JAHANGIR KHAN

Infections and Lipids in Alcohol Induced Acute Pancreatitis

ACADEMIC DISSERTATION
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ABSTRACT

Acute pancreatitis is an inflammatory disease of the pancreas and a major cause of morbidity worldwide. The clinical course of acute pancreatitis ranges from mild abdominal discomfort to life threatening severe disease. The most common causes of acute pancreatitis are heavy alcohol consumption and gallstone disease, together accounting for the majority of all cases. In Finland, alcohol is the predominant cause of acute pancreatitis.

The mechanism by which alcohol causes acute pancreatitis remains unclear, and despite being a clear risk factor for acute pancreatitis, only a small proportion of all heavy drinkers develop acute pancreatitis. In animal studies alcohol alone does not cause acute pancreatitis but is associated with pancreatic irritation, and it has been postulated that some co-factor may be required to trigger the acute disease. So far, however, no such trigger factor has been discovered. The present studies sought factors associated with the onset or severity of alcohol induced acute pancreatitis in humans. The roles of Helicobacter pylori, a common infection causing chronic inflammation and changes in gastrointestinal physiology, enteroviruses, common acute infections which have rarely been associated with the development of acute pancreatitis, and the serum lipid profile, which could be affected by heavy alcohol consumption, were studied.

Helicobacter pylori infection was found to be no more prevalent in patients with alcohol induced acute pancreatitis than in patients with other etiologies for acute pancreatitis or in controls with similar high alcohol consumption. Neither was Helicobacter pylori infection associated with the severity of acute pancreatitis. No acute enterovirus infection was observed in patients with alcohol induced acute pancreatitis. The prevalence of previous enterovirus infections was high in patients with alcohol induced acute pancreatitis, but this was also true for the controls with similar high alcohol consumption and no significant differences were observed, nor any association with the severity of acute pancreatitis. The serum fatty acid profile was found to differ significantly between patients with alcohol induced acute pancreatitis and control alcoholics. Some of the differences were caused by the acute disease and normalized after follow-up, while some changes were evident even after hospitalization and might in these patients be associated with increased risk of developing acute pancreatitis. The serum lipid concentrations were found to react strongly during acute pancreatitis. The concentrations of serum total cholesterol, HDL-cholesterol and LDL-cholesterol dropped significantly during the acute disease and normalized after follow-

up. Furthermore, lower serum total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations were associated with the development of more severe acute pancreatitis and the difference could be observed early in the course of the disease.

On the basis of these studies, it was concluded that neither Helicobacter pylori nor enterovirus infections seem to be associated with the development of alcohol induced acute pancreatitis in humans. The serum fatty acid profile differs between patients with alcohol induced acute pancreatitis and controls with similar high alcohol use suggesting a potential role as a predisposing co-factor for the development of alcohol induced acute pancreatitis. Serum cholesterol concentrations are significantly altered during alcohol induced acute pancreatitis and changes in the serum cholesterol concentrations seem to be associated with the severity of the disease, suggesting a potential role in assessing the severity of an acute pancreatitis episode.

TIIVISTELMÄ

Äkillinen haimatulehdus on haiman tulehduksellinen sairaus ja maailmanlaajuisesti tärkeä sairastavuuden aiheuttaja. Sen taudinkuva vaihtelee lievästä vatsaoireilusta henkeä uhkaavaan vakavaan sairauteen. Äkillisen haimatulehduksen tärkeimmät aiheuttajat ovat runsas alkoholinkäyttö sekä sappikivitauti, jotka yhdessä selittävät valtaosan kaikista tapauksista. Suomessa tärkein aiheuttaja on alkoholi.

Mekanismi jolla alkoholi aiheuttaa äkillisen haimatulehduksen on edelleen epäselvä ja vaikka runsas alkoholinkäyttö onkin selkeä riskitekijä, vain pieni osa kaikista alkoholin suurkuluttajista sairastuu äkilliseen haimatulehdukseen. Eläinkokeissa alkoholi aiheuttaa haiman ärsytystä mutta ei yksinään laukaise äkillistä haimatulehdusta ja onkin ehdotettu että taudin synty saattaa vaatia toisenkin ärsykkeen. Toistaiseksi kuitenkaan tällaista ei ole löydetty. Näissä tutkimuksissa etsittiin tekijöitä jotka liittyvät äkillisen alkoholihaimatulehduksen syntyyn tai vaikeuteen ihmisissä. Osatöissä määritettiin helikobakteeri pylori -infektioiden, enterovirusinfektioiden sekä seerumin lipidiprofiilin osuus. Helikobakteeri pylori on yleinen kroonista ruoansulatuskanavan tulehdusta ja toiminnan muutoksia aiheuttava bakteeri. Enterovirukset puolestaan ovat tavallisia hengitysteiden ja ruoansulatuskanavan akuuttien infektioiden aiheuttajia, joiden on myös harvoin todettu olevan yhteydessä äkillisen haimatulehduksen syntyyn. Runsas alkoholinkäyttö saattaa olla yhteydessä seerumin lipidiprofiilin muutoksiin.

Helikobakteeri pylori infektion vallitsevuudessa ei todettu eroja runsaan alkoholinkäytön vuoksi äkilliseen haimatulehdukseen sairastuneiden potilaiden ja muista syistä äkilliseen haimatulehdukseen sairastuneiden potilaiden tai runsaasti alkoholia käyttävien verrokkien välillä. Helikobakteeri pylori infektio ei myöskään ollut yhteydessä syntyneen taudin vaikeuteen. Akuuttia enterovirusinfektiota ei todettu potilailla jotka sairastuivat äkilliseen alkoholihaimatulehdukseen. Edeltävien enterovirusinfektioiden vallitsevuus oli korkea alkoholihaimatulehduspotilailla, mutta myös runsaasti alkoholia käyttävällä verrokkiryhmällä, eikä merkittäviä eroja ryhmien välillä tai yhteyttä äkillisen haimatulehduksen vaikeuteen todettu. Seerumin rasvahappoprofiilin todettiin poikkeavan merkittävästi äkilliseen alkoholihaimatulehdukseen sairastuneiden potilaiden ja verrokkialkoholistien välillä. Osa muutoksista liittyi äkilliseen sairauteen ja korjaantui seurannassa, kun taas osa eroavaisuuksista oli todettavissa sairaalahoidon jälkeenkin saattaen olla yhteydessä lisääntyneeseen riskiin sairastua äkilliseen haimatulehdukseen näillä potilailla. Seerumin lipidipitoisuuksien havaittiin reagoivan voimakkaasti äkillisen haimatulehduksen aikana. Seerumin kokonaiskolesterolin,

HDL-kolesterolin ja LDL-kolesterolin pitoisuudet putosivat merkittävästi taudin aikana ja korjaantuivat seurannassa. Lisäksi seerumin matalien kokonaiskolesterolin, HDL-kolesterolin ja LDL-kolesterolin pitoisuuksien todettiin olevan yhteydessä vaikean haimatulehduksen kehittymiseen ja nämä muutokset olivat todettavissa jo taudin alkuvaiheissa.

Näiden tutkimusten yhteenvetona todettiin, etteivät helikobakteeri pylorin tai enterovirusten aiheuttamat infektiot vaikuta olevan yhteydessä äkillisen alkoholihaimatulehduksen syntyyn ihmisissä. Seerumin rasvahappoprofiili poikkeaa äkilliseen alkoholihaimatulehdukseen sairastuneiden potilaiden ja runsaasti alkoholia käyttävien verrokkien välillä, ollen mahdollinen äkillisen alkoholihaimatulehduksen syntyyn vaikuttava tekijä. Seerumin kolesterolipitoisuudet muuttuvat voimakkaasti äkillisen alkoholihaimatulehduksen aikana, nämä muutokset ovat yhteydessä äkillisen haimatulehduksen vaikeuteen ja niitä voidaan mahdollisesti käyttää äkillisen haimatulehduksen vaikeuden määrittämisessä.

LIST OF ORIGINAL COMMUNICATIONS

1. Khan J, Pelli H, Lappalainen-Lehto R, Järvinen S, Sand J and Nordback I (2009): Helicobacter pylori in alcohol induced acute pancreatitis. Scand J Surg 98:221-224.

2. Khan J, Nordback I and Sand J (2012): There is hardly any association between Helicobacter pylori infection and the severity of acute pancreatitis. Scand J Gastroenterol 47:1523-1524.

3. Khan J, Nordback I, Seppänen H, Lappalainen-Lehto R, Järvinen S, Oikarinen S, Tauriainen S, Räty S, Hyöty H and Sand J (2013): Is alcoholic pancreatitis associated with enteroviral infection? World J Gastroenterol 19:3819-3823.

4. Khan J, Solakivi T, Seppänen H, Lappalainen-Lehto R, Järvinen S, Ronkainen J, Sand J and Nordback I (2012): Serum lipid and fatty acid profiles are highly changed in patients with alcohol induced acute pancreatitis. Pancreatology 12:44-48.

5. Khan J, Nordback I and Sand J (2013): Serum lipid levels are associated with the severity of acute pancreatitis. Digestion 87:223-228.

ABBREVIATIONS

AUDIT	Alcohol use disorders identification test
CCK	Cholecystokinin
CI	Confidence interval
ERCP	Endoscopic retrograde cholangiopancreaticography
FAEE	Fatty acid ethyl ester
HDL	High-density lipoprotein
Ig	Immunoglobulin
IL	Interleukin
LDL	Low-density lipoprotein
NF-κB	Nuclear factor kappa B
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SIRS	Systemic inflammatory response syndrome
SPINK1	Serine protease inhibitor Kazal type 1
TNF-α	Tumor necrosis factor α

INTRODUCTION

Acute pancreatitis is an inflammatory disease of the pancreas and one of the most common acute gastrointestinal diseases leading to hospitalization (Peery et al. 2012), associated with significant morbidity and a mortality rate of approximately 5% (Gullo et al. 2002, Frey et al. 2006). Most cases are caused by either gallstones or excessive alcohol consumption. In Finland, approximately 70% of all cases are alcohol-related (Jaakkola and Nordback 1993). Although some of the risk factors and mechanisms by which alcohol causes pancreatic injury have been identified, exactly how alcohol causes acute pancreatitis and why some, but not all, alcoholics develop the disease, is still unknown. In animal studies, alcohol by itself does not trigger the acute disease but does lower the threshold for other pancreas-injuring or over-stimulating factors which may initiate acute pancreatitis. If such a mechanism, the priming of the pancreas by alcohol and subsequent offsetting of acute pancreatitis by a following insult, also exists in humans, it has not so far been discovered. The aim of this thesis was to find factors associated with the onset and severity of alcohol induced acute pancreatitis.

REVIEW OF THE LITERATURE

1 The human pancreas

1.1 Basic anatomy and physiology of the human pancreas

The human pancreas is a glandular, elongated organ located in the retroperitoneal space of the abdomen. It lies behind the stomach, in front of the abdominal aorta and inferior vena cava, and between the duodenum and the spleen. Its average length is 20cm and it weighs 85–90 grams. It is anatomically divided into four main parts; the head, neck, body and tail, without clear borders between the parts. It contains the main pancreatic duct, also called the duct of Wirsung, which begins in the tail of the pancreas and runs throughout the length of the organ joining up with the bile duct forming the hepatopancreatic ampulla, which opens into the descending part of the duodenum in the major duodenal papilla. The accessory pancreatic duct, also known as the duct of Santorini, drains part of the head of the pancreas and its uncinate process, opening separately into the duodenum at the minor duodenal papilla, approximately 2cm above the major papilla, though usually communicating with the main duct. Variations in pancreatic ductal anatomy are common. The arterial blood supply of the pancreas is derived mostly from the pancreatic branches of the splenic artery, but also from the branches of the gastroduodenal and superior mesenteric arteries. The venous drainage of the pancreas is mostly provided by branches of the splenic vein, but also by the branches of the superior mesenteric vein, leading to the portal vein. The pancreatic lymphatic veins follow the blood vessels. The pancreas is innervated by the branches of the vagus and thoracic splanchnic nerves, which reach the pancreas by following the arteries from the celiac plexus and superior mesenteric plexus. (Townsend et al. 2008.)

The human pancreas serves two main functions; it secretes the many pancreatic digestive enzymes together with sodium bicarbonate -rich fluid through the pancreatic ducts into the small intestine and also produces several important hormones. The most important digestive enzymes secreted by the pancreas are amylase, lipase, and trypsin. The proteolytic enzymes are first produced in their inactive form and become activated when they are secreted into the intestinal tract. The most important hormones secreted by the pancreas are insulin and glucagon. These two functions reside separately in the

pancreas; the exocrine secretions are produced in the acini and ducts of the pancreas, which comprise most of the pancreatic tissue, while the hormones secreted by the pancreas are produced in numerous small clusters called the islets of Langerhans amidst the pancreatic parenchyma. (Yamada et al. 2003.)

1.2 Regulation of pancreatic function

The secretion of pancreatic digestive enzymes and fluid is chiefly promoted by the neurotransmitter acetylcholine and digestive hormones cholecystokinin (CCK) and secretin in response to eating-related stimuli (Singer and Niebergall-Roth 2009). Of these, acetylcholine and cholecystokinin mainly induce the production of digestive enzymes from the acini, while secretin increases the production of sodium bicarbonate fluid in the pancreatic ducts that transports the digestive enzymes into the small intestine and also serves to neutralize the acidic gastric juice entering the small intestine (Chey and Chang 2001, Mössner 2010). Acetylcholine is released from the autonomous nerve endings in the pancreas, while cholecystokinin and secretin are released from the mucosa of the small intestine into the blood circulation when food enters the small intestine (Konturek et al. 2003).

The production and release of insulin increase rapidly within a matter of minutes when blood glucose levels are elevated and are similarly inhibited when blood glucose levels are low. Several gastrointestinal hormones, such as glucagon-like peptide 1, and sympathetic and parasympathetic neural modulation mediate the secretion of insulin (Ahren 2000, Leech et al. 2011, Meloni et al. 2013). Some amino acids and serum free fatty acids potentiate the release of insulin when combined with elevated blood glucose levels (Newsholme et al. 2007a, Newsholme et al. 2007b). Similarly, the blood glucose level is the most important regulator of glucagon secretion, but in contrast to insulin, high levels of blood glucose inhibit and low levels of blood glucose promote glucagon secretion. The secretion of glucagon is modulated by several other hormones such as leptin and ghrelin (Chuang et al. 2011, Marroqui et al. 2011). Similarly to insulin, some amino acids increase the production of glucagon (Li et al. 2013). The production of both insulin and glucagon is inhibited by somatostatin (Kailey et al. 2012).

2 Acute pancreatitis

Acute pancreatitis is an inflammatory disease of the pancreas and a major cause of morbidity worldwide. Furthermore, acute pancreatitis is one of the most common

gastrointestinal diseases leading to hospitalization (Peery et al. 2012). The clinical course of acute pancreatitis ranges from mild abdominal discomfort to life threatening severe disease with circulatory shock and multi-organ failure (Frossard et al. 2008). Initially in the course of the illness, the pancreas becomes edematous due to the inflammation and exhibits leukocyte infiltration and often focal fat necrosis inside the pancreas and in the peripancreatic tissues. The edema may progress to extensive necrosis of the pancreatic parenchyma, and is then called acute necrotizing pancreatitis. Furthermore, acute pancreatitis may also be associated with hemorrhage from the pancreas, often from a necrotic region, and is then called acute hemorrhagic pancreatitis. The inflamed pancreas is sterile at the beginning of acute pancreatitis, but may later be secondarily infected, usually at a necrotic region (Cicalese et al. 2001).

2.1 Etiology and epidemiology of acute pancreatitis

An attack of acute pancreatitis may result from numerous causes. The most important and frequent causes of acute pancreatitis are alcohol and gallstones. Together they account for 70–90% of all cases. In most regions, gallstones are the most common etiology, whereas in other regions, alcohol is the predominant cause of acute pancreatitis (Jaakkola and Nordback 1993, Gullo et al. 2002, Yadav and Lowenfels 2006, Bai et al. 2007). Less common etiologies of acute pancreatitis include tumors, hypertriglyceridemia, hypercalcemia, post-endoscopic retrograde cholangiopancreaticography (post-ERCP), pancreatic duct obstructions, pancreatic trauma, systemic diseases, infections, hereditary susceptibility and some drugs (Harper and Cheslyn-Curtis 2011). Abdominal obesity (Sadr-Azodi et al. 2011) and smoking (Sadr-Azodi et al. 2012) increase the risk for acute pancreatitis. In approximately 10–30% of all cases, the cause of acute pancreatitis remains unknown, though many of these could be caused by bile microlithiasis or sphincter of Oddi dysfunction (Coyle et al. 2002, Wilcox et al. 2006). The annual incidence of acute pancreatitis ranges from approximately 10–70 per 100,000 patient years in different countries and regions (Table 1). Of the most common etiologies of acute pancreatitis, acute gallstone pancreatitis is more frequent in females and alcohol induced acute pancreatitis in males, while idiopathic acute pancreatitis is equally common in both sexes (Yadav and Lowenfels 2006). The mortality rate in acute pancreatitis is approximately 5% (Table 1).

Table 1. Incidence of acute pancreatitis. Mortality rates and proportions of the most common etiologies are given if reported by the author.

Author	Region	Year	Incidence (per 100.000 patient years)	Mortality (%)	Etiology		
					Alcohol (%)	Gallstones (%)	Idiopathic (%)
Jaakkola and Nordback 1993	Finland	1989	73.4	2.6			
Eland et al. 2000	Netherlands	1995	15.9*	10.7			
Birgisson et al. 2002	Iceland	1998–1999	32.3	4.0	32	42	26
Floyd et al. 2002	Denmark	2000	32.5*	6.7			
Lankisch et al. 2002a	Germany	1988–1995	19.7*	7.3	32	40	20
Goldacre and Roberts 2004	England	1998	9.8*	6.7			
Lindkvist et al. 2004	Sweden	1999	35*	5.7	25	42	33
Frey et al. 2006	California	2001	43.8*	4.2	20.3	32.6	36.6
Fagenholz et al. 2007	USA	2002	73	2.0			
O'Farrel et al. 2007	Ireland	2004	23.6				
Roberts et al. 2008	England	1998–2003	22.4	6.7			
Spanier et al. 2008	Netherlands	2004	19.2				
Sand et al. 2009	Finland	1987–2007	60–102 in men** 5–21 in women**	2.1–3.7 in men 2.6–4.1 in women			
Omdal et al. 2011	Norway	1996–2006	14.6*	3.5	10	50	7
Shen et al. 2012b	Taiwan	2000–2009	36.9*	4.3–3.3			
Spanier et al. 2013	Netherlands	2005	14.7				
Stimac et al. 2013	Croatia	2000–2009	30.2		19	61	

*Incidence of the first attack of acute pancreatitis
**Incidence of alcohol induced acute pancreatitis

2.2 Diagnosis of acute pancreatitis

Acute pancreatitis typically presents with severe acute epigastric pain often radiating to the back, fever and nausea, and may include signs of gastrointestinal obstruction, jaundice and shock. The most common clinical findings are abdominal tenderness and guarding. (Malfertheiner and Kemmer 1991.) However, other diseases such as acute cholecystitis or gastrointestinal perforation may present with similar symptoms and findings. During the disease, digestive enzymes leak from the pancreas. Measurements of blood amylase and/or lipase activity are the most common tests used in diagnosing acute pancreatitis. Of these, amylase measurement is more widely used, although lipase measurement is more specific (Kiriyama et al. 2010). Similarly, trypsinogen-2 measurement from the urine can be used to diagnose acute pancreatitis (Kylänpää-

Bäck et al. 2000). In addition, abdominal imaging by computed tomography, magnetic resonance imaging or ultrasound may identify acute pancreatitis (Bollen et al. 2007). No gold standard for diagnosing acute pancreatitis exists, but general consensus states that acute pancreatitis may be diagnosed when the patient presents with two of the following: typical symptoms and findings of acute pancreatitis, elevated blood amylase and/or lipase activity, the most common cut-off value being ≥ 3 times greater than the upper limit of normal, and characteristic findings of acute pancreatitis in abdominal imaging (Banks and Freeman 2006, Banks et al. 2013, Kiriyama et al. 2010, UK Working Party on Acute Pancreatitis 2005).

