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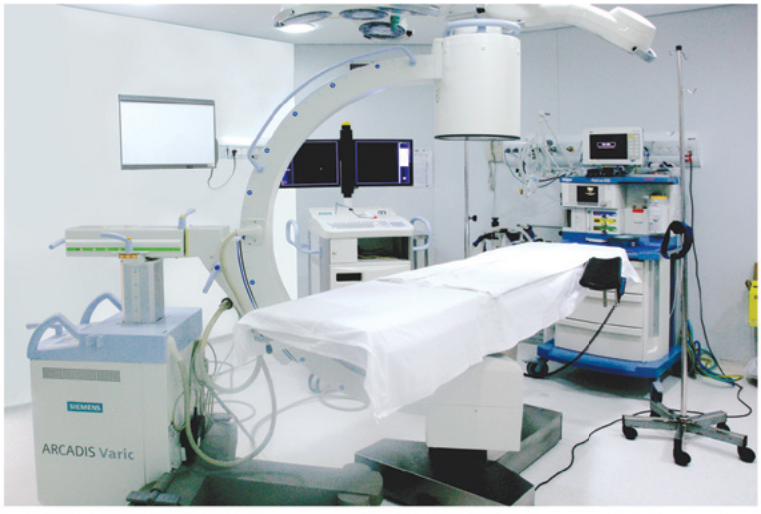

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Chronic hepatitis C is still a problem for the public health care system in Brazil

Strauss E. Chronic hepatitis C is still a problem for the public health care system in Brazil. Arq Gastroenterol. 2018;55(4):321-3.

Globally, about 80 million people are living with the hepatitis C virus (HCV)[1]. In 2016, the World Health Organization (WHO) adopted the Global Health Sector Strategy on Viral Hepatitis to eliminate hepatitis by 2030[2]. This commitment was proposed in response to the growing prevalence of chronic viral hepatitis worldwide The burden of the disease includes direct medical expenses for its hepatic and extrahepatic manifestations, as well as indirect costs related to impaired quality of life and loss of work productivity.

In Brazil, the Public Health Care System has been providing antiviral treatment for chronic hepatitis C for more than a decade with pegylated interferon and ribavirin. This now-outdated treatment, besides high costs, low efficacy, and prolonged duration, could not be administered in several clinical conditions, such as advanced cirrhosis (Child B and C), patients on the liver transplant waiting list (MELD>15), or after any type of organ transplantation. According to global and national recommendations, antiviral therapy could be prescribed only for patients with progressive fibrosis (F≥2). Special groups such as people with HIV/HCV coinfection, renal failure, people who inject drug, children, and others either went untreated or achieved less efficient outcomes.

The great advantage of all-oral treatment with direct-acting antivirals (DAAs) is the possibility of getting rid of interferon/ribavirin regimens, eliminating its adverse effects and achieving greater efficacy. DAAs also created the possibility of treating a wider range of patients, enabling a global campaign for HCV elimination despite the absence of a vaccine. The combination of preventive measures with a very efficacious treatment makes elimination an achievable goal, especially in countries where a public health system is aware of the problem and takes responsibility for addressing it.

In this volume, Minme et al. publish the article "Profile of patients with chronic hepatitis C in a public health program in Southern Brazil"[3]. The authors conducted a retrospective analysis of the main characteristics of 1,431 HCV patients listed for DAA therapy from 2015 to 2016. This is a huge population, the largest of the three publications on hepatitis C presented in this issue. The aims of the three studies are different, and we have chosen to comment on them in order of complexity and increasing interest.

Concerning sex and age, findings were comparable across different regions of the country. Men predominated in all regions and mean age ranged from 56 to 57.8 and 58.6 years, a somewhat older population than in previous Brazilian studies[4,5]. The period of analysis of the three articles covers 2014 to 2016 – the end of the interferon era and the start of the new all-DAA era. The predominance of cirrhosis was also a common factor in all three studies; one, in fact, included only cirrhotic patients. (This serves as a warning to readers, who should interpret these data with caution regarding the patients with early hepatitis C with little or no fibrosis).

The aim of Minme et al. was to present a profile of chronic hepatitis C in Southern Brazil. As geographical and regional differences, related to prevalence and some characteristics of hepatitis C, are well known around the world, it is valid to evaluate them in a very large regional case series. The take-home message of the article was that, although genotype 1 was predominant (60.5%), a high percentage of genotype 3 was found (33.8%) as a unique regional characteristic of the Brazilian South. Studying associations, the authors also found a greater prevalence of F4 (cirrhosis) in patients with genotype 3 and a higher viral load in those with genotype 1.

In this volume, Silva et al. publish the article "Waiting DAAs list mortality impact in HCV cirrhotic patients"[6]. The authors have followed a cohort of 129 patients with HCV cirrhosis for 11.2 months while on a waiting list for treatment with DAAs. During this period, the lethality rate was 6.9%, corresponding to the natural history of the disease. Risk factors for death were serum albumin level <2.9 g/dL, MELD score >15 and α-fetoproteins >40 ng/mL. Other factors, such as increased bilirubin levels, presence of ascites, bleeding esophageal varices or blood dyscrasia (measured by high INR) were not shown to be independent risk factors for death in this Brazilian cohort.

These data are particularly relevant because knowledge of the natural history of HCV is the cornerstone of any evaluation of the need for therapy and serves to justify the high costs of antiviral treatment. Here, the question is not about the progression of chronic hepatitis C to cirrhosis, but a possible modification of its natural course, once cirrhosis is fully installed. Besides raising the issue and providing some clues, the authors also open new perspectives for important future researches. There is a pressing need to stratify patients with cirrhosis (of any etiology), since prognosis is extremely variable. Besides the Child-Pugh classification and the MELD score, there is also another very simple and easily applicable stratification available. The BAVENO´s prognostic score considers the presence of varices, ascites, bleeding due to portal hypertension or the combination of other clinical complications related to portal hypertension[7].

The second point that must be highlighted is that antiviral treatment leading to SVR (sustained virological response) in patients with established cirrhosis may not only prevent complications, but also contribute to fibrosis regression[8]. On the other hand, follow-up of these patients is mandatory due not only to possible onset of hepatocellular carcinoma but also because, in more advanced cases, the disease may progress despite treatment and complications of cirrhosis develop[9].

In this volume, Castelo et al. publish their investigation "Hepatitis C in the Brazilian public health care system: burden of disease"[10]. In this multicenter study, the authors enrolled 313

patients with chronic genotype-1 hepatitis C. All grades of fibrosis (0–4) are covered, although the majority (42.8%) of patients had cirrhosis (F4). To evaluate disease burden, Castelo et al. used three questionnaires – the EQ-5D-3L, HCV-PRO, and WPAI:Hep C – to assess quality of life, functional status, and well-being. The EQ-5D-3L is a generic instrument assessing health status on five domains, whereas the HCV-PRO is a specific instrument to measure the effects of disease on function and well-being. The WPAI:Hep C questionnaire is designed to measure the effects of hepatitis C on productivity in the workplace and beyond. Comorbidities were highly prevalent in this cohort, with cardiovascular disease in 62.6% and metabolic disease in 50.5%. The leading complication of hepatic disease was presence of esophageal varices (54.5%), although bleeding from varices was present in only 7.3% of patients and hepatocellular carcinoma was diagnosed in a similar number of cases (7.3%). The questionnaires showed that anxiety and depression (53.9%) as well as pain and discomfort (47.5%) were very frequent. Productivity was the most commonly affected component of daily activity, affecting 23.5% of patients.

The key message of these data is extremely clear. Chronic hepatitis C, although apparently silent and most of the time asymptomatic, is associated with various and serious comorbidities and compromises health-related quality of life, as previously demonstrated[11]. Besides re-enforcing this often overlooked aspect, the authors evaluated medical costs, most of them covered by the Public Health Care System. The price of anti-virals corresponded to 95% of medical costs in the sample. As mentioned by the authors, the period of study corresponded to the advent of triple therapy with first-generation DAAs (telaprevir or boceprevir), just before the all-oral second generation of DAAs became available. At the time, severe treatment-emergent adverse reactions were still common and SVR rates were still low, around 65% to 70%. Soon after, starting in the end of 2015, all-oral combination treatment with DAAs achieved a real-world efficacy greater than 90% in Brazil. Side effects now tend to be mild, if present at all, and the mean duration of treatment has been shortened from 12 to 3 months.

Curing HCV infection is the first step to stopping the progression of liver disease. When this is achieved only after the onset of cirrhosis, patients must be staged and perspectives for regression of fibrosis is possible in less compromised patients. Conversely, development of hepatocellular carcinoma must be continuously evaluated. From this perspective, the cost burden of the disease cannot be limited to the time frame of antiviral treatment; a longer period of observation (years or decades) is desirable.

In Europe and United States DAAs became available 2 years before their launch in Brazil and clinical researches are already showing the good results of the new "elimination policy" for HCV hepatitis. A particularly good example is the transplant field, where fewer cases of decompensated HCV cirrhosis are being listed for liver transplantation. In Italy, an evaluation of 1,109 patients waitlisted for transplant before and after the introduction of DAAs revealed a significant decrease in HCV-related cirrhosis, especially for decompensated forms (from 24.2% to 15.9%)[12]. Similar results were found in a Spanish study of 2,379 patients waitlisted for liver transplant due to decompensated hepatic disease, with a significant decrease over time with large-scale use of DAAs[13]. More recently, a multicenter study including various European countries and the United States enrolled 60,527 liver transplant cases. Besides a decline in listing of patients with HCV-decompensated cirrhosis, the authors observed that post-liver transplant survival for HCV patients improved over the last 3 years due to the impact of DAAs[14].

Cost-effectiveness is an especially relevant issue in the treatment of hepatitis C, due mainly to the high cost of antiviral therapy, as noted by Castelo et al.; a growing body of research on this problem is being published worldwide. Some authors have described clinical, economic, and quality-of-life benefits[15]. In the first 5 years post-treatment, medical costs for patients achieving SVR are 13-fold lower than for patients not achieving SVR[16]. Different approaches for the cost-effectiveness analysis of hepatitis C treatment with DAAs were the subject of a recent systematic review of 36 publications, mainly from Europe and the U.S. Cost-effectiveness was largely rated as acceptable, from 67% to 100%[17].

In a Brazilian study of real-life cases of hepatitis C treated according to the Brazilian protocol of HCV treatment (PCDT), treatment costs for genotype-1 HCV patients were found to vary over time. Triple therapy with association of IFN/Riba and the first generation of DDAs was the most expensive whereas the new interferon-free regimens all-DAAs of second generation being the least expensive[18].

Although new treatment strategies yield excellent results in terms of efficacy and control, new problems have emerged and must be dealt with judiciously. The first one is the possibility of development of mutant HCV strains, which occur in patients who fail to achieve SVR. These may be related to the type of drug used or treatment period according to genotype, and usually occurs in the NS5A or NS3/4A regions of the HCV virus[19]. Nevertheless, recent clinical trial data show that most patients who fail HCV treatment with DAAs have excellent retreatment options since newly approved salvage therapies have become available[20].

In order to reduce the burden of chronic hepatitis C and improve treatment outcomes, the populations most difficult to treat or cure – such as those co-infected with HIV and people who inject drugs (PWID) – deserve specific measures to reduce ongoing exposure. Reinfection after successful HCV treatment is an important public health issue, and may impact efforts to control HCV transmission. As these groups usually went untreated, rates of HCV reinfection were low and seldom described in the interferon era; but now, with the advent of DAAs, these percentages have risen. In a recent study of 4,114 individuals, HCV reinfection was found to occur in 5.7% and 10.2% of recent PWID and patients with HCV-HIV co-infection. The authors of this remarkable and interesting study suggest multicomponent prevention strategies, such as continuous opioid-agonist treatment (OAT) for PWID following the end of successful DAA treatment, as reinfection was found to occur in only one patient on daily OAT[21].

In conclusion, cure of chronic hepatitis C is real and possible. There is a long way to go and plenty of hard work ahead on the road to 2030; Brazilian physicians must focus on treating as many HCV-infected patients as possible and on controlling HCV transmission. With the support of a strong and cooperative public health care system, this ambitious objective can be achieved.

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ORIGINAL ARTICLE

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Congenital hepatic fibrosis and obliterative portal venopathy without portal hypertension – a review of literature based on an asymptomatic case

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ABSTRACT – The disease and the case reported here are relevant especially because of their varied clinical presentation, possibility of being associated with other disorders affecting several organs and possible differential diagnoses. Congenital Hepatic Fibrosis is an autosomal recessive disease due to mutation in the PKHD1 gene, which encodes the fibrocystin/polyductine protein. It is a cholangiopathy, characterized by varying degrees of periportal fibrosis and irregular proliferation of bile ducts. Affected patients are typically diagnosed in childhood, but in some cases the disease may remain asymptomatic for many years. The exact prevalence and incidence of the disease are not known, but it is consider a rare disease, with a few hundred cases described worldwide. It can affect all ethnic groups and occur associated with various hereditary and non-hereditary disorders. The clinical presentation is quite variable, with melena and hematemesis being initial symptoms in 30%-70% of the cases. More rarely, they may present episodes of cholangitis. The disease has been classified into four types: portal hypertension, cholestasis / cholangitis, mixed and latent. Diagnosis begins with imaging tests, but the definition is made by the histopathological sample. So far, there is no specific therapy that can stop or reverse the pathological process. Currently, the therapeutic strategy is to treat the complications of the disease.

HEADINGS – Liver cirrhosis. Polycystic kidney diseases. Portal hypertension.

REPORT

Male patient, 47 years-old, underwent an abdominal tomography after an automotive accident, when was seen a liver with irregular borders, heterogeneous density and caudate lobe hypertrophy, all findings suggestive of chronic liver disease. Thence, the patient sought specialized medical support and reported he has low platelets value. He denied previous episodes of upper gastrointestinal bleeding, ascites, encephalopathy or any other complication attributable to cirrhosis. As a previous history, he reported follow up with an urologist due to kidney stones and mild dyslipidemia. There is no history of alcohol abuse. In the family there was no reported liver disease. He was taking fibrate to treat dyslipidemia. On physical examination he had no stigmas of liver disease. The complementary tests are described in TABLE 1 and FIGURE 1. Serological tests for viral hepatitis were negative, as were autoantibodies, except for the antinuclear antibody that showed a titration of 1:320, with a speckled pattern. Serum levels of alpha-1-antitrypsin, ceruloplasmin and ferritin were within normal range. With intention to clarify diagnosis, the patient underwent liver biopsy (FIGURES 2 and 3). The main findings were dense portal fibrosis, sometimes leading to portal-portal septa, but without formation of portal-central septa or nodules (FIGURES 2A and 2B). In these portal spaces were found malformed ductal structures, reminiscent of derangement of the ductal plate (FIGURE 3A). Supporting the elevations of aminotransferases and especially the higher levels of gamma glutamyl transferase (GGT), several bile ducts were surrounded or even permeated by inflammatory infiltrate with lymphocytes, macrophages and some polymorphonuclear cells (FIGURE 3B). Attention was also drawn to the obliteration of the portal venous branch, and sometimes there were several smaller caliber veins surrounding the main obliterated vein (FIGURES 2B and 3C). The diagnostic conclusion was portal fibrosis with evidence of ductal plate malformation, characterizing congenital

TABLE 1. Laboratory tests.

Test (reference value)	Result
Platelets (200.000-400.000)	176.000/mm^3
ALT (<49)	88 U/L
AST (<40)	54 U/L
Total bilirrubin (<1.0)	0.76 mg/dL
GGT (<40)	322 U/L
Alcaline phosphatase (<129)	110 U/L
Prothrombin time (<12 seconds)	13 seconds
Albumin	4 g/dL

ALT: alanine aminotransferases; AST: aspartate aminotransferases; GGT: gamma-glutamyl-transferase.

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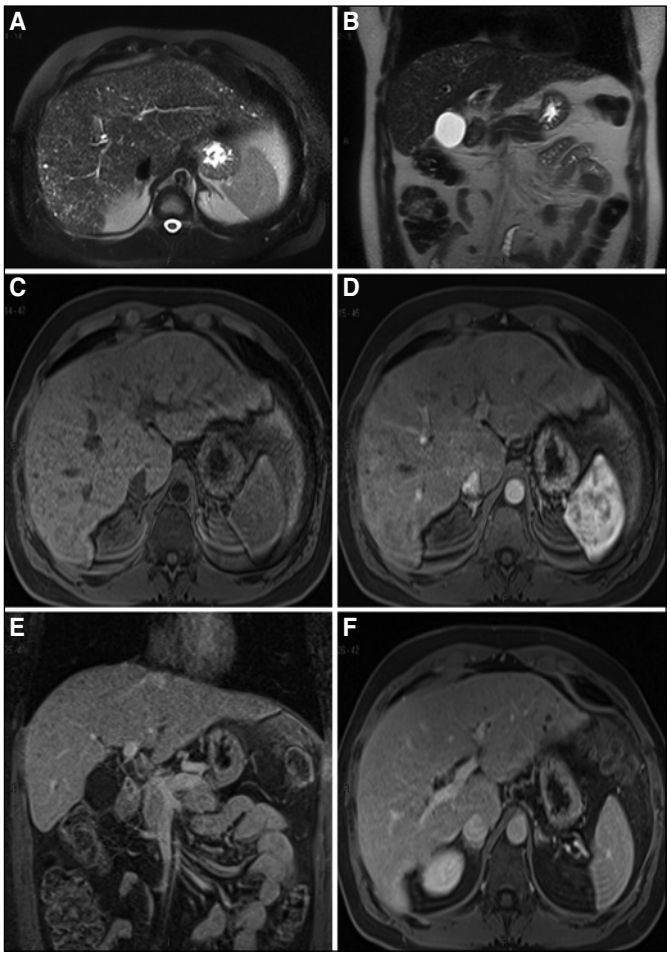

FIGURE 1. Magnetic Resonance images of the liver – Axial T2 HASTE (**A**), Coronal T2 HASTE (**B**), Axial T1 3D GRE pre-contrast (**C**) and in the late hepatic arterial phase post-gadolinium (**D**), Coronal (**E**) and Axial (**F**) T1 3D GRE in the venous phase post-gadolinium, show liver with irregular borders, heterogeneous signal intensity and hypertrophy of the caudate lobe, findings consistent with chronic liver disease. HASTE: half-Fourier acquisition Single-Shot Turbo Spin-Echo; GRE: Gradient-Echo.

