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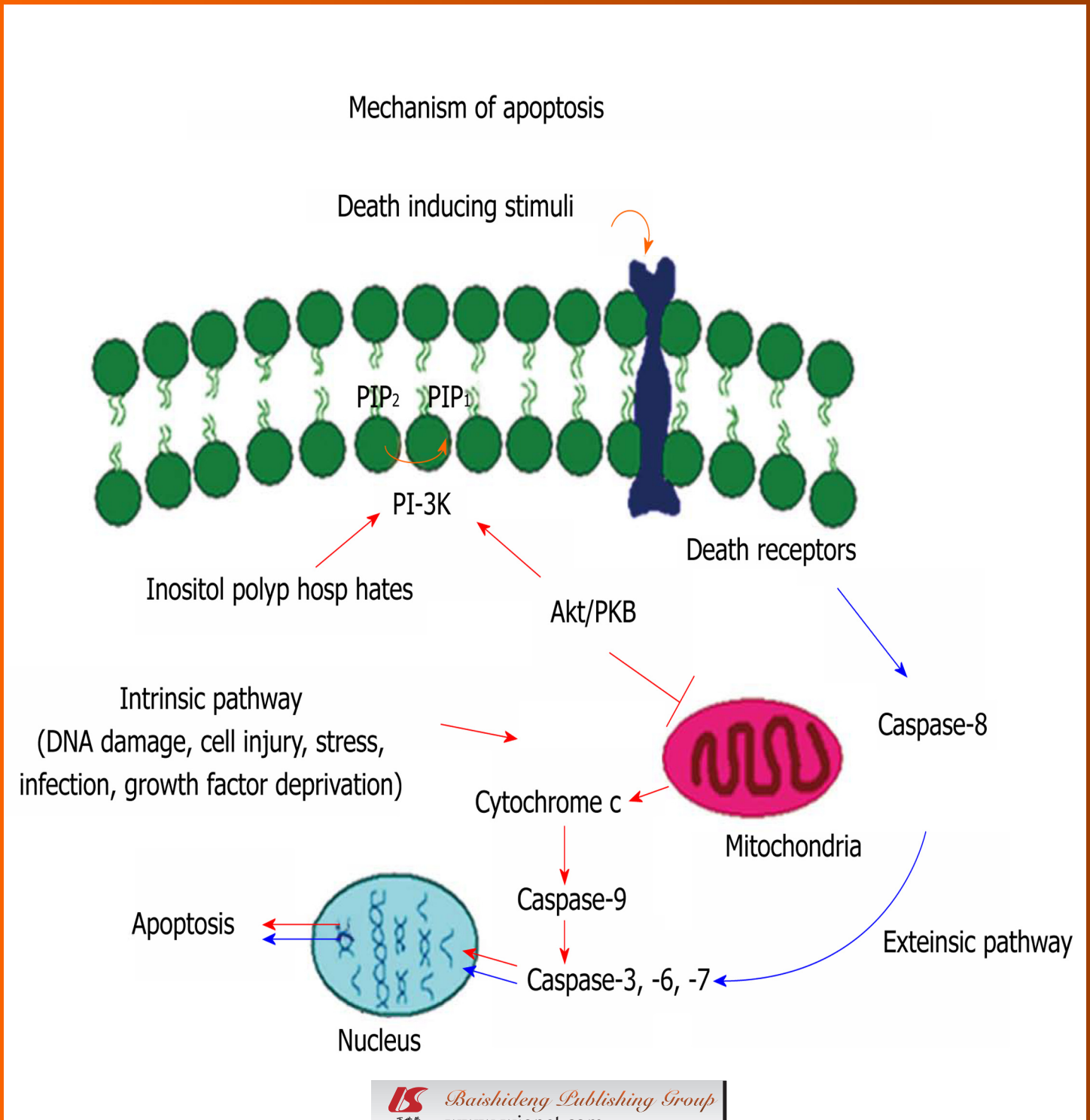

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EDITORIAL

Update on pathogenesis and clinical management of acute pancreatitis

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Abstract

Acute pancreatitis (AP), defined as the acute nonbacterial inflammatory condition of the pancreas, is derived from the early activation of digestive enzymes found inside the acinar cells, with variable compromise of the gland itself, nearby tissues and other organs. So, it is an event that begins with pancreatic injury, elicits an acute inflammatory response, encompasses a variety of complications and generally resolves over time. Different conditions are known to induce this disorder, although the innermost mechanisms and how they act to develop the disease are still unknown. We summarize some well established aspects. A phase sequence has been proposed: etiology factors generate other conditions inside acinar cells that favor the AP development with some systemic events; genetic factors could be involved as susceptibility and modifying elements. AP is a disease with extremely different clinical expressions. Most patients suffer a mild and limited disease, but about one fifth of cases develop multi organ failure, accompanied by high mortality. This great variability in presentation, clinical course and complications has given rise to the confusion related to AP related terminology. However, consensus meetings have provided uniform definitions, including the severity of the illness. The clinical management is mainly based on the disease´s severity and must be directed to correct the underlying predisposing factors and control the inflammatory process itself. The first step is to determine if it is mild or severe. We review the principal aspects to be considered in this treatment, as reflected in several clinical practice guidelines. For the last 25 years, there has been a global increase in incidence of AP, along with many advances in diagnosis and treatment. However, progress in knowledge of its pathogenesis is scarce.

Key words: Pancreas; Pancreatitis; Pathogenesis; Treatment

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INTRODUCTION

Acute pancreatitis (AP), defined as the acute nonbacterial inflammatory condition of the pancreas, is derived from the early activation of digestive enzymes found inside the acinar cells, with variable compromise of the gland itself, nearby tissues and other organs. AP is a disease with extremely different clinical expressions.

Most patients suffer a mild and limited disease but about one fifth of cases develop multiple organ disfunction syndrome (MODS), accompanied by high mortality. This great variability in presentation, clinical course and complications has given rise to the confusion related to AP related terminology. However, consensus meetings (Atlanta and later working groups) have provided more uniform definitions[1-3].

For the last 25 years, there has been a global increase in incidence of AP, along with many advances in diagnosis and treatment. However, progress in knowledge of its pathogenesis is scarce.

PATHOGENESIS

Given the great variability in the clinical manifestations of AP, there are many aspects that have been systematically reviewed and then reflected in consensus meetings and clinical guidelines[4-7]. It is well known that several situations may develop AP, but the innermost mechanisms and how they act to develop the disease are still unknown. Most concepts are based in experimental animal studies and relate to the mechanisms that originate the intracellular activation from trypsinogen to trypsin and, thus, the pancreas "self-digestion" that elicits the local and systemic inflammatory responses. However, these mechanisms are not strictly applicable to humans[8]. Two examples: biliary lithiasis and alcohol abuse are responsible for 70% to 75% of cases of AP in humans but no experimental animal model has reproduced the disease by these mechanisms. On the other hand, cerulein (a cholecystokinin analogue) and a diet supplemented with ethionine, deficient in choline, are very often used to induce pancreatitis in animals but are not accepted causes of AP in humans.

Biochemical and structural changes observed in the early stages of AP in different animal models, as well as in humans, are very similar. Multiple etiological factors involved generate these changes basically through three mechanisms: toxic-metabolic, genetic and mechanical (Table 1). What we do not know is why some individuals will develop an edematous pancreatitis and other individuals a much more severe necrotic pancreatitis[9]. An exhaustive review of the available literature about AP pathogenesis exceeds this article but it may be of interest to summarize some aspects known at present that have implications in the clinical management of AP. If we establish a phase sequence, we should mention some initial steps (alcohol abuse, the passage of calculi through the papilla, *etc*.) that can generate other steps inside the acinar cells (co-localization, zymogens activation, tissular damage, pro-inflammatory factors production) that favor AP development; besides some systemic events, such as chronic inflammation and fibrosis, that will favor chronic pancreatitis development[10,11].

Our current knowledge of the pathogenesis of AP can be summarized by the following points: (1) It has recently been confirmed that AP starts in acinar cells, as shown by animal models in which the main pancreatic duct was ligated[12]; (2) The initial mechanisms by which a diversity of situations develop AP and why they occur is not well known. Only a small percentage of individuals exposed to these developing situations will present with clinical manifestations of the disease. Not every patient with biliary lithiasis or hypercalcemia will develop AP; only 10% of alcohol abusers will develop the disease; (3) The exocrine pancreas synthesizes and secretes digestive enzymes that are mainly activated when they reach the duodenum. A small proportion of trypsinogen is activated spontaneously inside the acinar cells, although there are different substances and protective mechanisms that "wash out" a possible excess of activated trypsin (inhibitor of pancreatic trypsinogen secretion pancreatic secretory trypsin inhibitor or serine protease inhibitor Kazal type 1 (SPINK1), mesotrypsin, enzyme Y, α1-antitrypsin, α2-macroglobulin or autolysis of prematurely activated trypsin)[13]; (4) Once defensive mechanisms have been passed over, the situation favored by the co-localization with lysosomal enzymes, including catepsin B, intra acinar activation of proteolytic enzymes in excessive amounts, favors the pancreas self-digestion;

Table 1 Causes of acute pancreatitis[6,9]

Etiology of acute pancreatitis	
Toxic-metabolic	Alcohol
	Hyperlipidemia, hypercalcemia
	Drugs and pills
	Organophosphorus and other toxic substances
	Venoms (scorpion, spiders)
Mechanical	Biliary: lithiasis, microlithiasis, sludge
	Congenital malformations
	Pancreas divisum
	Annular pancreas
	Anatomical variants:
	Duodenal duplication
	Duodenal diverticulum
	Choledochal cyst
	Ampullary dysfunction and stenosis
	Trauma
Genetic	Familial
	Sporadic
Miscellanea	Vascular
	Hypotension
	Vasculitis
	Embolisms
	Hypercoagulability
	Autoimmune associated to other autoimmune disorders
	Sjögren syndrome
	Primary sclerosing cholangitis
	Celiac disease
	Autoimmune hepatitis
	Infections:
	Virus: mumps, Coxsackie A, HIV, CMV
	Bacteria: Mycobacterium tuberculosis
	Parasites: Ascaris
	Other: Mycoplasma
	Idiopathic

HIV: Human immunodeficiency virus; CMV: Cytomegalo virus.