2.3 Pathophysiology

Even though acute pancreatitis can be triggered by numerous causes, the resulting disease is surprisingly similar in all etiologies. The exact pathophysiologic process behind acute pancreatitis still remains somewhat controversial, and is most likely mediated by several factors.

2.4 Premature trypsin activation

A widely accepted theory suggests premature trypsin activation within the acini of the pancreas leading to organ auto-digestion as the cause of acute pancreatitis – a refined version of a theory originally suggested in the 19th century (Chiari 1896). Intra-acinar activation of trypsin during acute pancreatitis has been demonstrated in both animal models and in patients with acute pancreatitis and is associated with acinar cell death and the induction of acute pancreatitis (Willemer et al. 1989, Saluja et al. 1999, Laukkarinen et al. 2007, Gaiser et al. 2011). In addition to causing local tissue damage by itself, trypsin activates other pancreatic digestive enzymes, such as phospholipase, chymotrypsin, and elastase, which exacerbate the insult. Furthermore, the activation of trypsin within the acini promotes the production of cytokines and chemokines, such as tumor necrosis factor α (TNF-α) and interleukin (IL) 1β, from the acinar cells and macrophages and activates the complement cascade (Acioli et al. 1997, Lundberg et al. 2000). The mediator of intra-acinar trypsin activation seems to be pathologically elevated intracellular Ca^{2+}-levels and protein kinase C activation (Kruger et al. 2000, Thrower et al. 2008). Many intrinsic protective mechanisms are in place to inhibit premature trypsin activation. Trypsin, like other proteolytic enzymes secreted by the pancreas, is produced as an inactive precursor protein, trypsinogen, and is compartmentalized

within the acinus. Moreover, the acinar cells produce trypsin inhibitors such as serine protease inhibitor Kazal type 1 (SPINK1) and protease-activated receptor-2, while activated trypsin is degraded within the acinar cells and the intracellular Ca^{2+}-levels are constantly regulated (Hirota et al. 2006, van Acker et al. 2006, Sutton et al. 2008). Acute pancreatitis is thought to result from excessive pancreatic hyperstimulation or injury overrunning these protective mechanisms. However, it has also been shown in a cerulein-induced acute pancreatitis model in mice lacking the trypsinogen 7 gene, that even in the absence of premature trypsin activation, significant, but less, acinar cell death occurs (Dawra et al. 2011). In the same study, local and systemic inflammatory reactions were similar in mice lacking the trypsinogen 7 gene and in control mice.

2.5 Nuclear factor kappa-B activation

Nuclear factor kappa-B (NF-κB) is a transcription factor that promotes a large number of genes, many of them associated with inflammatory reactions. It normally lies inactive in the cytoplasm and becomes activated when its inhibitors, most importantly inhibitor κB-α and inhibitor κB-β, are degraded following stimuli by pro-inflammatory cytokines, lipopolysaccharides or reactive oxygen species (ROS) (Barnes and Karin 1997). The activation of NF-κB seems to be mediated by an increase in the intracellular Ca^{2+} levels, similarly to premature intra-acinar trypsin activation, by p38 mitogen-activated protein kinase and by protein kinase C activation (Tando et al. 1999, Satoh et al. 2006, Williard et al. 2010). During the early phase of pancreatic insult, the activity of NF-κB in acinar cells increases and this increase is parallel to, but independent of, intra-acinar trypsin activation (Gukovsky et al. 1998, Hietaranta et al. 2001). When activated, NF-κB promotes the production of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, together with chemokines and adhesion molecules leading to influx of inflammatory cells into the pancreas (Rakonczay et al. 2008). Furthermore, decrease or inhibition of NF-κB activity has been associated with less severe acute pancreatitis in many animal studies. Numerous antioxidants, for example N-acetylcysteine and lipid peroxidation inhibitors (Vaquero et al. 2001, Altavilla et al. 2003b, Virlos et al. 2003), and anti-inflammatory agents, such as peroxisome proliferator-activated receptor γ (Hashimoto et al. 2003) and cyclo-oxygenase 2 inhibitors (Song et al. 2002), have been shown to inhibit NF-κB activity and reduce the severity of acute pancreatitis in animal models (Rakonczay et al. 2008). Similarly, NF-κB knockout mice had less severe acute pancreatitis when compared to control mice in a cerulein-induced acute pancreatitis model (Altavilla et al. 2003a).

2.6 The inflammatory cascade in acute pancreatitis

A distinguishing feature in acute pancreatitis is the tendency to develop pro-inflammatory vicious cycles that may cause local pancreatic inflammation to progress into a severe acute systemic inflammatory response. For example, prematurely activated trypsin subsequently activates more trypsin and other pancreatic digestive enzymes (Gorelick and Otani 1999). After the initial insult, the local release of pro-inflammatory cytokines, chemokines and ROS further recruit and activate more leukocytes that migrate into the pancreas and secrete inflammatory mediators and ROS (Elfar et al. 2007, Vonlaufen et al. 2007). Released pancreatic digestive enzymes and lysosomal enzymes together with leukocyte activation and produced vasoactive factors, such as endothelin-1 and phospholipase A2 (Liu et al. 1995, Aufenanger et al. 2002), rapidly cause microcirculatory dysfunction leading to local ischemia, edema, necrosis and the production of ROS which in turn causes further acinar damage and feeds the pro-inflammatory cascade (Cuthbertson and Christophi 2006, Rakonczay et al. 2008). The release of pro-inflammatory mediators, the most important of which are IL-1β, IL-6, IL-8, IL-18 and TNF-α (Pooran et al. 2003, Bhatia et al. 2005, Malleo et al. 2007, Yuan et al. 2007), during acute pancreatitis can become systemic in nature and, if severe, lead to the development of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (Bhatia et al. 2000, Mayer et al. 2000, Elfar et al. 2007).

3 Alcohol induced acute pancreatitis

Heavy alcohol consumption is indisputably associated with an increased risk of developing acute pancreatitis and changes in alcohol consumption on a national level are reflected in the incidence of alcohol induced acute pancreatitis (Sand et al. 2009). However, only a small proportion of heavy drinkers develop acute pancreatitis even during long term follow up, the rate being 2–3% over 20–30 years (Lankisch et al. 2002b). Acute pancreatitis is considered as alcohol induced when other etiologies have been excluded by imaging and laboratory testing, and the patient reports heavy preceding alcohol consumption (Nordback et al. 2007). The risk of developing the first episode of acute pancreatitis increases concomitantly with the amount of alcohol ingested (Sand et al. 2007), and of patients who develop alcohol induced acute pancreatitis, those with higher alcohol consumption may have an increased risk of more severe disease (Jaakkola et al. 1994). The incidence of alcohol induced acute pancreatitis seems to be associated with heavy consumption of spirits or beer, but not wine (Sadr-Azodi et al. 2011, Roberts et al. 2013). Most patients report that the symptoms of acute pancreatitis

developed during the early withdrawal period after binge drinking rather than during the drinking period (Nordback et al. 2005). After the first episode of alcohol induced acute pancreatitis, approximately 50% of patients suffer a recurrent episode within 10-20 years (Appelros and Borgstrom 1999, Pelli et al. 2000, Gislason et al. 2004). Besides continued alcohol consumption, the most important risk factors for developing recurrent episodes are young age of the patient and a mild pancreatitis episode (Pelli et al. 2000). Approximately 15% of patients suffering an episode of alcohol induced acute pancreatitis are later diagnosed with chronic pancreatitis (Lankisch et al. 2009a), signifying irreversible pancreatic damage and insufficiency. The most important risk factors for chronic pancreatitis are heavy alcohol consumption and smoking (Yadav et al. 2009).

3.1 Mechanisms of alcohol related pancreatic injury

The exact pathophysiologic mechanism by which alcohol causes acute pancreatitis is still unknown. Heavy alcohol consumption is associated with both local pancreatic and systemic disturbances. Locally alcohol disturbs zymogen and lysosomal membrane stability in the acinar cells and increases their sensitivity to CCK (Haber et al. 1994, Gorelick 2003). Furthermore, ethanol upregulates intra-acinar digestive enzyme synthesis at the messenger ribonucleic acid (RNA) level (Apte et al. 1995) and inhibits digestive enzyme secretion resulting in increased digestive enzyme content in the acinar cells (Ponnappa et al. 1987). Similarly to hepatic cells in the liver, alcohol is metabolized in the pancreatic acinar cells through oxidative and non-oxidative pathways. The oxidative pathway involves alcohol dehydrogenase and produces acetaldehyde and ROS and accounts for the majority of ethanol metabolism in the pancreatic acini (Gukovskaya et al. 2002). The nonoxidative pathway involves fatty acid ethyl ester (FAEE) synthases and produces FAEEs (Hamamoto et al. 1990, Haber et al. 1998). Acetaldehyde may cause acinar damage (Nordback et al. 1991), inhibit acinar secretion resulting in the accumulation of intracellular enzymes (Sankaran et al. 1985, Ponnappa et al. 1987) and its metabolism is associated with further release of free radicals (Lieber 1991). FAEEs can cause pancreatic edema, trypsinogen activation and lysosomal instability in acinar cells (Haber et al. 1993, Werner et al. 1997), disturbances in Ca^{2+}-homeostasis together with the induction of pro-inflammatory transcription factors NF-κB and activator protein-1 (Gukovskaya et al. 2002, Criddle et al. 2006). In most animal models, alcohol alone does not cause acute pancreatitis but is associated with increased susceptibility to other factors which might trigger the onset of acute pancreatitis. In an experimental model of acute pancreatitis, acinar hyperstimulation with CCK or its analog cerulein leads

to premature trypsin activation and the subsequent development of acute pancreatitis (Steer and Meldolesi 1987, Willemer et al. 1990). In rats, ethanol sensitizes the acinar cells so that CCK stimulation causes increased NF-κB and cytokine activation and even low doses of CCK can initiate acute pancreatitis (Pandol et al. 1999, Gukovskaya et al. 2004). Therefore it is possible that alcohol primarily sensitizes the pancreas and that other, as yet unidentified factors that may be genetic or environmental, are required to trigger acute pancreatitis.

4 Acute gallstone pancreatitis

The prevalence of gallstones in western populations is 10–20% and is higher in females than males (Gibney 1990, Hopper et al. 1991, Muhrbeck and Ahlberg 1995). Approximately 20% of patients with gallstones become symptomatic (Sakorafas et al. 2007), and only a small percentage of patients with asymptomatic gallstones and a slightly greater number of patients with symptomatic gallstones develop serious complications such as acute cholecystitis or acute gallstone pancreatitis (Friedman 1993). In many countries, however, gallstones are the most common cause of acute pancreatitis (Table 1). Biliary etiology for acute pancreatitis may be confirmed by ultrasound and laboratory testing (Wang et al. 1988, Lévy et al. 2005). The risk of developing acute gallstone pancreatitis increases with small stones, <5mm, cystic duct diameter of >5mm and with a large number of stones in the gallbladder (Sugiyama and Atomi 2004). Gallstones may migrate into the bile ducts, causing bile and pancreatic juice flow obstruction and subsequent reflux into the pancreatic duct. Reflux of bile and pancreatic juice leads to impaired acinar secretion, intrapancreatic NF-κB and digestive enzyme activation, and may trigger acute pancreatitis (Lightner and Kirkwood 2001, Muili et al. 2013).

5 Post-ERCP acute pancreatitis

ERCP may be performed for diagnostic and/or therapeutic indications and is associated with a risk of developing acute pancreatitis (Nebel et al. 1975). The incidence of acute pancreatitis following ERCP is approximately 3–7% with patient and procedure related risk factors (Freeman et al. 2001, Dumonceau et al. 2010). Nonsteroidal anti-inflammatory drugs have been shown to reduce the risk of post-ERCP pancreatitis (Elmunzer et al. 2008) and their periprocedural rectal administration is recommended (Dumonceau et al. 2010). In some studies, the risk for developing post-ERCP pancreati-

tis has been reduced with prophylactic use of antibiotics (Räty et al. 2001, Brand et al. 2010), suggesting a possible role of bacterial contamination of the pancreatico-biliary tree in the pathogenesis of post-ERCP pancreatitis. However, routine use of antibiotics is not recommended based on current evidence (Dumonceau et al. 2010).

6 Hypertriglyceridemia-associated acute pancreatitis

Hypertriglyceridemia is a relatively rare cause of acute pancreatitis, accounting for up to 1–10% of all cases of acute pancreatitis admissions (Fortson et al. 1995, Anderson et al. 2009). Hypertriglyceridemia is a common finding during acute pancreatitis, as the levels of serum triglycerides tend to increase during the attack (Cameron et al. 1973, Dominguez-Munoz et al. 1991), but it is generally believed that significantly higher concentrations, >11mmol/L, are required to trigger acute pancreatitis (Toskes 1990), and the risk seems greatest in patients with extremely high, >20mmol/L, serum triglyceride concentrations (Sandhu et al. 2011). The pathophysiologic mechanisms by which elevated serum triglyceride concentrations can cause acute pancreatitis are not clear. It is thought that hypertriglyceridemia may cause capillary plugging leading to local ischemia and that hydrolysis of serum triglycerides by pancreatic lipase causes accumulation of free fatty acids causing local pancreatic injury (Havel 1969, Saharia et al. 1977, Morita et al. 1998).

7 Hypercalcemia-associated acute pancreatitis

Hypercalcemia is considered a rare cause of acute pancreatitis. The most common etiology of hypercalcemia is hyperparathyroidism, usually primary, but it can also be associated with renal failure, paraneoplastic syndromes and bone metastases or even iatrogenic causes such as intravenous calcium administration during cardiac surgery (Fernandez-del Castillo et al. 1991, Lafferty 1991). Hypercalcemia is often asymptomatic, but can cause a multitude of disorders in many organ systems including the cardiovascular, neuropsychological, gastrointestinal and renal systems. The risk of developing acute pancreatitis, however, seems to be only marginally increased in patients with primary hyperparathyroidism (Bai et al. 2012) and less than one percent of all cases of acute pancreatitis may be associated with primary hyperparathyroidism (Prinz and Aranha 1985). The concentration of serum calcium that may trigger acute pancreatitis and the pathogenic mechanism by which hypercalcemia causes acute pancreatitis are unclear. In animal models, intravenous administration of calcium leads to decreased pancreatic

secretion and accumulation of zymogen granules (Frick et al. 1995) and may lead to acute pancreatitis (Frick et al. 1994, Mithofer et al. 1995).

8 Infections as the cause of acute pancreatitis

The role of infections in causing acute pancreatitis is controversial. No single pathogen has been reported as regularly causing acute pancreatitis, but several bacteria, fungi, and especially viruses have been associated with acute pancreatitis, though mostly in case reports (Parenti et al. 1996). Besides mycoplasma pneumoniae, which have been suggested as rarely causing acute pancreatitis in association with lower respiratory infections (Mardh and Ursing 1974, Nakagawa et al. 2009), and tuberculosis, which may present with granulomatous infections in most organs including the pancreas (Vafa et al. 2013), viral infections, while still extremely rare, seem to account for the majority of cases of infection-triggered acute pancreatitis. The most frequently reported viruses that may cause acute pancreatitis are enteroviruses, mumps virus, hepatitis viruses, and cytomegalovirus. Enteroviruses typically cause mild respiratory or gastrointestinal infections but some, most importantly Coxsackie B -viruses, have been reported in several case reports to cause acute pancreatitis (Fechner et al. 1963, Lal et al. 1988, Coplan et al. 1996, Chrysos et al. 2004, Dettmeyer et al. 2006). Mumps virus typically causes parotitis and orchitis and has been identified as rarely causing acute pancreatitis (Witte and Schanzer 1968, Naficy et al. 1973, Feldstein et al. 1974, Vanlioglu and Chua 2011, Yung et al. 2011). Mumps infections are now rare due to extended vaccination programs. Acute pancreatitis may present during acute viral hepatitis caused by hepatitis A, B or E -viruses (Mishra et al. 1999, Jaroszewicz et al. 2005, Jain et al. 2007, Bhagat et al. 2008). Also, recurring hepatitis B infection after liver transplantation together with immunosuppressive medication may be associated with the development of acute pancreatitis (Alexander et al. 1988). Cytomegalovirus typically causes mononucleosis, but may lead to more severe infections in immunosuppressed patients, and has been associated with the development of acute pancreatitis (Parham 1981, Iwasaki et al. 1987, Wilcox et al. 1990, Osiro et al. 2012).

8.1 Helicobacter pylori

Since its discovery in 1984 (Marshall and Warren 1984), Helicobacter pylori has been identified as one of the most common chronic infections worldwide with a prevalence varying between 15 and 90% depending on the country and cohort studied (Brown

2000). It colonizes the gastric mucosa, can cause chronic gastritis and increases the risk for gastric and duodenal ulcers (Chan and Leung 2002) and gastric cancer (Marshall and Windsor 2005). Helicobacter pylori infection has been associated with increased pancreatic exocrine secretion (Dominguez-Munoz and Malfertheiner 2001) and may be associated with increased risk of pancreatic cancer (Trikudanathan et al. 2011) and the development of autoimmune pancreatitis (Kountouras et al. 2005a, Kountouras et al. 2005b). A peptide identified in the serum of most patients with autoimmune pancreatitis has been found to be homologous with an amino acid sequence of a plasminogen-binding protein of Helicobacter pylori and the serum of these patients had IgG antibodies against this protein (Frulloni et al. 2009). Furthermore, Helicobacter pylori infection has been associated with an increase in the local expression of proinflammatory cytokines (Lagunes-Servin et al. 2013) and slightly increased serum C-reactive protein concentrations (Oshima et al. 2005, Jackson et al. 2009), although not in all studies (Brenner et al. 1999). However, the association with Helicobacter pylori and alcohol induced acute pancreatitis has not been previously studied.