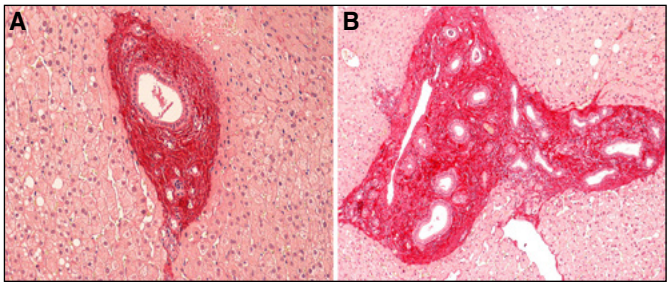

FIGURE 2. Anatomopathological examination. Histopathological aspects, evidenced by the red picrosirius histochemical reaction, highlighting portal fibrosis. **A**) Portal space expanded by dense fibrosis, highlighting the presence of small ductal structures beyond the original bile duct, located in the center of the figure. The portal venous branch is not evident (original x100). **B**) Portal space greatly enlarged by dense fibrosis, highlighting the presence of numerous bile ducts. The portal venous branch is elongated, with reduced lumen. In the lower face of the portal space, a branch of hepatic vein (centrolobular) is observed (original x100).

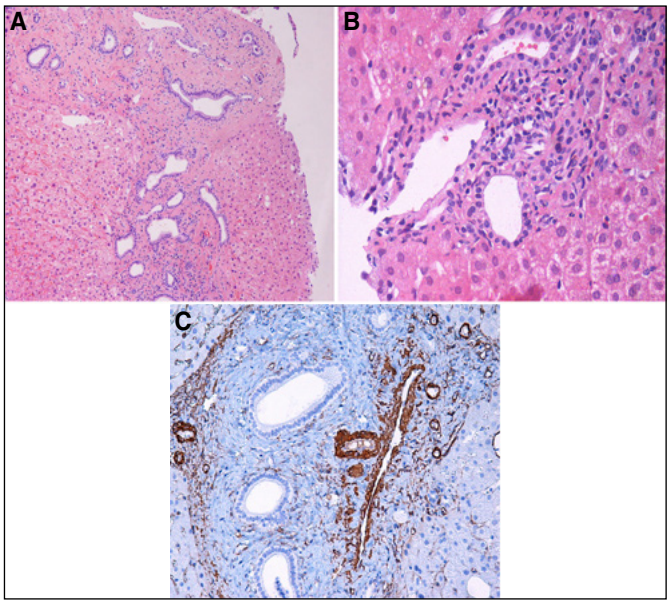

FIGURE 3. Anatomopathological examination. **A**) the hematoxylin-eosin staining shows the irregular contours of the various bile ducts, indicating ductal plaque remodeling disorders (he, original x100). **B**) mixed inflammatory infiltrate, variable in the portal spaces, is moderate in this figure, surrounding or even permeating the ductal epithelium (he, original x200). **C**) the immunohistochemical reactions for the detection of actin in the vessel wall demonstrate the obliteration of the portal vein branch, which is surrounded by several small venous branches (ihc, ac 1 to 4, original x100).

hepatic fibrosis with cholangitis component. At this moment, the patient is doing well and is has not been treated with any specific medication for congenital hepatic fibrosis.

DISCUSSION

Congenital hepatic fibrosis (CHF) is an autosomal recessive disease[1,2], due to a mutation on PKHD1, a gene encoding fibrocystin/polyductine[1,2,3], a ciliary protein expressed in cholangiocytes. CHF is defined by varying degrees of periportal fibrosis and irregular proliferation of bile ducts[1,4,5], being part of the so-called Fibropolycystic Diseases, which also include Caroli's Disease[6], Autosomal Dominant Renal Disease, Autosomal Recessive Polycystic Kidney Disease, Von Meyenburg Complex (bile duct hamartoma), and choledochal cyst[5,7,8]. Typically, CHF patients have been diagnosed in infancy or early childhood, but some recent data demonstrate that some patients remain asymptomatic for long periods[4], as the presented case here. The incidence and prevalence of CHF are not exactly known, but it is known to be a rare disease, with only a few hundred cases described in the literature[9]. The prevalence of congenital hepatic fibrosis in portal hypertensive children in a study from north India was 3%[7]. It is estimated that, when CHF occurs with Autosomal Recessive Polycystic Kidney Disease, the frequency is 1 in 20,000 live births, affecting all ethnic groups[8]. The disease may occur associated with other hereditary and non-hereditary diseases, with the most frequent associations being: Joubert Syndrome and Bardet-Biedl Syndrome[10]. The potential for involving several organs, requiring several medical specialties in differential diagnosis and treatment, reinforces the importance of this review article.

Pathophysiology

CHF is a genetic cholangiopathy caused by mutations in PKHD1, the gene located on chromosome 6p21-23[11], which encodes fibrocystin/polyductine (FPC), a protein of yet unknown function, expressed in the cilia and centromers of the bile ducts and renal tubule cells[2]. In others chronic hepatobiliary diseases, progression to fibrosis is due to a repair response against necro-inflammatory damage in hepatocytes and/or cholangiocytes. In CHF, the essential pathological processes appear to be genetically based, leading to ductal plate malformation, possibly beginning with maturation arrest and lack of ductal plate remodeling[7]. The ductal plate is an embryological structure formed by a cylindrical layer of epithelial cells that involve a branch of the portal vein[1] and is the embryological precursor of intrahepatic bile ducts[12]. As a result of this defect in remodeling the ductal plate, a persistence of immature embryological ductile structures occurs, which lead to the formation of portal fibrosis tissues. This portal fibrosis would ultimately lead to recurrent cholangitis or portal hypertension and associated symptoms[1]. Interestingly, our reported patient had no history of symptoms and complications at the time of diagnosis.

Although it is well established that chronic portal hypertension can lead to portal vein thrombosis with eventual cavernomatous transformation (CTPV)[13], it is believed that in the case of the CHF the lesions of the portal vein branches are a component of the disease, present from the beginning. The main support for this theory is based on the embryological relationship of the hepatic vessels and ducts[12]. Malformation of the ductal plate has a strong association with malformation of the portal vein, resulting in excessive small and branched branches of the portal vein[1,14]. A study from Barayktar et al. showed that almost 50% of CHF patients present CTPV and that patients with CTPV presented more prominent splenomegaly and a greater chance of varicose digestive hemorrhage[15]. Our patient also had no signs of CTPV.

The maturation stage of the ductal plate arrest would determine the final disease: either it involves the small interlobular bile ducts leading to CHF or involves the middle intrahepatic bile ducts causing Caroli's Disease[5]. The occurrence of simultaneous involvement of the two types of bile ducts (small and medium) results in Caroli Syndrome (FHC associated with Caroli Disease). In these cases, the clinic of Caroli's disease (recurrent cholangitis episodes) will be predominant, with CHF often underdiagnosed. Thus, hepatic biopsy becomes essential for differential diagnosis between Caroli's Disease or Syndrome[1].

In a more recent study, Locatelli et al. described that FPC deficiency would lead to the formation of microhamartomas and segmental dilations of the bile ducts with portal hypertension. Other studies have also identified various defects in intracellular signaling of FPC-deficient cells, including increased adenosine cyclic monophosphate (cAMP)/protein kinase A signaling and activation of β-catenin-dependent protein kinase A[16]. This, in turn, has appeared as a new regulator of inflammation, capable of influencing secretion of cytokines in liver cancer in experimental models. Hence, it has been proposed that persistent and unresolved cellular dysfunction may promote a chronic and low-grade inflammatory response and may lead to scarring (called "parainflammation")[2]. In this same study, the author showed evidence that this chronic and mild inflammatory response, generated by FPC-deficient cholangiocytes, secretes chemokines capable of recruiting macrophages derived from bone marrow, and responds to them through the activation of αvβ6 integrin, a local activator of TGFβ1. This theory proposes a new paradigm of biliary fibrosis and would be a model for understanding the relationship between cellular dysfunction, inflammation, hepatic fibrosis and macrophage polarization over time. This theory, however, contradicts the previously mentioned that the process of hepatic fibrosis in the CHF would occur without the presence of chronic inflammation.

Other authors have demonstrated and established that TGFβ is a potent growth inhibitory factor and a pro-fibrotic cytokine, with a central role in the physiological healing process as well as in the pathogenesis of organ fibrosis. The onset of activation of hepatic stellate cells (which are also at the center of the liver fibrosis process)[17,18] is primarily induced by TGFβ1 derived from Kupfer cells[1]. TGFβ1 would mediate its pro-fibrotic action through stimulation of fibroblasts and related cell types, including stellate cells, to secrete various extracellular matrix proteins[19]. Therefore, in pathological situations would lead to the accumulation of fibrotic matrix and in physiological situations would lead to efficient wound healing[1]. There are other studies that intend to establish the pathophysiological mechanism behind this abnormal and excessive fibrotic response associated with CHF, in which the possible involved ones would be plasminogen and tissue plasminogen activator (tPA), osteopontin and microRNA[1,20].

Clinical course

The onset of presentation and severity of symptoms is very variable. The majority manifests even in childhood[7,21]. In most patients, the first manifestations of the disease are signs and symptoms related to portal hypertension, often with gastrointestinal bleeding[5]. Hemathemesis and melena are initial symptoms in 30%-70% of cases[9]. More rarely, they may present episodes of cholangitis. Depending on the associated diseases, patients will have signs and symptoms related to other organs such as kidneys and central nervous system (CNS)[1]. These patients classically do not have cirrhosis, maintain normal hepatic lobular architecture and liver function[21].

CHF is classified into 4 types[5,7,9]:

1) Portal hypertension
2) Cholestatic / Cholangitis
3) Mixed
4) Latent

The reported case probably has the latent form. The portal hypertension type of CHF is the most common and becomes more severe in the presence of portal vein abnormality. Latent type presents in old age or is an incidental finding, as do our case. On physical examination the patient may present with hepatomegaly, especially of the left lobe, splenomegaly and nephromegaly. The liver is firm in consistency and has a slightly nodular surface. In laboratory tests, there is a slight elevation of liver enzymes, where in cholestatic conditions predominate elevations in alkaline phosphatase, gamma-glutamyltransferase and bilirubin. Cytopenias may occur as a consequence of splenomegaly and this is probably the cause of low levels of platelets in our patient. Changes in renal function tests occur when there is CHF associated with extensive cystic kidney disease, which may progress to end-stage renal failure. When kidney and liver disease are combined, the severity of each is independent[3].

Diagnosis

Ultrasound is usually the first method employed due to availability, low cost and harmlessness. It is able to detect alterations in the bile ducts and hepatic parenchyma, besides renal alterations. The most common findings are hypertrophy of the left lateral and caudate hepatic segments, right segment atrophy, splenomegaly, intra- and extrahepatic biliary dilation with concomitant cystic and solid lesions (regeneration nodules), periportal thickening, hepatic and renal cysts, and CTPV.

Computed tomography (CT) has advantages over ultrasound by better morphological representation, with accurate volumetric measurements, as well as by adequately showing the vessels and biliary tree. However, nuclear magnetic resonance imaging (MRI) with cholangioresonance is an important diagnostic alternative because it does not expose the patient to radiation and allows a very detailed evaluation of the biliary tree.

Anatomopathological examination, interpreted in the clinical-radiological context, is of great importance for a diagnostic definition of CHF. It is possible various degrees of fibrosis with nodular formations, and when diagnosed late, clinical and even histological findings may be confused with liver cirrhosis[1]. In addition, large fibrous bands are seen in the portal tract, with an increased number of proliferated irregular bile ducts with normal cubic epithelium. These findings were seen in our patient's liver biopsy, supporting the diagnosis. Hepatic lobes are normal with hepatocytes of usual morphology, especially in the early stages of the disease.

Among the major complications, signs of cholestasis and even recurrent cholangitis can be seen. The development of cholangiocarcinoma is another possible complication. When CHF is associated with Caroli's disease, cholangiocarcinoma occurs in 2.5%-16% of cases[1]. On the other hand, in cases of isolated CHF, there are few reports of cholangiocarcinoma[22,23]. It is suggested that malignant transformation via dysplasia occurs in abnormal intrahepatic bile trees in older patients with CHF[23]. There are also some cases of hepatocellular carcinoma associated with CHF reported in the literature[21].

Treatment

To date, there is no specific therapy capable of stopping or reversing the pathological process in CHF[1,18]. When considering the antifibrotic treatment, it is necessary to remember that fibrosis is a dynamic process in which the degradation of the extracellular matrix, and not only the matrix formation, is important[18]. Moreover, many studies in the literature emphasize that liver fibrosis is a reversible process[24]. Different researchers try to find a drug that can control the progression of the disease, stopping and even reversing fibrosis. Some drugs have shown success in animal studies[18], but have failed to present benefits in humans, including colchicine, angiotensin II blocker, interferon gamma and pirfenidone[1,18,24,25]. Therefore, the therapeutic strategy for CHF, so far, is to treat the complications of the disease[5].

Endoscopy is an important option for primary and secondary prophylaxis of bleeding esophageal and gastric varices, as well as for treatment of acute bleeding[26]. Likewise, it plays an important role in the management of recurrent cholangitis (especially in Caroli syndrome)[1,5].

Transjugular portosystemic intrahepatic shunt (TIPS) is indicated in cases not eligible for endoscopic sclerotherapy and also in cases of refractory upper gastrointestinal bleeding, such as a bridge to liver transplantation.

Surgical shunts for portal decompression are indicated in cases of upper gastrointestinal bleeding not amenable to upper endoscopy. In cases of CHF associated with Caroli's disease with recurrent cholangitis, partial hepatic resection may be indicated in cases of extensive heterogeneous involvement[1,5].

Hepatic transplantation is the only curative treatment for CHF. It is indicated in the advanced stages of the disease, with development of hepatic insufficiency, or in cases of recurrent cholangitis or malignant transformation[27]. The vast majority of cases of CHF who underwent liver transplantation, described in the literature, had Caroli's disease associated with recurrent cholangitis. On the other hand, Geramizadeh et al., in 2010, reported two cases of CHF patients who required hepatic transplantation due to hepatic insufficiency, without previous or current history of cholangitis[4]. In cases of associated renal and hepatic diseases, combined kidney and liver transplantation may be necessary. In addition, one study showed that in patients with liver and kidney disease who underwent only liver transplantation, there was an improvement in renal function[28].

CONCLUSION

The case reported herein corresponds to a form that is practically asymptomatic, but has a well-defined histological pattern of Congenital Hepatic Fibrosis (CHF), a rare or rarely diagnosed disease. The clinical spectrum of the CHF is very broad, and it can affect several organs, being a disease to be considered associated with other conditions, especially the kidney fibropolycystic disease.

Authors' contribution

Guerra JA and Kampa KC: text writing. Zapparoli M: images of magnetic resonance. Alves VAF: orientation, text correction and diagnosis based on histopathological findings. Ivantes CAP: orientation, patient care and text correction.

Guerra JA, Kampa KC, Zapparoli M, Alves VAF, Ivantes CAP. Fibrose hepática congênita e venopatia portal obliterativa sem hipertensão portal – revisão da literatura com base em um caso assintomático. Arq Gastroenterol. 2018;55(4):324-8.

RESUMO – A patologia e o caso aqui reportados são relevantes especialmente devido sua variada apresentação clínica, possibilidade de estar associada com outras desordens acometendo diversos órgãos e pelos possíveis diagnósticos diferenciais. A fibrose hepática congênita é uma doença autossômica recessiva, devido mutação no gene PKHD1, que codifica a proteína fibrocistina/poliductina. É uma colangiopatia, caracterizada por variados graus de fibrose periportal e proliferação irregular de ductos biliares. Os pacientes acometidos são tipicamente diagnosticados na infância, mas em alguns casos a doença pode permanecer assintomática por muitos anos. Exatas prevalência e incidência da doença não são conhecidas, mas sabe-se que é uma doença bastante rara, com algumas centenas de casos descritos no mundo. Pode afetar todos grupos étnicos e ocorrer associada com diversas desordens hereditárias e não-hereditárias. A apresentação clínica é bastante variável, com melena e hematêmese sendo sintomas iniciais em 30%-70% dos casos. Mais raramente, podem apresentar episódios de colangite. A doença tem sido classificada em quatro tipos: hipertensão portal, colestática/colangite, mista e latente. O diagnóstico inicia com exames de imagem, mas a definição é feita pela amostra histopatológica. Até o momento, não há terapia específica que possa parar ou reverter o processo patológico e a estratégia terapêutica atual é tratar as complicações da doença.

DESCRITORES – Cirrose hepática. Doenças renais policísticas. Hipertensão portal.