(5) On the other hand, trypsin will activate other pathways, such as complement, coagulation or fibrinolysis, extending the process outside the gland. The vascular endothelium and the interstitium are affected, which causes a microcirculatory damage that increases the vascular permeability, favoring the liberation of free radicals, pro-inflammatory cytokines (tumor necrosis factor (TNF)-α, interleukins (ILs) 1, 6 or 8), arachidonic acid metabolites (prostaglandins, platelet activator factor, leukotrines) or lipolytic and proteolytic enzymes, that can induce thrombosis and tissular hemorrhage and finally necrosis. Other substances that may be involved are substance P, kinases activated by stress (mitogen-activated protein kinase, extracellular signal-regulated kinase or JUNK), adhesion molecules (P-selectin or E-selectin) and cyclo-oxigenase-2 or heat shock proteins, the only ones that have a protective role[14,15]; (6) Although fortunately not common, occasionally an acute inflammatory process is associated with a systemic inflammatory response syndrome (SIRS) mediated by cytokines and pancreatic enzymes released in to general circulation that may affect distant organs, giving rise to respiratory distress, renal failure, myocardial depression and shock or metabolic alterations. Finally, a MODS may occur with vital risk of necrotic tissue infection, a situation where translocation of intestinal pathogens plays an important role[16,17]; (7) Our understanding of the implication of genetic factors in pathogenesis or the clinical course of AP is poor, but several clear examples of the importance of genetic variability have been reported. The prototype susceptibility genes include the cationic trypsinogen gene (*PRSS1*) and the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), as well as polymorphisms in SPINK1. Premature activation of pancreatic zymogens within the pancreas has also been proposed as the pathogenic mechanism for the acute attacks of pancreatitis seen in patients with hereditary pancreatitis but originated by these mutations. Mutations in at least one allele of the CFTR have been demonstrated in more than 35% of patients with idiopathic chronic pancreatitis and recurrent AP; also, in similar proportions, in patients with AP related to pancreas divisum. How these mutations might produce AP is unclear. One possible explanation is the production of a more concentrated pancreatic juice, leading to ductal obstruction or altered acinar cells function[18,19]; and (8) Genetic modifying factors are another interesting point: in clinical practice, the most important may be those that modify the severity of inflammatory response or increase the risk of specific complications. Some examples are the polymorphisms described in some pro-inflammatory or anti-inflammatory cytokines (TNF-α, IL-8, Il-10)[20].

Thus, AP is a disease that progresses through different phases. The initial step is triggered by an initial event (exposure to different and recognized etiological factors). This generates diverse changes inside acinar cells that produce digestive enzyme inhibition, associated with the co-localization of zymogens of digestive enzymes and lysosomal hydrolases. This generates the activation of zymogens inside the damaged acinar cells. This zymogens activation originates the release of different inflammatory mediators. These mediators regulate the severity of the disease, including its involvement in the development of a systemic inflammatory response. Repeated attacks of AP can promote the development of intrapancreatic fibrogenesis and chronic inflammation, which ultimately will generate chronic pancreatitis.

AP pathogenesis knowledge may have important implications in prevention and treatment. If the early events that generate the inflammatory process are understood, and if pro- and anti-inflammatory factors that modulate the severity of the disease are known, treatment will be implemented so the process will not happen or, at least, the possible associated complications will be minimized.

CLINICAL MANAGEMENT

The clinical management of AP is mainly based on the disease's severity. Two types of pancreatitis were defined at the Atlanta symposium in 1992: one light form, usually auto limited; and the other severe, where local complications may appear, such as necrosis and distant organ failure (OF) (Table 2). Fortunately, these complications are uncommon, occurring in approximately 15% of the cases; mortality, mainly when infected necrosis is present, is very high. The situation's severity will be determined by clinical, analytical and radiological criteria. Because some complications do not appear immediately (necrosis or pseudocysts), a severity definition will be made adequately at the end of the process[1,6]. The first step in the clinical management of AP is to estimate if it will progress as light or severe. The treatment of AP must correct the underlying predisposing factors and control the inflammatory process itself.

Patient's evaluation and prediction of illness severity

So far, there has been no precise method for this purpose, although in daily practice, following several clinical guidelines, a number of criteria are being used[5-7].

Prognosis scales and multiparametric methods: The most commonly used scales are characterized by having a high negative predictive value (NPV), i.e., the process considered mild will evolve in a favorable manner. At the same time, the positive predictive value (PPV) is not that high; many patients considered to suffer a severe disease will also evolve in a favorable manner. The Glasgow and Ranson scales have been and still are being used; they are easy to use, although they require 48 h for a complete evaluation[21-23]. The Acute Physiology and Chronic Health Evaluation (APACHE) Ⅱ scale and its modification for obese patients, is currently the most commonly used scale; a score higher than 8 indicates severe illness. The problem is that 14 variables must be recorded, but it can be useful to assess severity of illness at patient's ad-

Table 2 Definitions for acute pancreatitis according to the Atlanta classification[1,6]

Criteria of illness severity in acute pancreatitis	
Local complications	Necrosis: focal or diffuse area of non viable pancreatic parenchyma, with necrosis of peripancreatic fat (> 30% of the gland or > 3 cm)
	Pseudocyst: pancreatic juice collection surrounded by a wall of granulation or fibrous tissue that is developed as a consequence of acute or chronic pancreatitis or pancreatic traumatism
	Abscess: pus collection well defined that has scarce or no amount of pancreatic necrosis
Systemic complications (organic failure)	Respiratory failure: PaO2 < 60 mmHg
	Shock: systolic BP < 90 mmHg
	Renal failure: creatinine > 2 mg/dL after rehydration
	Upper gastrointestinal bleeding: > 500 mL/24 h
Bad prognosis data	Ranson's scale ⩾ 3
	APACHE II scale ⩾ 8

APACHE: Acute physiology and chronic health evaluation.

mission. More recently, the bedside index for severity in AP system has been developed (Table 3) with a predictive value similar to APACHE Ⅱ, but much simpler to implement because it only reflects five variables[24,25].

Clinical evaluation of MODS: The presence and severity of MODS is not a predictive method by itself, but it is the best indicator of AP severity and mortality, mainly if it appears early, persists for more than 48 h or is multi organic. At the Atlanta symposium, it was defined as shock, pulmonary insufficiency, renal failure and gastrointestinal bleeding; it can be quantified through diverse systems, but in our area, Sequential Organ Failure Assessment is perhaps the most commonly used.

The development of SIRS, characterized by tachycardia, tachypnea, hypocapnia, hyper or hypothermia, leucocytosis or leucopenia, can be recognized with a simple physical exam and often proceed to MODS. The patients that developed SIRS on admission and which persisted during their hospital stay, often developed MODS, with a mortality of 25%. In fact, some studies have assessed the predictive value of the clinical evaluation on admission, pointing out that this is comparable to some of the above mentioned parametric methods applied 48 h later.

Lab tests: The C reactive protein (CRP) is broadly recognized as an indicator of severity. Its serum peak appears 48 h after the disease onset and currently its precision as a prognostic factor is high. Values higher than 150 mg/L have a sensitivity of 80%, specificity of 76%, PPV of 76% and NPV of 86%, as an indicator of severe AP, even when correlated with necrosis. Marked hemoconcentration appears when a large amount of liquid has been accumulated in a third space. A prospective study showed that a hematocrit of 44%, together with the inability to decrease this level in 24 h, were good predictors of MODS and indicators of pancreatic necrosis. In fact, hematocrit NPV at 24 h was very high in predicting pancreatic necrosis and MODS. However, other authors do not report such results. Finally, we should mention that if the blood urea nitrogen (BUN) is increased at admission (> 20 mg/dL) or elevated 24 h later, it indicates poor progress.

Table 3 The bedside index for severity in acute pancreatitis prognosis system[25]

Parameters	
Blood urea nitrogen	BUN > 25 mg/dL
Impaired mental status	Conscious status impairment
Systemic inflammatory response	SIRS criteria presence[1]
Age	> 60 yr
Pleural effusion	Pleural effusion at X ray

[1]Systemic inflammatory response syndrome: presence of ⩾ 2 criteria. Heart rate > 90 bpm; Temperature > 38 ºC or < 36 ºC; Respiratory rate > 20 bpm or PaCO2 < 32 mmHg; Leucocytes > 12.000 or < 4.000/mm3 or > 10% immature forms. BUN: blood urea nitrogen; SIRS: Systemic inflammatory response syndrome.

Imaging studies: It is well known that a pleural effusion, seen in a chest X-ray on admission, predicts poor progress. However, it is more important to focus on the abdominal computed tomography (CT) scan findings, mainly when intravenous contrast administration has been completed, which will show the existence of necrosis, a severe criteria in the Atlanta classification.

A gradation system, used according to CT findings, was developed by Balthazar and has been broadly extended[26]. This, together with a score depending on necrosis extension, allows the calculation of a radiological severity index (CT Severity Index)[27]. Patients with a score higher than 5 had higher mortality, longer hospital stays and required more necrosectomies (Tables 4 and 5).

Not all patients with the diagnosis of AP require an abdominal CT scan. This should be reserved for those with severe AP or that show an evident deterioration during their stay. If a CT is to be obtained, it will preferably be done between the fourth and tenth day after the disease onset. Classically, it used to be said that a very early CT was not very helpful, but for some authors its utility has been demonstrated in the first 36 h to 48 h.

Treatment

The main causes of mortality in AP are MODS and infection of necrotic tissue. Prevention or diagnosis and early correction will be the first goal in the manage-

Table 4 Balthazar score system[26]

Grade	Computer tomography findings
A	Normal pancreas
B	Pancreatic focal or diffuse bigger size, including irregular contour or nonhomogeneous attenuation
C	Grade B + pancreatic inflammation
D	Grade C + fluid collection
E	Grade D + 2 or more fluid collections with or without the presence of gas in the pancreas or next to it

Table 5 Computer tomography index of illness severity for acute pancreatitis[27]

Balthazar's CT grade	Score	Necrosis at CT (%)	Score
A	0	None	0
B	1	< 30	2
C	2	30-50	4
D	3	> 50	6
E	4	-	-

CT index is obtained by the sum of the score obtained applying the Balthazar scale plus the score corresponding to the percentage of necrosis (maximum score = 10). CT: Computer tomography.

ment of these patients. Thus, support measures are very important, including an aggressive hydro-electrolytic replacement, analgesic control and nutritional support, as well as avoiding the recurrence of the process.

Support measures: In any AP patient, even in those that appear to present with light clinical AP, vital signs must be monitored and lab tests must be obtained periodically (oxygen saturation, respiratory and cardiac frequency, blood pressure, diuresis, red blood cell count, white blood cell count, BUN, blood glucose and electrolytes). In this way, SIRS or MODS may be detected, hydro-electrolytic derangements corrected and metabolic complications avoided.

Blood gases monitoring: Hypoxia is common in AP. In fact, O_2 saturation in arterial blood is one of the criteria included in multiparametric systems to assess severity of illness. Its origin is multifactorial and some studies have shown that its effect is similar to that of hypovolemia in the intestinal tissue; thus, it is essential to keep it above 95%.