8.2 Enteroviruses

Enteroviruses are RNA viruses belonging to the family of picornaviruses. They are further divided into Coxsackie A and B viruses, enteric cytopathogenic human orphan (ECHO) viruses, polioviruses and numbered serotypes (e.g. enterovirus 70), comprising more than 100 serotypes. The spectrum of diseases caused by enteroviruses in humans ranges from mild respiratory and gastrointestinal infections to severe diseases such as aseptic meningitis, encephalitis and perimyocarditis. Enteroviruses may even cause persistent infections in immunocompromised patients (McKinney et al. 1987). Elevated serum enterovirus antibodies have been reported in patients with acute pancreatitis (Capner et al. 1975, Arnesjo et al. 1976, Imrie et al. 1977). Besides being associated rarely with acute pancreatitis, enteroviruses may have a role in the development of type 1 diabetes (Tauriainen et al. 2011). In animal studies, enteroviruses can cause acute pancreatitis and combined with an alcohol diet, the course of the disease is more severe and even typically avirulent strains might trigger severe acute pancreatitis (Jerrells et al. 2003).

9 Other causes of acute pancreatitis

Blunt or penetrating abdominal trauma can lead to the development of acute pancreatitis, most commonly seen in children (Akhrass et al. 1997, Kao et al. 2003, Lautz et al. 2011). Iatrogenic trauma, such as surgery or biopsies, may also trigger acute pancreatitis (Lightwood et al. 1976, Eloubeidi et al. 2006). Many common drugs have been suggested as a possible cause of acute pancreatitis although the risk seems low considering their widespread use (Nitsche et al. 2012). Furthermore, a majority of the cases previously considered idiopathic could be caused by bile microlithiasis unidentified in abdominal ultrasound or by sphincter of Oddi dysfunction (Coyle et al. 2002, Wilcox et al. 2006). Hereditary pancreatitis is rare and most commonly associated with mutations in the serine protease 1 gene that encodes for cationic trypsinogen (Howes et al. 2004). Other known mutations associated with hereditary pancreatitis are those of SPINK1 gene and cystic fibrosis transmembrane conductance regulator gene (Chandak et al. 2004, Keiles and Kammesheidt 2006, Joergensen et al. 2010).

10 Classifying and predicting the severity of acute pancreatitis

Classifying and predicting the severity of acute pancreatitis enables more accurate prognosis, may help clinicians determine a course of treatment and aids comparing different treatment modalities. The clinical course of acute pancreatitis is most importantly determined by the development of local pancreatic and/or systemic complications. The majority of deaths occurring during the first two weeks are caused by severe systemic inflammatory reactions leading to multi-organ failure, while late mortalities are usually associated with sepsis related multi-organ failure caused by infectious complications, such as pancreatic abscesses (Renner et al. 1985, Mutinga et al. 2000, Carnovale et al. 2005). Known risk-factors for severe pancreatitis are chronic alcohol consumption (Papachristou et al. 2006), diabetes (Shen et al. 2012a), obesity (Chen et al. 2012), and possibly chronic renal failure (Pitchumoni et al. 1996). A certain gene allele encoding monocyte chemotactic protein-1 has been associated with increased risk of severe pancreatitis (Papachristou et al. 2005). Persistent (>48h) SIRS is associated with the development of multi-organ failure and mortalities (Johnson and Abu-Hilal 2004, Mofidi et al. 2006). In patients with severe acute pancreatitis, advanced age is a risk factor for mortality (Halonen et al. 2000, Gardner et al. 2008).

Many, both acute pancreatitis-specific and nonspecific clinical scoring systems are used to classify and/or predict the severity of acute pancreatitis. The most important scoring systems specific to acute pancreatitis are the Ranson and Glasgow scores

(Ranson et al. 1974, Imrie et al. 1978), which comprise routine clinical and laboratory parameters obtained at the time of admission and during the following 48 hours, the Balthazar score and computed tomography severity index (Balthazar et al. 1985, Balthazar et al. 1990), which score the severity of acute pancreatitis according to computed tomography morphology, and the original Atlanta classification introduced in 1992 and the revised Atlanta classification introduced in 2012 (Bradley 1993, Banks et al. 2013), which combine clinical, imaging, and laboratory parameters in establishing the severity of acute pancreatitis. The most important scoring systems used in measuring and predicting the severity of acute pancreatitis but not specific to the disease, are the Acute Physiology and Chronic Health Examination II (APACHE-II) (Knaus et al. 1985), which is a multi-parameter monitoring and severity-of-disease scoring system for critically ill patients, and the Marshall score and the Sequential Organ Failure Assessment (SOFA) score (Marshall et al. 1995, Vincent et al. 1996), which assess the incidence and severity of organ failure in one or more organ systems. Many other scoring systems as well as the use of certain biochemical markers such as hematocrit, C-reactive protein, procalcitonin and concentrations of some cytokines, such as IL-6 and hepatocyte growth factor, have been proposed for assessing and predicting the severity of acute pancreatitis (Alsfasser et al. 2013, Nieminen et al. 2014). According to the Harmless Acute Pancreatitis Score, absence of abdominal guarding or rebound tenderness, normal serum creatinine level and normal hematocrit level at the time of admission suggest a mild course of acute pancreatitis (Lankisch et al. 2009b).

Perhaps the most widely used system for assessing the severity of acute pancreatitis is the Atlanta classification. In the original Atlanta classification (Bradley 1993), acute pancreatitis is considered to be either mild, or, when local pancreatic and/or systemic complications develop, severe. Local complications include acute fluid collections, pancreatic necrosis, acute pseudocyst and pancreatic abscess. Systemic complications are defined as the development of shock (systolic blood pressure < 90mmHg), pulmonary insufficiency (arterial blood $p0_2 \leq 8.0$kPa), renal failure (creatinine ≥ 170µmol/L after rehydration), gastrointestinal bleeding (> 500ml in 24 hours), disseminated intravascular coagulation (platelet count $\leq 100*10^9$/L, fibrinogen < 1.0g/L and fibrin-split products > 80µg/L) and/or severe metabolic disturbances (calcium ≤ 1.87mmol/L). In the revised Atlanta classification (Banks et al. 2013), the severity of acute pancreatitis is classified as mild in the absence of organ failure and local pancreatic or systemic complications, moderately severe when associated with transient organ failure, local pancreatic and/or systemic complications, and severe when associated with persistent organ failure of one or more organ systems. Organ failure is defined according to the modified Marshall scoring system (Marshall et al. 1995) and described as transient if the organ failure resolves within 48 hours and persistent when lasting over 48 hours.

Local complications are defined as acute peripancreatic fluid collections and acute necrotic collections. The severity of post-ERCP acute pancreatitis is classified as mild when requiring hospitalization for 2–3 days, moderate when requiring hospitalization for 4–10 days, and severe when requiring hospitalization for over 10 days, interventions, or when local pancreatic complications, such as hemorrhage, necrosis or pseudocyst, develop (Cotton et al. 1991).

11 Alcohol and lipid metabolism

Heavy alcohol consumption is associated with many changes in the lipid metabolism. Together with metabolic syndrome, alcohol, by increasing the synthesis of triglycerides in the liver, is a leading cause of hypertriglyceridemia and associated with increased serum HDL-cholesterol and decreased serum LDL-cholesterol concentrations (Sane et al. 1984, Hata and Nakajima 2000, Brien et al. 2011). Certain essential fatty acids, linoleic acid and alphalinolenic acid, cannot be synthesized in the human body and must be obtained in the diet. Deficiencies in these essential fatty acids may, for example, cause impaired wound healing. Linoleic acid and alphalinolenic acid may be further elongated to form longer chain polyunsaturated fatty acids, some of which, most importantly arachidonic acid, serve as precursors to various eicosanoids which regulate many cellular responses such as those associated with inflammation (Calder 2008). Chronic alcohol consumption may be associated with malnutrition (Sarin et al. 1997) and changes in the serum fatty acid profile, such as deficiencies in polyunsaturated fatty acids and increased amounts of saturated and monounsaturated fatty acids (Ristic-Medic et al. 2013). Elevations in the serum triglyceride concentrations could increase the risk for acute pancreatitis, while HDL-cholesterol and polyunsaturated fatty acids of the n-3-series seem to have anti-inflammatory and polyunsaturated fatty acids of the n-6-series proinflammatory properties (James et al. 2000, Sundrarjun et al. 2004, Murphy and Woollard 2010, Bugdaci et al. 2011, de Batlle et al. 2012). Alterations in the serum lipid concentrations could therefore be associated with increased risk of acute pancreatitis.

AIMS OF THE STUDY

1. To evaluate whether heavy users of alcohol with concomitant Helicobacter pylori infection are at increased risk of developing alcohol induced acute pancreatitis and whether the course of acute pancreatitis is more severe in these patients.
2. To evaluate whether acute or subacute enterovirus infection is associated with the onset of acute pancreatitis in patients with heavy alcohol consumption.
3. To measure the serum lipid concentrations and fatty acid profile of patients with alcohol induced acute pancreatitis during acute pancreatitis and after follow-up.
4. To measure whether serum lipid concentrations during alcohol induced acute pancreatitis are associated with the severity of acute pancreatitis.

MATERIAL AND METHODS

1 Hypotheses and study setting

Study 1

Hypothesis: patients with Helicobacter pylori infection are more susceptible to developing alcohol induced acute pancreatitis

The prevalence of Helicobacter pylori infection was ascertained in 50 patients suffering their first alcohol induced acute pancreatitis and compared to that of 50 control subjects with similar high alcohol consumption and no history of acute pancreatitis.

Study 2

Hypothesis: patients with Helicobacter pylori infection suffer from more severe acute pancreatitis

The prevalence of Helicobacter pylori infection in 231 patients hospitalized for acute pancreatitis was measured. All etiologies were included, as were patients suffering their first or recurrent episode of acute pancreatitis. The course of acute pancreatitis, its severity, development of complications, need for intensive care unit treatment or surgery and mortalities were recorded and compared between patients with and without Helicobacter pylori infection.

Study 3

Hypothesis: acute or subacute enteroviral infection triggers the onset of alcohol induced acute pancreatitis

Evidence of simultaneous or preceding enteroviral infection was ascertained from 40 patients hospitalized for their first alcohol induced acute pancreatitis and compared to that of 40 control subjects with similar heavy alcohol consumption but no history of acute pancreatitis. In addition, biopsy samples from one patient with acute pancreatitis

and nine patients with chronic pancreatitis were analyzed for evidence of persistent enteroviral genome in the pancreas and compared to that of control samples obtained from 10 patients operated for pancreatic carcinoma.

Study 4

Hypothesis: the serum lipid and fatty acid profile is altered in patients with alcohol induced acute pancreatitis

The serum lipid and fatty acid profiles were measured from 19 patients hospitalized for their first alcohol induced acute pancreatitis and compared to those of 20 controls with similar heavy alcohol consumption and no history of acute pancreatitis. Additionally, late follow-up samples obtained 18-24 months after hospitalization were analyzed from 16 patients.

Study 5

Hypothesis: serum lipid concentrations react during acute pancreatitis and are associated with the severity of the disease

The serum lipid concentrations were measured from 233 patients hospitalized for acute pancreatitis. All etiologies were included as were patients suffering from their first or a recurrent episode of acute pancreatitis. Samples obtained within a few days of admission as well as follow-up samples during the hospitalization were analyzed and the association of serum lipid concentrations and the severity of acute pancreatitis were measured.

2 Recruitment of patients and controls

The patients and controls in Studies 1, 3 and 4 were recruited between January 2001 and November 2005. Patients hospitalized for their first alcohol induced acute pancreatitis and treated in Tampere University Hospital were included. The control material comprised heavy drinkers of alcohol recruited from an alcohol detoxification center during their stay there. None of the controls had a history of acute pancreatitis. Both the patients and controls had been previously prospectively recruited for a separate study. The pancreas biopsy samples in Study 3 were obtained during surgery performed between December 2001 and March 2006.

The patient material in Studies 2 and 5 comprised patients hospitalized for acute pancreatitis and treated in Tampere University Hospital between August 1995 and September 1999. The material had been collected in a separate prospective study (Nordback et al. 2001). All etiologies of acute pancreatitis were included as were patients suffering their first or a recurrent episode of acute pancreatitis.

3 Sample and data collection

Acute pancreatitis was diagnosed when the patient reported acute epigastric pain that led to hospitalization and clinical signs consistent with acute pancreatitis together with serum amylase activity of at least three times the upper normal range and elevated serum inflammation markers (C-reactive protein concentration and leukocyte count). Abdominal ultrasound was performed on every patient to confirm the diagnosis and to detect gallstones. If the diagnosis was uncertain or pancreatic complications were suspected, abdominal computed tomography was performed.

The length of hospitalization, development of complications, need for surgery or treatment in the intensive care unit, and mortalities were recorded. Acute pancreatitis was considered severe when it met the original Atlanta criteria (Bradley 1993); the patient was classified as having severe pancreatitis when local pancreatic complications, necrosis, abscess or pseudocyst developed, and in the presence of systemic complications such as shock, pulmonary insufficiency, renal failure and severe metabolic disturbances.

Basic demographic and clinical data was collected during the hospitalization or stay at the alcohol detoxification center. Acute pancreatitis was considered to be alcohol induced (Nordback et al. 2007) when the patient reported high alcohol intake (288g ethanol/week in males and 192g ethanol/week in females) in the Alcohol Use Disorders Identification Test (AUDIT) or in a thorough interview with the patient or the family and when other etiologies were excluded by laboratory testing and imaging. Gallstone pancreatitis was diagnosed when gallstones were detected in imaging and no other predisposing factor was detected. Acute pancreatitis caused by hypertriglyceridemia, hypercalcemia or other metabolic etiology was detected with laboratory testing, while post-ERCP pancreatitis or pancreatitis due to surgery or trauma was diagnosed when acute pancreatitis followed the aforementioned intervention or other insult to the pancreas. Some of the patients were classified as having multiple predisposing factors, such as heavy alcohol consumption and gallstones. In some patients, no clear etiology was found and these patients were classified as having idiopathic acute pancreatitis. The patients were treated according to clinic standards. To detect complications of

acute pancreatitis, abdominal computed tomography was performed when clinically indicated.

In all the studies, the serum samples in both patients and controls were obtained during the first days of hospitalization or stay at the alcohol detoxification center respectively. The serum samples were stored frozen during the interval between their acquisition and analysis and were thawed just prior to analysis. The long storage time should not have altered the results to any clinically significant extent (Tiedink and Katan 1988, Blaser et al. 1991, Zivkovic et al. 2009, McLeish et al. 2012). In Study 3 the pancreas biopsy samples were immediately frozen fresh with liquid nitrogen and stored frozen until analysis.

4 Laboratory analysis

Helicobacter pylori analysis

The prevalence of Helicobacter pylori infection was obtained by measuring the immunoglobulin (Ig) G antibodies from the serum samples using a commercial Enzygnost Anti-Helicobacter pylori II/IgG enzyme immunoassay kit (Dade Behring) with a cut-off value of 10IU/ml. The manufacturer's reported sensitivity and specificity were 93.4% and 98.8% respectively.

Enteroviral analysis

Evidence of acute enterovirus viremia was analyzed from the serum by detecting enteroviral RNA using a highly sensitive reverse transcription polymerase chain reaction (RT-PCR) method which amplifies a sequence common to all known enterovirus serotypes. The pancreas biopsy samples were disrupted and homogenized using the TissueRuptor homogenizator (Qiagen, Hilden, Germany). RNA was extracted from the homogenized sample by RNeasy mini kit (Qiagen) and analyzed similarly. Subacute and previous infections were identified by measuring the IgM, IgG and IgA antibodies from the serum samples. IgM class enterovirus antibodies were measured against a mixture of three enterovirus antigens: coxsackie virus B3, coxsackie virus A16 and echovirus 11 using capture EIA. IgG and IgA class antibodies were measured against a synthetic enterovirus peptide antigen which is a common epitope for several enteroviruses. The samples were considered positive when the antibody titers were ≥15 EIU.

Serum lipid and fatty acid profile analysis

The serum fatty acid profile was measured using gas chromatography (Hewlett-Packard 5890A gas chromatograph). The serum concentrations of total cholesterol, HDL-cholesterol, and triglycerides were measured enzymatically. Serum LDL-cholesterol concentrations were calculated using the Friedewald formula.

5 Demographic data of patients and controls

The demographic data of the patients in Studies 1–5 is presented in Table 2.

Table 2. Demographic data of patient and control material.

	Male/female N	Median age (range)	Median BMI (range)	Median AUDIT
Study 1. Helicobacter pylori in alcohol induced acute pancreatitis				
Alcohol (n=50)	38/12	45 (18–73)	26 (19–34)	22 (5–38)
Controls (n=50)	31/19	46 (21–66)	25 (16–34)	28 (15–36)
Study 2. There is hardly any association between Helicobacter pylori infection and the severity of acute pancreatitis				
Alcohol (n=130)	111/19	44 (22–68)	N/A	N/A
Gallstone (n=46)	23/23	65 (25–90)	N/A	N/A
Idiopathic (n=38)	22/16	53 (17–79)	N/A	N/A
Other (n=17)	12/5	60 (30–83)	N/A	N/A
Study 3. Is alcoholic pancreatitis associated with enteroviral infection?				
Alcohol (n=40)	32/8	47 (18–73)	26 (19–34)	22 (5–38)
Controls (n=40)	25/15	46 (22–66)	26 (16–34)	29 (15–36)
Pancreatitis biopsy (n=10)	4/6	52 (37–71)	N/A	N/A
Biopsy control (n=10)	5/5	72 (36–81)	N/A	N/A
Study 4. Serum lipid and fatty acid profiles are highly changed in patients with alcohol induced acute pancreatitis				
Alcohol (n=19)	15/4	45 (28–60)	25 (20–33)	20 (9–35)
Control (n=20)	16/4	47 (20–56)	25 (19–34)	30 (18–39)
Study 5. Serum lipid levels are associated with the severity of acute pancreatitis				
Alcohol (n=131)	111/20	44 (22–68)	N/A	N/A
Gallstone (n=48)	23/25	64 (25–90)	N/A	N/A
Idiopathic (n=36)	20/16	53 (17–79)	N/A	N/A
Other (n=18)	12/6	58 (25–80)	N/A	N/A

6 Statistical testing

All the statistical testing was performed with SPSS 16.0 statistical software. The Chi Square test and Fishers Exact test were used to compare categorical data between groups. The Mann-Whitney U-test and Wilcoxon signed-rank test were used to compare

the medians of nonparametrical scale variables between groups. Pearsons correlation coefficient was used to detect and quantify linear correlation between scale variables. All statistical tests were two-sided. P values ≤0.05 were considered statistically significant.

7 Ethical aspects

None of the patients or controls were subjected to any intervention apart from the routine care of acute pancreatitis for these studies. The studies were performed according to the Helsinki Declaration and were approved by the Ethics Committee of Tampere University Hospital. Written informed consent was obtained from every patient and control subject.