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ORIGINAL ARTICLE

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Hepatitis C in the Brazilian public health care system: burden of disease

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ABSTRACT – **Background** – Infection by hepatitis C virus is one of the leading causes of chronic hepatitis C and cause severe burden for patients, families and the health care system. **Objective** – The aims of this research were to assess the severity of liver fibrosis, comorbidities and complications of hepatitis C virus; to examine health-related quality of life (HRQoL), productivity loss and resource use and costs in a sample of Brazilian chronic hepatitis C, genotype 1, patients. **Methods** – This was a cross-sectional multicenter study performed in genotype-1 chronic hepatitis C patients to assess disease burden in the Brazilian public health care system between November 2014 and March 2015. Patients were submitted to a liver transient elastography (FibroScan) to assess liver fibrosis and answered an interview composed by a questionnaire specifically developed for the study and three standardized questionnaires: EQ-5D-3L, HCV-PRO and WPAI:HepC. **Results** – There were 313 subjects enrolled, with predominance of women (50.8%), caucasian/white (55.9%) and employed individuals (39.9%). Mean age was 56 (SD=10.4) years old. Moreover, 42.8% of patients who underwent FibroScan were cirrhotic; the most frequent comorbidity was cardiovascular disease (62.6%) and the most frequent complication was esophageal varices (54.5%). The results also showed that "pain and discomfort" was the most affected HRQoL dimension (55.0% of patients reported some problems) and that the mean HCV-PRO overall score was 69.1 (SD=24.2). Regarding productivity loss, the most affected WPAI:HepC component was daily activity (23.5%) and among employed patients, presenteeism was more frequent than absenteeism (18.5% vs 6.5%). The direct medical costs in this chronic hepatitis C sample was 12,305.72USD per patient in the 2 years study period; drug treatment costs represented 95.9% of this total. **Conclusion** – This study showed that most patients are cirrhotic, present high prevalence of cardiometabolic diseases and esophageal varices, reduced HRQoL mainly in terms of pain/discomfort, and work productivity impairment, especially presenteeism. Additionally, we demonstrated that hepatitis C virus imposes an economic burden on Brazilian Health Care System and that most of this cost is due to drug treatment.

HEADINGS – Hepatitis C. Liver cirrhosis. Quality of life. Cost of illness.

INTRODUCTION

Infection by hepatitis C virus (HCV) is one of the leading causes of chronic liver disease; however, the majority of the infected individuals is not diagnosed. It is estimated that there are approximately 170-200 million people infected globally and 2.5 million people in Brazil[1-4].

The prevalence of HCV genotypes vary across countries[5]. Given Brazil's continental size, it is expected that this prevalence also varies among regions. A comprehensive study enrolling 1,688 sequential samples from chronic HCV patients found a statistical significant difference regarding patterns of genotypes distribution among regions (P=0.00017). However, genotype 1 was the most frequent in all regions, ranging from 51.7% in the South to 74.1% in the North[6].

HCV symptoms may include malaise, weakness, anorexia, and jaundice, although the illness rarely causes acute symptomatic infection[7]. There is a lot of evidence showing that hepatitis C can lead to persistent infection in a high proportion of infected individuals, and it can progress to chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC)[8]. Chronic hepatitis C (CHC) causes severe burden for patients, families, and the health care system, once the economic implications include not only costs related to disease management, but also consequences of productivity loss[4-9]. It is estimated that CHC is responsible for approximately 350,000 deaths per year in United States[10].

In Brazil, Ministry of Health provides access to HCV treatment since 2002, following a National Guideline applied only to the Brazilian public healthcare system that includes interferon or pegylated interferon, ribavirin, sofosbuvir, simeprevir, daclatasvir and ombitasvir/veruprevir/ritonavir plus dasabuvir[11-14]. Patients receiving those therapies through government funding need to be attending a public medical facility specialized in HCV treatment. Other therapies not currently available in this National Guidelines can be obtained in the private setting through health insurance coverage or as a patient out-of-pocket expense[13].

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Thus, the aims of this report were to describe the severity of liver fibrosis, comorbidities and complications of HCV; health-related quality of life (HRQoL), productivity loss and resource use and costs for the public healthcare system, in a sample of Brazilian Chronic Hepatitis C, genotype 1, patients, as investigated in a multicenter study focused on treatment patterns and disease burden.

METHODS

Study design

This was a cross-sectional multicenter study investigating genotype-1 CHC patients to assess treatment patterns and burden of hepatitis C in the Brazilian public healthcare system between November 2014 and March 2015. The study was conducted in eight specialized centers in CHC treatment located in Southeast (Cities: Belo Horizonte, Botucatu, Rio de Janeiro, São Paulo, and Vitória), South (City: Porto Alegre), and Northeast (City: Recife) Brazilian regions. Patients were submitted to a liver transient elastography (FibroScan) to assess liver fibrosis grade, according to METAVIR score[15], and to an interview composed by a questionnaire specifically developed for the study and three standardized questionnaires: Euroqol 5-Dimension Questionnaire 3 level version (EQ-5D-3L)[16], Hepatitis C virus patient-reported outcomes instrument (HCV-PRO)[17], and HCV-specific Work Productivity and Activity Impairment (WPAI:HepC)[18].

Subject selection, recruitment and inclusion

Patients were randomly selected to be invited to the study screening process from a list of CHC patients who have attended at least one outpatient visit within the year before the study initiation, as provided by the study sites. Patients ranked by alphabetic ordered were numbered, then a computer generated random number list was used to select patients for screening. The selected patients received a phone call inviting to participate in the study. The patients willing to participate had a study visit scheduled to receive detailed information about the study protocol, signing the informed consent, and initiating the study procedures. Eligible patients were those with diagnosis of CHC genotype 1 as described in medical charts, with at least one outpatient visit during the year previous to study enrollment and aged at least 18 years old. Patients unable to provide informed consent, answer the interview and/or patients who had been enrolled in a clinical trial were excluded.

Data sources and collection methods

The structured interview included questions about sociodemographic and clinical characteristics, and consumption of out-of-pocket resources using a recall period of 12 months prior to inclusion in the study, except for medicines that considered a recall period of three months. Information about medical resources utilization, comorbidities and complications were abstracted from medical charts. Outpatient and hospital resources related to HCV and its complications were collected for a recall period up to 24 months prior to study entry. If the FibroScan test could not be performed at the same day as the interview, a second study visit with a maximum interval of eight weeks was scheduled.

To assess quality-of-life and function and well-being of patients, the following instruments were used, respectively: EQ-5D-3L and HCV-PRO. The EQ-5D-3L is a generic instrument assessing health status through five domains (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). Each dimension has three levels: no problems, some problems, extreme problems, accordingly, 3^5=243 combinations of health states are possible. Each of the health states was converted into a utility score between 0 and 1 (representing a scale between death=0 and perfect health=1), using the United Kingdom algorithm which was the standard reference at the time of the study protocol preparation[16,19]. The questionnaire also includes a Visual Analogue Scale (VAS-EQ) which records respondent's self-rated health, according to endpoints labeled from "Best imaginable health state" to "Worst imaginable health state"[16]. The HCV-PRO is a specific instrument that measures the effects of the disease upon function and well-being. It contains 16 items about how often an experience or limitation was perceived, with levels of response choices ranging from 1=all of the time to 5=none of the time. The total score is the conversion of 16 results into a 0-100 scale. A greater HCV-PRO total score indicates greater levels of function and well-being[17].

The WPAI:HepC measures the effects of hepatitis C on productivity in the workplace and beyond. It is a questionnaire that contains four questions and from these questions it is possible to derivate four domains: percentage of work time missed due to ill-health (absenteeism), percentage of impairment while working due to ill-health (presenteeism), percentage of overall work impairment due to ill-health (absenteeism and presenteeism), and percentage of daily activity impairment due to ill-health. These domains are converted into a 0-100% scale where high percentages represent more impact on productivity[18].

Costs were estimated by multiplying the amount of consumed resources (as self-reported by patients or abstracted from medical charts) for their unit costs. The unit costs sources of tests, outpatient visits, non-drug treatments, and emergency room visits were SIGTAP (System List of Procedures Management, Medicines, Prosthetics and Orthotics, and Specialty Materials of SUS; competency – December, 2015)[20]. The unit cost of hospitalizations/surgical treatments were obtained in SIH (Hospital Information System; competency - 2014) of SUS - Unified Health System[21]. The unit costs of drugs were firstly searched in BPS (Health Prices Database)[22] by the lowest and latest purchase price accessible; if the unit cost was not available in BPS, the maximum selling price allowed for Government purchases (PMVG ICMS 0%) in the CMED (Medication Market Regulation Chamber - December 18, 2015)[23] list was used. The reasons for emergency room visits, hospitalizations, and surgical treatments were classified according to International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10)[24]. Tests were classified according to Clinical Protocol and Therapeutic Guidelines for Hepatitis C and Coinfections (PCDT)[25], and drugs according to Anatomical Therapeutic Chemical (ATC) code[26]. The unit costs were obtained between December, 2015 and January, 2016 and all data prices were collected in Brazilian Real (BRL) and then converted into American dollar values (USD) using the exchange rate of the date of consultation to the price list (16/Jan/2016), where 1.00 BRL corresponded to 0.2479 USD.

Sample size calculation

This analysis is a part of a larger study designed to estimate the frequency of any specific HCV therapy in the sample (treatment pattern). The original sample size calculation had the purpose to detect a frequency of at least 10% of the triple therapy (pegylated interferon, ribavirin, telaprevir or boceprevir) recommended for HCV patients with METAVIR F3-F4 (that represents 33% of

the total population of HCV patients, according to Poynard et al.[27]). Thus, considering an assumed distribution of triple therapy among all HCV patients of 3% (10% of 33%), a margin of error of 3.0%, and α=0.05, a sample of 318 HCV patients was required. However, 313 patients were included in the study, which provided a 95% confidence interval (CI) with a margin of error of 3.3%. The data presented here are related to secondary outcomes of the aforementioned study.

Statistical analysis

The descriptive analysis was performed through tabulation measures of central tendency (mean) and dispersion (standard deviation-SD) to quantitative variables, and frequency to qualitative variables. The analyses were conducted using Stata (version MP 12®) and R Project (version 3.1.2©) to provide a 95% CI and *P*-value ≤0.05.

Ethical approval

The research was reviewed and approved by Brazilian Independent Ethics Committees of each participating site (Supplementary material 3). The coordinator center approval was obtained in September 12, 2014 (*Comitê de Ética em Pesquisa do CRT DST/Aids*, no. 789.165). All procedures were in accordance with the ethical standards of the institutional and national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards.

RESULTS

Sample characterization

Three hundred and eighteen patients were considered potentially eligible; however, five patients did not meet inclusion criteria, resulting in 313 subjects enrolled. TABLE 1 shows the sociodemographic characteristics among included patients. The sample was mainly composed by females (50.8%), Caucasian/white (55.9%) and employed (39.9%). Mean age was 56 (SD=10.4) years old and the predominant educational level was complete high school (29.1%). Only 1.6% and 6.4% of sample presented coinfection HCV/hepatitis B virus (HBV) and HCV/human immunodeficiency virus (HIV), respectively.

Liver fibrosis severity, comorbidities and complications

Cirrhosis was present in 42.8% of HCV patients who underwent FibroScan (FIGURE 1). Cardiovascular (62.6%), metabolic (50.5%), and mental (23.8%) diseases were the most frequent comorbidities, while esophageal varices (54.5%) and portal hypertension (43.6%) were the most frequent complications (TABLE 1).

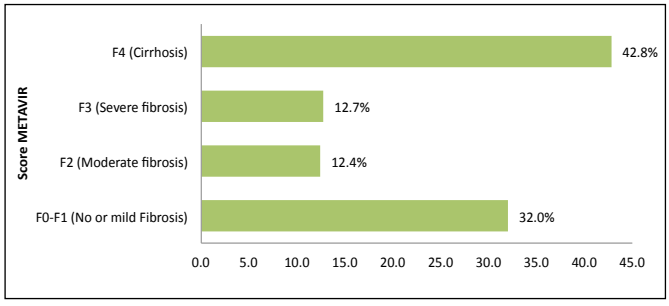

FIGURE 1. FibroScan test results according to METAVIR score (N=306).

TABLE 1. Sociodemographic and clinical characteristics of HCV patients.

Characteristic	N	%
Age (N=313)		
18–29 years old	1	0.3
30–39 years old	25	8.0
40–49 years old	54	17.3
50–59 years old	101	32.2
≥ 60 years old	132	42.2
Gender (N=313)		
Female	159	50.8
Male	154	49.2
Race (N=313)		
Caucasian/White	175	55.9
Brown	96	30.7
Black	39	12.5
Oriental	2	0.6
Indigenous	1	0.3
Educational level (N=313)		
No education	3	1.0
Incomplete elementary school	76	24.3
Complete elementary school	50	16.0
Incomplete high school	33	10.5
Complete high school	91	29.1
Incomplete graduation	22	7.0
Complete graduation	25	8.0
Employment (N=313)		
Employed	125	39.9
Retired	97	31.0
Unemployed	72	23.0
Pensioner	5	1.6
Autonomous	4	1.3
Absent from work	4	1.3
Student	3	1.0
NI	3	1.0
Comorbidities (N=206)		
Cardiovascular diseases	129	62.6
Metabolic diseases	104	50.5
Mental disorders	49	23.8
Extrahepatic manifestations	7	3.4
Coagulation disorders	7	3.4
Hepatocellular carcinoma	3	1.5
Cryoglobulinemia	2	1.0
Late cutaneous porphyria	1	0.5
Other[a]	39	18.9
Complications (N=55)		
Esophageal varices	30	54.5
Portal hypertension	24	43.6
Splenomegaly	20	36.4
Thrombocytopenia	20	36.4
Ascitis	10	18.2
Other liver diseases	9	16.4
Hepatic encephalopathy	5	9.1
Bleeding esophageal varices	4	7.3
Hepatocellular carcinoma	4	7.3
Spontaneous bacterial peritonitis	1	1.8
Hepatorenal syndrome	1	1.8
Other[b]	7	12.7

[a] Adenocarcinoma infiltrative of large intestine, leg amputation, sickle cell anemia, asthma, severe asthma, breast cancer, cirrhosis, cholelithiasis, chemical dependency, neuropathic pain, gastroesophageal reflux, epilepsy, hepatoesplenic schistosomiasis form, hepatic steatosis, fibromyalgia, antral erosive moderated gastritis associated with *H. pylori*, supraumbilical hernia, hemangioma, urinary incontinence, peripheral venous insufficiency, chronic renal insufficiency, lactose intolerance, labyrinthitis biliary lithiasis, myoclonus of soft palate, nefrolithiasis, neurocysticercosis, neurotoxoplasmosis, pangastritis, psoriasis, ulcerative colitis, syphilis, pulmonary sarcoidosis, esophageal varices, vitiligo.
[b] Lower limb edema, esophagitis, hyperferritinemia, icterus, cutaneous vasculitis.

Health-Related Quality of Life (HRQoL)

There were 305 valid responses for EQ-5D-3L. "Pain and discomfort" and "anxiety and depression" were the dimensions with highest HRQoL impairment, in which 55.0% and 47.0% of patients reported to have problems, respectively. "Self-care" was the least affected dimension, with 95.0% of responders reporting absence of problems (FIGURE 2). The mean overall EQ-VAS score was 75.1 (SD 21.4). Patients who reported problems to perform usual activities also presented the lowest score in this dimension according to EQ-VAS – TABLE 2.

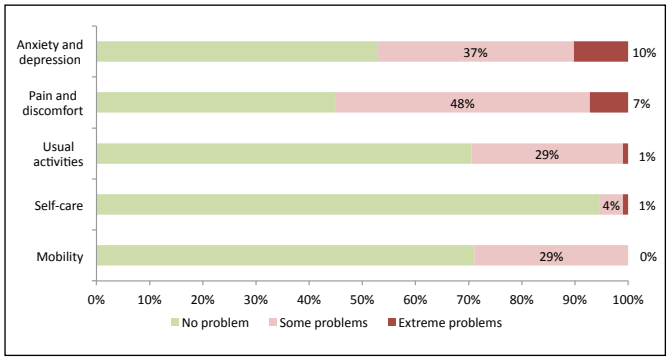

FIGURE 2. Limitations in health-related quality of life among HCV patients according EQ-5D-3L dimensions (N=305).

TABLE 2. Results of the Visual Analog Scale (EQ-VAS) among HCV patients according to EQ-5D dimensions (N=305).

Dimensions	No Problems		Some Problems		Extreme Problems	
	Mean	SD	Mean	SD	Mean	SD
Mobility	80.6	19	60.8	20.9	–	–
Self-care	76.1	20.6	60.6	20.5	50.3	30.5
Usual activities	82.2	17.2	58.8	21.1	39.3	17.6
Pain and discomfort	86.6	13.5	68.5	20.8	47.5	23.5
Anxiety and depression	84.9	14.4	67.2	20.7	53.9	26.4
Overall Score 0-100 [mean/SD]	75.1	[21.4]				

Function and well-being

The mean HCV-PRO overall score was 69.1 (SD=24.2). The dimensions presented similar mean scores. The most affected items in patients' perception were those regarding "tiredness" (Q1. mean=3.4; SD=1.3) and "sleep" (Q15. mean=3.4; SD=1.5) – TABLE 3.

Productivity loss

The productivity loss results showed that 79.9% of patients were currently remunerated, 12.4% of patients stopped working due to hepatitis C, and the mean age of work interruption was 49.7 (SD=9.5) years old. HCV caused temporary and permanent income reductions for 11.2% and 5.6% of employed patients, respectively. In addition, 14.4% of patients needed a sick leave in the past 12 months due to hepatitis C, of whom 61.1% received sick leave paid benefits (TABLE 4).

TABLE 3. Mean scores of HCV-PRO questions among HCV patients (N=313).

Question	Mean	SD
Q1. I feel too tired during the day to get done what I need (1–5)	3.4	1.3
Q2. I have to pace myself to finish what I had planned (1–5)	3.7	1.4
Q3. I feel forced to spend time in bed (1–5)	4.1	1.1
Q4. My muscles feel weak (1–5)	3.7	1.4
Q5. During the day, I cannot get comfortable (1–5)	3.7	1.2
Q6. I am unable to think clearly or focus on my thoughts (1–5)	4.0	1.2
Q7. I am forgetful (1–5)	3.5	1.2
Q8. Having hepatitis C has affected my sex life (1–5)	3.9	1.4
Q9. I feel bothered by pain or physical discomfort (1–5)	3.5	1.4
Q10. Because of my hepatitis C, I find it hard to meet people or make new friends (1–5)	4.5	1.2
Q11. Having this illness is very stressful to me (1–5)	3.6	1.4
Q12. I feel downhearted and sad (1–5)	3.5	1.4
Q13. I feel restless or on edge (1–5)	3.9	1.3
Q14. I feel little interest in doing things (1–5)	3.7	1.3
Q15. I have had difficulty sleeping or sleeping too much (1–5)	3.4	1.5
Q16. Hepatitis C lowers my quality of life (1–5)	3.7	1.5
Overall score (0-100)	69.1	24.2

TABLE 4. Productivity loss among HCV patients (N=313).