Hydro-electrolytic replacement: This is a crucial aspect in the patient's outcome to which much attention is being paid. Vomiting, diaphoresis, fever, fluid sequester in a third space and the vessel's increased permeability, give rise to hypovolemia that must be replaced early and adequately. Hypovolemia compromises pancreatic circulation, favoring the development of necrosis. Similarly, hypovolemia compromises the bowel, allowing for bacterial translocation and endotoxin production which, in turn, facilitates the infection of necrotic tissues[28].

The amount and composition of fluids used for replacement is not standardized, but resuscitation must be aggressive from the beginning and the patient's response carefully monitored; urine output, hematocrit and BUN are used as an indirect measurement of hypovolemia, mainly in the first 12-24 h if they were elevated at the beginning (hematocrit > 44% and BUN > 20 mg/dL)[29,30]. In patients with a risk of fluid overload, it is necessary to monitor the central venous pressure or even to insert a pulmonary artery catheter (Swan-Ganz) to monitor the cardiac preload.

Over the last years, different studies evaluating fluid therapy effect on AP prognosis have been published. We mention a recent review paper[31] that includes most of these studies, including randomized controlled trials demonstrating the importance of hydro-electrolytic resuscitation in the initial 72 h, but with greater risk of infection complications in the case of too rapid hemodilutions. In this way, we must take into account the results obtained by de-Madaria *et al*[32] in a prospective controlled study: an aggressive fluid therapy during the initial 24 h of admission in patients without signs of fluid depletion may be detrimental.

Some recent studies have shown that, for fluid replacement in AP, Ringer's lactate is superior to normal saline, as assessed by CRP measurements and the development of SIRS. However, in AP secondary to hypercalcemia, Ringer's solution would be contraindicated because of the high calcium content[33,34]. So, fluid therapy remains the main goal of early management in AP, but it is necessary to review actual data for development of guided protocols.

Analgesia: Usually, abdominal pain is the main symptom in AP and its control is an essential goal of treatment. There is no evidence confirming the superiority of any analgesic. The treatment must be gradual and several drugs may be used, such as pirazolones (metamizol) or opioids (meperidine, morphine, tramadol), which are usually administered intravenously. Pump analgesia, instead of bolus, is a good option when the pain is intense.

It is controversial whether morphine is used or not; only elevated doses produce hypertony of Oddi's sphincter. There are no studies showing that morphine worsens the clinical course of AP. On the other hand, repeated doses of meperidine may generate the accumulation of normeperidine (a meperidine metabolite) than can produce neuromuscular irritation[35]. The use of phentanile has also been proposed. Phentanile, administered either subcutaneously or i.v., gives good

results in terms of pain control and security profile.

In patients with severe pain or difficult analgesic control with standard measures, the epidural administration of opioids or local anesthetics has been used with good results in terms of gas exchange and bowel motility. Similarly, clinical trials using bupivacaine have shown the improvement of pancreatic microcirculation, together with a lower development of necrosis and systemic complications[36].

Nutritional support: Patients with light AP generally respond to fluid replacement in a few days without any repercussions on nutritional status. Oral feeding is recommended when vomiting or ileus is not present. Occasionally, oral feeding may elicit pain and should be stopped. However, when pain remits, usually between 24-48 h after the onset, oral feeding should be resumed. Classically, a fluid diet is followed by a low fat diet (below 30% of total calories), progressing to adequate. Some authors have recently suggested providing a solid and low fat diet earlier in the course of the disease less gradually, since the standard way does not offer advantages and may increase the length of stay in hospital[37,38].

However, in severe AP, characterized by a hypercatabolic state that affects nutritional status, it seems reasonable to provide nutritional support together with other measures of treatment. Moreover, in severe AP, pain, vomiting and ileus take longer to disappear. At the same time, the external compression of the digestive tract by collections or inflammation may prevent the reintroduction of an oral diet.

There are a large number of scientific papers trying to establish when, how and what kind of nutritional support should be provided to AP patients and occasionally results are contradictory. Some recommendations, based on meta-analysis and controlled studies, are given in clinical guides. Evidence comparing enteral nutrition (EN) through a naso-jejunal tube with total parenteral nutrition (TPN) has been pursued. It has been shown that EN, compared to TPN, is associated with a lower incidence of metabolic complications and infection, since the integrity of the intestinal barrier is kept. On the other hand, EN is cheaper and requires a shorter hospital stay. Besides, EN avoids some mechanical and septic complications related to central venous catheters that may reduce mortality[39-43].

If required, nutritional support should be provided early in the course of AP, as soon as in the first 48 h. Once the severity of the disease has been assessed, it is preferable to use semi elemental formulas with high protein and low lipid content, increasing the amount according to tolerance. EN tolerance is variable and depends on the infusion's rate, nutrient's concentration, place of delivery (stomach, jejunum) and the phase of inflammatory response of AP. If the placement of a postduodenal tube is not possible, a nasogastric tube may be used. Some studies have shown no difference between both ways of administration. There are few studies assessing the influence of different formulas in the course of AP, but it is thought that supplements might be helpful, such as immunostimulants (arginine, glutamine, omega-3 fatty acids, vitamins C and E, beta-carotenes), micronutrients (zinc, selenium, cromium) or even pro-biotic components.

Currently, the presence of intra abdominal fluid collections or persistently elevated pancreatic enzymes is not a contraindication for EN. However, it is true that in some patients, pain reappears and pancreatitis worsens, increasing the size of collections, when oral feeding is resumed or EN is set up. In these cases, TPN should be used.

Admission to an intensive care unit: AP mortality is generally a consequence of MODS. In the first two weeks, this risk is mainly related to a systemic inflammatory response. Then, mortality is usually associated with pancreatic necrosis and infection. Intensive care unit admission must be considered under the following circumstances: (1) Persistent MODS for more than 48 h and early onset (during the first week) because it is associated with 50% mortality; (2) Clinical manifestations predicting MODS development, according to clinical status, multiparametric systems (more than 3 Glasgow or Ranson analytical criteria at 48 h or APACHE Ⅱ higher than 8), biochemical data (riboflavin carrier protein > 150 mg/dL at 48 h), radiological data (persistent pleural effusion for more than 48 h after admission) or associated obesity; and (3) Development of local complications.

Clinical management of local complications of AP

Similar to the presentation of MODS, hemodynamic instability or severe metabolic derangements, local complication developments requires the coordinated efforts of a multidisciplinary team, including gastroenterologists and other medical specialists, radiologists, intensive care specialists and surgeons.

Pancreatic necrosis: The presence of pancreatic necrosis is an inscrutable marker of illness severity. Often, necrosis is followed by early or late OF development, due to the inflammation itself or its associated infection. Necrosis infection is the most severe local complication that can appear and is associated with 40% mortality. According to these facts, prophylactic antibiotics have been assessed to reduce mortality.

Antibiotic prophylaxis: Antibiotic prophylaxis is one of most controversial matters of the clinical management of AP and nowadays it is not possible to make clear recommendations. A number of studies have been published with contradictory results that can be explained by the inclusion of heterogeneous patients, different antibiotic regimes, questionable designs of study, and different study objectives. At present, the American Association of Gastroenterology recommends antibiotic prophylaxis in extended necrosis, according to abdominal CT (involving more than 30% of gland). It is

recommended that prophylaxis should not extend longer than 14 d because then it would favor fungus infection. Recent studies, some of them meta-analysis of previous studies, as well as other well designed studies do not approve routine use of prophylactic antibiotics because there are no significant differences related to surgery or mortality. We must pay special attention to identify some subgroups of patients that might obtain a benefit with this antibiotic prophylaxis[6,44-51].

Sterile necrosis: It was classically advised to remove necrotic tissue to prevent the development of MODS. At present, there is broad consensus to try to manage the situation conservatively, at least for the first 3 or 4 wk; delayed necrosectomy is associated with lower morbidity and mortality. In this timeframe, a spontaneous resolution of necrosis may occur or necrosis may organize, giving the opportunity of minimally invasive therapy. A percutaneous or endoscopic treatment may be attempted, but the material density may prevent complete drainage. Open or laparoscopic surgery can removed necrotic tissue and effective drainage and washing can be established[52,53].

Infected necrosis: Around one third of necrotic AP are infected, a complication that may appear during the second week of evolution. This situation should be suspected if a systemic inflammatory response persists for more than two weeks after admission, clinical course worsens or air bubbles appear at CT. After excluding other infection origins, infected necrosis has to be confirmed by puncture guided ultrasonography or CT, followed by Gram smear and culture. If the initial puncture is not diagnostic, it can be repeated after a few days. While waiting for the culture's result, the intravenous antibiotic should be started. Carbapenem (imipenem or meropenem 1 g/8 h) or ciprofloxacin plus metronidazole will be maintained until obtaining the antibiogram result. If Gram positive bacteria are isolated, vancomycin (1 g/12 h) will be administered[54].

The standard treatment for infected pancreatic necrosis is open or laparoscopic surgical drainage. However, on occasions, percutaneous drainage may work well. As recommended by the International Association of Pancreatology Clinical Guideline, drainage should be effectively established when the patient is septic. A step by step treatment is proposed by which percutaneous or endoscopic drainage should be established first and then necrosectomy with drainage through a minimally invasive retroperitoneal access. When this method was compared with open surgery, it offered several advantages, including the chance to avoid surgery in some patients, less complications and lower cost[55-59].

The alternatives to open surgery should be considered, mainly in frail and critical patients that would not tolerate a more aggressive surgery. Some alternatives such as endoscopic necrosectomy or invasive percutaneous drainage should be evaluated through controlled trials. In clinical practice, it is important to consider the importance of a multidisciplinary management, considering the clinical situation as well as the comorbidity of the patient and the center experience.

Other local complications of AP: There are other situations that, although less common, should be considered.

Hemorrhagic complications: Hemorrhagic complications of AP are fortunately rare; however, they may present in a diversity of forms. Sometimes, upper or lower gastrointestinal bleeding occurs due to gastroduodenitis secondary to adjacent inflammation, bleeding peptic ulcer, pseudocyst rupture into the digestive tract or drainage of a pseudo aneurysm through the Wirsung. In severe cases of AP, bleeding may occur due to intra- or retroperitoneal erosion of the vessels of the celiac trunk, mainly the splenic artery. Diagnosis may be established by angiography or angio-CT. Angiography, besides identifying the bleeding point, sometimes allows embolization that may stop bleeding. If this method fails, the definitive treatment has to be surgery[60].