RESULTS

1 The prevalence of Helicobacter pylori in patients with acute pancreatitis

There was no significant difference in the prevalence of positive antibody titers between patients hospitalized for their first alcohol induced acute pancreatitis and controls with similar heavy alcohol consumption but no history of acute pancreatitis. In Study 1, 10/50 (20%, 95% confidence interval [CI] 9–31%) patients and 15/50 (30%, 95% CI 17–43%) controls tested positive for Helicobacter pylori, p=0.248. In Study 2, the overall prevalence of Helicobacter pylori infection in patients with acute pancreatitis was 45% (95% CI 39–52%). The rate of seroprevalence was similar in all etiologies of acute pancreatitis; 54/130 (42%, 95% CI 33–50%) in patients with alcohol induced acute pancreatitis, 25/46 (54%, 95% CI 39–69%) in patients with gallstone pancreatitis, 18/38 (47%, 95% CI 31–64%) in patients with idiopathic acute pancreatitis and 8/17 (47%, 95% CI 21–74%) in patients with other causes of acute pancreatitis. There were no significant differences according to gender in the seroprevalence of Helicobacter pylori in patients with alcohol induced acute pancreatitis in Studies 1 and 2, but was higher in males than females in patients with acute gallstone pancreatitis, 18/23 (78%) vs. 7/23 (30%), respectively, p=0.001, in Study 2.

Patients with acute pancreatitis and Helicobacter pylori infection tended to be somewhat older than those testing negative for Helicobacter pylori. In Study 1, the median age was 48 (range 32–73) in patients testing positive and 44 (range 18–60) in patients testing negative for Helicobacter pylori infection, p=0.097. In Study 2, the overall median age was 50 (range 25–90) in patients testing positive and 45 (range 17–80) in patients testing negative for Helicobacter pylori infection, p=0.001, 47 (range 25–63) vs. 42 (range 22–68) in patients with alcohol induced acute pancreatitis, p=0.043, 69 (range 30–90) vs. 54 (range 25–80) in patients with acute gallstone pancreatitis, p=0.004, 53 (range 31–79) vs. 53 (range 17–66) in patients with idiopathic acute pancreatitis, p=0.456 and 61 (range 36–83) vs. 56 (range 30–75) in patients with acute pancreatitis due to other causes, p=0.456 respectively.

In Study 2, 152 (66%) patients had their first acute pancreatitis and 73 (32%) had a recurrent acute pancreatitis. The information was missing for six patients. There

were no statistically significant differences in the seroprevalence of Helicobacter pylori between patients with their first or recurrent acute pancreatitis (Table 3).

Table 3. Distribution of Helicobacter pylori seroprevalence in patients suffering their first or recurrent acute pancreatitis in Study 2.

Etiology	Helicobacter pylori N (%; 95% CI)
First acute pancreatitis	
All (n=152)	73 (48%; 40–56%)
Alcohol (n=75)	32 (43%; 31–54%)
Gallstone (n=40)	23 (58%; 41–74%)
Idiopathic (n=24)	12 (50%; 28–72%)
Other (n=13)	6 (46%; 15–78%)
Recurrent acute pancreatitis	
All (n=73)	31 (42%; 31–54%)
Alcohol (n=53)	22 (42%; 28–55%)
Gallstone (n=4)	1 (25%; 0–100%)
Idiopathic (n=13)	6 (46%; 15–78%)
Other (n=3)	2 (67%; 0–100%)

2 Helicobacter pylori and the severity of acute pancreatitis

In Study 1, patients testing positive for Helicobacter pylori had statistically significantly longer hospital stay compared to patients testing negative, 11 days vs. 6 days, p=0.013. However, in Study 2 with a larger patient cohort, no statistically significant differences in the length of hospital stay, severity of acute pancreatitis according to the original Atlanta criteria, development of individual complications, need for treatment in the intensive care unit or mortalities were observed. For detailed information on the course of acute pancreatitis and its complications according to Helicobacter pylori seropositivity in Studies 1–2, see Table 4.

Table 4. The course, severity and individual complications of acute pancreatitis in relation to Helicobacter pylori seropositivity.

Etiology of acute pancreatitis	Length of hospitalization Median days (range)	Atlanta[1] severe N (%)	Pancreatic necrosis N (%)	Pancreatic abscess N (%)	Pancreatic pseudocyst N (%)	ICU-treatment N (%)	Mortalities N (%)
Study 1. Helicobacter pylori in alcohol induced acute pancreatitis							
Alcohol							
H.pylori+ (n=10)	11 (6–25)*	4 (40%)	2 (20%)	1 (10%)	1 (10%)	0	0
H.pylori- (n=40)	6 (3–47)*	6 (15%)	4 (10%)	0	3 (8%)	2 (5%)	0
Study 2. There is hardly any association between Helicobacter pylori infection and the severity of acute pancreatitis							
All							
H.pylori+ (n=105)	8 (3–150)	28 (27%)	18 (17%)	4 (4%)	5 (5%)	20 (19%)	8 (8%)
H.pylori- (n=126)	8 (3–46)	36 (29%)	26 (21%)	3 (2%)	10 (8%)	14 (11%)	4 (3%)
Alcohol							
H.pylori+ (n=54)	8 (3–150)	15 (28%)	10 (19%)	2 (4%)	3 (6%)	8 (15%)	3 (6%)
H.pylori- (n=76)	7 (3–46)	25 (33%)	20 (26%)	2 (3%)	5 (7%)	10 (13%)	2 (3%)
Gallstones							
H.pylori+ (n=25)	8 (3–36)	6 (24%)	4 (16%)	2 (8%)	0	6 (24%)	2 (8%)
H.pylori- (n=21)	10 (3–21)	3 (14%)	1 (5%)	0	2 (10%)	1 (5%)	1 (5%)
Idiopathic							
H.pylori+ (n=18)	8 (3–29)	4 (22%)	2 (11%)	0	1 (6%)	3 (17%)	2 (11%)
H.pylori- (n=20)	6 (3–18)	3 (15%)	2 (10%)	1 (5%)	2 (10%)	0	0
Other							
H.pylori+ (n=8)	13 (7–41)	3 (38%)	2 (25%)	0	1 (13%)	3 (38%)	1 (13%)
H.pylori- (n=9)	13 (5–41)	5 (56%)	3 (33%)	0	1 (11%)	3 (33%)	1 (11%)

Atlanta1 = According to the original Atlanta classification (Bradley 1993)
H.pylori = Helicobacter pylori
ICU = Intensive care unit
* p≤0.05

3 Enterovirus infections in acute pancreatitis

In Study 3, no enterovirus RNA was detected by RT-PCR in the serum of any patients suffering their first alcohol induced acute pancreatitis or in any control subjects. IgM class antibodies tested positive in 5/40 (13%) vs. 4/40 (10%), p=0.723, IgG class antibodies tested positive in 15/40 (38%) vs. 19/40 (48%), p=0.366 and IgA class antibodies in 25/40 (63%) vs. 33/40 (83%), p=0.045, in patients and controls respectively. The severity of acute pancreatitis or length of hospitalization was not associated with enteroviral antibodies.

Three pancreatic biopsy samples from patients with pancreatic carcinoma and two biopsy samples from patients with chronic pancreatitis tested positive for enteroviral RNA. The etiology of chronic pancreatitis was alcohol consumption in both patients. The tissue specimen from the patient with alcohol induced acute pancreatitis was negative for enteroviral RNA.

4 Serum fatty acid profile in acute pancreatitis

The results of Study 4, the total proportions of serum saturated, monounsaturated and polyunsaturated fatty acids and the proportions of individual fatty acids in patients with their first alcohol induced acute pancreatitis – during hospitalization and after 18–24 month follow-up – and those of controls with similar high alcohol use but no history of acute pancreatitis, are presented in table 5. Patients had statistically significantly lower proportions of saturated C14:0 fatty acids, polyunsaturated C18:2, C18:3 and C20:3 fatty acids of the n-6-series and C18:3 fatty acids of the n-3-series than controls. In contrast, patients had higher percentages of saturated C16:0 fatty acids and monounsaturated fatty acids C18:1 of the n-9-series than controls. Mead acid, C20:3 of the n-9-series, a marker of essential fatty acid deficiency, was lower in patients than in controls. After the 18-24 month follow-up, the proportions of C14:0, C20:3n6 and C18:3n3 remained significantly lower and the proportion of C20:3n9 significantly higher in patients than in controls.

Table 5. Proportions of serum fatty acids in patients with alcohol induced acute pancreatitis during hospitalization, after 18–24 month follow-up, and controls with similar heavy alcohol consumption but no history of pancreatitis.

	Patients during hospitalization % (range)	Patients after 18–24 month follow-up % (range)	Non-pancreatitis controls % (range)
Saturated fatty acids	30.73 (28.04–35.43)	29.07 (25.74–36.22)	29.60 (27.21–39.16)
Myristic acid C14:0	0.62** (0.29–1.67)	0.61* (0.35–1.87)	0.85 (0.59–2.12)
Palmitic acid C16:0	23.39* (20.85–27.01)	22.02 (18.31–26.21)	22.10 (19.34–30.15)
Stearic acid C18:0	6.85 (5.54–7.97)	6.29* (5.64–8.14)	7.12 (5.48–8.19)
Monounsaturated fatty acids	33.05** (28.74–43.17)	30.89 (25.95–38.09)	29.79 (24.52–38.11)
Palmitoleic acid C16:1n7	3.97 (2.82–6.79)	3.78 (2.40–5.75)	4.00 (2.65–8.28)
Oleic acid C18:1n9	28.52** (25.52–37.81)	27.38 (22.80–33.48)	25.45 (21.62–33.21)
n-6 polyunsaturated fatty acids	25.76 (16.92–32.70)	30.87 (22.18–34.11)	29.38 (19.25–36)
Linoleic acid C18:2	18.03* (11.58–23.78)	21.84 (16.05–27.39)	21.89 (13.36–28.73)
Gammalinolenic acid C18:3	0.25*** (0.11–0.46)	0.33 (0.15–0.56)	0.40 (0.27–0.83)
Homogammalinolenic acid C20:3	1.35* (0.99–2.25)	1.37* (0.72–1.85)	1.49 (1.08–2.10)
Arachidonic acid C20:4	6.30 (2.96–7.87)	5.90 (3.67–8.64)	5.00 (3.20–7.60)
n-3 polyunsaturated fatty acids	4.27 (2.87–8.93)	4.47 (3.27–8.54)	4.49 (3.91–7.27)
Alphalinolenic acid C18:3	0.50*** (0.36–1.02)	0.73* (0.39–1.06)	0.90 (0.54–1.09)
Eicosapentaenoic acid C20:5	0.90 (0.44–3.59)	1.06 (0.41–2.90)	1.25 (0.77–2.35)
Docosapentaenoic acid C22:5	0.67 (0.42–0.89)	0.62 (0.41–0.81)	0.65 (0.49–0.81)
Docosahexaenoic acid C22:6	2.28 (1.28–3.88)	2.15 (1.39–4.46)	1.82 (1.15–3.46)
Mead acid C20:3n9	0.14* (0.10–0.44)	0.15* (0.07–0.41)	0.21 (0.10–0.46)

*p≤0.05, ** p<0.01, ***p<0.001 when compared to controls

5 Serum lipids in acute pancreatitis

The concentrations of serum total cholesterol, LDL-cholesterol, and HDL-cholesterol react during acute pancreatitis. In Study 4, the median serum total cholesterol concentration was 3.31mmol/L (range 1.96–5.22mmol/L) in patients suffering their first alcohol induced acute pancreatitis measured on the second day of hospitalization and 5.06mmol/L (range 3.18–6.99mmol/L) in non-pancreatitis controls with similar heavy alcohol consumption but no history of acute pancreatitis, $p<0.001$. Similarly, the median serum HDL-cholesterol concentrations were 0.76mmol/L (range 0.38–1.18mmol/L) vs. 1.23mmol/L (range 0.43–2.69mmol/L), $p<0.001$, and the median serum LDL-cholesterol concentrations were 1.84mmol/L (range 0.87–3.87mmol/L) vs. 3.01mmol/L (range 1.84–4.03mmol/L), $p<0.001$, in patients and controls, respectively. The serum triglyceride concentrations were not significantly altered: 1.03mmol/L (range 0.57–4.13mmol/L) vs. 1.28mmol/L (range 0.64–4.88mmol/L), $p=0.242$, in patients and controls, respectively. In these same patients after 18–24 month follow-up, no statistically significant differences in the serum total cholesterol, HDL-cholesterol, LDL-cholesterol or triglyceride concentrations were observed when compared to controls.

In Study 5, 203 out of 233 (87%) patients' serum samples were available from the first two days of hospitalization. The results of the lipid analysis are shown in Table 6. The concentrations of serum total cholesterol, HDL-cholesterol and LDL-cholesterol measured during the first two days of hospitalization were statistically significantly lower in patients who developed severe acute pancreatitis according to the Atlanta criteria when compared to those of the other patients; 3.20mmol/L (range 1.10–9.40mmol/L) vs. 3.80mmol/L (range 1.70–8.60mmol/L), $p<0.001$, 0.72mmol/L (range 0.07–1.74mmol/L) vs. 1.05mmol/L (range 0.12–2.55mmol/L), $p<0.001$, and 1.60mmol/L (range (0.34–4.69mmol/L) vs. 2.14mmol/L (range 0.29–6.21mmol/L), $p<0.001$, respectively. The serum triglyceride concentrations tended to be higher in patients with severe pancreatitis, 1.20mmol/L (range 0.40–12.66mmol/L) vs. 0.95mmol/L (range 0.33–8.47mmol/L), but this difference did not reach statistical significance, $p=0.057$. Similarly, in patients with alcohol induced acute pancreatitis, the concentrations of serum total cholesterol, HDL-cholesterol and LDL-cholesterol, measured within two days after hospitalization, were significantly lower in patients who developed severe pancreatitis, $p=0.028$, $p=0.001$ and $p=0.009$ respectively. In other patient subgroups, no significant differences were observed, but when all patients with non-alcoholic acute pancreatitis were analyzed as a single group, the serum total cholesterol and LDL-cholesterol concentrations were significantly lower in patients who developed severe pancreatitis, $p=0.010$ and $p=0.032$ respectively. Low serum total

cholesterol, HDL-cholesterol and LDL-cholesterol concentrations measured during the first two days of hospitalization were associated with in-hospital mortalities in all patients, p=0.09, p=0.002 and p=0.008 respectively, and, in subgroup analysis, in patients with alcohol induced acute pancreatitis, p=0.038, p=0.003 and p=0.005 respectively, but not in other patient subgroups. Furthermore, the serum total cholesterol, HDL-cholesterol, LDL-cholesterol and serum triglyceride concentrations measured during the first two days of hospitalization correlated with the length of hospitalization; r=-0.161, p=0.022; r=-0.264, p<0.001; r=-0.174, p=0.013 and r=-0.147, p=0.036 respectively. In subgroup analysis, the serum HDL-cholesterol and triglyceride concentrations had a significant correlation with the length of hospitalization in patients with alcohol induced acute pancreatitis, r=-0.265, p=0.004 and r=0.206, p=0.026 respectively.

Table 6. Median serum lipid concentrations measured within two days after hospitalization in patients with acute pancreatitis.

	Total cholesterol (mmol/L)	HDL-cholesterol (mmol/L)	LDL-cholesterol (mmol/L)	Triglyceride (mmol/L)
All patients (n=203)	3.70	0.95	2.00	1.01
Mild (n=154)	3.80***	1.05***	2.14***	0.95
Severe (n=49)	3.20***	0.72***	1.60***	1.20
Alcoholic (n=117)	3.50	0.93	1.82	1.20
Mild (n=84)	3.70*	1.01**	1.92**	1.08
Severe (n=33)	3.00*	0.61**	1.41**	1.42
Gallstone (n=44)	3.80	1.01	2.33	0.75
Mild (n=36)	3.95	1.09	2.50	0.75
Severe (n=8)	3.40	0.81	2.14	0.79
Idiopathic (n=31)	3.80	1.03	2.30	0.92
Mild (n=28)	3.95	1.04	2.37	0.93
Severe (n=3)	3.00	0.71	1.65	0.89

*p≤0.05, ** p<0.01, ***p<0.001
Note: Severity is presented according to the original Atlanta classification (Bradley 1993).

DISCUSSION

Acute pancreatitis is an important cause of morbidity and mortality and, despite being relatively common, many aspects of its pathophysiology remain unclear. Several reasons make studying acute pancreatitis challenging. The retroperitoneal location of the human pancreas and complications associated with invasive procedures, such as biopsies, make it relatively inaccessible during life. Furthermore, most patients with acute pancreatitis present after a delay following the onset of symptoms making it difficult to study the initial phases of the disease. Many animal models of acute pancreatitis have been developed and much of our knowledge of acute pancreatitis and its cellular mechanisms is actually derived from animal studies. Animal models of acute pancreatitis, however, are not entirely analogous to the disease in humans.

Heavy alcohol consumption and gallstone disease are the most common causes of acute pancreatitis. In some countries, such as Finland, alcohol is the leading cause of acute pancreatitis. Despite this, surprisingly little is known about the actual mechanism by which alcohol causes acute pancreatitis and most alcoholics do not develop the disease. Furthermore, in animal studies, simply feeding animals with alcohol does not induce acute pancreatitis. It does, however, cause pancreatic irritation and makes the pancreas susceptible so that further stimulation or insult may trigger acute pancreatitis. Similar pancreatic insult after alcohol consumption has been observed even in humans (Nordback et al. 1995). This makes it tempting to speculate that a separate factor besides alcohol might also trigger acute pancreatitis in humans made susceptible by heavy alcohol consumption, but so far no such trigger-factor has been found. The objective of these studies was to identify factors associated with the onset and severity of alcohol induced acute pancreatitis. The roles of both a common chronic and a common acute infection, represented by Helicobacter pylori and enterovirus infections, and a metabolic cause, represented by the serum lipid studies, were studied.

1 Helicobacter pylori

In the studies summarized here, Helicobacter pylori infection was not more prevalent in patients suffering their first alcohol induced acute pancreatitis than in controls with similar heavy alcohol consumption but no history of acute pancreatitis, suggesting that this does not predispose to the development of alcohol induced acute pancreatitis, at least to a clinically significant extent. Neither was Helicobacter pylori infection associated with the development of severe acute pancreatitis, any of its individual complications nor the length of hospitalization in patients with alcohol induced acute pancreatitis or acute pancreatitis due to other causes. The prevalence of Helicobacter pylori infection was obtained by measuring the IgG antibodies from serum samples. Due to the retrospective setting of these studies and the use of previously collected serum samples, other modalities, such as urea breath test, stool antigen or histology analysis could not be used. Helicobacter pylori antibody titers remain elevated for several months to years after successful eradication (Veenendaal et al. 1991, Bergey et al. 2003). However, as spontaneous healing of Helicobacter pylori infection is rare (Xia and Talley 1997, Kumagai et al. 1998), serum IgG antibodies should reflect the current or recent status accurately. Furthermore, treatment with antibiotics or proton pump inhibitors does not cause false negative results in the antibody analysis. We conclude that Helicobacter pylori infection does not increase the risk of alcohol induced acute pancreatitis or affect its course.