Productivity Loss		
Patients currently remunerated [a]	N (%)	250 (79.9)
Stopped working due to hepatitis C	N (%)	39 (12.4)
Age of work interruption	USD (mean/SD)	49.7 (9.5)
Permanent income reduction caused by HCV	N (%)	7 (5.6)
Monthly income before the permanent reduction	USD (mean/SD)	974.99 (1,023.31)
Monthly income after the permanent reduction	USD (mean/SD)	508.20 (481.15)
Need of sick leave in the last 12 months	N (%)	18 (14.4)
Temporary income reduction caused by HCV in the last 12 months	N (%)	14 (11.2)
Monthly income before the temporary reduction	USD (mean/SD)	707.46 (412.28)
Monthly income after the temporary reduction	USD (mean/SD)	428.82 (233.72)

[a] Patients who reported to receive any kind of income, despite their employment status.

There were 312 valid responses for WPAI:HepC. The most affected component was daily activity (23.5%). Among employed patients, presenteeism was more frequent than absenteeism (18.5% vs 6.5%) and the overall work impairment was 15.9%.

Health care resource use and cost

• Out-of-pocket (OOP) expenses

TABLE 5 shows that 40.3% of patients had private health insurance. The average monthly cost of health insurance was USD 90.71 per patient. The cost component with highest annual cost was visits with other health care professionals (mean=USD 562.11 per patient). Besides, only 11.5% of patients had OOP expenses with medicines in the past three months. The mean OOP cost of medicines in this period was USD 42.66.

TABLE 5. Out-of-pocket medical resources due to hepatitis C (N=313).

Resources	N	%
Private health insurance	126	40.3
Acquisition of private health insurance due to hepatitis c (n=126)	10	7.9
Monthly expenses with health care insurance (mean/sd)		
USD	90.71	52.26
Medical tests in the past 12 months	60	19.2
Expenses with tests (mean/sd)		
USD	170.36	258.29
Medical visits in the past 12 months	21	6.7
Expenses with visits (mean/sd)		
USD	194.97	274.90
Visit with other health care professional in the past 12 months	12	3.8
Expenses with visits (mean/sd)		
USD	562.11	671.81
Medicines in the past 3 months	36	11.5
Expenses with medicines (mean/sd)		
USD	42.66	40.63

Regarding non-medical expenses related to hepatitis C, all resources had low frequency of use among patients, except for the need of transportation in the past 12 months (93.9%). The mean number of trips was 11.3 (SD=14.9) and each travel costed USD 8.83 on average (TABLE 6).

TABLE 6. Non-medical resources due to hepatitis C (N=313).

Resources	N	%
Need of assistant in the past 12 months	23	7.3
Housekeeper/Nanny	17	65.3
Caregiver	1	4.3
Driver	2	8.7
Other*	4	17.4
Expenses with assistant in the past 12 months (mean/SD)		
USD	163.19	141.95
Need of support equipment in the past 12 months	4	1.3
Crutch/cane/adult walkers	2	50.0
Sphygmomanometer	1	25.0
Washing machine	1	25.0
Expenses with support equipment in the past 12 months (mean/SD)		
USD	166.09	128.56
Home adaptations in the past 12 months	7	2.2
Grab rails	6	85.7
Air conditioner	1	14.3
Stair	1	14.3
Bedroom remodeling	1	14.3
Ramps	2	28.6
Expenses with home adaptations in the past 12 months (mean/SD)		
USD	339.97	331.59
Need of transportation in the past 12 months	294	93.9
Number of travels to the hospital due to hepatitis C in the past 12 months (mean/SD)	11.3	14.9
Expenses in each travel (mean/SD)		
USD	8.83	23.25

*General Helper, Assistant, Seller, Mason.

• Healthcare system expenses

The total direct medical costs with this CHC sample were USD 3,851,691.23 for the past two years, which represented USD 12,305.72 per patient in the 2-year study period (per capita annual costs of USD 6,152.85 – TABLE 7). Drug treatment accounted for 95.9% of the total costs.

TABLE 7. Cost according to each type of health care resource used by HCV patients in the last 2 years.

Resource	Patient (N=313)		Cost (USD)			Cost (%)
	N	%	Per patient		Total	
			Mean	SD		
Drug treatment	179	57.2	20,636.76	26,681.42	3,693,980.03	95.9
Directly related to HCV	111	62.0	32,410.11	27,483.02	3,597,522.34	93.4
Not-directly related to HCV	147	82.1	656.17	1,643.84	96,457.69	2.5
Test	307	98.1	244.43	170.45	75,041.13	1.9
Diagnostic/complementary test recommended	306	99.7	217.37	150.96	66,514.62	1.7
Other	290	94.5	29.40	48.04	8,526.51	0.2
Hospitalization/Surgery	16	5.1	4,499.92	8,954.80	71,998.73	1.9
Outpatient visit	313	100.0	33.22	28.73	10,397.27	0.3
Physician	313	100.0	28.58	23.96	8,945.47	0.2
Other health care professional	128	40.9	11.34	14.61	1,451.80	0.0*
Non-drug treatment	19	6.1	9.71	13.39	184.42	0.0**
Emergency visit	16	5.1	5.60	4.82	89.65	0.0***
Total	313	100.0	12,305.72	22,692.31	3,851,691.23	100.0

*0.037. **0.004. ***0.002.

DISCUSSION

Accurate assessment of liver fibrosis stage has important implications for prognostic, monitoring purposes and is essential for a rational therapeutic decision-making in hepatitis C[28]. FibroScan results showed that most of our sample had cirrhosis. Thus, it is reasonable to consider that most Brazilian HCV patients attending CHC specialized centers have indication to antiviral treatment and require interventions to control known negative cofactors for disease progression. These include life style modifications, as weight loss, alcohol and drug abstinence[28].

There are scarce data regarding the prevalence of liver fibrosis stages determined by FibroScan in Brazil. In fact, our frequencies of severe fibrosis and cirrhosis were much higher than results found by Fernandes et al. Those authors conducted a study in 120 CHC patients and reported the following findings: 54%, 30%, 9%, and 7% for METAVIR stages F0F1, F2, F3, and F4, respectively[29]. Even considering some false positive results of FibroScan or the higher frequency of severe patients in our sample due to the fact that the study sites are specialized centers to where high complexity patients are referred to, it is possible that this higher prevalence of cirrhosis is related to a progressively worse scenario of liver fibrosis among Brazilian patients.

Regarding comorbidities, the frequencies of HCV/HBV and HCV/HIV confections in our sample were lower than those observed in previous Brazilian studies[30,31]. In a study performed by Carvalho-Filho et al.[30] with 581 CHC patients, 59 (10.2%) individuals had HIV coinfection and 31 (5.3%) had HBV coinfection. Both frequencies were higher than observed in the present study. Another study, carried out by Moia et al. in HCV patients, found a prevalence of HCV/HIV coinfection more than two-fold higher than the one described in this sample (23.9%)[31].

HCV has significant hepatic implication, however it has also been involved in derangements of multiple other organ systems including the muscular, skeletal, nervous, endocrine, cardiovascular, respiratory, and urinary systems[32]. The most common comorbidities in our study were those related to cardiovascular, metabolic and mental diseases, which is consistent with literature data[31,33-36].

Previous studies have addressed the role of HCV infection on cardiovascular-related complications[34,36], and suggest a strong relationship between HCV infection and the atherogenic process, with high risk of coronary heart disease, carotid atherosclerosis, peripheral artery disease and, ultimately, cardiovascular-related mortality[36]. Regarding metabolic diseases, evidences suggest that HCV interference with glucose and lipid metabolism leads patients to acquire diabetes more frequently[35,36]. Also, CHC present considerable psychological burden, particularly depression and anxiety[33,37], which justifies the presence of mental disorders as a frequent comorbidity.

The EQ-5D-3L index found in the present study was 0.733 [SD 0.28] and is consistent with previously researches conducted in France (0.764 [SD 0.283]) and Canada (0.76 for patients with non-cirrhotic chronic HCV, 0.74 for patients with compensated cirrhosis, and 0.66 for patients with decompensate cirrhosis)[38,39].

Andrade and collaborators conducted a study using EQ-5D-3L in Brazilian general population. The descriptive analysis showed the following frequencies of patients that presented no problems: mobility (91.23%), self-care (97.59%), usual activities (89.85%), pain/discomfort (57.71%) and anxiety/depression (64.92%)[40]. Thus, except for self-care, HCV patients seem to present higher impairment in HRQoL in all EQ-5D-3L dimensions as compared to the Brazilian general population.

More than 10% of our sample stopped working due to hepatitis C. The mean age of work interruption was 10 years earlier than the regular age of retirement in Brazil for women and 15 years earlier for men. Current literature confirms that CHC patients experience increased work productivity impairment[41,42]. Besides, our data suggested that presenteeism seems to be more relevant than absenteeism in CHC patients. A similar result was found by DiBonaventura et al. in a European study involving CHC patients[42].

Drugs for CHC are part of the Drug Dispensing Program of the Brazilian Ministry of Health, but are also purchased by

State Departments of Health[43]. Therefore, the introduction of new costly technologies implies an economic impact on multiple levels of medical care in the Brazilian healthcare system. In this scenario, real-world data on CHC costs are extremely relevant to support planning and funding of HCV management strategies, for example highlighting disease aspects that are more costly and can be addressed by effective treatment and monitoring, reducing the overall CHC cost for the society.

The HCV per capita annual costs in the present cohort was USD 6,152.85 (BRL 4,819.93). These costs were higher than direct costs of other chronic condition in Brazilian populations, such as diabetes (USD 1,012)[44], and non-melanoma skin cancer (BRL 1,172)[45]; but lower than rheumatoid arthritis (BRL 19,860.16)[46]. In addition, the results showed that drug treatment was the costliest component, accounting for 95.9% of total HCV costs; accordingly, a Brazilian study conducted prior to the incorporation of protease inhibitors have shown that drug treatment represented 88.2% of the total costs[47].

We acknowledge some limitations of our study, mainly the possibility of missing data in the patients' medical records. Because it applies to all participants, it is unlikely to result in systematic bias among the different treatments or groups of patients. Secondly, some data were cross-sectionally obtained, which does not allow asserting a temporality between exposure and outcome. In addition, the study patients were from tertiary care centers; consequently, there are limitations regarding the understanding of the disease features in other levels of care. Thirdly, an important caveat is that new DAA prices decreased recently in the country, mainly within the Brazilian public healthcare system, what could lead to lower final costs in future analysis. Another important limitation is the fact that North and Midwest regions were not represented in the sample, which restricts the external validity of the results for the whole country. Finally, this study was conducted between 2014 and 2015 and since then new HCV treatments were made available in SUS, thus the costs exposed here may lack representativeness of currently available therapeutic strategies.

CONCLUSION

To our knowledge, this study presents the most detailed information on HCV Brazilian patients in a real-world setting. The results evidenced that most patients had cirrhosis, with high prevalence of cardiometabolic diseases, esophageal varices, reduced HRQoL mainly in terms of pain/discomfort, and work productivity impairment, especially presenteeism.

Additionally, we demonstrated that HCV imposes an economic burden on Brazilian Healthcare System, and that most of this cost is related to drug treatment. These results have strong implications for clinicians and policy makers, especially with the expanding of the availability of novel therapies in Brazil.

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Authors' contribution

The authors Castelo A, Brandão Mello EB, Teixeira R, Madruga JVR, Reuter T, Pereira LMMB, Silva GF, Alvares da Silva MR was were principal investigators. The author Zambrini H participated in the study design, planning, conduct and interpretation of data. The author Ferreira PRA performed the liver transient elastography (FibroScan).

Departments where the work was performed

Centro de Referência e Treinamento DST/AIDS, São Paulo. Universidade Federal de São Paulo. Universidade Estadual Paulista, Botucatu, São Paulo. Hospital Universitário Gafrée e Güinle, Rio de Janeiro. Universidade Federal de Minas Gerais, Faculdade de Medicina. Universidade Federal do Espírito Santo. Universidade Federal do Rio Grande do Sul, Hospital de Clínicas de Porto Alegre. Instituto do Fígado e Transplante de Pernambuco.

Conflict of interests

Castelo A: research grants received from BMS and AbbVie, travel grant received from Gilead and BMS.

Brandão Mello EB: speaker for and Consultant to AbbVie, Merck, Gilead, Janssen and BMS; member: National and State of Rio de Janeiro Committee of Viral Hepatitis and Liver Diseases.

Teixeira R: clinical research activities to BMS, AbbVie, Fiocruz/Brazil; speaker Gilead, Bristol, Janssen, AbbVie, Fiocruz/Brazil.

Madruga JVR: participation in Advisory Boards for AbbVie, BMS, Gilead, GSK/ViiV and MSD; lectures for AbbVie, BMS, Gilead, GSK/ViiV, MSD, and Janssen; conduct clinical trials for AbbVie, BMS, Gilead, GSK/ViiV, MSD, and Janssen.

Reuter T: gilead speaker; researcher at NIH–START Study; GSK– principal investigator for the SAILING study.

Pereira LMMB: member of the MSD board, clinical research: MSD, Jansen, AbbVie; member of the Advisory Committee of the Viral Hepatitis Program (Ministry of Health).

Silva GF: companies for whom he provides consulting / medical education (last 5 years): MSD, Janssen, Bayer, Shering, Pharma, Roche, Bristol Myers-Squibb, Boehringer Ingelheim, AbbVie, Ferring, Gilead; Public service: Prof. of Gastroenterology of FMB–UNESP; Chief of the FMB Viral Hepatitis Clinic – UNESP; clinical research: MSD, Janssen, Roche, Bristol Myers – Squibb, AbbVie.

Alvares-da-Silva MR: classrooms, advisory board and/or clinical research for AbbVie, Alexion, Bayer, BMS, Fiocruz/Biomanguinhos, Eisai, Gilead, Janssen, Merck; member of the Advisory Committee on Viral Hepatitis and the National Technical Chamber of Liver Transplantation of the Ministry of Health of Brazil.

Zambrini H: AbbVie employee and may own AbbVie stock or stock options.

Ferreira PRA: clinical research activities: BMS, Janssen, AbbVie; speaker: Janssen, AbbVie, GSK, Gilead, BMS; support for medical education activities: Janssen, AbbVie, GSK, MSD; performing hepatic elastography by FibroScan in public and private medicine; Federal and state civil servant; Member of the SBI Viral Hepatitis Committee; member of the technical advisory committee of the State Program of Viral Hepatitis in São Paulo.

Castelo A, Brandão Mello EB, Teixeira R, Madruga JVR, Reuter T, Pereira LMMB, Silva GF, Alvares-da-Silva MR, Zambrini H, Ferreira PRA. Hepatite C no sistema público de saúde brasileiro: impacto da doença. Arq Gastroenterol. 2018;55(4):329-37.

RESUMO – **Contexto** – A infecção pelo vírus da hepatite C (HCV) é uma das principais causas de hepatite C crônica e provoca implicações graves para pacientes, familiares e sistema de saúde. **Objetivo** – Os objetivos deste estudo foram: analisar a gravidade da fibrose hepática, comorbidades e complicações da hepatite C; examinar a qualidade de vida relacionada à saúde (QVRS), a perda de produtividade e o uso de recursos e custos no sistema público por pacientes brasileiros com hepatite C crônica, genótipo tipo 1. **Métodos** – Foi realizado um estudo transversal, multicêntrico em pacientes com hepatite C crônica genótipo-1 para avaliar a carga da doença no sistema público de saúde brasileiro entre novembro de 2014 e março de 2015. Os pacientes foram submetidos a uma elastografia hepática transitória (FibroScan) para avaliar a fibrose e a uma entrevista composta por um questionário desenvolvido para o estudo e cinco questionários padronizados: EQ-5D-3L, HCV-PRO, e WPAI:HepC. **Resultados** – Foram recrutados 313 pacientes. A amostra foi composta predominantemente por mulheres (50,8%), caucasianos/brancos (55,9%) e indivíduos empregados (39,9%). A média de idade foi 56 (DP=10,4) anos. Em média, os pacientes com HCV esperaram 40,6 (DP=49,6) meses entre o diagnóstico e o primeiro tratamento. Ademais, 42,8% dos pacientes que realizaram o FibroScan tinham cirrose; a comorbidade mais frequente foi doença cardiovascular (62,6%) e a complicação mais comum as varizes esofágicas (54,5%). Os resultados também mostraram que "dor e desconforto" foi a dimensão de QVRS mais afetada (55,0% dos pacientes relataram alguns problemas) e que a média do escore do HCV-PRO foi 69,1 (DP=24,2). Em relação à perda de produtividade, o componente do WPAI:HepC mais afetado foi atividade diária (23,5%) e entre os pacientes empregados, presenteísmo foi mais frequente do que absenteísmo (18,5% vs 6,5%). Os custos diretos médicos totais com essa amostra foi de 12.305,72USD por paciente em um período de dois anos; o tratamento medicamentoso representou 95% desse total. **Conclus**ão – Esse estudo mostrou a maioria dos pacientes possui cirrose, apresenta alta prevalência de doenças cardiometabolicas e varizes esofágicas, QVRS reduzida principalmente em termos de dor/desconforto e dano na produtividade, especialmente presenteísmo. Adicionalmente, nós demonstramos que o HCV impõe uma carga econômica no sistema de saúde brasileiro e que os medicamentos correspondem à maioria dos custos.

DESCREITORES – Hepatite C. Cirrose hepática. Qualidade de vida. Efeitos psicossociais da doença.