Pancreatic duct breaking: Generally this is produced in the context of pancreatic necrosis due to erosion of the duct. In cases of necrosis, complete or partial pancreatic duct breaking occurs in about 60% of cases. The pancreatic juice often accumulates inside the abdomen, in the neighborhood of the pancreas, originating a pseudocyst. However, pancreatic juice can also flow to other locations, causing pancreatic ascites, pleural effusion, distant pseudocyst or cutaneous fistula. To assess this situation, wirsungraphy by using CT, nuclear magnetic resonance (spectroscopy) or endoscopic retrograde cholangiopancreatography (ERCP) can be performed. This latter method may be associated with the placement of a stent, which will favor definitive resolution. Nutritional support and potent antisecretors such as octreotide should be associated. Collections can be removed by percutaneous or endoscopic drainage. Successful fistula sealing has been described by using cyanoacrylate or fibrine. If other treatments fail, which is common, surgery is indicated. If the duct is opened at the pancreatic tail, a distal pancreatic resection may be curative. Otherwise, internal drainage, through a pancreatic-digestive anastomosis, may be necessary[61,62].

Abdominal and retroperitoneal collections: They are only treated if they are symptomatic or complicated (infection, rupture, pseudoaneurysm). The treatment will depend on whether or not the collection communicates with the duct of Wirsung, the collection has a firm wall, the duration of process and the presence of necrosis or detritus inside the collection. For collections less than four weeks, the treatment with percutaneous or endoscopic drainage is preferable. However, the presence of semisolid detritus may require a surgical treatment as the best option.

Splenic venous thrombosis: This occurs in about 20% of AP. Thrombosis is usually resolved when pancreatitis heals but if the thrombus migrates or extends to the portal or superior mesenteric veins, intestinal perfusion can be compromised or liver failure may appear. When thrombosis is diagnosed, platelet antiaggregant treatment may be instituted. However, the theoretical risk of bleeding in necrotic AP should be considered.

Treatment of pancreatic pseudocysts: Fluid collections that appear during AP disappear spontaneously in 40% to 50% of cases. In about 10% to 15% of cases, these collections persist and become encapsulated, generating pancreatic pseudocysts (PP). A "true" PP (i.e. without an epithelial lining; the counterpart would be a pancreatic cyst) takes at least 4 to 6 wk from the beginning of symptoms to be encapsulated by a wall formed by inflammatory fibrosis of the adjacent tissues. Few studies have documented the natural evolution of PP. Classically, it was considered that PP more than 6 cm in diameter or those that persisted for more than 6 wk should be operated on. Currently, it has been shown that about half of all PP can be solved spontaneously; thus, the attitude has shifted towards a more conservative approach.

Asymptomatic PP may be followed-up for periods of six month or longer, if they do not grow, become symptomatic or present complications such as hemorrhage, infection or mechanical compromise of adjacent organs. In these situations, percutaneous, endoscopic or surgical drainage should be considered. Its election depends on multiple factors: patient's general status, size, number and location of PP, communication or not with the main pancreatic duct, solid necrosis inside or not and possible complications. At the same time, a differential diagnosis between PP and another kind of cystic lesion is essential[63,64]. No controlled study has compared these three options of treatment, but intramural or transpapillar endoscopic drainage seems to be the preferred technique. The availability of sono endoscopes facilitates drainage of PP, even in cases of associated segmentary portal hypertension. Percutaneous drainage should be chosen in complicated PP or in patients with high surgical risk. In turn, percutaneous drainage of PP may be complicated by a pancreatic fistula (up to 20% of cases) or infection. A percutaneous drainage should be avoided in cases of hemorrhage or pancreatic ascites. At present, surgical treatment (mainly by internal drainage) is reserved for patients that percutaneous or endoscopic treatment failed in, those with complications from chronic pancreatitis, those with multiple or giant PPs, or when malignancy cannot be ruled out[65-67].

Some considerations about treatment of biliary AP: ERCP and timing for cholecystectomy

Gallstones are the most common cause of AP in most countries. This is important since cholecystectomy prevents recurrences. What is the right thing to do once the patient has improved after the acute episode? Or when is the best moment for surgery? Since the development of laparoscopic surgery and ERCP, some situations have been reviewed, analyzed in meta-analyses and the conclusions reflected in clinical guidelines.

After a first episode of AP, recurrence ranges between 25% and 60%. One fourth of these recurrences appears in the first six weeks and the percentage increases with time[68]. If pancreatitis has been light and the patient has satisfactorily recovered, ideally, cholecystectomy should be performed before the patient's hospital discharge. Alternatively, patients should have definitive surgical treatment in the next 2-4 wk. If pancreatitis has been severe, with associated collections, surgery should be delayed until the collections have been resolved or are not clinically relevant[55,69,70].

Another important aspect to be considered in the management of these patients is the possibility of residual choledocholithiasis and, thus, the need to explore the main biliary tree. In light AP, it was questioned whether to perform a preoperative ERPC plus endoscopic sphincterotomy and calculi extraction (if adequate) or, alternatively, to treat a possible residual lithiasis at surgery if this was discovered through the intraoperative cholangiography. After evaluating some experiences with mild to moderate AP patients, it can be established that it is preferable to choose cholecystectomy with intraoperative cholangiography plus calculi extraction (if these are present), limiting the practice of ERCP if calculi extraction has not been completed at surgery[71-73]. On the other hand, if AP is severe or courses with associated cholangitis or jaundice, ERCP plus sphincterotomy is advisable early during the patient's admission. Then the question arises as to whether or not to operate later. There is not enough data to make a categorical recommendation. However, if the patient does not have a high surgical, i.e. ASA Ⅰ-Ⅲ [according to the American Society of Anesthesiologists (ASA) physical status classification system] it seems reasonable to operate since a new episode of AP might imply a greater risk than surgery. Contrarily, in high risk patients (ASA Ⅳ-Ⅴ), it may be preferable to "wait and see." Occasionally, in selected patients, ERCP plus sphincterotomy may be considered, along with a posterior treatment with ursodeoxicholic acid to treat gallstones[74-76].

CONCLUSION

Each of these sections could probably lead to a review with more comprehensive comments. We recall the usefulness of the recommendations reflected in several clinical guidelines, although it is necessary to review some topics, such as fluid therapy or pancreatic necrosis management. Knowledge of the environment in which we operate and the limitations, and this approach to current recommendations should be converging lines in the

management of patients with AP in daily clinical practice.

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REVIEW

Molecular signaling mechanisms of apoptosis in hereditary non-polyposis colorectal cancer

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Abstract

Colorectal cancer is the second most leading cause of cancer related deaths in the western countries. One of the forms of colorectal cancer is hereditary non-polyposis colorectal cancer (HNPCC), also known as "Lynch syndrome". It is the most common hereditary form of cancer accounting for 5%-10% of all colon cancers. HNPCC is a dominant autosomal genetic disorder caused by germ line mutations in mismatch repair genes. Human mismatch repair genes play a crucial role in genetic stability of DNA, the inactivation of which results in an increased rate of mutation and often a loss of mismatch repair function. Recent studies have shown that certain mismatch repair genes are involved in the regulation of key cellular processes including apoptosis. Thus, differential expression of mismatch repair genes particularly the contributions of *MLH1* and *MSH2* play important roles in therapeutic resistance to certain cytotoxic drugs such as cisplatin that is used normally as chemoprevention. An understanding of the role of mismatch repair genes in molecular signaling mechanism of apoptosis and its involvement in HNPCC needs attention for further work into this important area of cancer research, and this review article is intended to accomplish that goal of linkage of apoptosis with HNPCC. The current review was not intended to provide a comprehensive enumeration of the entire body of literature in the area of HNPCC or mismatch repair system or apoptosis; it is rather intended to focus primarily on the current state of knowledge of the role of mismatch repair proteins in molecular signaling mechanism of apoptosis as it relates to understanding of HNPCC.

Key words: Colorectal cancer; Hereditary non-polyposis colorectal cancer; Apoptosis; Molecular signaling mechanisms; DNA mismatch repair proteins

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INTRODUCTION

DNA mismatch repair (MMR) system consists of several genes that play a crucial role in correcting DNA errors arising during DNA replication of the cell division. Besides their established role in DNA repair, MMR genes are also involved in programmed cell death or apoptosis, for example, apoptosis induced by DNA damage. Two major protein complexes namely MutL and MutS are

derived following formation of unique combination of MMR proteins including MLH1, MSH2 and MSH6. These MMR protein complexes (MutL and MutS) work together in a concerted manner to form a DNA repair machinery that are responsible for majority of DNA repair whether it is base-base mismatch or insertion/deletion mutation. This machinery repairs DNA not only during cell division but also during DNA damages induced by a number of environmental factors including treatment with several types of cytotoxic drugs used as anti-cancer agents[1].

Mismatch repair genes are highly conserved from prokaryotic to eukaryotic cells. The first indication of mismatch repair was obtained from *Streptococcus pnemoniae* and then in *Escherichia coli*[2]. Loss of mismatch repair genes is associated with increased risk of cancer, destabilization of the genome and an increased rate of mutation particularly in microsatellite sequences[3]. Inherited mutations in mismatch repair genes especially *MLH1* and *MSH2* are associated with hereditary non-polyposis colorectal cancer (HNPCC)[4]. It has been reported that somatic mutations and epigenetic silencing of MLH1 promoter gene are observed in sporadic cancer[5]. Several studies have reported that MMR system is also involved in mediating the activation of cell cycle check points and apoptosis in response to various anti-cancer drugs that act on DNA[6,7]. Thus, cells that have deficiency in one of the mismatch repair genes would be resistant to apoptosis than cells that are proficient in mismatch repair genes[8].

Apoptosis can occur through two different pathways; extrinsic pathway or intrinsic pathway. The extrinsic pathway is activated *via* ligation of death receptors on cell surface membrane leading to activation of caspase 8, followed by caspase 3. This pathway bypasses mitochondria. The intrinsic pathway, on the other hand, involves depolarization of mitochondrial membrane leading to the release of cytochrome C from mitochondrial intermembrane space. Intrinsic pathway is activated *via* apoptotic signals produced within the cell due to developmental cues or cell stress. Proteins such as cytochrome c released from mitochondria bind to apoptotic protease activating factor 1 (Apaf1) and caspase 9. This results in activation of caspase 3, and commitment to cell death. This pathway is regulated by the B-cell lymphoma 2 family of proteins. Accumulation of Bcl-2-associated X protein or Bcl-2 homologous antagonist killer on the mitochondrial outer membrane results in a conformational change allowing for membrane insertion and pore formation. A basic description of apoptosis and apoptotic pathways is provided here before providing its link to HNPCC and DNA mismatch match repair system. Relatively detailed description of apoptotic mechanisms in relation to carcinogenesis has been reported elsewhere[9].