2 Enteroviruses

Enteroviruses have been reported as rarely causing acute pancreatitis in humans, mostly in case reports. Recent animal studies have suggested that, when combined with preceding alcohol consumption, enterovirus infections could trigger acute pancreatitis. Earlier studies on the association of enteroviruses and acute pancreatitis in humans did not include modern PCR techniques nor controls with similar alcohol consumption. In the present study, no acute enterovirus viremia was detected by RT-PCR in any patients presenting with their first alcohol induced acute pancreatitis. Some patients had positive IgM antibodies related to subacute disease, but the occurrence was similar in the non-pancreatitis controls. The prevalence of positive enterovirus IgA and IgG antibody titers was high, but this was also true for the control group, and no statistically significant differences were observed between patients and controls. Surprisingly many pancreas tissue samples tested positive for enteroviral RNA in RT-PCR. We could not ascertain whether enteroviral genome was present in the pancreatic acini or the islets of

Langerhans, as reported by some authors (Ylipaasto et al. 2004, Dotta et al. 2007), and the clinical implication of this finding remains unclear. In conclusion, the rate of acute, subacute and previous enterovirus infections was not increased in patients hospitalized for their first alcohol induced acute pancreatitis when compared to alcoholics with no history of acute pancreatitis, suggesting that enterovirus infections do not trigger the acute disease in humans.

3 Serum fatty acid profile

Alcohol consumption is associated with changes in the serum fatty acid profile. Some fatty acids may promote or alleviate inflammatory reactions or even be toxic, and could therefore be associated with inflammatory diseases such as acute pancreatitis. The anti-inflammatory effects of fatty acids of the n-3-series are mediated partly by attenuation of NF-κB activation (Hassan et al. 2010, Siriwardhana et al. 2012).

We sought to measure whether the serum fatty acid profile differs between patients who developed alcohol induced acute pancreatitis and controls with similar alcohol consumption but no history of acute pancreatitis. The proportion of oleic acid, which can damage acinar cells (Yang et al. 2009) and has been used to induce experimental pancreatitis (Nordback et al. 1994), was increased in patients with alcohol induced acute pancreatitis during hospitalization but not after follow-up when compared to controls, suggesting that the changes are secondary to the acute disease. The proportion of alphalinolenic acid, an essential fatty acid of the n-3-series, was lower in patients than controls both during acute pancreatitis and after follow-up, which may be associated with increased risk of developing acute pancreatitis. The concentrations of most polyunsaturated fatty acids of the n-6-series except arachidonic acid were lower in patients with alcohol induced acute pancreatitis during the course of the disease but not after follow-up, suggesting that these changes were secondary to the acute disease, possibly due to increased cytokine production. Other authors have reported a decrease in the serum n-3-series fatty acids during allergic inflammatory reactions (Johansson et al. 2010). We conclude that alcohol induced acute pancreatitis results in many changes in the serum fatty acid profile, and that most of these changes normalize after follow-up, suggesting that they are secondary to the acute disease rather than the cause of it. The lower proportion of alphalinolenic acid that was observed both during the acute disease and after follow-up might be associated with increased risk of alcohol induced acute pancreatitis.

4 Serum lipids

Severe acute disease may alter the serum lipid concentrations (Wu et al. 2004, Jahangiri 2010), and low serum lipid concentrations have moreover been associated with worse prognosis in critically ill patients (Stachon et al. 2008a, Stachon et al. 2008b). Other authors have reported an association with low serum HDL-cholesterol concentrations and longer hospitalization in patients with acute pancreatitis (Bugdaci et al. 2011). In Study 4, significantly lower concentrations of serum total cholesterol, HDL-cholesterol, and LDL-cholesterol were observed in patients suffering their first alcohol induced acute pancreatitis during hospitalization than after follow up or in controls with similar heavy alcohol consumption, but no history of acute pancreatitis. There were no statistically significant differences in the serum triglyceride concentrations. Since the changes in the serum lipid concentrations disappear after follow-up, they seem to be secondary to the acute disease and/or its treatment. In Study 5 we report similar low concentrations of serum total cholesterol, HDL-cholesterol and LDL-cholesterol in patients with acute pancreatitis and that the finding was similar in all etiologies of acute pancreatitis. Furthermore, in Study 5, patients who later developed severe acute pancreatitis had statistically significantly lower concentrations of serum total cholesterol, HDL-cholesterol and LDL-cholesterol than other patients in samples obtained during the first days of hospitalization, thereby suggesting a prognostic value. Differences in the treatment of acute pancreatitis, such as more intensive fluid resuscitation according to disease severity, may explain some of the difference, but usually treatment is similar in all patients during the first days of the disease. The finding remained statistically significant in patients with alcohol induced acute pancreatitis, but not in other etiologies of acute pancreatitis, probably due to limited sample size.

The mechanism causing lower serum cholesterol concentrations in severe disease is somewhat unclear. It has been suggested that the rate of lipoprotein synthesis in the liver, general catabolic metabolism and inflammatory activation lead to lower concentrations of serum lipids. HDL-cholesterol has been reported to have anti-inflammatory properties (Murphy and Woollard 2010, Bugdaci et al. 2011) and it is possible that patients with lower HDL-cholesterol concentrations develop more severe inflammatory reactions. However, as there were no differences in Study 4 after follow-up, the changes in the serum lipid profile seem to be secondary to the acute disease and are simply more profound in severe cases.

5 Study strengths and limitations

The main strength of these studies was the inclusion of a control group with no history of acute pancreatitis but with prior alcohol consumption similar to that of patients hospitalized for alcohol induced acute pancreatitis, thus allowing comparison between the two groups. After the first episode of alcohol induced acute pancreatitis, recurrent episodes are not as clearly related to the amount of alcohol consumed, suggesting increased or altered susceptibility to the disease. Most patients in these studies were suffering from their first alcohol induced acute pancreatitis, eliminating the possible confounding factor. Another strength of these studies was the relatively large study material. The main limitation was the retrospective setting of these studies. However, as the prevalence of Helicobacter pylori is decreasing in developed countries, this also offered more statistical power in the Helicobacter pylori analysis. Another drawback in these studies was that the severity of acute pancreatitis was classified according to the original Atlanta classification, which was later revised. A possible source of error in these studies was that abdominal computed tomography was not routinely performed on all patients, possibly resulting in some patients with otherwise clinically mild disease but with local complications such as pancreatic necrosis being erroneously classified as having mild acute pancreatitis. In Study 5, most (87%), but not all, patients had serum samples available from the first two days following hospitalization, limiting the conclusions that can be drawn from this study. A further methodological weakness in this study was that fluid and oral feeding therapy was according to clinic standards and, athough probably similar in all patients, not controlled.

SUMMARY AND CONCLUSIONS

On the basis of these studies the following conclusions may be drawn.

1. Helicobacter pylori infection neither increases the risk of developing alcohol induced acute pancreatitis nor affects the course of the disease.

2. Enterovirus infections do not seem to trigger alcohol induced acute pancreatitis in humans.

3. The serum fatty acid profile is significantly altered during alcohol induced acute pancreatitis. Some differences persist after follow-up compared to non-pancreatitis controls, which may be associated with increased risk of alcohol induced acute pancreatitis.

4. Serum total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations drop significantly during alcohol induced acute pancreatitis and lower concentrations are associated with the development of more severe disease.

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ORIGINAL COMMUNICATIONS

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HELICOBACTER PYLORI IN ALCOHOL INDUCED ACUTE PANCREATITIS

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ABSTRACT

Background and Aims: **The objective of this study was to measure the association of Helicobacter pylori infection with alcoholic acute pancreatitis.**

Material and Methods: **This study comprised of 50 patients with their first alcoholic pancreatitis and 50 alcoholic controls with no history of pancreatitis but similar alcohol use recruited from an alcohol rehabilitation center. Helicobacter infection was measured using Enzygnost EIA IgG-test. Complications and length of hospital stay were also recorded in patients with alcoholic pancreatitis.**

Results: **The seroprevalence of Helicobacter pylori was 10/50 (20%) in the pancreatitis group and 15/50 (30%) in the control group (p = NS). The median length of hospital stay of pancreatitis patients was 7 days, 11 days for those tested positive (range 6–25) and 6 days for those tested negative (range 3–47) for Helicobacter pylori, p = 0.013. As determined with the Atlanta criteria, seropositive patients tended to have more often severe pancreatitis, 4/10 (40%) vs. 6/40 (15%), OR 3.78 (95% CI 0.815–17.52), p = 0.097.**

Conclusions: **This study suggests that Helicobacter pylori infection is not associated clinically significantly with the development of alcoholic pancreatitis. However, Helicobacter pylori infection may be associated with longer hospital stay due to more severe disease, which needs to be studied in a larger series of patients.**

Key words: Alcoholism; alcohol-induced disorders; Helicobacter infections; pancreatitis, acute necrotizing; pancreatitis, alcoholic; prevalence; serology

INTRODUCTION

The worldwide incidence of acute pancreatitis (AP) ranges from 5 to more than 100 per 100 000 patient years (1–7). The proportions of the two most common etiological factors, alcohol and gallstones, vary considerably in different countries and regions (3, 8–10). In Finland, alcohol is the most common etiological factor, accounting for about 70% of all AP (4). The cellular mechanisms and inflammatory responses of acute pancreatitis have recently been reviewed (11, 12).

Even though heavy alcohol use has been shown to be a clear risk factor for AP, the exact pathophysiological mechanism triggering the onset of alcohol induced acute pancreatitis (AIAP) remains unclear. Most heavy alcohol consumers do not develop pancreatitis – only 2–3% even in the group of the highest alcohol intake may develop an AP during a 20–30 year follow up period (13).

Helicobacter pylori (HP) has been shown to be the most important causative agent in peptic and duodenal ulcer formation (14), and has been clearly linked

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to increased risk of gastric cancer (15). The prevalence of HP in Finland is similar with other industrialized nations, being 6–70%, depending on the age of the cohort studied (16, 17). Recently Domínguez-Muñoz et al (18) showed that the interdigestive exocrine pancreatic secretion and postprandial release of gastrin were increased in HP positive subjects. Manes et al (19) reviewed studies addressing HP and pancreatic diseases and the mechanisms through which HP might affect pancreatic physiology. In summary, release of ammonia and lipopolysaccharides, activation of leukocytes and subsequent release of proinflammatory cytokines together with hypergastrinemia and duodenal acidification associated with HP infection might trigger or modulate AIAP, but the association has not been previously extensively studied.

Furthermore, it was unexpectedly suggested that the prevalence of HP infection was lower in patients suffering from their first episode of AIAP compared to an age and alcohol consumption matched control group of alcoholics with no history of AP (20). This was, however, a pilot study addressing a different question with very low sample sizes. The aim of this study was to compare HP seropositivity between AIAP patients and alcoholics with no history of AIAP. We hypothesized that the seroprevalence of HP would considerably differ between the two groups, which might suggest that HP infection has an effect on the development of AP. Furthermore, we also studied the association between the seropositivity and the severity of AIAP and the length of hospital stay.

MATERIAL AND METHODS

Based on the previous study (20), we expected prevalences of about 10% and 40%, for HP infection in the AIAP-patients and alcoholics with no history of AIAP, respectively. When the power was set at 90% and alpha at 0.05, a sample size of 100 (n = 50 for both groups) was calculated to be required (21).

This study was performed as a retrospective analysis of previously collected serum samples in a prospective study (22). Our study subjects were recruited between January 2001 and November 2005. The first group consisted of 50 patients hospitalized for their first AIAP attack. Their serum samples were obtained during the hospitalization. AP was diagnosed when the patient experienced epigastric pain resulting in hospitalization and had the clinical signs consistent with AP together with serum amylase activity of at least three times the upper normal range and elevated inflammation markers (serum C-reactive protein concentration and/or blood leukocyte count) and/or the radiologic findings of AP during hospitalization. AP was considered as alcohol induced (23) when other etiological factors were excluded by laboratory testing and imaging and the patient reported high consumption of alcohol in the Alcohol Use Disorders Identification Test (AUDIT), or by further interviewing of the patient in the two cases with 7 or less points in the AUDIT test. The patients had not reported to be treated for abdominal complaints previously.

AP was considered severe when it met with the Atlanta criteria (24). Basic information, such as age, gender and Body Mass Index (BMI) were recorded. The length of hospital stay, need and lenght of intensive care unit treatment and the development of complications were recorded. Alcohol use, including the length of the last drinking session prior to AIAP attack, was measured by interviewing the patients. This was performed by a person specialized to dependence problems.

The control group consisted of 50 alcoholics recruited from an alcohol detoxification centre whose serum samples were obtained during their stay in the centre. We matched the control group according to age and reported amount of alcohol use. None of the control subjects had a history of AP.

The median age in the AIAP group was 45 years (range 18–73). The group consisted of 38 males and 12 females. The median AUDIT score was 22 (range 5–38) and the median BMI was 26 (range 19–34). Likewise, the median age in the control group was 46 (range 21–66) and the group consisted of 31 males and 19 females. The median AUDIT score was 28 (range 15–36) and the median BMI was 25 (range 16–34).

The serum samples were stored frozen in –70°C the time between their acquisition and analysis. The samples were analysed using a commercial Enzygnost Anti-Helicobacter pylori II/IgG EIA kit (Dade Behring) with a cut-off value of 10 U/ml. The reported sensitivity and specificity by the manufacturer were 93.4% and 98.8%, respectively.

Statistical testing was performed with the SPSS statistical software using Pearsons correlation, the Chi-Square test, Mann-Whitney U-test and Fisher's Exact test. P values ≤0.05 were considered significant. This study was performed according to the Helsinki declaration and was approved by the Ethical Committee of Tampere University Hospital.

RESULTS

Both groups were high alcohol consumers and there were no significant differences in the age, gender distribution or BMI between the two groups. Ten out of 50 (20%; 95% CI 9–31%) in the AIAP group and 15 out of 50 (30%; 95% CI 17–43%) in the control group tested positive for HP, p = 0.248. Thus HP serology did not differ significantly between the study groups. HP was not associated with the reported amount of alcohol use in either group.

The median length of hospital stay for all AIAP patients was 7 (range 3–47) days. Two patients, both HP negative, required intensive care unit treatment. The median length of hospital stay for patients tested negative was 6 (range 3–47) days and 11 (range 6–25) days for patients tested positive for HP, p = 0.013. Ten out of 50 (20%) patients had a severe pancreatitis according to the Atlanta criteria. There were no mortalities. Four out of ten (40%) patients seropositive for HP had a severe pancreatitis compared to six out of 40 (15%) patients negative for HP (OR 3.78 [95% CI 0.815–17.52], p = 0.097). In HP positive patients, two patients had necrotizing pancreatitis, one patient developed a pancreatic abscess and one pancreatic pseudocysts. In HP negative patients, four patients had necrotizing pancreatitis, one of which also developed a pseudocyst, one had respiratory failure and developed a pseudocyst and one later developed pseudocysts. There was no statistically significant correlation between HP serology and the development of individual complications. The median peak C-reactive protein concentration during the first days after admission in all patients was 168mg/L and was

significantly increased in patients with severe pancreatitis, 307mg/L vs. 164mg/L, p = 0.010. The respective figures for patients positive and negative for HP were 213mg/L and 166mg/L, p = 0.576.

The median age for patients tested positive and negative for HP, was 48 and 44 (p = 0.096) in the AIAP group and 46 and 46 (p = 0.589) in the control group, respectively. Reported alcohol use, age, nor BMI, were associated with the length of the hospital stay, or other markers of severity studied.

DISCUSSION

We sought to evaluate whether the prevalence of HP seropositivity differs between AIAP patients with their first AIAP and alcoholics with no history of AIAP and could thus be a factor affecting the onset of AIAP. Furthermore, we compared the course and length of hospital stay in AIAP patients according to HP serology.

We obtained the prevalence of HP by measuring the IgG-class antibodies from the sera of the patients. The Enzygnost test itself has been validated by other authors, reporting sensitivities and specificities of 79–93% and 79–100%, respectively (25–27). Freezing and thawing of the serum samples should not affect the results (28). Because the titers remain elevated for several months to years after the eradication of HP (29, 30) we could eliminate the confounding factors associated with the treatment of AIAP, such as possible use of antibiotics and proton pump inhibitors during the acute course of the disease. Thus the seropositivity should reflect the situation preceding the onset of AIAP. While it would have been optimal to include the urea breath test, stool antigen, or histology analysis in addition to serology, they were not included in the original study, and due to the retrospective setting of this study, could not be performed. Spontaneus healing of HP infection is rare (31, 32) and therefore is unlikely to affect the results. The patients did not report recent treatment of HP, but of course it is possible that some had undergone such therapy without proper recognition. However, we anticipate that if such had happened, it would have happened at the same rate both in the study group and the control group.

The seroprevalence of HP in this study was 20% in AIAP patients and 30% in alcoholics with no history of AIAP, p = 0.248. This difference was much less than in the earlier pilot study (20), and suggests that HP is not associated with AIAP in such quantity, that it would be a clinically significant determinant in the genesis of AIAP. To establish this difference (20% vs. 30%) significantly in the seroprevalence of HP between the two groups with 90% power would have required a sample size of 820 patients. Despite the possible statistical significance then found, the clinical significance of such a small difference would still have remained minimal.

The median length of hospital stay was somewhat long in our study, 7 days, though most cases were not severe according to the Atlanta criteria. The relatively high median peak C-reactive protein concentrations also indicate at least intermediate severity of most cases while actual organ failure was rare in our population. The long hospital stay was mostly due to pain management and difficulties in eating.

HP was associated with a somewhat extended hospital stay. This was possibly due to the slight albeit not significant association between the severity of AIAP and HP seropositivity. HP positive patients also had somewhat increased median peak C-reactive protein concentration, but the difference was not statistically significant. However, based on these results, it is impossible to determine whether HP is affecting the pathophysiology of AIAP or whether this suggested association with the length of stay is just a coincident. There is also a possibility that some cofactor exists that disposes patients to both HP seropositivity and somewhat more severe AIAP. Therefore a separate study addressing primarily the association of HP and the severity of AP is required to answer this question.

In conclusion this study suggests that HP infection is not clinically significantly associated with the development of AIAP. However, HP infection might prolong the length of hospital stay due to more severe disease, which needs to be further investigated.

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LETTERS TO THE EDITOR

There is hardly any association between *Helicobacter pylori* infection and the severity of acute pancreatitis

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Helicobacter pylori infection is one of the most common chronic infections worldwide, being most prevalent in developing countries and lower socio-economic classes. Its prevalence ranges from 7% to 90%, depending on the cohort studied [1]. In addition to causing gastric and duodenal ulcers [2], it increases the risk of gastric cancer [3]. Furthermore, *H. pylori* infection causes changes in the physiology of the gastrointestinal tract. *H. pylori* infection is associated with duodenal acidification, increased gastrin secretion, and increased exocrine pancreatic secretion [4]. Although these changes – such as increased pancreatic stimulation – could be associated with an increased risk of acute pancreatitis or a more severe course of the disease, the association between *H. pylori* infection and acute pancreatitis has not been thoroughly studied.

In a previous study, we measured the prevalence of *H. pylori* infection in patients with their first alcohol-induced acute pancreatitis and compared it to that of controls with similar high alcohol use but no history of acute pancreatitis [5]. The rate of infection was similar in both groups studied. The objective of this study was to ascertain whether *H. pylori* infection is associated with a more severe course of acute pancreatitis.