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ORIGINAL ARTICLE

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Evaluation of the behavior of levels of HMGB1 and IL6 as predictors of infection, acute kidney injury and mortality in cirrhotic patients with variceal bleeding

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ABSTRACT – **Background** – Gastroesophageal varices and associated bleeding are a major cause of morbidity and mortality in cirrhotic patients. **Objective** – To evaluate the potential role of the biomarkers HMGB1 (High Mobility Group Box 1) and IL-6 (Interleukin-6) as predictors of infection, acute kidney injury and mortality in these patients. **Methods** – It is a prospective, observational study that included 32 cirrhotic patients with variceal bleeding. **Results** – The subjects'mean age was 52±5 years and 20 (62.5%) were male. The average MELD was 17.53±5 and the average MELD-Na was 20.63±6.06. Thirty patients (93.3%) patients were Child-Pugh class B or C. Infection was present in 9 subjects (28.1%), acute kidney injury was present in 6 (18.1%) and 4 (12.5%) patients died. The median serum levels of HMGB1 were 1487 pg/mL (0.1 to 8593.1) and the median serum level of IL-6 was 62.1 pg/mL (0.1 to 1102.4). The serum levels of HMGB1 and IL-6 were significantly higher in patients who developed infection, acute kidney injury and death ($P<0.05$). The Spearman's correlations for HMGB1 and IL-6 were 0.794 and 0.374 for infection, 0.53 and 0.374 for acute kidney injury and 0.467 and 0.404 for death, respectively. **Conclusion** – Serum levels of HMGB1 and IL-6 were higher in patients with the three studied outcomes. HMGB1 serum levels showed a high correlation with infection and a moderate correlation with acute kidney injury and death, while IL-6 showed a moderate correlation with infection and death and a low correlation with acute kidney injury.

HEADINGS – Liver cirrhosis. Esophageal and gastric varices. Biomarkers.

INTRODUCTION

The development of variceal hemorrhage is a direct consequence of portal hypertension[1]. It occurs in 25 to 40% of patients with cirrhosis, and each episode of active variceal hemorrhage is associated with a 10% to 20% of mortality[2,3]. Complications related to bleeding and the treatment of bleeding contribute substantially to mortality from active hemorrhage[4]. The principal complications that cause death are aspiration pneumonia, sepsis and renal failure[5,6].

At the epithelial level, specifically in the small intestine, patients with liver cirrhosis present changes in barrier function. Increased intestinal permeability has been found in cirrhotic patients compared with healthy controls[7,8]. This increased permeability appears to be a result of the loosening of tight junctions associated not only with increased resistance to portal venous flow but also as a result of systemic circulatory dysfunction[9,10]. This circumstance favors the translocation of gram-negative bacteria across the intestinal barrier and may lead to infection and others complications. Most often, the bacteria are killed, but bacterial byproducts known as PAMPs (pathogen-associated molecular patterns) such as lipopolysaccharide and DAMPs (damage-associated molecular patterns) are released. PAMPs and DAMPs are spontaneously recognized by PRRs (pattern recognition receptors) expressed in immune and other types of cells. PRR engagement may result in the release of pro-inflammatory cytokines/chemokines, leading to systemic inflammation[11].

Among various mediators of the acute or chronic inflammatory conditions that accentuate systemic vasodilation are stand HMGB1 (High Mobility Group Box 1) and IL-6 (Interleukin-6)[12,13]. HMGB1 is a type of DAMP, a pro-inflammatory nuclear protein actively secreted by the cells of the innate immune system and the hepatocytes, released as a result of apoptotic phenomena during the cell death process[12]. When interacting with toll-like receptor 4 (TLR-4) - a type of PPR transmembrane - in one of its various signaling pathways, HMGB1 induces the activation of nuclear factor kappa B, producing immunostimulatory responses through transcriptional pro-inflammatory genes, including tumor necrosis factor, Interleukin-1 and ultimately IL-6, the most important cytokyne in sepsis[13,14].

The grade of inflammation parallels the severity of liver, circulatory and renal dysfunction and acute on chronic liver failure (ACLF)[15,16]. Its deleterious effect on organ function may derive from reduced organ perfusion and/or the effects of cytokines and reactive oxygen species on cell function and apoptosis[17,18].

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The aim of this study is to evaluate the association of the biomarkers HMGB1 and IL-6 with infection, acute kidney injury (AKI) and mortality in cirrhotic patients with variceal bleeding and determine the usefulness of these mediators as potential predictors of major outcomes.

METHODS

This was a prospective, observational study that included 32 patients diagnosed with liver cirrhosis with active bleeding resulting from rupture of gastroesophageal varices between June 2014 and March 2016. All the patients underwent endoscopic treatment via the ligation of esophageal varices or GOV1-type gastric varices associated with an intravenous bolus of octreotide 50 mcg followed by 50 mcg/hour for 5 days. Antimicrobial prophylaxis included norfloxacin 400 mg every 12 hours for 7 days or ceftriaxone 1 g every 24 hours for same period, when appropriate. During the hospitalization period, demographic, clinical and laboratory data were collected to investigate the predictors of infection, AKI and mortality. Spontaneous bacterial peritonitis was defined as the presence of at least 250 polymorphonuclear leukocytes/mm^3 in ascitic fluid analyzes, in the absence of a source of peritoneal cavity infection. The criteria for the pneumonia diagnosis were symptoms of acute respiratory tract disease associated with at least one systemic finding - confusion, headache, sweating, chills and radiological infiltrate not previously present. Urinary tract infection was characterized by bacterial growth of at least 10^5 colony forming units/mL of urine (100.000 cfu/mL) associated with tract urinary symptoms. The diagnosis of AKI was defined by the presence of two creatinine values, with a difference of at least 0.3 mg/dL or the elevation of at least 50% of its baseline value. Baseline creatinine was defined as the most recent and stable value prior to hospital admission within a maximum interval of seven days. Four participants were excluded. Three of them decided not to sign the consent form and one patient was submitted to liver transplant in the acute phase of bleeding. The demographic variables included age and gender and the clinical variables were Child-Pugh classification, Model for End-Stage Liver Disease (MELD), MELD sodium, presence of ascites and length of hospitalization. The examined laboratory variables were hemoglobin level at admission and after 48 hours, platelet count on admission and after 48 hours, serum levels of HMGB1 and IL-6, serum creatinine, albumin, total bilirubin, prothrombin activity with INR, activated partial thromboplastin time, fibrinogen and sodium on admission. The need for blood components was also recorded.

Statistical analyses were performed using the Statistical Package for Social Sciences version 21.0 (SPSS, Inc., Chicago, IL). Numerical variables are presented as means and standard deviations or as minimums, maximums and medians if they did not present normal distribution (Kolmogorov-Smirnov test). Categorical variables were presented as percentages. The Mann-Whitney test was used to assess the associations between variables and Spearman's test was used to evaluate the correlation between serum levels of HMGB1 and IL-6 and infection, AKI and death.

Ethics

The study was approved by the Ethics Committee of the Federal University of Minas Gerais and was conducted in accordance with the 1975 Declaration of Helsinki (6th revision, 2008). All the patients signed a free informed consent form before beginning the study.

RESULTS

A total of 32 patients with mean age of 52 years ± 10 were included; 20 (62.5%) were male. Alcohol and hepatitis C were the main causes of cirrhosis (TABLE 1). Seventeen patients (53.1%) were classified as Child-Pugh class C, 13 (40.6%) were Child-Pugh class B, and 2 (6.3%) were Child-Pugh class A. The mean MELD was 17.53±5, and MELD-Na was 20.63±6.06. The median hospitalization period was 8 days[5-17]. There was need for red blood cell transfusion in 11 patients (34.4%) to maintain hemoglobin levels between 7 and 8 g/dL. Seven patients received platelet transfusions, and fresh frozen plasma and cryoprecipitate were used for one patient each. Infectious complications were present in 9 (28.1%) patients (pneumonia in five, spontaneous bacterial peritonitis in three and urinary tract infection in one). AKI was observed in 6 (18.1%) patients, and 4 (12.5%) patients died. The median serum level of HMGB1 was 1487 pg/mL (0.1–8593.1); for IL-6, it was 62.1 pg/mL (0.1–1102.4).

TABLE 1. Etiology of cirrhosis (n=32).

Etiology	Frequency (n)	Percentage
Alcohol	11	34.4
Hepatitis C	10	31.3
Auto-immune hepatitis	4	12.5
Hepatitis B	2	6.3
Primary sclerosing colangitis	2	6.3
Primary biliar colangitis	2	6.3
Non-alcoholic stetohepatitis	1	3.1

After the univariate analysis for the evaluation of factors associated with mortality was performed, the variables MELD, MELD-Na, red blood cell transfusion, plasma transfusion, transfusion of cryoprecipitate, acute renal injury, sodium, serum HMGB1 and infection were entered into the initial model; however, none remained at the end of the multivariate analysis. For the outcomes infection and AKI, the variables HMBGB1, plasma transfusion, cryoprecipitate, albumin, sodium and length of hospitalization entered the initial model but did not remain with significance at the end of the model.

To evaluate the behavior of HMGB1 and IL-6 serum levels as outcome predictors, their medians were compared between patients who developed infection, AKI and mortality and those who did not. HMGB1 serum levels were higher in patients who developed infection (P=0.000), AKI (P=0.004) and death (P=0.011). Regarding serum levels of interleukin-6, P-values were 0.002 for infection, 0.044 for AKI and 0.003 for mortality. Results are showed in TABLES 2, 3 and 4.

TABLE 2. Median serum HMGB1 and IL6 values in cirrhotic patients with and without infection.

Serum levels (pg/mL)	Infection		*P* value
Median (Range)	Yes	No	
HMGB1	4635 (3535–8593)	496 (0.1–2536)	0.000
IL-6	132 (80–1102)	21.7 (0.1–269)	0.002

TABLE 3. Median serum HMGB1 and IL6 values in cirrhotic patients with and without acute kidney injury.

Serum levels (pg/mL)	Acute kidney injury		*P* value
Median (Range)	Yes	No	
HMGB1	4222 (2063–8593)	548 (0.1–5175)	0.002
IL-6	123 (33.4–743)	27 (0.1–1102)	0.044

TABLE 4. Median serum HMGB1 and IL6 values in cirrhotic patients who had or did not evolve to death.

Serum levels (pg/mL)	Death		*P* value
Median (range)	Yes	No	
HMGB1	5446 (1592–8593)	596 (0.1–4645)	0.011
IL-6	123 (33.4–743)	27 (0.1–1102)	0.003

The correlation between HMGB1 and IL-6 values and the outcomes was determined using Spearman's coefficient (rho). The value of rho for HMGB1 for infection was 0.794; for AKI, the value was 0.530; and for mortality, the value was 0.467. Regarding the rho coefficients for interleukin-6, the values were 0.567 for infection, 0.374 for AKI and 0.404 for mortality (TABLE 5). These results evidenced that the serum levels of HMGB1 showed a high correlation with infection and moderate correlations with AKI and mortality. Serum IL-6 levels showed a moderate correlation with infection and mortality and a low correlation with AKI.

TABLE 5. Spearman's coefficient (rho) between HMGB1 and Il-6 values and the outcomes.

	Infection	Acute kidney injury	Death
HMGB1	0.794	0.530	0.467
IL-6	0.567	0.374	0.404

Spearman's coefficient reference values (rho). R=1: perfect; R=0.80 to <1: very high; R=0.60 to <0.80: high; R=0.40 to <60: moderate; R=0.20 to <0.40: low; R=0 to <0.20 very low; R=0: null.

DISCUSSION

Variceal hemorrhage is one of the most important consequences of portal hypertension. One-fourth of the patients with varices develop hemorrhage within 2 years (2). The prognosis of patients with variceal hemorrhage has improved over the last two decades as the understanding of the pathophysiology of portal hypertension has improved, but mortality remains at 10% to 20%[2,3].

Interest in the use of inflammatory mediators as prognostic biomarkers is increasing. In Hepatology, interleukin-6 has been studied more frequently, but HMGB1 is not still recognized as a possible biomarker in these patients. Cai et al.[19] evaluated 50 patients with hepatitis B virus (HBV)-related ACLF, 35 patients with liver cirrhosis, 35 patients with chronic hepatitis B and 35 healthy controls. HMGB1 concentrations continually declined in the survivors and increased in the nonsurvivors. Duan et al.[20] evaluated the HMGB1 levels in the serum of 60 patients with HBV-related ACLF, 30 with chronic hepatitis B and 24 healthy individuals and investigated its potential relationship to the clinical features of these patients. Enhanced serum levels of HMGB1 were associated with the development of HBV-related ACLF in CHB patients. No study assessing HMGB1 as a prognostic marker for acute bleeding in cirrhotic patients was found. In the acute bleeding phase, circulatory dysfunction in cirrhotic patients worsens and is associated with a higher incidence of infection and other complications, such as AKI and hepatic encephalopathy, possibly via mechanisms that involve weakening of the gut barrier and bacterial translocation[16].

In patients with liver disease, Kao et al.[21] evaluated 158 naïve liver cirrhosis patients and 144 non-liver cirrhosis individuals and tested the correlations among the mediators IL-6, interleukin-27, tumor necrosis factor-α (TNFα) and vascular endothelial growth factor with STAT proteins at diverse clinical-pathologic stages in the cirrhotic patients. Over-expression of IL-6 reflects hepatic dysfunction and varices bleeding with mortality, as well as correlates p-STAT3 expression. A study published in 2012 evaluated the role of TNF-α and IL-6 in cirrhotic patients who had hepatic and renal impairment with spontaneous bacterial peritonitis[22]. Approximately 40% (n=48) of cirrhotic patients with SBP developed renal and hepatic impairment and showed significantly higher plasma and ascitic fluid cytokine levels upon the diagnosis of infection[22]. In contrast, Elsing et al.[23] did not find higher levels of IL-6 in patients with acute decompensation compared with non-decompensated patients. There was also no difference between the patients with and without bleeding.

In this study, it was established that serum levels of HMGB1 are higher in patients who developed infection, AKI and death compared with patients who did not have these outcomes and that the correlation was high for infection and moderate for AKI and death. Similar results were observed regarding serum IL-6 levels; however, the correlation was moderate for infection and death and low for AKI outcome.

Infectious complications and AKI are directly associated with cirrhotic circulatory dysfunction, a phenomenon whose main triggering factor is splanchnic arterial vasodilation secondary to portal hypertension[24]. However, these two outcomes, as well as other forms of organic dysfunction in cirrhosis, can occur even without the progression of circulatory dysfunction because they also result from the complex interaction between the innate immune system and bacterial components – also called PAMPs – translocated from the intestinal lumen and DAMPs[15,25,26]. Once linked to PRRs, PAMPs and DAMPs activate several cascades of intracellular and extracellular signaling, producing proinflammatory responses that, when excessive or chronic, can cause tissue damage[27,28]. Thus, infection complications and AKI in cirrhosis should be interpreted as resulting not only from arterial vasodilation but also from exacerbated systemic inflammation[27]. Among various mediators of acute or chronic inflammatory states that accentuate systemic vasodilation and are associated with organ failure, HMGB1 and IL-6 are the most prominent[29]. HMGB1, a type of DAMP, is a proinflammatory nuclear protein that is actively secreted by cells of the innate immune system and released during apoptosis, including hepatocytes in the process of cell death[30]. By interacting with the toll-like receptor 4 – a type of transmembrane PRR - in one of its several signaling pathways, HMGB1 induces the activation of nuclear factor kappa B, producing immunostimulatory responses via the transcription of proinflammatory genes, including tumor necrosis factor, interleu-

kin-1 and, also, IL-6[31]. Likewise, HMGB1 can accumulate in the renal tissue and urine, stimulating the production of inflammatory cytokines, including IL-6, through interaction with toll-like receptors 4[32]. Elevated levels of toll-like receptors 4 are found in the renal tubular cells of cirrhotic patients with AKI, suggesting that these receptors may mediate renal injury in the context of infection or systemic inflammation[33].

CONCLUSION

The intrinsic relationship between these inflammatory mediators and the endpoints studied strengthens the likelihood that they may also function as prognostic markers. The higher performance of HMGB1 may be related to its function as DAMP at the beginning of the inflammatory cascade independent of the stimulus of other mediators, as with IL-6.

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Authors' contribution

Vilela EG performed the data collection and the statistical analysis. Vilela EG, Saturnino SF and Andrade MVM wrote, edited and formatted the article. Gomes CGO and Pinheiro CS reviewed the article and Nascimento VC performed the exams.

Vilela EG, Pinheiro CS, Saturnino SF, Gomes CGO, Nascimento VC, Andrade MVM. Avaliação do comportamento dos níveis séricos de HMGB1 e IL-6 como preditores de infecção, injúria renal aguda e mortalidade em pacientes cirróticos com varizes sangrantes. Arq Gastroenterol. 2018;55(4):338-42.

RESUMO – **Contexto** – Varizes esofagogástricas são a maior causa de morbimortalidade em pacientes cirróticos. **Objetivo** – Avaliar o papel de biomarcadores, High Mobility Group Box 1 (HMGB 1) e interleucina-6 (IL-6) como preditores de infecção, injúria renal aguda e mortalidade nestes pacientes. **Métodos** – Estudo prospectivo, observacional que incluiu 32 pacientes com cirrose hepática na fase aguda do sangramento. **Resultados** – A média de idade dos pacientes foi de 52±5 anos sendo 20 (62,5%) do gênero masculino. A média do MELD foi de 17,53±5 e a média do MELD-Na 20,63±6,06. Trinta (93,3%) pacientes foram classificados como Child B ou C. Complicação infecciosa esteve presente em 9 (28,1%) pacientes, injúria renal aguda em 6 (18,1%) e 4 (12,5%) evoluíram para o óbito. A mediana do nível sérico de HMGB 1 foi de 1487 pg/mL (0,1- 8593,1) e da IL-6 foi de 62,1pg/mL (0,1-1102,4). Os níveis séricos de HMGB 1 e IL-6 foram significativamente maiores nos pacientes que evoluíram com infecção, injúria renal aguda e óbito (P<0,05). Os valores da correlação de Spearman para os níveis séricos de HMGB 1 e IL-6 foram de 0,794 e 0,374 para infecção, 0,53 e 0,374 para injuria renal aguda e 0,467 e 0,404 para óbito, respectivamente. **Conclusão** – Níveis séricos de HMGB 1 e IL-6 foram maiores nos três desfechos estudados. Níveis séricos de HMGB 1 apresentaram alta correlação para com o desfecho infecção e moderada correlação para com injúria renal aguda e óbito, enquanto os níveis séricos de IL-6 apresentaram moderada correlação para com infecção e óbito e baixa correlação para com injúria renal aguda.