APOPTOSIS

Apoptosis or programmed cell death plays an important role in tissue development and homeostasis[9]. Apoptosis was first described in 1927 by Currie *et al*[10]. In apoptosis, cells undergo a series of biochemical and morphological changes including cell shrinkage, chromatin condensation, cell membrane blebbing, formation of apoptotic bodies, and finally ending with engulfment of apoptotic bodies by macrophages or neighboring cells[11]. A detailed description of morphological changes and activation of cellular signaling pathways that occur during apoptosis has been published in an earlier report[9]. This report also provides an in-depth analysis of intracellular signaling molecules that trigger apoptotic events and that can be exploited for chemoprevention to carcinogenesis. Apoptosis can be triggered by various stimuli from outside or inside the cell, for example, DNA damage due to defect in DNA repair mechanism, treatment with cytotoxic drugs, or by deployment of death signals[12].

APOPTOTIC PATHWAYS

In mammals, there are two main apoptotic pathways, extrinsic pathway (death receptor mediated pathway) and intrinsic pathway (mitochondrial mediated pathway). As shown in Figure 1, the extrinsic pathway is mediated by cell surface death receptors. The death ligands bind and ligate with death receptors such as Fas, tumor necrosis factor receptor, or tumour necrosis factor-related apoptosis-inducing ligand receptors. This results in recruitment of adaptor protein Fas-associated death domain and caspase-8 forming a death inducing signaling complex (DISC). The auto activation of caspase-8 causes the activation of other caspases (caspase-3, -6, -7) in the caspase cascade process[13] that ultimately lead to cellular destruction. Caspases are aspartate-specific cysteine proteases and members of the interleukin-1β converting enzyme family[13]. So far, 14 mammalian caspases have been identified. Caspases are synthesized as inactive zymogen, which upon proteolytic cleavage become active.

The intrinsic pathway is mediated by different apoptotic stimuli. Most intrinsic signals induce depolarization of mitochondrial membrane and the release of cytochrome C into the cytoplasm. The release of cytochrome C initiates a series of biochemical events including activation of caspase cascade and thus cellular destruction. One of the most important events is that the released cytosolic cytochrome C binds to Apaf-1 and procaspase-9 that result in the formation of an intracellular DISC-like complex, apotosome. Activation of procaspase-9 leads to proteolytic processing and activation of procaspase-3, -6, and 7 resulting in an activation of caspase cascade and cell death[14]. The progression of apoptosis is highly regulated by a series of signaling pathways including those that involve in caspase cascade and PI3 kinase/AKT/PKB pathways. The caspase-cascade plays an important role in stimulation and transduction of apoptotic signals. The activation of the caspases is considered as a hallmark of apoptosis[14].

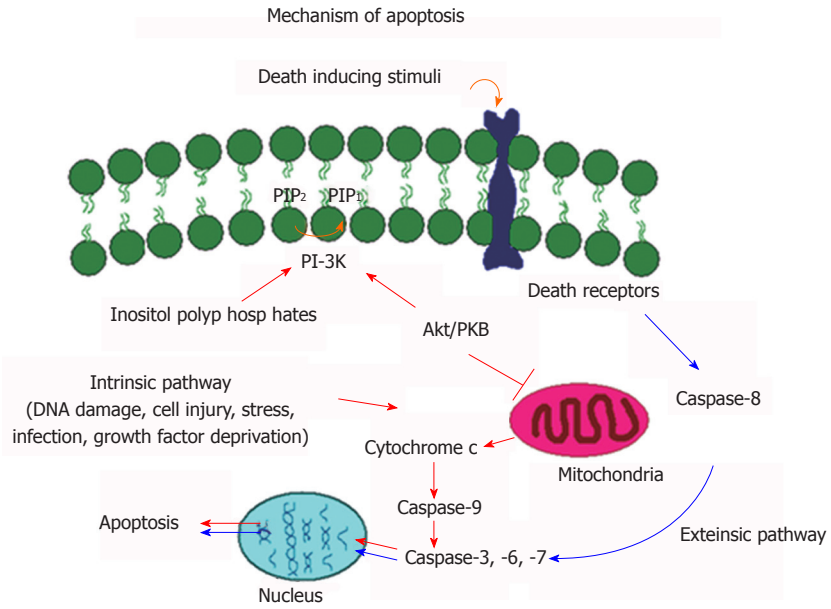

Figure 1 Molecular signaling mechanisms of apoptosis. Schematic representation of extrinsic and intrinsic apoptotic pathways that lead to activation of caspase cascade and programmed cell death.

COLORECTAL CANCER

A number of gastrointestinal cancers specifically associated with various regions of the intestinal tract starting from esophageal to cancers of anus have been identified. Among gastrointestinal cancers, colorectal cancer is by far the most studied intestinal cancer type. In general, cancer is a disease caused by defective genes that transforms a normal cell into a cancerous cell in such a way that is unable to control cell growth, and thus continue to proliferate in an irregular fashion. Cancerous cells are no longer responsive to apoptotic signals, thus escaping programmed death process (apoptosis). Colorectal cancer is one of the leading causes of deaths in the world. Colorectal cancer is the second most common cause of cancer related deaths in Western countries including the United States. Colorectal cancer was reported to be responsible for 9% of new cancer cases and 10% of cancer deaths in 2010 in the United States alone[15,16].

Colorectal cancer can develop as a disease if there is any genetic disorder; the most common cause is chromosomal instability[17]. There are two major types of colorectal cancers that are primarily regulated genetically; sporadic colon cancer caused by sporadic mutation and hereditary colon cancer caused by hereditary mutation. In sporadic case, the gene mutation is induced by exposure to different carcinogens. Sporadic cancer happens by chance and no family history can be tracked. In hereditary case, the gene mutations are found in the germ lines and the defect can pass from the parents to the children that result in an accumulation of cancer in the family; for example, HNPCC[17].

An important event to prevent cells from forming clonal growth that would lead to carcinogenesis is apoptosis[18]. During carcinogenesis, apoptotic process is deregulated and thus cells tend to escape natural death process to overcome any cellular damage. In other words, cells cannot perform its normal growth function if there is a mutation in certain cancer-related genes[19]. Mismatch repair genes may represent such a scenario. Among mismatch repair genes, *MLH1* and *MSH2* are most studied genes that have been linked to cause abnormalities in apoptotic process. Therefore, in this review article, our efforts will be focused to provide a possible linkage of these two mismatch repair genes in HNPCC and their manifestation of apoptosis. Furthermore, understanding of apoptotic mechanisms is of utmost importance because defect causes failure in treatment with anti-cancer agents, and may also cause a number of other human diseases[20].

HEREDITARY NON-POLYPOSIS COLORECTAL CANCER

HNPCC, also known as "Lynch Syndrome", is the most common form of hereditary colorectal cancer accounting for 5%-10% of all colon cancers. HNPCC is a dominant autosomal genetic disorder (affected person has one copy of the mutated gene) caused by germ line mutations in mismatch repair genes[21]. Patients with HNPCC show microsatellite instability due to mutations in DNA mismatch repair genes[22]. Microsatellites are repeated sequences of DNA usually 1 to 10 nucleotides long throughout the genome. Four genes are known to be responsible for HNPCC. These are *MLH1*, *MSH2*, *MSH6*, and *PMS2*. In early 1990s, the identification of genetic basis for HNPCC began with the localization of both *MLH1* and *MSH2* gene[23]. HNPCC has 80%-90% mutations in *MLH1* and *MSH2* genes. *MSH6* gene accounts for 10% of HNPCC whereas *PMS2* gene accounts only for 5% of HNPCC cases[23]. In HNPCC, a simple inser-

tion/deletion of a single nucleotide leads to frame shift mutation and thus truncated protein product formation. The frame shift mutation accounts for the majority of the mutations that have been identified in HNPCC[24]. HNPCC is also characterized by development of extra colonic tumor formation. The average age of diagnosis of HNPCC is approximately 45 years. HNPCC is subdivided into Lynch syndrome I (colorectal cancer only) and Lynch syndrome Ⅱ (colorectal cancer and extra colonic tumors), the extra colonic tumors that are associated with HNPCC are cancers of endometrium, stomach, small bowel, urinary tract, and ovaries[25].

DIAGNOSIS OF HNPCC PATIENTS

Several diagnostic criteria have been developed to help identify HNPCC[26]. Amsterdam Criteria I developed in 1991 was the first international criteria used for the diagnosis of HNPCC. Amsterdam Criteria I require three observations, colorectal cancer in three or more relatives, in at least two generations, and one or more relatives diagnosed before the age of fifty years[26]. Amsterdam Criteria I led to the development of Amsterdam Criteria Ⅱ to include the extracolonic malignancies[26].

Microsatellite instability (MSI) screening is used as an added criterion to establish defective DNA mismatch repair system. Microsatellites are short, tandemly repeated DNA sequences. MSI is a change in length of microsatellite allele due to insertion/deletion of repeating units during DNA replication[27]. This was used as a primary method for screening HNPCC[28,29] after its discovery in proximal colon tumors[30]. In 1997, the National Cancer Institute Workshop proposed a panel of five markers for microsatellite that could be used to detect MSI. This panel is called Bethesda panel, and includes two mononucleotide (BAT-25 and BAT-26) and three-di nucleotide (D5S346, D2S123, and D17S250) repeats. Samples are classified as MSI-high, if two or more of the five markers show instability, whereas those with one unstable marker are classified as MSI-low. Samples with no alteration are considered as MSI-stable[30]. In 2002, the National Cancer Institute workshop recommended a second panel of mononucleotide markers such as BAT-40 for detection of microsatellite instability high (MSI-H) because mononucleotide appears to be more sensitive to detect MSI-H than dinucleotide markers. Since both MSI and HNPCC are caused by mismatch repair defects, MSI can be used as primary method for screening population at risk for HNPCC. There is limitation for using MSI because MSI is not specific for HNPCC but it could exist in 10% to 15% of sporadic colorectal cancers. Hypermethylation of MLH1 promoter causes MSI in sporadic cancer. It is recommended that the patient who meets the Bethesda guidelines would be tested for MSI followed by immunohistochemistry (IHC) for the MSI-H tumors[27].

Use of IHC for MLH1, MSH2 proteins and MSI is a yet another good criterion to some extent because they are complimentary to each other in identifying HNPCC patients. IHC helps identify the mutated gene, and may detect MMR deficient case that can be missed by MSI testing[31]. MSH2 forms a dimer with MSH6 while MLH1 forms a dimer with PMS2. So a mutation in MSH2 or MLH1 will result in the loss of MSH2/MSH6 or MLH1/PMS2 staining by using IHC method. However; the reverse is not true.