We reanalyzed prospectively collected patients of a previous study [6] hospitalized for acute pancreatitis. All etiologies of acute pancreatitis and both patients with their first or recurrent episode were included. Altogether 231 patients were studied. One hundred sixty-eight (73%) were male and the median age of the patients was 48 (range 17–90). Acute pancreatitis was caused by alcohol in 130 (56%) patients, gallstones in 46 (20%), other known causes in 17 (7%), while 38 (17%) remained idiopathic. One hundred fifty-two (66%) patients had their first acute pancreatitis. The prevalence of *H. pylori* infection was obtained by measuring the IgG antibodies from the sera using EIA antigen test.

Table I. The severity and individual complications of acute pancreatitis in patients with or without *Helicobacter pylori* infection.

Etiology		Mortality	Severe*	Pancreatic necrosis	Pancreatic abscess	Pancreatic pseudocyst
All patients (n = 231)	*H. pylori*+	8% (8/105)	27% (28/105)	17% (18/105)	4% (4/105)	5% (5/105)
	H. pylori-	3% (4/126)	29% (36/126)	21% (26/126)	2% (3/126)	8% (10/126)
Alcohol (n = 130)	*H. pylori*+	6% (3/54)	28% (15/54)	19% (10/54)	4% (2/54)	6% (3/54)
	H. pylori-	3% (2/76)	33% (25/76)	26% (20/76)	3% (2/76)	7% (5/76)
Gallstones (n = 46)	*H. pylori*+	8% (2/25)	24% (6/25)	16% (4/25)	8% (2/25)	0% (0/25)
	H. pylori-	5% (1/21)	14% (3/21)	5% (1/21)	0% (0/21)	10% (2/21)
Other (n = 17)	*H. pylori*+	13% (1/8)	38% (3/8)	25% (2/8)	0% (0/8)	13% (1/8)
	H. pylori-	11% (1/9)	56% (5/9)	33% (3/9)	0% (0/9)	11% (1/9)
Idiopathic (n = 38)	*H. pylori*+	11% (2/18)	22% (4/18)	11% (2/18)	0% (0/18)	6% (1/18)
	H. pylori-	0% (0/20)	15% (3/20)	10% (2/20)	5% (1/20)	10% (2/20)

*Severe according to the Atlanta criteria.

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One hundred five (46%) patients tested positive for *H. pylori*. The rate of infection was similar in all etiologies of acute pancreatitis; 42% in patients whose pancreatitis was caused by alcohol, 54% in those with gallstone pancreatitis, 47% in patients with other causes, and 47% in patients with idiopathic acute pancreatitis. We observed no significant associations of *H. pylori* infection and the severity of acute pancreatitis, its complications, length of hospitalization, need of intensive care unit treatment, or mortalities. The findings were similar in all etiologies and in patients with their first or recurrent episode of acute pancreatitis (Table I).

We observed no significant association between *H. pylori* infection and the severity of acute pancreatitis in any patient subgroup in this study. In light of these and previous studies, we conclude that *H. pylori* infection scarcely has any impact on the genesis or course of acute pancreatitis.

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BRIEF ARTICLE

Is alcoholic pancreatitis associated with enteroviral infection?

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Abstract

AIM: To investigate whether enteroviral infection might trigger acute pancreatitis in patients made susceptible due to high alcohol consumption.

METHODS: Patients with alcohol-induced acute pancreatitis were analyzed for signs of simultaneous or preceding enteroviral infection. We studied the serum samples of 40 patients hospitalized for alcohol-induced acute pancreatitis and 40 controls recruited from an alcohol detoxification center. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect enterovirus RNA and diagnose acute viremia. Immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) enteroviral antibodies were measured using enzyme immunoassay to detect subacute and previous infections. The samples were considered positive when the antibody titers were ⩾ 15 IU. Furthermore, using RT-PCR, we studied pancreatic biopsy samples obtained during surgery from nine patients with chronic pancreatitis, one patient with acute pancreatitis and ten control patients with pancreatic carcinoma for evidence of persisting enteroviral RNA in the pancreatic tissue.

RESULTS: No enterovirus RNA indicating acute viremia was detected by RT-PCR in the serum samples of any patient or control. A high incidence of positive antibody titers was observed in both study groups: IgM antibodies had positive titers in 5/40 (13%) *vs* 4/40 (10%), $P = 0.723$; IgG in 15/40 (38%) *vs* 19/40 (48%), $P = 0.366$; and IgA in 25/40 (63%) *vs* 33/40 (83%), $P = 0.045$, patients and controls, respectively. Ten (25%) patients had severe pancreatitis and two (5%) required treatment in intensive care. The median length of hospitalization was 7 d (range: 3-47 d). The severity of acute pancreatitis or the length of hospitalization was not associated with enteroviral IgM, IgG or IgA antibodies. Five pancreatic biopsy samples tested positive with RT-PCR, three (8%) in the control group and two (5%) in the patient group ($P = 0.64$).

CONCLUSION: The rate of enteroviral infection is not increased in patients with alcohol-induced acute pancreatitis when compared to alcoholics with similar high alcohol use.

Key words: Pancreatitis; Alcoholic; Pancreatitis; Acute necrotizing; Enterovirus

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INTRODUCTION

Although heavy alcohol consumption is known to be associated with the development of acute pancreatitis, surprisingly little is known of the actual mechanism behind this association. Furthermore, only a small proportion of heavy drinkers ever develop acute pancreatitis even during long-term follow up[1]. Excessive alcohol consumption has been reported to cause 9%-70% of all cases of acute pancreatitis[2-7]; being predominant in some countries (*e.g.*, United States, Hungary and Finland), whereas gallstones are predominant in many other countries such as China, Greece and Italy. While alcohol remains a clear risk factor for acute pancreatitis, a multitude of other factors that may be genetic or environmental could be involved in triggering or modulation of the disease.

One previously suggested co-factor possibly associated with the induction of acute alcohol-associated pancreatitis is enteroviral infection. Human enteroviruses typically cause mild respiratory or gastrointestinal infections, but are also associated with myocarditis and aseptic meningitis. Over 100 enterovirus serotypes have been identified, including the polio virus. Other enteroviruses are classified as *coxsackie A* and *B* viruses, *enteric cytopathogenic human orphan* viruses or as numbered serotypes (*e.g.*, *enterovirus 70*). The evidence suggesting an association between enteroviruses and acute pancreatitis is mostly derived from case reports[8-12] and historical serological studies[13,14]. Evidence of enterovirus infection in the pancreatic beta cells has been reported by several authors[15-17]. More recently, Ozsvár *et al*[18] reported a significant rise in *coxsackie B* virus antibody titers in acute and chronic pancreatitis patients. Recent animal studies further support a possible connection between enteroviral infection and pancreatitis[19-22]. Jerrells *et al*[23] reported that mice on an alcohol diet and infected with a strain of *coxsackie B* virus developed more severe pancreatitis than control mice, and that even typically avirulent strains produced severe pancreatitis in these mice. Clemens *et al*[24] showed that the pancreas of mice on an alcohol diet had impaired regeneration potential compared to control mice which may be associated with the severity of acute pancreatitis and the development of chronic pancreatitis. These studies suggest that enteroviruses may play a triggering role in at least a portion of human alcoholic pancreatitis.

To the best of our knowledge, there are no studies addressing the association between enteroviral infection and alcohol-associated acute pancreatitis in humans, where the alcohol intake of the non-pancreatitis controls has been comparable. The aim of this study was to ascertain whether patients suffering from alcohol-associated acute pancreatitis show evidence of simultaneous or preceding enteroviral infection in greater numbers than control subjects with similar recent alcohol consumption, but no previous or current pancreatitis. In addition, we analyzed pancreatic biopsy samples obtained from chronic pancreatitis patients and control patients during surgery to evaluate whether chronic pancreatitis specimens showed signs of persistent enteroviral genome in the pancreas.

MATERIALS AND METHODS

This study was a retrospective analysis of previously collected serum samples from a prospective study[25]. The study patients were recruited between January 2001 and November 2005. The samples for the first group, 40 patients hospitalized due to their first alcohol-associated acute pancreatitis, were collected during the first days of hospitalization. The samples for the control group, 40 alcoholics recruited from an alcohol detoxification center, were collected during their stay in the center. The patients were diagnosed with acute pancreatitis when they met the following criteria: acute epigastric pain that led to hospitalization, clinical signs consistent with acute pancreatitis together with serum amylase activity of at least three times the upper normal range, elevated serum inflammation markers (C-reactive protein concentration and leukocyte count), and/or the findings of acute pancreatitis on imaging. Alcohol was considered the probable etiology when the patient reported high alcohol intake during the alcohol use disorders identification test (AUDIT) or in a thorough interview of the patient or the family and other etiologies were excluded by laboratory testing and imaging[26]. Heavy alcohol consumption was similarly identified in the control subjects. Previously diagnosed pancreatitis or any acute illness were exclusion criteria when recruiting the control subjects.

The length of hospitalization, the development of complications and the need for and duration of treatment in the intensive care unit in alcohol-associated acute pancreatitis patients were recorded together with basic information such as body mass index (BMI), age and gender. Acute pancreatitis was considered severe when it met the Atlanta criteria[27]. The AUDIT questionnaire, amount of alcohol consumption (g/wk) preceding hospitalization and amount of smoking were elicited by a person specialized in addiction problems. The control group was matched according to age and reported amount of alcohol consumption. Thirty-two (80%) of the patients were male with a median age of 47 years (range: 18-73 years) and median BMI of 26 kg/m^2 (range: 19-34 kg/m^2). In the control group, 25 (63%) patients were male with a median age of 46 years (range: 22-66 years) and median BMI of 26 kg/m^2 (range: 16-34 kg/m^2). The median AUDIT scores were 22 (range: 5-38) in the patient group and 29 (range: 15-36) in the control group.

The biopsy samples were collected from 20 patients who underwent pancreatic surgery: one with alcohol-associated acute pancreatitis, nine with chronic pancreatitis and ten with pancreatic carcinoma. The development of chronic pancreatitis was alcohol associated in five and idiopathic in four patients. None of the patients with pancreatic carcinoma had a history of acute pancreatitis or excessive alcohol consumption. They were operated on between December 2001 and March 2006. The biopsy

samples were analyzed for the presence of enteroviral RNA using a highly sensitive reverse transcription-polymerase chain reaction (RT-PCR) method which amplifies a sequence common to all known enterovirus serotypes. The details of this method have been described earlier[28]. Frozen tissue samples were disrupted and homogenized using the TissueRuptor homogenizator (Qiagen, Hilden, Germany). RNA was extracted from the homogenized sample using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions.

The serum samples were stored at -70 ℃ during the interval between their acquisition and analysis. Evidence of enteroviral infection was analyzed by detecting immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) class antibodies by enzyme immunoassay and by detecting enteroviral-RNA using the RT-PCR method described above. IgM class enterovirus antibodies were measured against a mixture of three enterovirus antigens (coxsackie virus B3, coxsackie virus A16 and echovirus 11) using a capture enzyme immunoassay as previously described[29]. IgG and IgA class antibodies were measured against a synthetic enterovirus peptide antigen (sequence KEVPALTAVETGAT-C derived from an immunodominant region of capsid protein VP1, which is a common epitope for several enteroviruses) as described earlier[30-32]. The samples were considered positive when the antibody titers were ⩾ 15 EIU.

Statistical analysis

Statistical testing was performed with SPSS statistical software using Pearson's correlation, χ^2 test, Mann-Whitney *U*-test and Fisher's Exact test. *P* values ⩽ 0.05 were considered statistically significant. This study was performed according to the Helsinki Declaration and was approved by the Ethics Committee of Tampere University Hospital.

RESULTS

Ten (25%) patients had severe pancreatitis according to the Atlanta criteria. Of these, six patients had necrotizing pancreatitis, one developed infected necrosis and three developed pseudocysts. Two patients required treatment in intensive care. The median length of hospitalization was 7 d (range: 3-47 d).

No enterovirus RNA was detected by RT-PCR in any patient or control subject. IgM antibodies had positive titers in 5/40 (13%) *vs* 4/40 (10%), *P* = 0.723; IgG in 15/40 (38%) *vs* 19/40 (48%), *P* = 0.366; and IgA in 25/40 (63%) *vs* 33/40 (83%), *P* = 0.045, patients and controls, respectively. The severity of acute pancreatitis or the length of hospitalization was not associated with enteroviral IgM, IgA or IgG antibodies.

Three pancreatic biopsy samples from patients with pancreatic carcinoma and two biopsy samples from patients with chronic pancreatitis tested positive for enteroviral RNA. The etiology of chronic pancreatitis was alcohol consumption in both patients. The tissue specimen from the patient with alcohol-induced acute pancreatitis was negative for enteroviral RNA.

DISCUSSION

In this study, we ascertained whether patients hospitalized for their first alcohol-induced acute pancreatitis had evidence of simultaneous or preceding enteroviral infection. In animal studies, enterovirus infection has been found to cause pancreatitis and, furthermore, simultaneous consumption of alcohol has been found to exacerbate the pancreatic insult. We hypothesized that enteroviral infection might be the triggering factor in at least some of the patients with their first alcohol-induced acute pancreatitis.

All the samples analyzed in this study were stored frozen. To the best of our knowledge, no studies have been reported on the possible adverse effects of prolonged storage and thawing of samples of enteroviral antibodies or on RT-PCR sensitivity. In general, repeated freezing and thawing may slightly alter the results observed, but the cycles generally do not affect samples to any clinically significant extent[33-35].

No evidence of acute viremia was found in any of the patients. Positive IgM antibodies reflect subacute disease and 13% of our patients tested positive, with a similar rate in the control group. We also report a relatively high number of patients with positive IgA and IgG antibody titers. However, this was also the case in the control group. IgG antibodies remain elevated long after the infection, while IgA antibodies usually disappear within a few months. Therefore, we suspect that this finding reflects the fact that our patients and controls were of lower socio-economic background with a tendency to acquire such infections at an increased rate when compared to the general population. An association between lower socio-economic status and increased enteroviral infection rate has previously been reported[36,37]. Thus, our findings do not suggest a role for enteroviral infection in the pathogenesis of alcohol-induced acute pancreatitis in humans, at least to a clinically significant extent. In fact, IgG and IgA class enterovirus antibodies tended to be at lower levels in the pancreatitis group, which may reflect the general immunosuppression associated with this disease.

A surprisingly high percentage of pancreatic tissue samples obtained during surgery, from patients operated on either for chronic pancreatitis or carcinoma of the pancreas, tested positive for enteroviral RNA in RT-PCR. In an earlier study, Lászik *et al*[38] studied pancreatic tissue specimens obtained during surgery for acute pancreatitis using *in situ* hybridization and reported no evidence of enteroviral infection in any of the samples. In the present study, we did not investigate whether enteroviral genome was present in the acini or the islets of Langerhans in the pancreas. Recent studies suggest a role for enteroviral infection in the genesis of type 1 diabetes[39], and, furthermore, direct beta cell involvement[15-17]. It is therefore possible, although not certain, that the high percentage

of enteroviral genome observed in the tissue samples in our study also came from the islets of Langerhans in this patient material.

In conclusion, we report no evidence of an increased rate of enteroviral infection in patients hospitalized for their first alcohol-induced acute pancreatitis when compared to alcoholics with similarly heavy alcohol consumption, but with no history or signs of acute or chronic pancreatitis. The rate of positive results in pancreatic tissue samples was clearly higher in our study than reported elsewhere, although the sample size was small.

COMMENTS

Background

Although heavy alcohol consumption is known to be associated with the development of acute pancreatitis, surprisingly little is known of the actual mechanism behind this association. Furthermore, only a small proportion of heavy drinkers ever develop acute pancreatitis even during long-term follow up.

Research frontiers

One previously suggested co-factor possibly associated with the induction of acute alcohol-associated pancreatitis is enteroviral infection. Human enteroviruses typically cause mild respiratory or gastrointestinal infections, but are also associated with myocarditis and aseptic meningitis.

Innovations and breakthroughs

There are no studies addressing the association between enteroviral infection and alcohol-associated acute pancreatitis in humans, where the alcohol intake of the non-pancreatitis controls has been comparable.

Applications

The aim of this study was to ascertain whether patients suffering from alcohol-associated acute pancreatitis show evidence of simultaneous or preceding enteroviral infection in greater numbers than control subjects with similar recent alcohol consumption, but no previous or current pancreatitis.

Peer review

This study partially answered a question in etiology of acute pancreatitis. It was well designed retrospective study.

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Original article

Serum lipid and fatty acid profiles are highly changed in patients with alcohol induced acute pancreatitis

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A B S T R A C T

Background/aims: Hyperlipidemia is one known etiology of acute pancreatitis. Alcohol use is known to induce changes in lipid metabolism and might alter the serum lipid and fatty acid profile. We hypothesized that these changes may explain individual susceptibility of developing acute pancreatitis. We compared lipid and fatty acid profiles of patients with acute alcoholic pancreatitis and alcoholic controls.
Methods: 19 patients with their first alcoholic pancreatitis and 20 controls were included. Late follow-up samples were obtained from 16 patients. Serum lipids were analyzed enzymatically and the fatty acid profile using gas chromatography.
Results: The concentrations of serum total cholesterol, LDL-cholesterol and HDL-cholesterol were markedly lower in patients than in controls during the acute disease but normalized after follow-up. Patients had statistically significantly lower fatty acid proportions of saturated C14:0, polyunsaturated C18:2, C18:3 and C20:3 of the n-6-series and C18:3 of the n-3-series than controls. In contrast, patients had higher percentages of saturated C16:0 and monounsaturated C18:1n9 fatty acids than controls. Mead acid, C20:3n9, marker of essential fatty acid deficiency, was lower in patients than in controls. C14:0, C20:3n6, C18:3n3 and C20:3n9 remained altered after follow-up.
Conclusion: Serum lipid and fatty acid levels were significantly altered during the acute disease and returned toward normal after 18–24 months, suggesting that the changes are secondary to acute pancreatitis. They are unlikely to be the much sought 'trigger factor' of pancreatic necro-inflammation. However, further studies are warranted to fully establish this point.

1. Introduction

Acute pancreatitis is a significant cause of morbidity and mortality world-wide. Besides gallstones, its main cause is alcohol. Together they account for the majority of all cases, 75–90% [1–3].

The mechanisms by which alcohol causes acute pancreatitis are still far from clear. The metabolites of ethanol; acetaldehyde and fatty acid ethyl esters, and the release of free radicals associated with ethanol oxidation, may cause acinar insult [4,5] and induce transcription factors NF-κB and AP-1 that mediate the production of proinflammatory cytokines [6]. Fatty acid ethyl esters cause elevations in the intracellular calcium concentrations that can result to premature digestive enzyme activation, activation of the proinflammatory cascade and even cell death [7,8]. Although the risk of developing the first pancreatitis is thought to increase concomitantly with the amount of alcohol ingested [9], only a small proportion of all alcoholics ever develop acute pancreatitis [10]. Therefore individual differences have been sought to explain the varying susceptibility, but without success.

