Nox activation in halting the metabolic defects, thereby retaining normal β-cell mass. In addition, a recent study from Zhou and colleagues also confirmed that the treatment with selective inhibitor NSC23766 attenuated Rac1 expression and oxidative stress in the pancreas in ob/ob mice.[98] These findings provide insights into potential therapeutic targets and interventional modalities to prevent the metabolic defects.

POTENTIAL THERAPEUTIC TARGETS AND INTERVENTIONAL MODALITIES

Based on the above discussion and published evidences, it is clear that Nox-derived reactive oxygen species have both positive and negative roles in the islet β-cell function. Targeting Nox holoenzyme complex could be beneficial in subsiding the excessive generation of ROS during oxidative stress milieu. In this context, a recent study proposed that $gp91^{phox}$, $p47^{phox}$ and $p67^{phox}$ might serve as potential drug targets due to their selective association in the Nox holoenzyme complex.[99] On the contrary, peptide inhibitors blocking Rac1/2 activation and $p47^{phox}$ translocation might not be a good approach, since they are integral members of other NADPH oxidasecomplexes too. However, Mizrahi, et al. developed $p47^{phox}$-$p67^{phox}$-Rac1 chimera as a quintessential single molecule activator of Nox[100] to study the

effects of Nox activation regulatory roles for Rac1. These observations are in agreement with the findings, where researchers have demonstrated a decrease in glucose-mediated Nox-induced ROS generation in the presence of prenylation inhibitors. Developing inhibitors for such quintessential single molecule activators might provide a novel therapeutics to minimize excessive generation of ROS Nox-mediated pancreatic β-cell dysfunction. Furthermore, an alternate approach to minimize the excessive generation of ROS is to enrich the antioxidant capacity of the islet β-cells. As reviewed by Acharya and Ghaskadbi,[101] pancreatic islet β-cells hold a poor antioxidant defense mechanism. And counterbalancing oxidative environment by antioxidant treatment or overexpressing antioxidant enzymes might prove to be successful in regulating islet β-cell function. Indeed, such modalities have been shown to work efficiently both *in vivo* and *in vitro*. Along these lines, treatment with antioxidant, α-lipoic acid has been demonstrated to improve insulin sensitivity in type 2 diabetic subjects.[102] Moreover, researchers have also shown that vitamin E treatment improves pancreatic physiology under diabetic state.[103] Asayama, et al. found that rats deficient in vitamin E, selenium, or both had decreased insulin secretory reserves, suggesting that vitamin E status can directly affect pancreatic islet function. In a mouse model of type 2 diabetes, treatment with vitamin E combined with vitamin C and n-acetyl cysteine resulted in large number of pancreatic islets than controls.[104] Furthermore, a recent study in humans has shown that, taurine affectively restored β-cell function and improved insulin sensitivity.[105] Together these studies further highlight antioxidant therapy as one of the feasible options in attenuating excessive generation of ROS and subsequent reduction in oxidative stress environment in the islet β-cells.

In addition to the above mentioned strategies for attenuating oxidative stress, inhibitors blocking Tiam1/Rac1/Nox signaling axis,[57,94,106] polyphenolic extracts supplementation,[107] stress activated kinase inhibitors,[108-111] and angiotensin receptor antagonists[112] have proven efficaciously to reduce oxidative stress and improve islet β-cell function.

CONCLUSION

Glucose stimulate insulin secretion (GSIS) involves a series of metabolic events involving interaction between a variety of signaling pathways to facilitate the transport of insulin-laden granules to the plasma membrane for fusion and subsequent insulin release. Compelling evidence supports involvement of small G-proteins like Rac1 and Cdc42 in the cytoskeletal reorganization, which is necessary for GSIS to occur. Recent findings further validate that Tiam1 represents one of the GEFs for Rac1 and that Tiam1/Rac1 signaling axis is requisite for GSIS. Nox appears to be an effector protein for Tiam1/Rac1 signaling and that its activation leads to a tonic increase in the generation of ROS under the stimulatory conditions of glucose and fatty acids leading to insulin release. In addition to this, Tiam1/Rac1 signaling axis appears to play a vital role in Nox-mediated ROS generation under the duress of excessive glucose, palmitate, ceramide and cytokines culminating in oxidative stress and metabolic dysfunction of islet β-cells. Together, these findings suggest positive and negative modulatory roles for Tiam1-Rac1-Nox signaling pathway in islet function. The Figure 3 depicted below is indicative of potential effects of ROS on islet β-cells at different stages. Low levels of generated ROS have a positive effect on glucose stimulated insulin secretion, and as the levels of the ROS increases it causes detrimental effects and β-cell dysfunction.[113] Therefore, it may be challenging to draw a line as to how much of ROS generation is beneficial for the normal function of islets as opposed to how much is bad to elicit damaging effects on the pancreatic β-cell. It is likely that there may be a "window of opportunity" or "point of return" for the islet β-cell to recover from the noxious effects of excessive ROS due to accelerated Tiam1-Rac1-Nox signaling pathway in the diabetic states.

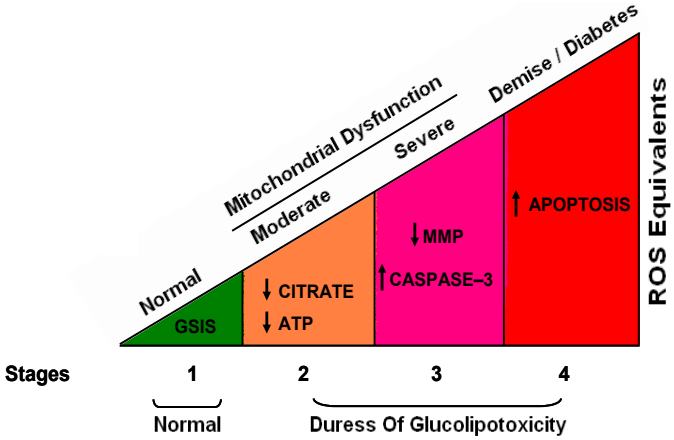

Figure 3: Hypothetical model for ROS generation in identifying the effects on pancreatic islet β-cells.

CONFLICTS OF INTEREST

The authors declare that they dont have any conflicts of interest or any acknowledgements for this submission.

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