DNA MISMATCH REPAIR SYSTEM

DNA Mismatch repair system consists of several genes that encode nuclear proteins responsible for maintaining genetic stability by repairing base-to-base mismatches and insertion/deletion loops that arise during S phase of the DNA replication. In eukaryotic cells, MMR repair systems include MutS and MutL proteins complexes. The genetic stability is normally dependent upon the ability of these MMR protein complexes to recognize DNA damages and repair them; failure to which causes genetic instability[32,33]. Eukaryotic DNA mismatch repair is initiated when MutS alpha (MSH2-MSH6) or MutS beta (MSH2-MSH3) recognizes and binds to mismatched DNA. The binding of these heterodimers to the mismatches recruits MutL including MutL alpha (MLH1-PMS2) or MutL beta (MLH1-PMS1)[7]. Human MutL alpha has an ATPase activity that regulates the termination of mismatch-provoked excision[7]. The formation of MutS:MutL mismatch DNA complex leads to strand discrimination and removal of the errors that are made. MMR process includes other factors that help correct these errors. These include proliferating cell nuclear antigen (PCNA), replication factor C (RF-C), exonuclease1 (Exo1), and DNA polymerase. PCNA interacts with both MSH2 and MLH1 and is thought to play a role in the initiation and DNA re-synthesis steps of the mismatch repair[7]. When there is deficiency in mismatch repair system, the replication errors in the genome are not repaired, As a result, mutations accumulate throughout the genome[34]. Thus mutations in mismatch repair genes cause predisposition to cancer.

Germ line mutation of MMR genes specially *MLH1* and *MSH2* were identified in the families with colorectal cancer; 70%-80% population have mutations in these two genes[35,36]. Therefore, cells that have mutation(s) in either one of these genes show mutator phenotypes and display MSI[7]. MLH1 and MSH2 are nuclear proteins with 756 and 934 amino acids that respectively encode proteins of approximately 80 KDa and 100 KDa. The *MLH1* gene is located on chromosome 3p21 with nineteen exons, while MSH2 gene is on chromosome 2p16 with sixteen exons[37].

ROLE OF DNA MISMATCH REPAIR PROTEINS IN HNPCC

The DNA mismatch repair system is considered as sensory system that can scan DNA, and when there is any

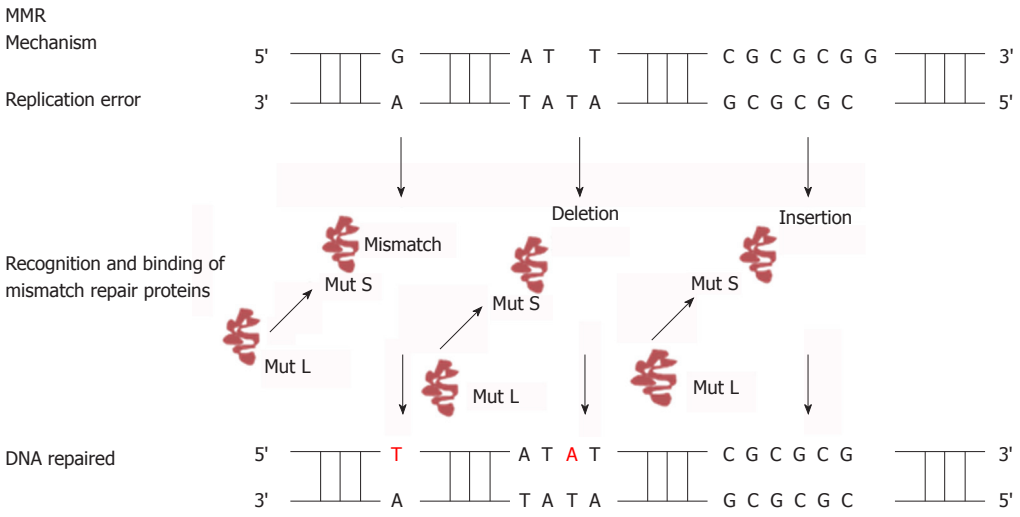

Figure 2 Mechanism of DNA mismatch repair. Figure shows how DNA mismatch repair system (MutS and MutL) detects DNA mismatches as well as insertion/deletion mutations during DNA replication and repairs it with the help of the DNA mismatch repair system.

insertion, deletion, or mispaired nucleotide, this system is able to detect and remove the errors[38]. DNA mismatch repair system can be divided into four phases: first recognition of mismatch base by MutS, second recruitment of MutL, third removal of incorrect base, fourth re-synthesis of corrected DNA by DNA polymerase enzyme using the parent strand as a template[39]. DNA mismatch repair was first discovered in bacteria where it was identified. Inactivation of this system increases mutation rates and fails to repair the DNA replication error[39]. In early 1990s, the importance of this system was further appreciated with the identification of genetic basis for HNPCC[23]. HNPCC is a caused by inherited germ line mutations in one of the mismatch repair genes especially *MLH1* and *MSH2*[31,40]. About 90% of HNPCC cases display MSI. MSI has been uniquely linked with mismatch repair defects, so MSI can be used as a marker for mutator phenotype to diagnose patients at high risk for HNPCC[40].

MECHANISMS OF DNA MISMATCH REPAIR

The human mismatch repair proteins are responsible for recognition and correcting errors that are made during DNA replication. A simplified version of how DNA mismatch repair system functions to correct DNA repair during replication is shown in Figure 2. The function of MutS-α (*MSH2/MSH6*) is to repair base-base mismatches. The function of MutS-β (*MSH2/MSH3*) is to repair insertion/deletion loop that arise during replication. MSH proteins have ATPase activity, one adenosine triphosphate (ATP) binding site present at each molecule[41]. In the presence of adenosine diphosphate (ADP) MutS protein will bind tightly to the mismatches on the DNA strand, while in the presence of ATP the MutS will act as sliding clamp[38]. MutS protein will move along to the DNA to identify which strand needs to be repaired. It has been proposed that the feature of the newly synthesized strand is a single strand nick for example the gaps between Okazaki fragments in the lagging strand[38]. So MMR will excise the strand containing a nick, which needs exonuclease activity. After the binding of MutS to the mismatches, MutS:ATP complex will recruit MutL proteins. MutL binds to the complex and interact with MutS at the site of the mismatches. MutL protein will transfer DNA polymerase, PCNA, and recruit exonuclease I for excision up to kilobase of DNA[38]. Excision of the mismatches can be either 5' to 3' or the opposite. 5' to 3' requires MutS, Exo1, and replication protein A. Whereas 3' to 5' excision requires MutL RFC and PCNA. After the mismatches have been removed, the re-synthesis step starts by the involvement of DNA polymerase[41].

There are two proposed models for the signaling of the downstream mismatch repair processes after the mismatch recognition; stationary (Trans) model and moving (Cis) model. The moving model includes translocation and molecular switch models[42]. In stationary model, the binding of MSH proteins to the DNA strand considered as protein -protein interaction, this cause DNA to bend and bring the two distant sites together. In moving model, MSH proteins will bind to the mismatch on the DNA and then this protein will move away from the site to look for the strand discrimination signal. Translocation model suggests that hydrolysis of ATP drives unidirectional movement of MSH proteins, and this will result in the creation of α-loop[7]. Molecular switch model hypothesized that the binding of MSH proteins to the mismatch will trigger an ADP to ATP exchange that will support the bi-directional sliding of MSH proteins away from the mismatch. After the movement of the MSH proteins from the site another MSH proteins will occupy the empty site. When MSH proteins reach the strand break, the excision step begins[7].

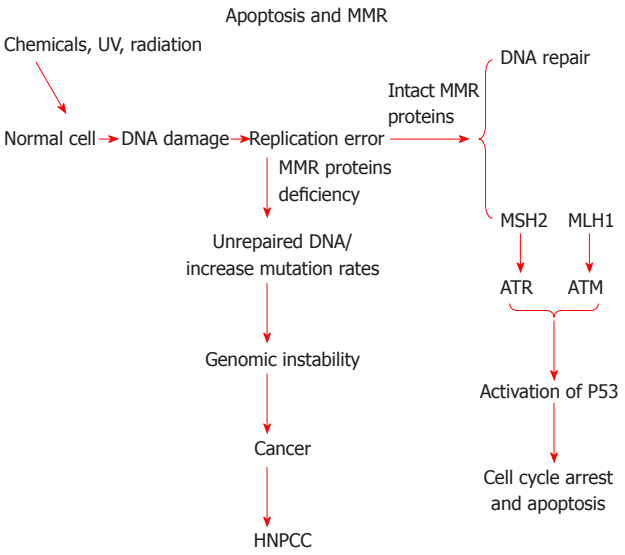

Figure 3 Relationship of mismatch repair proteins with apoptosis. This diagram depicts possible relationship between DNA mismatch repair proteins and HNPCC, and its possible link with apoptosis. MMR: DNA mismatch repair; ATR: Ataxia telangiectasia and Rad3-related protein; ATM: Ataxia telangiectasia mutated; HNPCC: Hereditary non-polyposis colorectal cancer.

ROLE OF MISMATCH REPAIR PROTEINS IN APOPTOSIS

As mentioned before, DNA mismatch repair system has been implicated in correction of base/base mismatches and insertion/deletion loops (Figure 2) that arise during DNA replication[43]. The inactivation or defects in MMR, usually MSH2 or MLH1, is associated with HNPCC and responsible for microsatellite instability[44]. A recent study shows that MMR system plays an important role in apoptotic machinery, activation of cell cycle check points[45], and in cytotoxicity induced by certain types of DNA damaging drugs. A simplified relationship of DNA mismatch repair system with apoptosis is shown in Figure 3. However, the exact mechanism by which the mismatch repair proteins mediate apoptosis is not yet understood[46]. It has been shown that loss of DNA mismatch repair causes resistance to certain types of DNA damaging agents because MMR deficient cells display defects in G2/M cell cycle arrest when treated with these agents[47,48]. MMR have been linked to resistance to a number of chemotherapeutic drugs such as 6-thioguanine and DNA methylating agents. Loss of MMR proteins also result in low level resistance to cisplatin. Cisplatin works by binding with DNA and creating DNA adducts that lead to intrastrand or interstrand cross-links which disrupt the structure of DNA helix. Proficient MMR system is important to recognize the damaged DNA created by cisplatin. The complex (DNA and cisplatin) interferes with the normal activity of MMR and prevents the repair process. Therefore the inability to complete the repair of damaged DNA caused by this drug leads to apoptosis whereas in MMR deficient system, cells continue to proliferate and cause resistance to the drug[49].