Alcohol use is known to induce changes in lipid metabolism in many ways although acute and chronic effects differ [11]. Together with metabolic syndrome, alcohol is the leading cause of hypertriacylglycerolemia [12,13]. It increases serum HDL-cholesterol levels and synthesis of triacylglycerols and VLDL particles in the liver [11,14,15]. Heavy, chronic alcohol use, however, is associated with increased lipoprotein lipase activity and subsequently increased VLDL turnover rate and decreased serum LDL-cholesterol levels [15–18].

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The fatty acid composition of serum lipoproteins and cellular membranes is determined by both dietary intake and endogenous metabolism. Heavy alcohol use might be associated with poor nutrition which in turn might change the serum fatty acid profile [19–21]. The simplest of the polyunsaturated fatty acids, linoleic acid (18:2n6) and α-linolenic acid (18:3n3) cannot be synthesized by the human body and hence must be obtained from the diet. All long chain fatty acids are modified by the same set of enzymes, stearoyl-CoA desaturase (delta-9 desaturase), delta-5 and delta-6 desaturases and elongation reactions in the tissues, especially the liver, that give rise to longer and more highly unsaturated derivative fatty acids. Some 20-carbon polyunsaturated fatty acids, especially arachidonic acid, serve as precursors for synthesis of eicosanoids which regulate various cellular responses [22].

Alcohol abuse might interfere with fatty acid metabolic pathways. Pawlosky and Salem reported a lower total amount of fatty acids and that of many nonessential and essential (16:0, 18:0, 18:1n9, 18:2n6, 18:3n6, 20:3n6, 18:3n3, 22:5n3 and 22:6n3) fatty acids in the liver of patients with alcohol liver disease than in controls [23]. They also reviewed studies addressing the mechanisms by which alcohol might cause changes in the fatty acid metabolism. Shortly, while in vitro studies indicated that prolonged alcohol use might decrease the delta-5 and delta-6 desaturase activities, which are involved in the desaturation of fatty acids, in vivo the use of alcohol led to higher production of polyunsaturated fatty acids. The lower amount of tissue polyunsaturated fatty acids might be due to the increased rate of catabolism caused by lipid peroxidation. In turn, Boros et al. studied the tissue composition and metabolic rates of certain fatty acids in the liver, pancreas and serum in rats with chronic ethanol consumption [24]. They report increased desaturase activity and fatty acid uptake in the pancreas and lower desaturase activity together with decreased synthesis and transportation of unsaturated C16:0, C18:0 and C18:1n9 fatty acids in the liver associated with chronic ethanol consumption. Marosvolgyi et al. studied the serum fatty acid profile in patients with chronic alcoholic pancreatitis and reported higher percentages of monounsaturated fatty acids and lower levels of arachidonic acid in patients with chronic pancreatitis than in controls [25].

The serum fatty acid profiles have not been previously studied in patients with alcohol induced acute pancreatitis. We hypothesized that the fatty acid profiles differ in patients who developed acute pancreatitis from those with high alcohol consumption but without pancreatitis. The objective of this study was to measure the fatty acid and lipid profiles in patients hospitalized for their first alcohol induced acute pancreatitis during the course of the acute disease and after discharge and follow-up in comparison with control subjects who had high alcohol use but no history of pancreatitis.

2. Methods

Twenty patients hospitalized for their first alcohol induced acute pancreatitis and twenty controls from an alcohol detoxification center with no history of pancreatitis but similar alcohol use were recruited. One patient was excluded from the analysis due to hereditary lipid metabolism disorder diagnosed with these studies. Demographic data of patients and controls is presented in Table 1. The Alcohol Use Disorders Identification Test (AUDIT) was used to evaluate alcohol consumption. In addition, the amount of alcohol used was approximated by interviewing the patients and controls by a person specialized in dependence problem. Serum samples were obtained during the second day of hospitalization (group I) and during the few days stay in the detoxification center (controls). Follow-up samples were available from 16 acute pancreatitis patients and were obtained 18–24 months after hospitalization (group II). All samples were stored frozen in −70 °C the time between their acquisition and analysis.

Table 1
Demographic data of patients and controls.

	Patients	Controls
Male %	79%	80%
Median age (Range)	45 (28–60)	47 (20–56)
Median BMI (Range)	24.6 (19.6–33.1)	24.6 (19.0–34.0)
Median AUDIT score (Range)	20 (9–35)	30 (18–39)
Reported median alcohol intake >288 g/week N (%)	15/19 (79%)	18/20 (90%)

Acute pancreatitis was considered as alcohol induced [26] when the patient reported high consumption of alcohol in the AUDIT and other causes were excluded by laboratory testing and imaging. The serum total cholesterol, HDL-cholesterol and serum triacylglycerol levels were measured enzymatically and LDL-cholesterol levels were calculated using the Friedewald formula. The serum fatty acid profile was analyzed using gas chromatography as previously described [27]. The activity of desaturase enzymes was approximated by calculating the respective indexes; proportion of C20:4n6 divided by C20:3n6 for delta-5 desaturase, proportion of C18:3n6 divided by C18:2n6 for delta-6 desaturase and proportion of C18:1 divided by C18:0 for delta-9 desaturase.

Statistical testing was performed on the SPSS statistical software using the chi-square test, Fishers exact test, Mann–Whitney *U*-test and Wilcoxon signed-rank test, when applicable. P values ≤ 0.05 were considered statistically significant. This study was performed according to the Helsinki Declaration and was approved by the Ethical Committee of Tampere University Hospital. Each individual signed a written consent.

3. Results

Only one of our patients had a severe pancreatitis according to the Atlanta criteria while others suffered from a mild to moderate disease. The median hospitalization was six days (range 3–11 days), none required intensive care treatment and thus the association between the severity of pancreatitis and serum lipid and fatty acid profiles could not be assessed. Furthermore, data concerning the length and amount of drinking, nutritional intake and length of abstinence prior to the onset of symptoms could not be obtained with sufficient reliability from the patient material to warrant statistical analysis.

Serum lipid levels of acute pancreatitis patients and controls are shown in Table 2. The serum total cholesterol, LDL-cholesterol and HDL-cholesterol levels were significantly lower in patients during hospitalization than in controls. Values normalized during follow-up, without further differences between the groups.

The proportions of individual fatty acids and the total proportions of saturated, monounsaturated and polyunsaturated fatty

Table 2
Serum lipid levels of patients during hospitalization (group I) and after 18–24 month follow-up (group II) and controls.

	Group I Median (range)	Group II Median (range)	Controls Median (range)
Total cholesterol (mmol/L)	3.31*** (1.96–5.22)	4.95 (2.47–6.51)	5.06 (3.18–6.99)
LDL-cholesterol (mmol/L)	1.84*** (0.87–3.87)	2.85 (1.38–4.61)	3.01 (1.84–4.03)
HDL-cholesterol (mmol/L)	0.76*** (0.38–1.18)	1.32 (0.81–2.98)	1.23 (0.43–2.69)
Triacylglycerol (mmol/L)	1.03 (0.57–4.13)	1.13 (0.62–3.97)	1.28 (0.64–4.88)

*$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$ when compared to controls.

acids are presented in Table 3. Patients had statistically significantly lower proportions of saturated C14:0 fatty acids, polyunsaturated C18:2, C18:3 and C20:3 fatty acids of the n-6-series and C18:3 fatty acids of the n-3-series than controls. In contrast, patients had higher percentages of saturated C16:0 fatty acids and monounsaturated fatty acids C18:1 of the n-9-series than controls. Mead acid, C20:3 of the n-9-series, a marker of essential fatty acid deficiency, was lower in patients than in controls. C14:0, C20:3n6, C18:3n3 and C20:3n9 remained altered at follow-up.

The calculated desaturase indexes are presented in Table 4. The delta-6-desaturase index was significantly lower and delta-5-desaturase and delta-9-desaturase indexes were significantly higher in patients than in controls. The delta-5-desaturase index remained significantly higher after follow-up.

Most of our patients continued drinking after the first episode of acute pancreatitis. At the end of follow-up, nine patients (47%) had suffered a recurrent episode of acute pancreatitis. There were no statistically significant differences in the serum lipid or fatty acid profiles between these patients and those with no recurrences.

Table 4
The desaturase indexes of patients during hospitalization (group I) and after follow-up (group II) and controls.

	Group I Median (range)	Group II Median (range)	Controls Median (range)
Delta-5 desaturase	4.44* (2.40–7.57)	4.19* (2.85–8.08)	3.50 (1.88–5.18)
Delta-6 desaturase	0.013** (0.006–0.023)	0.015 (0.005–0.033)	0.017 (0.012–0.051)
Delta-9 desaturase	4.29* (3.30–5.41)	4.22 (3.46–5.47)	3.51 (3.15–5.59)

*$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$ when compared to controls.

4. Discussion

In this study the serum fatty acid and lipid profile of patients hospitalized for their first alcohol induced acute pancreatitis was measured and compared to those of alcoholics with no history of pancreatitis. Control subjects were recruited from an alcohol detoxification center and also had heavy preceding alcohol use. Furthermore, the length of abstinence in both groups after a sustained period of drinking was similar. To ascertain whether changes in the fatty acid and lipid profile were constitutional or due to changes during the pancreatitis episode, follow-up samples were obtained 18–24 months after hospitalization for analysis.

We report significant and profound decreases in the serum total cholesterol, LDL-cholesterol and HDL-cholesterol levels of patients hospitalized for alcohol induced acute pancreatitis during hospitalization but not at follow-up suggesting these changes to be pancreatitis associated and not constitutional. The levels of serum triacylglycerol tended to be somewhat lower during hospitalization, but this difference was not statistically significant. Low serum cholesterol and triacylglycerol levels have previously been reported to be indicators of poor prognosis in patients treated in intensive care unit [28] but have not been extensively studied in patients hospitalized for acute alcohol induced pancreatitis. In contrast to our results, Balachandra et al. reported higher mean levels (7.2 mmol/l) of serum total cholesterol in a relatively small sample, of patients with alcohol induced acute pancreatitis and no significant difference when compared to patients with nonalcoholic acute pancreatitis [29]. In another study, Krikava et al. report normal mean serum total cholesterol and LDL-cholesterol and significantly increased HDL-cholesterol and triacylglycerol levels, 4.6 mmol/l and 5.1 mmol/l, respectively, in patients hospitalized for acute pancreatitis, though the sample size was small [30]. Our results of significantly lowered cholesterol concentrations in acute alcohol induced pancreatitis patients accords closely with the concept that the acute phase response caused by any injurious stimulus results in significantly lowered LDL and HDL concentrations [31]. The discrepancy of our findings and those of Balachandra et al. and Krikava et al. might be related to the timing of acquisition of the samples; in the previous studies the samples were obtained at the time of admission and in the present study on the second day after admission. It is thought that lipid changes manifest themselves fairly early during the course of the acute condition and for example after an acute myocardial infarction LDL and HDL-cholesterol concentrations reach their maximal reductions on day 7 [32]. To the best of our knowledge, the association of serum cholesterol and triacylglycerol levels and the severity of acute pancreatitis have not been previously extensively studied and in light of these results warrant further research, for example to be tested as a simple marker of prognosis.

The serum fatty acid profile was significantly altered in patients hospitalized for alcohol induced acute pancreatitis compared with alcoholics with no history of pancreatitis. The increased proportion of palmitic acid (C16:0) during hospitalization might be due to poor nutrition and, furthermore, has been associated with heavy alcohol use [33]. The proportion of oleic acid (C18:1) during hospitalization

Table 3
The fatty acid profile of acute alcoholic pancreatitis patients during hospitalization (group I) and after 18–24 month follow-up (group II) and alcoholic controls.

	Group I % (range)	Group II % (range)	Controls % (range)
Saturated fatty acids	30.73 (28.04–35.43)	29.07 (25.74–36.22)	29.60 (27.21–39.16)
Myristic acid C14:0	0.62** (0.29–1.67)	0.61* (0.35–1.87)	0.85 (0.59–2.12)
Palmitic acid C16:0	23.39* (20.85–27.01)	22.02 (18.31–26.21)	22.10 (19.34–30.15)
Stearic acid C18:0	6.85 (5.54–7.97)	6.29* (5.64–8.14)	7.12 (5.48–8.19)
Monounsaturated fatty acids	33.05** (28.74–43.17)	30.89 (25.95–38.09)	29.79 (24.52–38.11)
Palmitoleic acid C16:1n7	3.97 (2.82–6.79)	3.78 (2.40–5.75)	4.00 (2.65–8.28)
Oleic acid C18:1n9	28.52** (25.52–37.81)	27.38 (22.80–33.48)	25.45 (21.62–33.21)
n-6 polyunsaturated fatty acids	25.76 (16.92–32.70)	30.87 (22.18–34.11)	29.38 (19.25–36)
Linoleic acid C18:2	18.03* (11.58–23.78)	21.84 (16.05–27.39)	21.89 (13.36–28.73)
Gammalinolenic acid C18:3	0.25*** (0.11–0.46)	0.33 (0.15–0.56)	0.40 (0.27–0.83)
Homogammalinolenic acid C20:3	1.35* (0.99–2.25)	1.37* (0.72–1.85)	1.49 (1.08–2.10)
Arachidonic acid C20:4	6.30 (2.96–7.87)	5.90 (3.67–8.64)	5.00 (3.20–7.60)
n-3 polyunsaturated fatty acids	4.27 (2.87–8.93)	4.47 (3.27–8.54)	4.49 (3.91–7.27)
Alphalinolenic acid C18:3	0.50*** (0.36–1.02)	0.73* (0.39–1.06)	0.90 (0.54–1.09)
Eicosapentaenoic acid C20:5	0.90 (0.44–3.59)	1.06 (0.41–2.90)	1.25 (0.77–2.35)
Docosapentaenoic acid C22:5	0.67 (0.42–0.89)	0.62 (0.41–0.81)	0.65 (0.49–0.81)
Docosahexaenoic acid C22:6	2.28 (1.28–3.88)	2.15 (1.39–4.46)	1.82 (1.15–3.46)
Mead acid C20:3n9	0.14* (0.10–0.44)	0.15* (0.07–0.41)	0.21 (0.10–0.46)

*$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$ when compared to controls.

was greater than previously elsewhere reported [27,34,35] and has been reported to increase with heavy use of alcohol [33]. In the study by Boros et al., the amount and uptake of oleic acid in the pancreas was higher in the group with ethanol consumption [24]. Interestingly, oleic acid has been used to induce experimental pancreatitis [36]. These changes observed may be related to enhanced fatty acid synthesis. The changes were associated with acute pancreatitis, either its induction or its consequence, since the changes disappeared during follow-up. As a result of ethanol metabolism excess acetyl-CoA is generated and serves as substrate for de novo lipogenesis in the liver. In addition, ethanol, possibly via its metabolite acetaldehyde, has been shown to activate the fatty acid synthesis controlling transcription factor sterol regulatory element binding protein 1c (SREBP-1c) [37]. SREBP-1c also activates the transcription of the genes coding for fatty acid desaturase mRNAs [38]. This might suggest changes being rather secondary to ethanol consumption than pancreatitis. It remains open, why alcoholic pancreatitis patients differed from control alcoholics without pancreatitis.

The polyunsaturated fatty acids of the n-3-series are precursors of eicosapentaenoic acid (C20:5) and the polyunsaturated fatty acids of the n-6-series of arachidonic acid (C20:4), respectively. They, in turn, can be further metabolized into several series of prostaglandins, thromboxanes and leukotrienes that either aggravate or attenuate the inflammatory process [39]. The significantly lower proportion of α-linolenic acid (C18:3), an essential fatty acid of the n-3-series, in patients during hospitalization and after follow-up might indicate poor nutrition. In fact, essential fatty acid insufficiency has been found to be associated with enhanced conversion of n-3, n-6 and n-9 fatty acids to their derivatives and accumulation of monounsaturated fatty acids 16:1n7, 18:1n9 and 20:3n9 [40]. With the exception of arachidonic acid, the proportions of all fatty acids of the n-6-series were significantly lower in patients hospitalized for alcohol induced acute pancreatitis. After follow-up, only homo-gammalinolenic acid remained at significantly lower proportion. In addition to dietary factors, these changes might be associated with increased production of cytokines during the disease. It has been shown that TNFα, interleukin-1 and interleukin-6 enhance the de novo synthesis of fatty acids and inhibit their oxidation, although the mechanisms are not known, and thus might magnify the effects caused by heavy alcohol use [41].

Another major etiology for acute pancreatitis is gallstone disease. Due to changes in lipid metabolism caused by alcohol intake, patients with acute gallstone pancreatitis were not considered as appropriate control subjects for this study. Furthermore, the age and gender distribution among these patients differs greatly from those with acute alcohol induced pancreatitis. However, in future studies, the prognostic value of serum lipid levels should be considered in these patients also.

In conclusion, we report profound changes in the serum lipid levels in patients with acute alcohol induced pancreatitis. The levels of serum total cholesterol, LDL-cholesterol and HDL-cholesterol were significantly lower in acute pancreatitis patients than in controls during the acute disease but not after follow-up. Furthermore, we report lower proportion of several poly-unsaturated fatty acids of the n-6 and n-3 −series, which are precursors of inflammatory as well as anti-inflammatory mediators, and higher concentrations of other fatty acids, which might have a role in the pathophysiology of developing acute pancreatitis.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This study was supported by the competitive research funding of the Pirkanmaa Hospital District.

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Serum Lipid Levels Are Associated with the Severity of Acute Pancreatitis

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Key Words
Lipid metabolism · Lipids · Pancreatitis · Pancreatitis, acute necrotizing · Pancreatitis, alcoholic

Abstract
Background/Aims: Serum lipid concentrations react during acute disease. We sought to measure changes in the serum lipid profile during acute pancreatitis and ascertain whether these changes were associated with the severity of the disease. ***Methods:*** A total of 233 patients (71% male, median age 48 years) hospitalized for acute pancreatitis were included in the study. The most common etiology for acute pancreatitis was alcohol (n = 131, 56%), followed by biliary (n = 48, 21%) and idiopathic pancreatitis (n = 36, 16%). Serum lipid levels were measured enzymatically. We analyzed samples obtained during the first days of hospitalization and later follow-up samples to measure changes during the course of the disease. ***Results:*** We report profound changes in the serum lipid concentrations during acute pancreatitis. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol concentrations measured within 2 days of admission (n = 203) were significantly lower in patients who developed severe pancreatitis (3.20 vs. 3.80 mmol/l, p = 0.001; 0.72 vs. 1.05 mmol/l, p < 0.000, and 1.60 vs. 2.14 mmol/l, p < 0.000, respectively). Low serum total cholesterol, HDL cholesterol and LDL cholesterol concentrations were moreover associated with in-hospital mortalities and longer hospitalization (p < 0.05). In the subgroup analysis, the findings remained statistically significant in patients with alcohol-induced acute pancreatitis. ***Conclusion:*** Levels of serum total cholesterol, HDL cholesterol and LDL cholesterol are significantly lower in patients with severe acute pancreatitis and are associated with longer hospitalization.