DESCRITORES – Cirrose hepática. Varizes esofágicas e gástricas. Biomarcadores.

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ORIGINAL ARTICLE

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Waiting DAAs list mortality impact in HCV cirrhotic patients

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ABSTRACT – **Background** – The infection for the hepatitis C virus (HCV) is a leading cause of liver-related morbidity and mortality through its evolution to liver cirrhosis, end-stage liver complications and hepatocellular carcinoma. Currently, the new drugs for the HCV infection, based on direct antiviral agents, have changed the outcomes in this setting. **Objective** – To assess death incidence, during the wait for the treatment with the new drugs, and to analyze which independent variable (age, sex, ascite, HDA, albumin, α-fetoprotein, platelets and Meld score) had relation with death. **Methods** – Prospective study with cirrhotic patients by HCV. Inclusion: cirrhotic patients by hepatic biopsy (METAVIR), clinic or image, detectable RNA (HCV). Exclusion: Other stages of hepatic fibrosis and hepatocellular carcinoma. Descriptive statistic in continue variables. Fisher Exact and Kaplan Meier and Cox Regression Analysis to assess the association of variables studied with death. $P<0.05$. **Results** – A total of 129 patients were included. Of this, 73% were men. Mean age was 57.8±12.1, albumin of 3.5±0.6 mg/dL, platelets of 123.4±59.6 and Meld score of 10.59±3.56. The time of observation was 11.2±3.26 months, and the number of death 9/129 (6,9%). The Kaplan-Meier showed association between death with albumin lower than 2.9 (0.0006), MELD score higher than 15 (0.007) and α-fetoprotein higher than 40 ng/mL (<0.0001). Adjusted Cox Regression Analysis showed that α-fetoprotein higher than 40 ng/ml could be considered an independent risk for death. **Conclusion** – We conclude that, patients with advanced cirrhosis should be prioritized for treatment with direct antiviral agents.

HEADINGS – Hepacivirus. Liver cirrhosis, drug therapy. Liver cirrhosis, mortality.

INTRODUCTION

The infection for the hepatitis C virus (HCV) is a leading cause of liver-related morbidity and mortality through its evolution to liver cirrhosis, end-stage liver complications and hepatocellular carcinoma. Currently, the new drugs for the HCV infection, based on direct antiviral agents (DAAs), have changed the outcomes in this setting. These drugs have been used safely and effectively in cirrhotic patients, including decompensated cirrhosis which is responsible for significant morbidity and mortality, along with healthcare costs[1]. It took a long time for DAA therapy to become available in Brazil, leaving patients (including cirrhotics) with no effective treatment to their condition; For this reason, it was possible to observe the disease's natural progression in many patients. The aim of this study is to evaluate mortality rate in cirrhotic patients while awaiting the DAAs.

METHODS

This was a prospective study of HCV-RNA positive patients with liver cirrhosis, admitted to the University Hospital of Botucatu (Botucatu School of Medicine, São Paulo State University). Data collection was undertaken between April 2015 and May 2016, and was approved by Ethics Committee of this Hospital (4378-2012); all patients' data were anonymized. A diagnosis of cirrhosis was made if the patient had a positive liver biopsy on record, or if they had clinical features of cirrhosis along with any of the following: evidence of portal hypertension, ascites, encephalopathy or signs of liver cirrhosis on ultrasound, CT scan or liver elastography examination. Exclusion criteria for this study were: Active hepatocellular carcinoma at baseline, and contraindication to DAAs therapy. Data were collected and recorded prospectively. Demographic data collected included: gender, age, viral genotype, platelet count, liver function tests (albumin, bilirubin and INR), MELD score and alpha-fetoprotein. We collected and recorded the treatment´s experience and ascites. The history of bleeding from varices was also registered. Outcomes of interest were time of death, or beginning of treatment with DAAs. Data was recorded and analyzed using SPSS 17.0, Chicago, IL, USA. Nominal variables are presented in absolute and relative frequencies. For continuous variables, descriptive statistics are presented as mean and standard deviation, with minimum and maximum values. The associations between selected factors and death were compared using the Fisher's exact test or Chi-squared test for association or trend, as appropriate. A Kaplan-Meier survival analysis was performed to evaluate the influence of the variables on outcomes. Cox's regression analysis was also performed. The level of statistical significance for all tests was set at $P=0.05$.

Declared conflict of interest of all authors: none
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RESULTS

Demographics: Of 214 patients evaluated during the study period, 129 patients met the diagnostic criteria for cirrhosis (therapy with DAAs was also indicated): 73% male (94/129), the mean patient age of 57.8±12.1 yrs, mean serum albumin of 3.5±0.6 mg/dL, mean platelet count of 123.4±59 and mean MELD score of 10.59±3.56. The mean time follow up was 11.2±3.26 months. The number of deaths was 9/129 (6.9%), these patients had lower levels of serum albumin and bilirubin associated with a higher MELD score than the survivors (TABLE 1). We observed no statistically significant differences among death and gender; genotype 1 and 3; history of ascites or bleeding varices. However, there were clear associations between death and: albumin levels lower than 3.5 mg/dL (0.03), or lower than 2.9 mg/dL (0.006), when comparing naïve patients with experienced ones (0.0002), and also between death and MELD scores higher than 15 (0.017). Analysis of the Kaplan-Meier survival curve showed a significant association between death in naive patients (0.0005), albumin lower than 2.9 (0.0006), MELD score higher than 15 (0.007) and α-fetoprotein higher than 40 ng/mL (<0.0001). No significant differences were found regarding bilirubin, past of ascites or bleeding varices, as well as Inr. Adjusted Cox Regression Analysis showed that only levels of α-fetoprotein higher than 40 ng/mL could be considered an independent risk for death.

TABLE 1. Collection parameters and comparison with survivor population.

Parameter	Mean avarage baseline (All population)	Variables	Censored	Death
Gender	Male: 73% (94/129) Female: 27% (35/129)			
Age (years)	57.8 ± 12.1	Age (years)	57.7 ± 11.8	58.4 ± 16.3
Albumin (mg/dL)	3.5 ± 0.6	Albumin (mg/dl)	3.6 ± 0.5	2.9 ± 0.6
Platelet Count	123.4 ± 59,6	Bilirrubin	1.4 ± 1.2	3.7 ± 5.0
MELD Score	10.59 ± 3.56	MELD score	10.3 ± 3.2	13.7 ± 5.5
		Inr	1.21 ± 0.1	1.36 ± 0.3

MELD: Model for End-Stage Liver Disease; Inr: International Normalized Ratio.

DISCUSSION

The virus of hepatitis C affects around 200 million people worldwide, and is the leading cause of cirrhosis, hepatocellular carcinoma, and liver transplantation in western countries[2]. HCV-related cirrhosis has a 1%-5% annual risk of leading to liver cancer and 3%-6% risk of clinical decompensation, after which, patients have a 4%-67% chance of dying[3]. During the interferon-based therapy, Sustained Virologic Response (SVR) was around 40%-80%, but in cirrhotics it was approximately 20%[4]. However, its use in patients with decompensated cirrhosis was not permitted, mainly due to the risk of infection and death[5]. Nowadays, the safety and efficacy of DAAs has enabled the treatment of cirrhosis, including decompensated cirrhosis. For this reason, patients with advanced liver disease have been included in the waiting list for DAA treatment. Recently, D'Amico et al.[6] proposed a new classification for cirrhosis, based on clinical criteria, and their prognosis. In this classification, there are 4 stages: 1 (no ascites, no varices); 2 (varices, no ascites); 3 (ascites ± varices) and 4 (variceal bleeding ± ascites). The estimated mortality rates per year are 1%, 3.4%, 20% and 57%, respectively[6]. More recently, other authors proposed a 5th stage, due to infection and renal failure, with an increase on mortality rate from 1 year to 67%[7]. Recently published data showed that the 5 year cumulative incidence of liver-related mortality in decompensated cirrhotics was 61.3%[8]. Patients studied in our work were predominantly male, mean age of 57.8 years old. The mortality rate at the mean time of 11.2 months was 6.9%. Although we did not use the previous classification described, we observed in our study patients with advanced liver disease, with previous ascites (27.9%) and in 9% past of variceal bleeding. On the other hand, 41% them had platelets less than 100.000/mm^3; α-fetoprotein above 20 and 40 ng/dL were observed in 21% and 11% respectively, and also albumin less than 3.5 and 2.9 were seen in 43% and 17%, respectively. The analysis showed that in naive patients, albumin less than 3.5 and 2.9 mg/dL, MELD score above 15 and α-fetoprotein above 20 and 40 ng/dL were associated with death. Cox regression analysis showed that the only independent factor associated with death was α-fetoprotein greater than 40 ng/dL. The public health system in Brazil began to offer treatments with DAAs in December 2015. Before that we could not offered any treatment for 1.5 years, and therefore, we were able to observe the natural evolution of cirrhosis.

CONCLUSION

With this study we concluded that, patients with advanced liver disease should be prioritized for treatment with DAAs.

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Authors' contribution

Silva GF: designed the study, wrote the paper and revised the manuscript for final submission. Andrade VG and Moreira A: performed the research.

Silva GF, Andrade VG, Moreira A. Impacto da lista de espera para AADs em pacientes cirróticos por VHC. Arq Gastroenterol. 2018;55(4):343-5.

RESUMO – **Contexto** – A infecção pelo vírus da hepatite C (VHC) é uma das principais causas de morbidade e mortalidade relacionada ao fígado, através de sua evolução para cirrose hepática, complicações hepáticas em estágio terminal e carcinoma hepatocelular. Atualmente, os novos fármacos para a infecção pelo VHC, baseados nos novos antivirais de ação direta (AADs), modificaram os resultados nesse cenário. **Objetivo** – Avaliar a incidência de morte, durante a espera pelo tratamento com as novas drogas, e analisar quais variáveis independentes (idade, sexo, ascite, HDA, albumina, α-fetoproteína, plaquetas e escore de MELD) tiveram relação com o óbito. **Métodos** – Estudo prospectivo com pacientes cirróticos pelo VHC. Inclusão: pacientes cirróticos por biópsia hepática (METAVIR), clínica ou imagem, RNA detectável (VHC). Exclusão: Outras fases de fibrose hepática e carcinoma hepatocelular. Estatística descritiva em variáveis contínuas. Exato de Fisher e Kaplan Meier e Análise de Regressão de Cox para avaliar a associação das variáveis estudadas com o óbito. *P*<0,05. **Resultados** – Um total de 129 pacientes foram incluídos. Destes, 73% eram homens. A idade média foi de 57,8±12,1, a albumina de 3,5±0,6 mg/dL, as plaquetas de 123,4±59,6 e o escore de MELD de 10,59±3,56. O tempo de observação foi de 11,2±3,26 meses e o número de mortes 9/129 (6,9%). O Kaplan-Meier mostrou associação entre o óbito com albumina menor que 2,9 (0,0006), escore MELD maior que 15 (0,007) e α-fetoproteína maior que 40 ng/mL (<0,0001). A análise de regressão de Cox ajustada mostrou que α-fetoproteína maior que 40 ng/mL poderia ser considerada um risco independente para morte. **Conclusão** – Concluímos que pacientes com cirrose avançada devem ser priorizados para tratamento com AADs.

DESCRITORES – Hepacivirus. Cirrose hepática, tratamento farmacológico. Liver cirrhosis, mortalidade.

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ORIGINAL ARTICLE

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Immune mediated diseases in patients with celiac disease and their relatives: a comparative study of age and sex

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ABSTRACT – **Background** – Up to 15% of other immune-mediated diseases (IMDs) can occur in patients with CD throughout their lives and are associated with multiple factors, including sex and sex hormone levels. Moreover, sex is associated with differences in clinical presentation, onset, progression, and outcomes of disorders. **Objective** – To investigate the prevalence of IMDs at diagnosis in patients with celiac disease (CD) and their first-degree relatives and to compare the findings between female and male patients of different age. **Methods** – A retrospective study including Brazilian patients with CD who visited the same doctor during January 2012 to January 2017 was performed. Demographic and medical history data were collected through self-administered questionnaires and medical charts of the patients. In total, 213 patients were examined at diagnosis: 52 males (mean age, 40.0 years) and 161 females (mean age, 41.4 years). The patients were divided into two groups according to sex and age. **Results** – IMDs were observed in 60.2% of the female (97/161) and 42.3% of the male patients (22/52; P=0.22). However, the frequency of IMDs was significantly higher in females aged 51-60 years than in males with same age (P=0.0002). Dermatitis herpetiformis (DH) was significantly more prevalent in males (P=0.02), whereas atopy was more prevalent in females (P=0.02). IMDs observed in first-degree relatives were similar to those observed in patients (70.9%; P<0.001), with a higher number observed in female relatives. **Conclusion** – The frequency of IMDs in CD patients was similar in all age groups and both sexes, except women diagnosed with CD after 51 years of age presented with an increased frequency of IMDs compared with males. Dermatitis herpetiformis was more prevalent in males, whereas atopy was more prevalent in females. No difference was observed in the type of IMDs between the first-degree relatives of both sexes.

HEADINGS – Celiac disease. Autoimmune diseases. Sex factors. Age factors. Dermatitis herpetiformis.

INTRODUCTION

Celiac disease (CD) is an autoimmune-mediated systemic disorder triggered by the ingestion of gluten – the protein fraction of wheat, barley, and rye – in genetically susceptible individuals. Up to 15% of other immune-mediated diseases (IMDs) can occur in patients with CD throughout their lives[1]. Diseases of the skin, endocrine, nervous system, liver, and rheumatological or connective tissues, among many other, are directly or indirectly related to the immune function[2-5]. Moreover, a significantly increased prevalence of CD (10–30-fold) has been documented in individuals with different IMDs[2-5].

IMDs and autoimmune diseases (AIDs) are associated with multiple factors, including sex and sex hormone levels. Moreover, sex is associated with differences in clinical presentation, onset, progression, and outcomes of disorders. Although the precise underlying reason remains unknown, sex hormone and chromosome abnormalities have been implicated to play roles in these sex-based differences. In addition, effects of environmental factors should be considered[6]. Sex-based differences in immune responses, which can affect both the innate and adaptive immune responses, contribute to differences in AID prevalence. In general, females exhibit a higher frequency of AIDs than males[7]. Several reports have shown a significantly increased prevalence of IMDs in the first-degree relatives of patients with CD compared with controls[8-10].

In the present study, we investigated the prevalence of IMDs at diagnosis in patients with CD and in their first-degree relatives and compared the findings between female and male patients in different age groups.

METHODS

The research was approved by the Pontifical Catholic University of Paraná Ethical Committee. We performed a retrospective study including Brazilian patients with CD who visited a single private clinic over five years (2011-2016). Demographic and medical data were collected using patients' medical charts following clinical protocols and through self-administered questionnaires. Data regarding the family history of diseases, including IMDs previously reported in the literature[11,12], were also collected.

All patients included in this study were newly diagnosed with CD on the basis of clinical complaints, positive screening with au-

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toantibodies (anti-tissue transglutaminase and/or anti-endomysial antibodies), and confirmation by histological findings of the duodenal mucosa, in accordance with the Marsh classification[13].

Frequency analyses were performed through Chi-squared and Fisher's exact tests using Graph Pad Prism 5.0 (GraphPad software Inc., La Jolla, CA, USA). A $P<0.05$ was considered statistically significant.

RESULTS

A total of 213 patients with CD at diagnosis were examined: 52 males (mean age, 40.0 years; range, 12-70 years) and 161 females (mean age, 41.4 years; range, 12-87 years). The proportion of females and males with CD was 3:1.

The patients were divided into the following groups according to sex and age:

FEMALES: Group FI (12-20 years), Group FII (21-30 years), Group FIII (31-40 years), Group FIV (41-50 years), Group FV (51-60 years), and Group FVI (more than 61 years).

MALES: Group MI (12-20 years), Group MII (21-30 years), Group MIII (31-40 years), Group MIV (41-50 years), Group MV (51-60 years), and Group MVI (more than 61 years)

TABLE 1 summarizes the data regarding age, sex, and occurrence of IMDs the study cohort. In total, 54.4% of the patients with CD presented with IMDs. No significant difference was observed in the total prevalence between females and males (P=0.22). However, female patients aged 51-60 years exhibited significantly higher frequency of IMDs than males (P=0.0002).

TABLE 1. Distribution of age, gender and immune mediated diseases (IMDs) in the groups of the 213 studied patients.

Group/Age	Female (n=161)	Male (n=52)	IMDs at diagnosis Female n (%)	IMDs at diagnosis Male n (%)	*P**
I (12-20 years) n=33	25	8	12 (48.0)	2 (25.0)	0.41
II (21-30 years) n=33	26	7	15 (57.7)	6 (85.7)	0.22
III (31-40 years) n=48	36	12	17 (47.2)	7 (58.3)	0.73
IV (41-50 years) n=48	37	11	20 (54.0)	6 (54.5)	0.72
V (51-60 years) n=29	21	8	20 (95.2)	3 (18.8)	0.002
VI (> 61 years) n=22	16	6	8 (50.0)	0	0.05
Total	n=161	n=52	92 (57.1)	24 (46.1)	0.22

* Fisher's Exact Test comparing gender.

TABLE 2 presents the number and percentages of reported IMDs according to sexes. IMDs were significantly more frequent in females [97/161 (60.2%)] than in males [22/52 (42.3%)] (P=0.025). Statistical analysis revealed that dermatitis herpetiformis (DH) was significantly more prevalent in males (P=0.029), whereas atopy was significantly more prevalent in females (P=0.024).