There are two models that described the role of mismatch repair system in DNA damaging signaling[7]. The first model is direct signaling model; this model propose that MSH-MLH complex identify DNA adduct, and this will recruit ataxia telangiectasia and Rad3-related protein (ATR) or ataxia telangiectasia mutated (ATM) to the adduct site as a result activation of downstream damage signaling. The second model named as futile DNA repair cycle[7]. This model suggests that DNA adducts will trigger the strand specific MMR which targets the newly replicated DNA. The adduct in the template strand cannot be removed, and this will provoke new cycle of MMR. If futile repair cycle persists this will activate ATM/ATR to promote cell cycle arrest and apoptosis[7].

In 1999, Zhang *et al*[50] found that over expression of MSH2 or MLH1 induced apoptosis[50]. Over expression of MSH2 was toxic to the cells, and develop severe nuclear abnormalities that caused cells to undergo apoptosis. They explained that the over expression of MLH1 or MSH2 in cells causes apoptosis because of the increased levels of these two proteins may alter or sequester other proteins such as PCNA that have a role in cell cycle progression or induction of apoptosis. Therefore, over expression of one of these two proteins might sequester PCNA from its role in DNA synthesis. As a result, apoptosis may be induced. Later, Chen *et al*[51] (2004), have shown that the mismatch repair protein MLH1 acts as a substrate for caspase-3 which proteolyzed MLH1 in cancer cells that are treated with anti-cancer drugs that inhibit topoisomerase Ⅱ. The cells, in turn, undergo apoptosis.

Human MLH1 is cleaved by caspase-3 at Asp418 residue to produce a proapoptotic carboxyl- terminal product[51] which partially redistributes from the nucleus to the cytoplasm. Losses of mismatch repair proteins (MLH1 or MSH2) cause resistant to cisplatin. Aebi *et al*[52] (1996)found that human colon cancer cell line HCT116 that is deficient in hMLH1 protein was 2 fold resistant to cisplatin when compared to cells that express hMLH1. Similar results were found when hMSH2 deficient cell lines were compared with hMSH2 proficient cell lines against resistance to cisplatin[52] indicating that both MLH1 and MSH2 proteins play a role in apoptotic cell signaling. Wu *et al*[53] (2008), has shown that human MLH1 protein is involved in the cellular response to psoralen interstrand crosslinks (ICLs). It has been found that MLH1 deficient cells are more resistant to psoralen ICLs than cells that have deficiency in MSH2[53]. In cells that have deficiency in MLH1 protein, apoptosis was not induced by psoralen ICLs, as well as CHK2 checkpoint homolog phosphorylation was undetectable when they compare with MLH1 proficient cells. This indicate that MLH1 is involved in signaling psoralen ICL- induced check point activation[53].

Thus, cell lines that are proficient or deficient in expression of MLH1 or MSH2 proteins can serve excellent model systems to study the role of these proteins in signaling mechanisms of apoptosis in relation to their involvement in HNPCC. In our laboratory, we have used

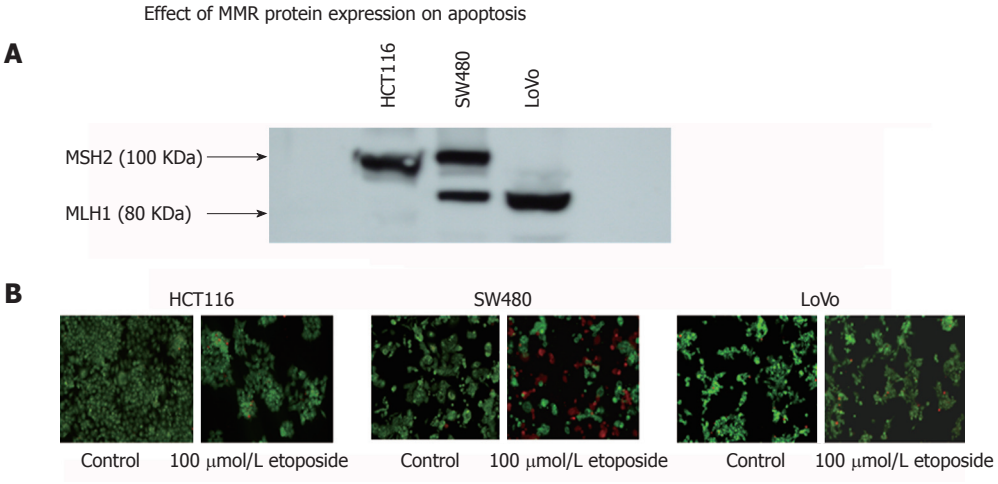

Figure 4 Possible link of the expression of mismatch repair proteins MLH1 and MSH2 with apoptosis. A differential expression of MSH2 and MLH1 mismatch repair proteins in SW480, HCT116 and LoVo colorectal carcinoma cell lines (A). These cells also show variation in etoposide induced apoptosis (B) suggesting a possible link of mismatch repair proteins and apoptosis.

such a model cell lines (SW480, HTC116 and LoVo) to accomplish just that. Figure 4 shows that, in a Western blotting experiment using specific monoclonal antibodies to MLH1 and MSH2 protein on the same blot, colorectal adenocarcinoma cell lines SW480 expresses both full length MLH1 (80 KDa) and MSH2 (100 KDa) proteins. HCT116 expresses only full length MSH2 and LoVo expresses only full length MLH1. Similar data has also been reported earlier[54] in a collaborative publication with Dr. Bruce Boman's group at Helen F Graham Cancer Center, Newark DE. These cell lines were then used to induce apoptosis by treatment with 100 μmol/L etoposide, a known cancer treatment drug used to induce apoptosis. It is apparent that the cell lines that are deficient in one of the mismatch repair proteins (HCT116 for MLH1 and LoVo for MSH2) are resistant to apoptosis (diminished red staining) than the cell line (SW480) which expresses both these proteins. This clearly links the role of these mismatch proteins to apoptosis. Further studies in evaluating the molecular signaling mechanism of apoptosis using these cell lines are in progress.

CONCLUSION

Indeed, colorectal cancer biology especially hereditary carcinogenesis such as HNPCC is a challenging area of research to gain insight into molecular events that leads to this disease. We have described the genetic basis of HNPCC and the role of mismatch repair proteins in onset of the syndrome. While a lot has been learnt about the mechanism of DNA mismatch repair system and how it regulates HNPCC, a lot more still need to be learnt about this syndrome. Many fundamental questions still remain unanswered; besides mismatch repair genes, what other genes are involved in the pathogenesis of this disease? Are there adequate methods available to screen population using genetic markers that may predispose HNPCC? How crucial are signaling molecules in mediating actions of DNA mismatch repair genes to HNPCC and apoptosis? So far we have only learnt a linkage of HNPCC to apoptotic pathway. It is not yet clear how one can exploit apoptotic mechanism to therapeutic intervention of HNPCC? A better understanding of the apoptotic signaling pathways that link mismatch repair genes such as *MLH1* and *MSH2* to HNPCC are attractive approaches to answering many of these and other burning questions. Clearly, this is an exciting time for HNPCC research and discovery as more efforts with improved methodologies especially those that dissect out apoptotic signaling pathways linking this disorder become available.

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BRIEF ARTICLE

Gender-associated differences in urea breath test for *Helicobacter pylori* infection referrals and results among dyspeptic patients

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Abstract

AIM: To verify whether there is a gender difference in the ^{13}C-urea breath test results in a large cohort.

METHODS: The test results of dyspeptic patients referred for ^{13}C-urea breath testing between January and December, 2007 were evaluated. Testing was carried out at the health insurance organization branches and evaluated at a central laboratory in Israel.

RESULTS: Of a total of 28 746 test results, 18 122 (63.04%) were from females and 10 624 (36.95%) from males. Overall, 10 188 (35.4%) results [expressed as delta over baseline (DOB)] were positive (DOB ^{13}C > 5), 18,326 (63.7%) were negative (DOB ^{13}C < 3.5) and 232 (0.8%) were borderline (DOB ^{13}C 3.5-5). There was a significant difference between the total positive rate among females and males (34.8% *vs* 37.2%, respectively, $P = 0.0003$). The mean test value was increased by approximately 10 units for females compared to males ($P < 0.01$) and this difference was consistent for all age groups (i.e., between 10-80 years of age, $P < 0.01$).

CONCLUSION: More females were referred to ^{13}C-urea breath testing. More males had positive results. The mean test values were significantly higher among females of all age groups, possibly representing an increased bacterial load among females and suggesting gender-associated differences in *Helicobacter pylori* host interactions.

Key words: *Helicobacter pylori*; Urea breath test; Gender; Dyspepsia

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is the major cause of peptic ulcer disease as well as being implicated in the pathogenesis of gastric cancer[1]. The ^{13}C-urea breath test (^{13}C-UBT) is considered the most accurate non-invasive diagnostic tool for the presence of *H. pylori*[2,3] and one that is widely used in clinical practice. By measuring the intragastric urease activity, potential advantages of ^{13}C-UBT are threefold: it allows assessment of the *H. pylori*

bacterial load, which, according to several reports, might be a risk factor in the development of peptic ulcer disease[4-7]; it serves to determine the severity of gastritis activity[4-6]; and it influences the efficacy of *H. pylori* eradication therapy[8-11]. A significant elevation of ^{13}C-UBT values among females infected with *H. pylori* compared to males was recently reported, suggesting gender-associated differences in *H. pylori* host interaction[12]. The aim of our current study was to evaluate the pattern of ^{13}C-UBT referrals among a large cohort of dyspeptic males and females and to verify whether or not there is such a difference in ^{13}C-UBT results.

MATERIALS AND METHODS

Maccabi Health Services is the second largest health insurance organization (HMO) in Israel, providing health services to approximately 2 million citizens. Its central laboratory provides ^{13}C-UBTs for its subscribers nationwide. The sample for the current study consists of ^{13}C-UBTs collected at the HMO branches and evaluated at MHC's central laboratory from January to December, 2007. The ^{13}C-UBT was performed with a mass spectrometer (Analytical Precision 2003, UK) using 75 mg of urea labeled with ^{13}C in 200 mL of orange juice. Breath samples were collected twice from each patient (at 0 and 30 min) and the ratio of ^{12}C to ^{13}C was measured at both time points. The difference was calculated by subtraction and termed the excess delta or the delta over the baseline (DOB). A DOB > 5.0 was considered positive for *H. pylori* infection, a DOB < 3.5 was considered negative for *H. pylori* infection and a DOB of 3.5-5 was considered as a borderline result. All the study patients were asked to stop the use of H_2 antagonists, proton pump inhibitors or any antibiotics one week prior to undergoing the breath test.

Statistical analysis

Categorical variables were summarized with number and percentage of patients. The χ^2 and Fisher exact tests were used to compare categorical variables and the Kruskal-Wallis one-way analysis of variance was used to analyze the demographic data. Significance was set at a P value < 0.05. The data were analyzed using SPSS version 15.0 (SPSS Inc. Chicago, IL).