Introduction

Acute pancreatitis is an inflammatory condition with a varying clinical course ranging from mild abdominal discomfort to life-threatening severe disease [1]. Its incidence varies in different regions, ranging from 5 to 70 per 100,000 patient years [2–8]. Alcohol and gallstones are its main causes, and together they account for approximately 75–90% of all cases [8–10]. Their relative incidence varies in different regions, alcohol being the most common cause in some countries such as Finland and USA, while gallstones are predominant in other regions, such as China [8–12].

Acute pancreatitis is often self-limiting, requiring only a few days of hospitalization, but can lead to intensive care unit (ICU) treatment with severe, even fatal, multiorgan failure and sepsis. Acute pancreatitis is classified as severe when it meets the Atlanta criteria [13]. Of all cases, approximately 25% are considered severe, while the mortality rate ranges from 2 to 10% [1, 8, 14]. Most cases of severe pancreatitis are associated with the development of necrotizing pancreatitis, although not all of these patients suffer from clinically severe disease.

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In this study, we sought to ascertain changes in the serum lipid profile in a large cohort of patients with acute pancreatitis of varying etiology soon after admission and throughout hospitalization and whether these changes were associated with the development of complications, the severity of pancreatitis or mortality. So far, the relationship between serum lipids and the severity of acute pancreatitis has not been extensively discussed in the literature.

Materials and Methods

This study was performed as a retrospective analysis of serum samples prospectively collected in an earlier study [15] from patients hospitalized for acute pancreatitis. Acute pancreatitis was diagnosed when the patient had acute epigastric pain, elevated serum inflammation markers and serum amylase activity ≥3 times the upper normal range. Abdominal ultrasound was performed on every patient to confirm the diagnosis and to detect gallstones. If the diagnosis was uncertain or pancreatic complications were suspected, abdominal CT was performed. The length of hospitalization, severity of acute pancreatitis, need for surgery or ICU treatment and mortality rate was recorded. The severity of acute pancreatitis was defined according to the Atlanta criteria; the patient was classified as having severe pancreatitis when local pancreatic complications, necrosis, abscess or pseudocyst developed and in the presence of organ failure and systemic complications such as shock, pulmonary insufficiency, renal failure and severe metabolic disturbances. In statistical analysis, we automatically classified patients needing surgery or ICU treatment and those who died as severe cases.

We included all etiologies of acute pancreatitis and patients suffering either from their first episode or recurrent acute pancreatitis. The etiology was considered biliary when gallstones were diagnosed with abdominal ultrasound either in the gallbladder or in the bile ducts; as alcohol induced when the patient reported heavy alcohol consumption (288 g of ethanol/week in males and 192 g of ethanol/week in females) without other predisposing findings; as hypertriglyceremic or hypercalcemic when suggested by laboratory findings, or as post-endoscopic retrograde cholangiopancreaticography (post-ERCP) when new or worsening abdominal pain and suggestive laboratory findings (elevation of serum inflammation markers and serum amylase activity ≥3 times the upper normal range), confirmed by abdominal CT imaging when the diagnosis was uncertain, presented shortly after the aforementioned intervention. When no clear etiology was diagnosed, the patient was classified as having idiopathic acute pancreatitis. In some patients, more than one possible etiology was discovered.

The serum samples were collected during hospitalization, each after at least overnight fasting, and stored frozen during the time between their acquisition and analysis. The first sample was obtained within a few days after admission, and further follow-up samples were obtained during the course of the disease. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were measured enzymatically, and serum low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.

Statistical testing was performed with SPSS 16.0 statistical software using the χ^2 test and Fisher's exact test to compare proportions in categorical data and the Mann-Whitney U test to compare the differences in medians between groups. For unevenly distributed values, including those that were unevenly distributed in at least one subgroup, the median values and range are presented. Pearson's correlation coefficient was calculated to detect and quantify linear correlation between variables. p values ≤0.05 were considered statistically significant. This study was performed according to the Helsinki Declaration and was approved by the Ethics Committee of Tampere University Hospital. Each individual provided written consent.

Results

Patients included in this study numbered 233. Alcohol-induced acute pancreatitis was the most common etiology, occurring in 131 patients (56%). Forty-eight patients (21%) had gallstone pancreatitis, 5 (2%) had post-ERCP pancreatitis and 36 (16%) had idiopathic acute pancreatitis. Thirteen patients (6%) had other etiologies. Of these, 3 patients had concurrent excessive alcohol consumption and gallstones, 3 had postoperative pancreatitis, 2 had hereditary pancreatitis, 1 had unclear intestinal necrosis with pancreatitis, 1 had hyperparathyroidism with hypercalcemia, 1 had hyperparathyroidism with hypercalcemia and gallstones, 1 had hypertriglyceridemia and 1 patient had a stenosis of the sphincter of Oddi as the cause of acute pancreatitis. The demographic data are presented in table 1.

In total, 155 patients (68%) were suffering from their first episode of acute pancreatitis and 73 (32%) from a recurrent episode. The information was unreliable in 5 patients. Of patients with their first episode of acute pancreatitis, 78 (50%) had pancreatitis caused by alcohol consumption, 41 (27%) by gallstones, 3 (2%) had post-ERCP pancreatitis, 10 (7%) had other etiologies and 23 (15%) had idiopathic pancreatitis. Of the patients with recurrences, alcohol was the most common etiology, in 52 patients (71%). Five patients (7%) had gallstones, 1 (1%) had post-ERCP pancreatitis and 3 (4%) had other etiologies as the cause of their recurrent acute pancreatitis. Sixteen cases of recurrences (17%) remained idiopathic. Detailed information on the severity, mortality rate, length of hospital stay and the need for and length of ICU treatment is presented in table 2.

The first serum samples were obtained within 2 days after admission in 203 patients (87%). Follow-up samples were available from 85 patients (37%). The results from the lipid analysis are presented in table 3.

The concentrations of serum total cholesterol, HDL cholesterol and LDL cholesterol in serum samples obtained during the first 2 days of hospitalization (n = 203)

Table 1. The demographic data of all patients and patient subgroups

	All patients (n = 233)	Alcohol-induced acute pancreatitis (n = 131)	Gallstone pancreatitis (n = 48)	Idiopathic pancreatitis (n = 36)	Post-ERCP pancreatitis (n = 5)	Other causes of pancreatitis (n = 13)
Males, n	166 (71%)	111 (85%)	23 (48%)	20 (56%)	3 (60%)	9 (69%)
Median age, years	48 (17–90)	44 (22–68)	64 (25–90)	53 (17–79)	36 (30–71)	60 (25–80)

Values in parentheses represent ranges, except where indicated otherwise.

Table 2. Information on the clinical course in all patients and different etiologies of acute pancreatitis

	All patients (n = 233)	Alcohol-induced pancreatitis (n = 131)	Gallstone pancreatitis (n = 48)	Idiopathic pancreatitis (n = 36)	Post-ERCP pancreatitis (n = 5)	Other causes of pancreatitis (n = 13)
Mortality, n	13 (6%)	6 (5%)	3 (6%)	2 (6%)	1 (20%)	1 (8%)
Severe pancreatitis according to the Atlanta criteria[1], n	64 (28%)	40 (31%)	9 (19%)	6 (17%)	2 (40%)	7 (54%)
Necrosis, n	44 (19%)	31 (24%)	5 (11%)	3 (9%)	1 (20%)	4 (31%)
Abscess, n	8 (4%)	5 (4%)	2 (4%)	1 (3%)	0	0
Pseudocyst, n	13 (6%)	6 (5%)	2 (4%)	2 (6%)	0	3 (23%)
ICU treatment, n	35 (15%)	19 (15%)	7 (15%)	3 (8%)	1 (20%)	5 (38%)
Median length of hospital stay, days	8 (3–150)	7 (3–150)	8 (3–36)	7 (3–29)	13 (11–41)	10 (5–41)
Median peak C-reactive protein, mg/l	174 (0–446)	177 (5–446)	150 (1–329)	149 (0–350)	178 (147–306)	226 (24–404)

Values in parentheses represent ranges, except where indicated otherwise. [1] 'Severe' according to the Atlanta criteria: local pancreatic complications; necrosis, abscess or pseudocyst development and/or the presence of organ failure and systemic complications; shock, pulmonary insufficiency, renal failure or severe metabolic disturbances.

Table 3. Serum lipid concentrations in all patients and patient subgroups and according to the Atlanta severity classification of acute pancreatitis

	Total cholesterol, mmol/l			HDL cholesterol, mmol/l			LDL cholesterol, mmol/l			Triglycerides, mmol/l		
Days after admission:	0–2 (n = 203)	3–4 (n = 73)	5–6 (n = 29)	0–2	3–4	4–5	0–2	3–4	4–5	0–2	3–4	4–5
All patients	3.70	3.20	3.10	0.95	0.53	0.46	2.00	2.03	2.13	1.01	1.22	1.29
Nonsevere	3.80	3.50	3.60	1.05	0.64	0.47	2.14	2.32	2.33	0.95	1.23	1.40
Severe	3.20	2.80	2.80	0.72	0.43	0.43	1.60	1.64	1.76	1.20	1.19	1.19
Alcoholic pancreatitis	3.50	3.30	3.35	0.93	0.66	0.66	1.82	1.94	2.21	1.20	1.40	1.40
Nonsevere	3.70	3.65	4.10	1.01	0.69	0.73	1.92	2.33	2.37	1.08	1.40	1.47
Severe	3.00	2.85	3.00	0.61	0.46	0.53	1.41	1.62	1.79	1.42	1.48	1.19
Gallstone pancreatitis	3.80	3.70	3.00	1.01	0.46	0.29	2.33	2.32	2.04	0.75	1.15	1.32
Nonsevere	3.95	3.80	3.95	1.09	0.50	0.32	2.50	2.44	2.92	0.75	1.15	1.59
Severe	3.40	2.55	2.55	0.81	0.29	0.29	2.14	1.74	1.74	0.79	1.16	1.16
Idiopathic pancreatitis	3.80	2.80	2.45	1.03	0.71	0.51	2.30	1.74	1.48	0.92	0.89	1.03
Nonsevere	3.95	2.95	2.45	1.04	0.72	0.51	2.37	1.89	1.48	0.93	0.90	1.03
Severe	3.00	2.40	–	0.71	0.71	–	1.65	1.45	–	0.89	0.89	–

Subgroups with few patients were omitted. 'Severe' according to the Atlanta criteria: local pancreatic complications; necrosis, abscess or pseudocyst development and/or the presence of organ failure and systemic complications; shock, pulmonary insufficiency, renal failure or severe metabolic disturbances.

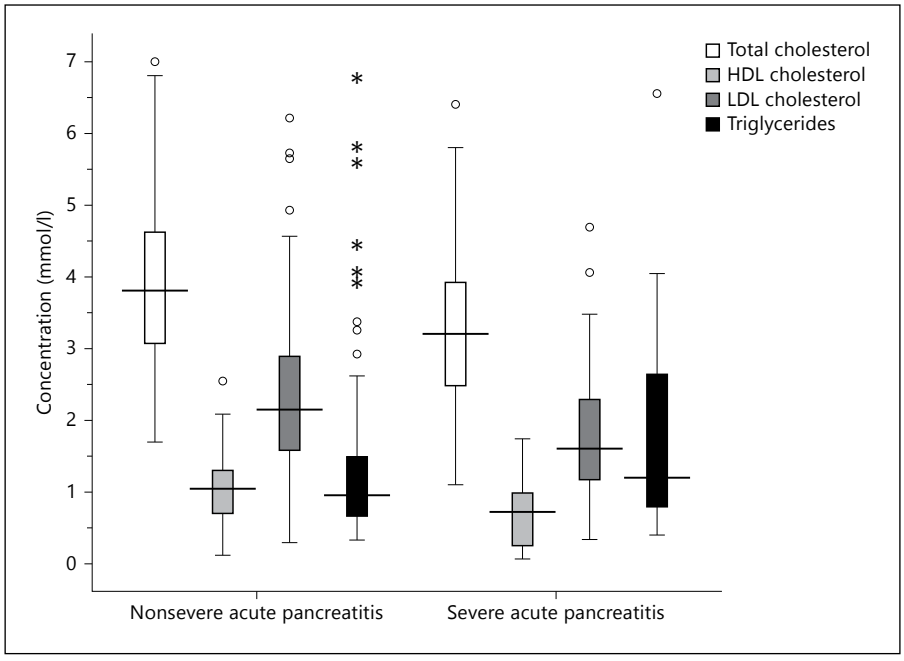

Fig. 1. The concentrations of serum lipids in samples obtained during the first 2 days after hospitalization from patients with nonsevere and severe acute pancreatitis. The circles and asterisks represent extreme observed values.

were significantly lower in patients who developed severe pancreatitis according to the Atlanta criteria (fig. 1; 3.20 vs. 3.80 mmol/l, p = 0.001; 0.72 vs. 1.05 mmol/l, p < 0.000, and 1.60 vs. 2.14 mmol/l, p < 0.000, respectively). The serum triglyceride concentration tended to be higher in patients with severe pancreatitis (1.20 vs. 0.95 mmol/l), but this difference was not statistically significant (p = 0.057). In the subgroup analysis of samples obtained during the first 2 days of hospitalization, serum total cholesterol, HDL cholesterol and LDL cholesterol remained statistically significantly lower in patients with severe acute pancreatitis caused by alcohol (p = 0.028, p = 0.001 and p = 0.009, respectively), but not in other patient subgroups. When samples acquired during the first 2 days of hospitalization from patients with nonalcoholic pancreatitis were analyzed as a single group, serum total cholesterol and LDL cholesterol were significantly lower (p = 0.010 and p = 0.032, respectively) in patients with severe acute pancreatitis, but the difference in serum HDL cholesterol concentration was not statistically significant (p = 0.063).

In all patients, in-hospital mortalities were associated with lower serum total cholesterol, HDL cholesterol and LDL cholesterol levels in samples obtained during the first 2 days of hospitalization (p = 0.09, p = 0.002 and p = 0.008, respectively). These differences remained statistically significant in patients with alcohol-induced acute pancreatitis (p = 0.038, p = 0.003 and p = 0.005, respectively), but not in other patient subgroups.

The serum total cholesterol, HDL cholesterol, LDL cholesterol and serum triglyceride concentrations measured from samples obtained during the first 2 days of hospitalization correlated with the length of hospitalization in all patients (r = –0.161, p = 0.022; r = –0.264, p < 0.001; r = –0.174, p = 0.013, and r = –0.147, p = 0.036, respectively). The differences remained statistically significant for HDL cholesterol and triglyceride concentrations in patients with alcohol-induced acute pancreatitis, HDL cholesterol levels in idiopathic acute pancreatitis and total cholesterol in acute gallstone pancreatitis in the subgroup analysis.

The serum total cholesterol and LDL cholesterol concentrations measured from serum samples obtained 3–4 days after hospitalization (n = 73) were statistically significantly lower in patients who developed severe pancreatitis (p = 0.003 and p = 0.002, respectively). However, in patients with samples available from the first 2 days and days 3–4 (n = 49), the change in the concentrations of serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides did not correlate with the severity of acute pancreatitis.

Discussion

In this study, we sought to ascertain changes in the lipid profile in patients hospitalized for acute pancreatitis and whether these changes are associated with the sever-

ity of the disease. We included all etiologies of acute pancreatitis and patients with their first episode or recurrent acute pancreatitis. Excessive alcohol consumption was the most common etiology for acute pancreatitis in this study, both in patients with their first episode of acute pancreatitis and even more so in patients with recurrences. Furthermore, severe cases were most prevalent in this patient group. None of our patients were diagnosed as having chronic pancreatitis at the time of the study, though it is possible that some of the cases with recurrences suffered from chronic pancreatitis that was not properly recognized. Repeated cycles of thawing and freezing serum samples may affect lipid analysis, but the serum samples used in this study were stored frozen and thawed just before the analysis, and the long storage time should not have altered the results to any clinically significant extent [16–18]. We report profound changes in serum lipid concentrations during hospitalization. Patients with severe pancreatitis had statistically significantly lower concentrations of serum total cholesterol, LDL cholesterol and HDL cholesterol when compared to other patients. These changes occurred early, during the first 2 days of hospitalization, indicating a possible role in predicting the severity of the disease, and moreover persisted even later during hospitalization. In the subgroup analysis, the changes in the serum cholesterol concentrations were statistically significant in patients with alcohol-induced acute pancreatitis but not in other subgroups, which was probably due to the limited sample size. While it is possible that some of the differences in the serum lipid concentrations observed between patients with nonsevere and severe disease were caused by more intensive treatment, such as fluid resuscitation, in the latter group, its role in the early phase in particular should be minimal as the treatment was similar in all patients at that stage.

It has long been known that severe acute disease can alter serum lipid levels [19, 20]. In myocardiac ischemia, for example, the concentrations of serum cholesterol drop to very low levels during the first days of hospitalization [21]. Stachon et al. [22, 23] reported lower serum lipid levels during admission to the ICU in patients who later died. In an earlier and separate study, we reported exceptionally low serum cholesterol levels in patients with their first episode of alcohol-induced acute pancreatitis [24], but the sample size was small and the study did not include other etiologies of acute pancreatitis. The mechanisms causing low serum cholesterol levels remain largely unknown but are thought to be associated with a lower rate of lipoprotein synthesis in the liver, the general catabolic metabolism and the activation of the inflammatory system during the acute stage of the disease [21, 25]. In contrast, during the acute phase, production of triglycerides in the liver increases. When combined with lower lipoprotein lipase activity, this might lead to higher serum concentrations of triglycerides [25], a tendency observed in this study also. Bugdaci et al. [26] have previously studied the lipid profile in patients with acute pancreatitis. They reported an association with low serum HDL cholesterol concentration and longer hospitalization. Their study material was somewhat smaller and consisted mainly of patients with biliary and nonsevere cases of acute pancreatitis, while the most numerous subgroup in our study comprised patients with alcohol-induced acute pancreatitis, with a high percentage of severe cases. Chikamune et al. [27] studied the lipid profile in an oleic acid-induced animal model of pancreatitis. They reported changes in the apolipoprotein compositions but not in the serum total cholesterol concentration.

A drawback of the present study was our inability to compare the changes in the serum lipid concentrations using established prognostic tools such as the Ranson score due to the retrospective nature of this study. Furthermore, this study does not address the mechanisms behind the association of serum lipid concentrations and the severity of acute pancreatitis. Other authors have suggested that low amounts of HDL cholesterol, which has anti-inflammatory properties, can lead to a more severe systemic response [26, 28], while it is possible that these changes are caused by the acute illness through the activation of the inflammatory system and impaired liver function and are simply more profound in more severe cases. It is also possible that some unrecognized confounding factor behind the association exists. Clearly, further studies are required to establish the pathophysiology behind this interesting association.

Conclusion

In conclusion, this study demonstrates that low concentrations of serum total cholesterol, LDL cholesterol and HDL cholesterol are associated with more severe acute pancreatitis. These changes are already present during the early stages of the disease and are similar in all etiologies of acute pancreatitis. Furthermore, the changes observed persist even later during the course of the disease. However, the mechanism behind this interesting association remains unclear, and further studies are needed.

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