TABLE 2. Immuno mediated diseases diagnosed in celiac patient in according to gender.

Immuno mediated disease	Female (n=161)	Male (n=52)	*P* value *
Hypothyroidism	36 (22.3)	6 (11.5)	ns
Dermatitis Herpetiformis	15 (9.4)	11 (21.1)	**0.029**
Atopy	15 (9.4)	0	0.024
Lymphocitic colitis	6 (3.7)	2 (3.8)	ns
Endometriosis	5 (3.1)	0	ns
Autoimmune gastritis	4 (2.5)	0	ns
Diabetes mellitus type 1	3 (1.9)	1 (1.9)	ns
Autoimmune hepatitis	2 (1.2)	0	ns
Psoriasis	2 (1.2)	0	ns
Lupus erythematosus	2 (1.2)	0	ns
Raynaud phenomem	2 (1.2)	0	ns
Alopecia areata	1 (0.6)	0	ns
Vitiligo	1 (0.6)	0	ns
Addison's disease	1 (0.6)	1 (1.9)	ns
Inflammatory bowel disease	1 (0.6)	1 (1.9)	ns
Lymphoma	1 (0.6)	0	ns
Total	97 (60.2)	22 (42.3)	0.025

* Fisher's exact test.

In addition, information regarding 151 first-degree relatives (n=103; 68.2% females) with IMDs, as reported by the patients at diagnosis, was also analyzed. TABLE 3 lists IMDs identified in the first-degree relatives of the study cohort. IMDs detected in the first-degree relatives were similar to those detected in the patients. CD was more prevalent in female first-degree relatives (P=0.045). Atopy was more frequent in male first-degree relatives (P=0.002).

The frequency of different IMDs was higher in female relatives (mothers, sisters, and daughters) than in male relatives (TABLE 4).

DISCUSSION

In the present Brazilian study, the proportion of females and males with CD was 3:1, which is consistent with that in an Italian study by Ciacci et al.[14]. Notably, females consult doctors more frequently than males, present with more symptoms, and are more prone to develop AIDs[15].

Certain sex-based immunological differences are present throughout life, whereas others are only apparent after puberty

TABLE 3. Immuno mediated diseases in relatives of the studied patients (n=52 males and n=161 females).

Immuno mediated diseases (IMDs)	Celiac patient's sex	First degree relative with IMDs n (%)	First degree relative with IMDs sex
Hypothyroidism	F	48 (29.8%)	43 F; 5 M
	M	12 (23.0%)	11 F; 1 M
Celiac disease *	F	30 (18.6%)	25 F; 5 M
	M	9 (17.3%)	8 F; 1 M
Atopy#	F	6 (3.7%)	3 F; 3 M
	M	7 (13.4%)	3 F; 4 M
Rheumatoid arthritis	F	4 (2.5%)	4 F; 0M
	M	4 (7.7%)	3 F; 1 M
Diabetes mellitus type 1	F	3 (1.8%)	0 F; 3 M
	M	3 (5.7%)	2 F; 1 M
Crohn's disease	F	3 (1.8%)	1 F; 2 M
	M	0	
Dermatitis herpetiformis	F	2 (1.2%)	1 F; 1 M
	M	2 (3.8%)	1 F; 1 M
Vitiligo	F	1 (0.6%)	1 F; 0 M
	M	1 (1.9%)	1 F; 0 M
Psoriasis	F	1 (0.6%)	0 F; 1 M
	M	0	0
Lupus erythematosus	F	1 (0.6%)	1 F; 0 M
	M	1 (1.9%)	1 F; 0 M
Lymphoma	F	1 (0.6%)	0 F; 1 M
	M	1 (1.9%)	1 F; 0 M
Total		151/213 = 70.9%	

* P=0.045 Chi-Square test. # P=0.002 Fisher Exact test. F: female; M: male. All the other comparison: P=not significant.

and before reproductive senescence, suggesting the involvement of both genes and hormones[16]. CD can be diagnosed at any age, but a delay in diagnosis is frequently unrecognized in young adults and older patients. In our investigation, 9.9% (16/161) of the females and 11.5% (6/52) of the males were diagnosed after 61 years of age[17]. Substantial diagnostic delay in CD is associated with poor clinical outcomes, and it is occurs more frequent in females[18]. Bai et al.[19] studied 323 patients (211 female and 112 men) in the USA and reported no sex-based differences in the age at diagnosis or the mode of presentation, which is consistent with our findings.

The total number of associated IMDs (57.1% in females and 46.1% in males) was higher than that described in previous reports (15%-30%)[20]. This high prevalence could be partially explained by the shared genes in immunological pathways between CD and other IMDs[21]. Based on the findings of between-group comparison of age, the diagnosis of CD in females later in their lives can hypothetically predispose them to more IMDs as a result of prolonged inflammation[22]. Sategna-Guidetti et al.[23] have reported that the duration of gluten exposure in adults with CD was not correlated with the risk of autoimmune disorders, which differs from our findings.

Notable, young patients in the present study (Groups FI and MI) were diagnosed with IMDs before the diagnosis of CD, which is consistent with a report by Demirezer et al. in Turkey[24]. Demirezer and colleagues identified female sex, family history of AIDs as risk factors for IMDs, as presented in the present study.

In the present study, DH was more prevalent in males than in females, which is similar to the findings of other studies[16,25]. Other dermatological diseases, such as psoriasis, vitiligo, and alopecia areata, are diagnosed before CD. Interestingly, benefits of a gluten-free diet have been described[26] in these diseases, although the cause is not known.

The close association between CD and endocrine autoimmunity has been frequently reported in the literature, thus warranting a broader immune genetic study given that patients with CD and their relatives present with endocrine autoimmune syndromes[27]. In the general population, thyroid disease (TD) is frequent in females after 40 years of age, as shown in this study. Conversely, TD is generally frequent in males after 60 years of age. However, in our study, TD was diagnosed relatively early, as described by another studies[28]. Diabetes mellitus type I was diagnosed before CD in two young male patients (age, 14 and 21 years). However, two females (age, 51 and 54 years) undergoing treatment for diabetes mellitus type I were referred to a gastroenterologist later in their life and were diagnosed as having CD. Both females were received treatments for hypothyroidism, and one of them developed DH. Interestingly, three or four IMDs occurring in the same patient is a rare association[29]. Compared with controls, patients with CD were 11.4 times more likely to develop Addison's disease before or after the diagnosis of CD[24]. In this study, one female (age, 49 years) and one male (age, 61 years) presented with Addison's disease at the diagnosis of CD.

Ludvigsson et al.[30] have reported that patients with CD were at a threefold increased risk of developing systemic lupus erythematous (SLE). In the present study, two patients were diagnosed with SLE before the diagnosis of CD was confirmed. In our previous study, the endomysial antibody was more frequently positive in patients with SLE than in controls, particularly those with discoid lesions[31]. Raynaud phenomenon was observed in two patients; both showed the classic tricolor phenomenon and symmetrical bilateral involvement of hands and feet[32]. No other autoimmune rheumatic disease was detected.

Concomitant CD and endometriosis may render the diagnosis difficult due to the overlapping symptoms, such as abdominal pain, bowel changes, spontaneous abortion, and infertility[33]. In the present study, endometriosis was diagnosed in 3.1% of the patients (5/161) at the diagnosis of CD. In another region of Brazil, Aguiar et al.[34] have reported 2.5% prevalence of CD in 120 females with endometriosis and 0.6% in 1500 healthy females. Thus, commonalities exist between CD and endometriosis by altered Th1 immune response pattern arising from the polymorphic genes of interleukin-18 and interferon-gamma[35]. Marziali et al.[35] have recommended a gluten-free diet as a new strategy for the management of painful endometriosis-related symptoms.

TABLE 4. Frequency of immuno mediated diseases in first-degree relatives of celiac patients.

Immuno mediated diseases	Celiac patient's sex (n=213)	Mother	Father	Sister	Brother	Daughter	Son
Hypothyroidism	F	30 (18.6%)	4 (2.5%)	13 (8.1%)	1 (0.6%)	0	0
	M	6 (11.5%)	1 (1.9%)	4 (7.6%)	0	1 (1.9%)	0
Celiac disease	F	12 (7.4%)	4 (2.5%)	13 (8.1%)	1 (0.6%)	0	0
	M	3 (5.8%)	1 (1.9%)	4 (7.6%)	0	1 (1.9%)	0
Rheumatoid arthritis	F	3 (5.8%)	0	1 (0.6%)	0	0	0
	M	2 (3.8%)	0	0	1 (1.9%)	1 (1.9%)	0
Atopy	F	2 (1.2%)	1 (0.6%)	1 (0.6%)	1 (0.6%)	0	1 (0.6%)
	M	1 (1.9%)	2 (3.8%)	0	1 (1.9%)	2 (3.8%)	1 (1.9%)
Dermatitis herpetiformis	F	1 (0.6%)	1 (0.6%)	0	0	0	0
	M	0	0	0	1 (1.9%)	1 (1.9%)	0
Diabetes mellitus type 1	F	0	0	0	2 (1.2%)	0	1 (0.6%)
	M	1 (1.9%)	0	0	0	1 (1.9%)	1 (1.9%)
Vitiligo	F	0	0	1 (0.6%)	0	0	0
	M	1 (1.9%)	0	0	0	0	0
Psoriasis	F	0	0	0	1 (0.6%)	0	0
	M	0	0	0	0	0	0
Lupus erythematosus	F	0	0	1 (1.6%)	0	0	0
	M	1 (1.9%)	0	0	0	0	0
Crohn's disease	F	0	1 (0.6%)	2 (1.2%)	0	0	0
	M	0	0	0	0	0	0
Lymphoma	F	0	1 (0.6%)	0	0	0	0
	M	1 (1.9%)	0	0	0	0	0
Total		64 (30.0%)	16 (7.5%)	37 (17.4%)	9 (4.2%)	7 (3.3%)	4 (1.9%)

The association between atopy and CD has not been satisfactorily established. Our patients reported asthma, eczema, rhinitis, or conjunctivitis. In a study in Sweden, Enroth et al.[36] have reported a high level of self-reported allergies (42.3%) in patients with CD; this finding was inversely correlated with age and was more frequent in young patients, emphasizing heritability. In our study, only females (9.4%) reported any atopic manifestations.

Several authors have shown that self-reports are often accurate[9], and the family history can be effectively captured based on patient reports[12]. Relatives of CD patients exhibit a higher prevalence of IMDs compared with the relative of controls. Moreover, relatives of CD patients with IMDs are at 25% higher risk of having undiagnosed CD, and they must be screened for CD[8]. However, Roy et al.[37] have described that physicians treating patients with CD seldom recommend routine screening of the first-degree relatives.

CD was present in first-degree relatives, being more prevalent in mothers, sisters, and daughters. These results are in accordance with previous reports of our group[38]. In a meta-analysis, Vaquero et al.[39] have described the prevalence of CD and its risk in the first-degree relatives according to geographic areas; a lower risk was observed in South America than in Asia, North America, and Europe. However, findings of a few studies, including two in Argentina (Buenos Aires 1977 and 1999) and five in Brazil, which showed differences in the regions of these countries and different racial mixtures of the population, should also be considered. We speculate that the findings of a majority of these studies were not applied in the clinical setting.

Diseases detected in the first-degree relatives were almost the same as those detected in patients with CD of both sexes. A higher frequency of IMDs was reported in females, which is in corroboration with the reported literature[7].

CONCLUSION

The frequency of IMDs in CD patients was similar in all age groups and both sexes, except women diagnosed with CD after 51 years of age presented with an increased frequency of IMDs compared with males. DH was more prevalent in males, whereas atopy was more prevalent in females. No difference was observed in the type of IMDs between the first-degree relatives of both sexes.

Authors' contribution

Kotze LMS and Kotze LR: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript. Moreno I: acquisition of data; analysis and interpretation of data; drafting of the manuscript. Nisihara R: study concept and design; analysis and interpretation of data; drafting of the manuscript; statistical analysis.

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RESUMO – **Contexto** – Até 15% das outras doenças imunomediadas (DIMs) podem ocorrer em pacientes com doença celíaca ao longo de suas vidas e estão associados a múltiplos fatores, incluindo sexo e níveis de hormônios sexuais. Além disso, o sexo está associado a diferenças na apresentação, início, progressão e desfecho das doenças. **Objetivo** – Investigar a prevalência de DIMs ao diagnóstico de doença celíaca e em seus familiares de primeiro grau e comparar os resultados entre sexo feminino e masculino em diferentes idades. **Métodos** – Estudo retrospectivo incluindo pacientes brasileiros com diagnóstico de doença celíaca que realizaram acompanhamento com o mesmo médico no período de janeiro 2012 a janeiro de 2017. Dados demográficos e histórico médico foram coletados através de um questionário auto administrado e prontuários médicos dos pacientes envolvidos. No total, 213 pacientes eram portadores de doença celíaca, dos quais 52 do sexo masculino (idade média 40,0 anos) e 161 do sexo feminino (idade média 41,4 anos). Os pacientes foram divididos em dois grupos de acordo com o sexo e idade. **Resultados** – DIMs foram observadas em 60,2% das pacientes femininas (97/161) e 42,4% dos pacientes masculinos (22/52; P=0,22). Entretanto, a frequência de DIMs foi significantemente maior em pacientes do sexo feminino com idade entre 51-60 anos que em pacientes masculinos da mesma idade (P=0,0002). Dermatite herpetiforme apresentou maior prevalência no sexo masculino (P=0,02), enquanto atopia obteve maior prevalência nas pacientes do sexo feminino (P=0,02). DIMs observadas em familiares de primeiro grau foram similares as encontradas nos pacientes (70,9%; P<0,001), com um maior número observado em familiares femininos. **Conclusão** – A frequência de DIMs em pacientes com doença celíaca foi similar nos grupos etários e ambos sexos, exceto as mulheres com diagnóstico de doença celíaca após a idade de 51 anos, as quais apresentaram um aumento na frequência de DIMs em comparação com os pacientes do sexo masculino. Dermatite herpetiforme apresentou maior prevalência em pacientes do sexo masculino, enquanto que atopia foi mais prevalente no sexo feminino. Em relação ao sexo, não foi observada diferença no tipo de DIMs observada entre os familiares de primeiro grau.

DESCRITORES – Doença celíaca. Doenças autoimunes. Fatores sexuais. Fatores etários. Dermatite herpetiforme.

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ORIGINAL ARTICLE

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Food intake, nutritional status and gastrointestinal symptoms in children with cerebral palsy

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ABSTRACT – **Background** – Cerebral palsy may be associated with comorbidities such as undernutrition, impaired growth and gastrointestinal symptoms. Children with cerebral palsy exhibit eating problems due to the effect on the anatomical and functional structures involved in the eating function resulting in malnutrition. **Objective** – The aim of this study was to investigate the association between food intake, nutritional status and gastrointestinal symptoms in children with cerebral palsy. **Methods** – Cross-sectional study that included 40 children with cerebral palsy (35 with spastic tetraparetic form and 5 with non-spastic choreoathetoid form of cerebral palsy, all requiring wheelchairs or bedridden) aged from 4 to 10 years. The dietary assessment with the parents was performed using the usual household food intake inquiry. Anthropometric data were collected. Gastrointestinal symptoms associated with deglutition disorders, gastroesophageal reflux and chronic constipation were also recorded. **Results** – The median of height-for-age Z-score (-4.05) was lower (P<0.05) than the median of weight-for-age (-3.29) and weight-for-height (-0.94). There was no statistical difference between weight-for-age and weight-for-height Z-scores. Three patients with cerebral palsy (7.5%) exhibited mild anemia, with normal ferritin levels in two. Symptoms of dysphagia, gastroesophageal reflux, and constipation were found in 82.5% (n=33), 40.0% (n=16), and 60.0% (n=24) of the sample, respectively. The patients with symptoms of dysphagia exhibited lower daily energy (1280.2±454.8 Kcal vs 1890.3±847.1 Kcal, P=0.009), carbohydrate (median: 170.9 g vs 234.5 g, P=0.023) and fluid intake (483.1±294.9 mL vs 992.9±292.2 mL, P=0.001). The patients with symptoms of gastrointestinal reflux exhibited greater daily fluid intake (720.0±362.9 mL) than the patients without symptoms of gastroesophageal reflux (483.7±320.0 mL, P=0.042) and a greater height-for-age deficit (Z-score: -4.9±1.7 vs 3.7±1.5, P=0.033). The patients with symptoms of constipation exhibited lower daily dietary fiber (9.2±4.3 g vs 12.3±4.3 g, P=0.031) and fluid (456.5±283.1 mL vs 741.1±379.2 mL, P=0.013) intake. **Conclusion** – Children with cerebral palsy exhibited wide variability in food intake which may partially account for their severe impaired growth and malnutrition. Symptoms of dysphagia, gastroesophageal reflux, and constipation are associated with different food intake patterns. Therefore, nutritional intervention should be tailored considering the gastrointestinal symptoms and nutritional status.

HEADINGS – Cerebral palsy. Gastrointestinal diseases. Nutritional status. Deglutition disorders. Gastroesophageal reflux. Constipation.

INTRODUCTION

Cerebral palsy is a chronic non-progressive encephalopathy that is caused by various agents and presents with heterogeneous clinical manifestations[1]. It may be associated with comorbidities such as undernutrition (46% to 90% of the patients)[2], impaired growth, mental retardation, epileptic seizures, communication disorders, visual and auditory defects[3], and gastrointestinal symptoms, including dysphagia, gastroesophageal reflux, and constipation[4,5]. Food intake is one of the factors that determine malnutrition[4]. Oropharyngeal dysphagia due to motor dysfunction[5-7] may reduce the food intake of patients, with consequent malnutrition, pulmonary aspiration, respiratory infection, and chronic lung disease[8-9]. Gastroesophageal reflux in cerebral palsy is associated with severe complications, such as esophagitis and esophageal dysphagia, and also with reduced food intake[3,7]. As for constipation, it is believed that alterations in the neural modulation are associated with reduced colonic motility[10]. Other factors may also contribute to the development of constipation, such as severe skeletal deformities, spasm, use of anticonvulsants[11], low levels of physical activity, and a low-fiber diet[7,12].