RESULTS

A total of 28 746 ^{13}C-UBTs were performed, 18 122 (63.04%) in females and 10 624 (36.95%) in males, during the one year study period. Figure 1 demonstrates the number of ^{13}C-UBT referrals according to the patients' age. Overall, 10 188 (35.4%) ^{13}C-UBTs were positive (Δ^{13}C > 5), 18 326 (63.7%) were negative (Δ^{13}C < 3.5) and only 232 (0.8%) were borderline (Δ^{13}C 3.5-5). The difference between the total positive rate among females and males (34.8% *vs* 37.2%) was highly significant (P = 0.0003) (Figure 2).

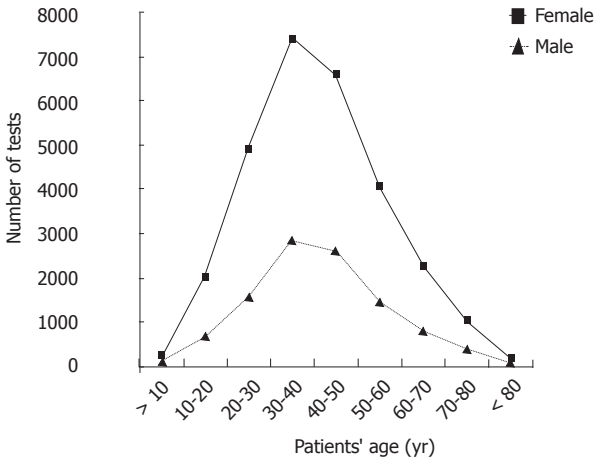

Figure 1 Number of ^{13}C-urea breath test referrals according to gender and age group.

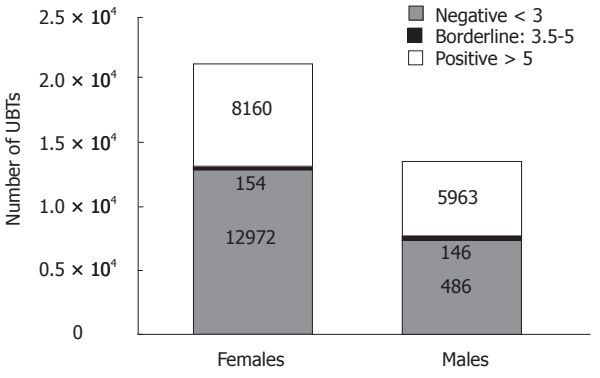

Figure 2 Distribution of ^{13}C-urea breath test results according to gender. UBT: Urea breath test.

We analyzed the mean ^{13}C-UBT values in both genders according to the patients' age (Figure 3). There was a significant increase of about 10 units in the mean ^{13}C-UBT value among females compared to males and that difference remained constant for all age groups between 10 years and 80 years of age (P < 0.01 for each).

DISCUSSION

The main findings of the present study are that more females are referred to ^{13}C-UBTs than males, that the rate of positive results is higher among males, and that there is a highly significant increased mean ^{13}C-UBT value for females in all age groups compared to age-matched males.

The numerical results of the ^{13}C-UBT are the function of total urease activity within the stomach, so the test might serve as a quantitative index of the density of gastric *H. pylori* colonization. Previous studies have reported inconsistent results about the relationship between ^{13}C-UBT findings and histology-based semi quantitative measures of bacterial infection. Several studies

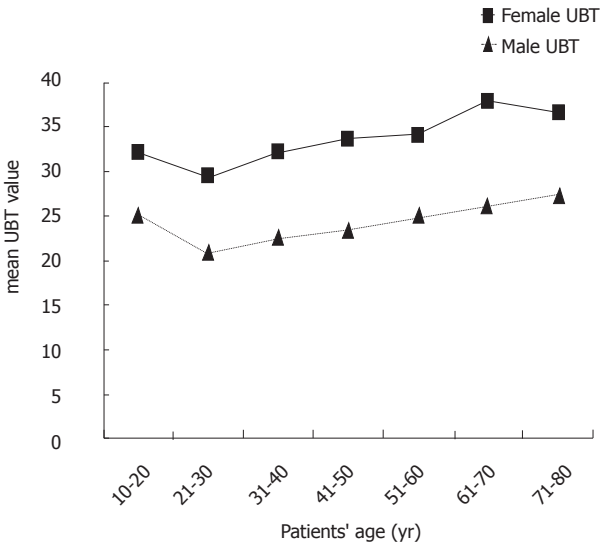

Figure 3 Mean ^{13}C-urea breath test values in males and females according to age group. DOB: Delta over baseline.

have demonstrated a correlation between the excess of delta (δ) $^{13}CO^{2}$ excretion and the *H. pylori* bacterial load[13-18], while others found that the ^{13}C-UBT value has only qualitative meaning, i.e., either positive or negative for *H. pylori* infection[19-21]. Kobayashi *et al*[22] reported that the gastric mucosal density of *H. pylori* as estimated by real-time polymerase chain reaction was significantly correlated with ^{13}C-UBT results and histological grading. Some groups have shown that ^{13}C-UBT-based or histologically estimated bacterial density in gastric mucosa can predict the extension of gastric inflammation and *H. pylori* eradication[5,6].

The observation in our study of the significantly increased mean ^{13}C-UBT value found in females of all age groups requires an explanation, whether the ^{13}C-UBT value represents bacterial load or urease enzyme activity. In ^{13}C-UBT, orally administered ^{13}C-labeled urea is hydrolyzed into ammonia and into $^{13}CO_2$ by urease in the presence of *H. pylori* infection. The results are expressed as DOB values. Since endogenous $^{12}CO_2$ production varies with age (i.e., adults more than children), weight, height and sex (i.e., males more than females), individuals with relatively lower body weight and height may produce smaller amounts of endogenous $^{12}CO_2$, whereupon their DOB values, expressed as a change in the $^{13}CO_2/^{12}CO_2$ ratio, may consequently increase[22]. This, however, can explain only a small part of the increased mean ^{13}C-UBT values among females and not the significantly increased values (approximately 10 units) in all age groups that were found in the present study.

Most *H. pylori*-related diseases are associated with male gender. The role of gender as a risk factor for *H. pylori* infection was reviewed by de Martel and Parsonnet in a meta-analysis of large, population-based studies[23]. Those authors found that male gender was significantly associated with *H. pylori* infection (OR: 1.16, 95% CI: 1.11-1.22) and that this male predominance of *H. pylori* infection was homogeneous and consistent across adult populations from various countries. They concluded that these findings may partially explain the male predominance of *H. pylori*-related adult diseases, such as duodenal ulcer and gastric adenocarcinoma.

Gender differences have also been found in response to treatment. Moayyedi *et al*[24] reported that anti-*H. pylori* therapy was significantly less successful in women than in men. They hypothesized that this may relate to an increased prevalence of 5-nitroimidazole-resistant organisms in women. Alternatively, there may be gender differences in acid output and gastric blood flow that influence treatment success[25]. The findings of the current study may provide another explanation: that the increased bacterial load among females causes the decreased response to antibiotic therapy.

Several studies have shown that the presence of *H. pylori* infection is a stronger predictor of gastric cancer in females compared to males[26-29]. Smoking and alcohol consumption were significantly more prevalent in males with gastric cancer than in males without it, and these differences were not present in females. It may therefore be considered that as risk factors, smoking and alcohol consumption have a stronger impact on males than on females. Here again, the finding that females might have an increased bacterial load may provide an explanation for *H. pylori* infection having been shown to be a stronger predictor of gastric cancer in females compared to males. Ohtani *et al*[30] have shown an effect of ovarian-dependent female hormones on *H. pylori*-induced gastric cancer in hypergastrinemic INS-GAS mice and Crabtree and colleagues have demonstrated that there are gender differences in the magnitude of the gastric cytokine responses to *H. pylori*[31].

An interesting observation in our study is that the number of total ^{13}C-UBT referrals was significantly higher among females than males in almost all age groups, but especially between the third and fifth decades (Figure 2). The increased number of ^{13}C-UBTs among females was also associated with a slightly increased rate of negative test results. Both observations might reflect the increased prevalence of functional dyspepsia among females compared to males[32-34]. It would appear that females, especially those between the third and fifth decades of life, tend to suffer more from functional disorders. This would serve to explain the increased number of females referred to *H. pylori* ^{13}C-UBTs. On the other hand, organic *H. pylori*-related diseases are more associated with male gender and this would explain the increased rate of positive ^{13}C-UBT results among males.

We found that the number of referrals to ^{13}C-UBTs was greater among females than males, especially among females between the third and fifth decades of life. This could be explained by the increased prevalence of functional dyspepsia among females. The rate of positive ^{13}C-UBT results, however, was greater among males. Another important observation was the significantly increased mean ^{13}C-UBT values among females in all age groups.

This may represent an increased bacterial load among females but this gender difference needs to be further investigated before any firm conclusions can be drawn.

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COMMENTS

Background

Helicobacter pylori (*H. pylori*) is the major cause of peptic ulcer disease and the ^{13}C-urea breath test (^{13}C-UBT) is considered the most accurate non-invasive diagnostic tool for the presence of *H. pylori*. A significant elevation of ^{13}C-UBT values among females infected with *H. pylori* compared to males was recently reported, suggesting gender-associated differences in *H. pylori* host interaction, and the aim of the current study was to evaluate the pattern of ^{13}C-UBT referrals among a large cohort of dyspeptic males and females and to verify whether or not there is such a difference in ^{13}C-UBT results.

Research frontiers

The main findings of the present study are that more females are referred to ^{13}C-UBTs than males, that the rate of positive results is higher among males, and that there is a highly significant increased mean ^{13}C-UBT value for females in all age groups compared to age-matched males.

Innovations and breakthroughs

The authors found significantly increased mean ^{13}C-UBT values among females in all age groups. This may represent an increased bacterial load among females.

Terminology

H. pylori is a spiral bacterium implicated in gastritis, gastric ulcer and peptic ulcer disease. UBT is a non-invasive diagnostic procedure used to identify infections by *H. pylori*. It is based upon the ability of the bacterial enzyme urease to convert urea to ammonia and carbon dioxide. UBT is recommended in leading society guidelines as a preferred non-invasive choice for detecting *H. pylori* before and after treatment.

Peer review

The study was performed on a large cohort of patients regarding the possibility of a gender difference in the ^{13}C-urea breath test. The analysis is well conducted and the results are interesting.

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Advances in Hepato Bilary Pancreatic Endoscopy
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San Juan, Puerto Rico

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Mayo Clinic 2012 Gastroenterology and Hepatology
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16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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