Children with cerebral palsy exhibit eating problems due to the effect on the anatomical and functional structures involved in the eating function[3], resulting in reduced energy and nutrient intake and in consequent malnutrition[12]. As a function of malnutrition, the body fat reserves become depleted, the muscle mass is reduced, and immune dysfunction occurs, with consequent increased risk of respiratory and urinary tract infections[13,14].

No study published up to the present time has sought to associate nutritional status with food intake and the presence of gastrointestinal symptoms in cerebral palsy[15,16]. Therefore, the aim of this study was to investigate the correlation of food intake and nutritional status with the presence of gastrointestinal symptoms in children with severe cerebral palsy.

Case series and methods

This cross-sectional study was approved by the Ethics Committee of the São Paulo School of Medicine, Federal University of São

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Paulo (*Universidade Federal de São Paulo*). Data were collected at the outpatient clinic of Our Home Spiritualist Center – André Luiz Houses (*Centro Espírita Nosso Lar – Casas André Luiz*), Guarulhos, and at Saint Francis Children's Home (*Lar Escola São Francisco*), São Paulo, Brazil. The children's guardians and institutional representatives were asked to sign an informed consent form.

The sample of this study was of convenience, which comprised 40 children with cerebral palsy, 23 (57.5%) male and 17 (42.5%) female. The patients socioeconomic level was established based on the classification formulated by the Brazilian Association of Market Research Companies (*Associação Brasileira de Empresas de Pesquisa* – ABEP)[17].

All of the patients had severe cerebral palsy, requiring wheelchairs or bedridden. The age varied from 4 to 10 years that was the selected range for inclusion in the study. All of the patients who met the inclusion criteria and had medical appointments on the days when the study was conducted were invited to participate in the study. According to the Oxford Feeding Study II classification[18], 87.5% (35/40) of the patients had the spastic tetraparetic cerebral palsy and 12.5% (5/40) had the non-spastic choreoathetoid form of cerebral palsy.

Information was registered in a form that included personal, socioeconomic, and anthropometric data, clinical history, gastrointestinal symptoms, feeding route, characteristics of oral motor function, and dietary survey.

The dietary assessment with the parents was performed using the usual household food intake inquiry. The dietary data was collected by one of the authors (Caramico-Favero DCO) who is an experienced nutritionist. Utensils and utensils album images have been used and sipped over the table (homemade measures). Posteriorly homemade measuring was converted to grams and milliliters. Hydric ingestion was calculated from the liquids intake of the diet (water, coconut water, juices, milk, carbonated drinks, jellies and teas). The water used for cleaning the enteral tube or gastrostomy was also computed. The type of enteral diet was also registered (homemade blended, commercial formula or both homemade and commercial). Information about noncommercial enteral feeding was collected. Nutrition calculations were performed using the NutWin Software for Support for Decision-Making in Nutrition version 2.5 by the Federal University of São Paulo[19].

The food intake values were compared to the references in Dietary Reference Intake (DRI) for the following parameters: Estimated Energy Requirements (EER); Estimated Average Requirement (EAR) of energy; Recommended Dietary Allowance (RDA) and Tolerable Upper Intake Level (UL) of carbohydrates and proteins[20]. Fiber intake was assessed based on the American Health Foundation's recommendation, according to which the minimum daily fiber intake (in grams) is equal to the child's age (in years) plus five[21].

To assess the participants' nutritional status, their body weight was measured, and their height was estimated. The body weight was assessed using a Toledo® (São Bernardo do Campo, São Paulo, Brazil) 500-kg capacity scale with a 100-g precision platform. This scale allowed the weighing of children on wheelchairs as needed. The children's current weight was calculated by subtracting the wheelchair weight from the combined child-wheelchair weight; the children were weighed wearing a minimum of clothing and without diapers. In the remaining cases, as the sample characteristically comprised children who cannot stand up by themselves, they were held by an adult and weighed, and their current weight was calculated by subtracting the adult's weight from the combined child-adult weight. The children's height was estimated based on the tibia length[22].

The anthropometric parameters weight-for-age, height-for-age, and weight-for-height were analyzed based on Z-scores relative to the reference values established by the World Health Organization (WHO). Values two standard deviations below the corresponding Z-score were considered to be indicative of nutritional deficit[23,24].

To assess the children's hemoglobin and ferritin levels, blood samples were collected by puncture of a forearm vein, which was performed by a duly trained professional at America Diagnosis Laboratory (*Laboratório Diagnósticos da América, Barueri*, São Paulo, Brazil). Sample analysis was performed using a Pentra ABX automated analyzer, Horiba Medical (Kyoto, Japan). The diagnosis of anemia and iron deficiency was based on comparing the measured blood hemoglobin and ferritin levels to the reference values formulated by WHO[25].

Symptoms suggestive of dysphagia, gastroesophageal reflux, or constipation were recorded in the patients' individual forms.

The symptoms of dysphagia were evaluated by speech therapists based on reported occurrence of cough, drooling, or choking; orofacial motor features registered during the study in ad hoc form; utensils used for feeding; and consistency of the food consumed[26]. The presence of nausea, vomiting, and regurgitation was considered symptoms of gastroesophageal reflux[27]. Constipation was defined as pain and/or strain to pass stools combined with fragmented and hard or cylindrical stools with diameter larger than a hotdog sausage with cracks in the surface, and/or two or fewer defecations per week. Having only two or fewer isolated bowel movements per week was considered to be indicative of constipation. Patients who did not meet any of those criteria but used laxatives were also characterized as having constipation. This definition was adapted from previous studies that assessed constipation in children with neurological diseases[10,28,29]. The symptoms of gastroesophageal reflux and constipation were assessed by a gastroenterologist.

The results were analyzed by means of parametric and non-parametric statistical tests depending on the distribution of the variables and are described together with the results. Analysis was performed using the software SigmaStat version 3.5. (Systat Software, San Jose, California, USA)[30] and Epi-Info version 3.2.2 (Atlanta, GA, USA)[31]. The value set to reject the null hypothesis was 0.05 or 5%.

RESULTS

The sample comprised 40 children with cerebral palsy with mean age of 6.7±2.4 years old, of whom 33 (82.5%) were fed by the oral route, five (12.5%) via a gastrostomy tube, and two (5.0%) by the oral route combined with a nasogastric or gastrostomy tube.

According to Friedman's test followed by Dunn's multiple comparison test, the height-for-age Z-score of the 40 children with cerebral palsy (median = -4.05; 25th and 75th percentiles: -5.30 and -2.89) exhibited a greater deficit ($P<0.05$) than weight-for-age (median = -3.29; 25th and 75th percentiles: -3.95 and -2.28) and weight-for-height (median = -0.94; 25th and 75th percentiles: -2.06 and 0.12). Weight-for-age and weight-for-height did not reach statistical significant difference ($P>0.05$).

TABLE 1 describes the data on daily carbohydrate, protein intake, distributed according to the RDA and EAR for age and gender. The protein and carbohydrate intake was above the RDA in 92.5% (37/40) and 85.0% (34/40) of the participants, respectively.

TABLE 1. Carbohydrate and protein intake according to Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), and Tolerable Upper Intake Level (UL) of children with cerebral palsy.

Nutrient	< 2 SD of EAR	From -2 SD of EAR to EAR	Within EAR and RDA	> RDA	> UL
Carbohydrates* (g/day)	1 (2.5%)	1 (2.5%)	4 (10.0%)	34 (85.0%)	–
Protein* (g/day)	–	–	3 (7.5%)	37 (92.5%)	–

*Mean intake (standard-deviation) of carbohydrates and protein were, respectively, 199.6±85.3 and 48.6±17.9 g.

In 30% (12/40) of the sample, the energy intake was less than 90% of the EER; in 20% (8/40) of the sample it was 91% to 120% of the EER; and in 50% (20/40) it was more than 120% of the EER.

Approximately 57.5% of the sample (n=23) consumed less than the minimum daily fiber intake recommended by the American Health Foundation (age in years + 5).

Regarding the prevalence of gastrointestinal symptoms, 82.5% (33/40) of the sample exhibited symptoms of dysphagia, 40.0% (16/40) symptoms suggestive of gastroesophageal reflux, and 60.0% (24/40) clinical evidence of constipation.

TABLE 2 compares the anthropometric and dietetic data, nutritional indexes and the intake of energy, macronutrients, fiber, and fluids as a function of the presence or absence of symptoms of dysphagia. The mean energy intake was significantly lower among the children with clinical signs of dysphagia (*P*=0.009). In regard to the macronutrients, the children with dysphagia exhibited a greater intake of carbohydrates and protein and lower intake of fat, but only the carbohydrate intake exhibited a significant difference (*P*=0.023). Fluid intake was significantly lower among the children with dysphagia (*P*=0.001).

TABLE 2. Comparison of anthropometric nutritional parameters and energy, macronutrient, fiber, and fluid intake between children with cerebral palsy with or without dysphagia.

Variable	Dysphagia		*P*
	Yes (n=33)	No (n=7)	
Height-for age Z-score*	-4.4 ± 1.6	-4.3 ± 1.5	0.833
Weight-for-height Z-score*	-1.0 ± 1.7	-0.6 ± 2.2	0.504
Weight-for-age Z-score*	-3.2 ± 1.0	-3.0 ± 1.4	0.637
Dietary intake			
Energy (kcal/day)*	1280.2 ± 454.8	1890.3 ± 847.1	0.009
Carbohydrates (g/day)**	170.9 (137.3; 214.3)	234.5 (187.1; 327.2)	0.023
Protein (g/day)*	46.0 ± 17.1	60.5 ± 17.9	0.051
Fat (g/day)*	41.4 ± 21.1	61.3 ± 34.1	0.050
Fiber (g/day)*	10.0 ± 4.4	12.6 ± 4.8	0.160
Iron**	9.0 (6.1;13.3)	12,0 (7.3; 16.2)	0.200
Fluids (mL/day)*	483.1 ± 294.9	992.9 ± 292.2	0.001

*Mean and standard deviation, Student's t-test; **Median and 25th and 75th percentiles, Mann-Whitney test.

TABLE 3 compares the anthropometric and dietary indicators as a function of the presence or absence of clinical manifestations compatible with gastroesophageal reflux disease. The children with signs and symptoms suggestive of gastroesophageal reflux disease exhibited greater fluid intake (*P*=0.042).

TABLE 3. Comparison of anthropometric nutritional parameters and daily energy, macronutrient, fiber, and fluid intake between children with cerebral palsy with or without clinical manifestations of gastroesophageal reflux.

Variable	Gastroesophageal reflux		*P*
	Yes (n=16)	No (n=24)	
Height-for age Z-score*	-4.9 ± 1.7	-3.7 ± 1.5	0.033
Weight-for-height Z-score*	-1.1 ± 1.7	0.9 ± 1.8	0.751
Weight-for-age Z-score*	-3.3 ± 1.1	-3.0 ± 1.0	0.514
Intake			
Energy (kcal/day)*	1276.9 ± 406.8	1460.4 ± 668.2	0.332
Carbohydrates (g/day)*	226.4 ± 111.6	181.7 ± 58.2	0.105
Protein (g/day)*	51.8 ± 19.3	46.5 ± 16.9	0.357
Fat (g/day)**	46.1 (30.2; 68.1)	35.5 (28.9; 48.1)	0.199
Fiber (g/day)*	8.7 ± 4.4	11.6 ± 4.3	0.052
Iron (mg/dia)**	8.0 (5.6; 11.7)	11.9 (6.7; 15.8)	0.071
Fluids (mL/day)*	720.0 ± 362.9	483.7 ± 320.0	0.042

*Mean and standard deviation, Student's t-test; **Median and 25th and 75th percentiles, Mann-Whitney test.

TABLE 4 compares the nutritional and dietary data as a function of the presence or absence of clinical evidences of constipation. The children with symptoms of constipation exhibited significantly lower daily intake of fiber (*P*=0.031) and fluids (*P*=0.013).

As TABLES 2, 3 and 4 show, the anthropometric parameters did not differ between the children with or without gastrointestinal symptoms, except, for patients with gastroesophageal symptoms who presented lower height-for-age Z-score.

The hemoglobin and ferritin levels shown that three male participants (7.5%) exhibited mild anemia, with normal ferritin levels in two. No child exhibited ferritin reduction not accompanied by a decreased hemoglobin level. The mean iron intake of the sample was 11.0±6.0 mg/daily.

TABLE 4. Comparison of anthropometric nutritional parameters and daily energy, macronutrient, fiber and fluid intake between children with cerebral palsy with or without constipation.

Variable	Constipation		*P*
	Yes (n=24)	No (n=16)	
Height-for-age Z-score*	-4.4 ± 1.6	-3.9 ± 1.7	0.337
Weight-for-height Z-score*	-0.9 ± 1.5	-1.0 ± 2.1	0.881
Weight-for-age Z-score*	-3.2 ± 1.0	-3.1 ± 1.2	0.927
Intake			
Energy (kcal/day)*	1488.8 ± 656.1	1234.3 ± 412.4	0.176
Carbohydrates (g/day)**	195.6 (158.6; 250.8)	166.6 (131.8; 197.0)	0.095
Protein (g/day)**	45.6 (35.6; 67.1)	40.2 (34.9; 57.3)	0.448
Fat (g/day)**	46.8 (29.5; 58.0)	33.9 (27.5; 45.9)	0.275
Fiber (g/day)*	9.2 ± 4.3	12.3 ± 4.3	0.031
Iron	8.0 (5.6; 12.9)	11.9 (8.1; 15.0)	0.062
Fluids (mL/day)*	456.5 ± 283.1	741.1 ± 379.2	0.013

*Mean and standard deviation, Student's t-test; **Median and 25th and 75th percentiles, Mann-Whitney test.

DISCUSSION

The results of this study show that children with cerebral palsy often exhibit inadequate food intake, severe anthropometric deficits, and a high frequency of gastrointestinal symptoms associated with certain diet characteristics.

Malnutrition and impaired growth are a common in children with cerebral palsy due to several factors, some of which are related to diet[3,16,32,33]. Although inadequate intake of energy, protein, essential fatty acids, vitamins, and minerals is considered to be the main cause of these conditions[15,34] few studies have assessed the food intake of children with cerebral palsy. A study conducted in Greece compared the energy intake of 16 children with cerebral palsy and 16 children without neurologic abnormalities (control group) and found it to be inadequate in both groups[15]. In addition to inadequate energy intake, muscle tone, level of physical activity, and the presence of involuntary motions may contribute to the incidence of malnutrition found in children with cerebral palsy by increasing their daily energy requirements[34].

A study conducted in Norway on 221 children with mental deficiency found that the presence of orofacial dysfunction is associated with reduced daily energy intake[35]. It should be observed that the reference values used in this study were based on the nutrient intake of children who did not present cerebral palsy and thus may not be fully appropriate for children with cerebral palsy. The EAR values correspond to the median distribution of the nutrient requirements of healthy individuals of the same gender and age range and meet the needs of 50% of the corresponding population[20]. In children with spastic paralysis, the muscles are hypertonic, which increases their energy requirements[5]. In addition, in such children, orofacial motor dysfunction interferes with sucking, chewing, and swallowing. The prevalence oforopharyngeal dysphagia varies from 16 to 99%[32]. Affection of the oral phase of swallowing is characterized by inability to control the food in the mouth due to problems with sealing the lips, loss of oral reflexes and of the motion of the anterior and dorsal parts of the tongue, and difficulties in chewing. Individuals with cerebral palsy have difficulty closing the lips while swallowing, which contributes to the food bolus escaping the mouth, aggravating malnutrition, and hinders the assessment of effective food intake[5,32]. In this study, clinical symptoms of dysphagia were found in most of the participants (33/40) who exhibited lower energy intake (TABLE 2) than the ones without evidence of dysphagia. A Brazilian study carried out in Santos (São Paulo, Brazil) included 90 children with quadriplegic cerebral palsy aged between 2 and 13 years showed high prevalence difficult to chewing (41%) and swallow (12.8%)[36].

In regard to macronutrient distribution, the intake of carbohydrates and protein was over the RDA in 85.0% (34/40) and 92.5% (37/40) of the sample, respectively, as shown in TABLE 1. The carbohydrate intake was significantly higher among the children without dysphagia (TABLE 2). A study conducted in Greece[15] with children with cerebral palsy up to 10 years old found that carbohydrates represented 47%, fat 36%, and protein 17% of the energy intake, and that this distribution was adequate in the case of carbohydrates and proteins but slightly above the recommended intake in the case of fat according to the Acceptable Macronutrient Distribution Range (AMDR), which indicates the ideal distribution of energy provided by each macronutrient relative to the total energy intake[20]. A previous study in Brazil found low consumption of carbohydrates (52%), adequate intake of protein (53%) and high intake of lipids (43%)[36]. In the Queensland, Australia, a study verified that non-ambulant, tube-fed cerebral palsy patients had significantly lower protein intakes compared to orally fed children[37]. There were no other differences in macronutrient intake between children with cerebral palsy and the control group.

The low prevalence of iron deficiency anemia found in this study is noteworthy. Only a single child with cerebral palsy had reduced hemoglobin and ferritin. The two patients presenting reduced hemoglobin did not have low serum ferritin levels.

Low fluid intake combined with insufficient dietary fiber intake may contribute to the development of constipation[5,7]. Approximately 60% of the sample in this study exhibited constipation, which agrees with the reports in the literature[5,12,38]. The average daily dietary fiber intake was lower among the participants with constipation (TABLE 4). A similar association was found with fluid intake, i.e., the amount of water consumed by the children with constipation was lower than the amount consumed by the children without constipation (TABLE 4). The reason for the lower dietary fiber intake exhibited by individuals with cerebral palsy may be that they consume foods with a low degree of consistency[5,12]. A Brazilian group of children with cerebral palsy also had low daily intake of fruits and vegetables (sources of dietary fiber) and liquids (less than three cups of 200 mL/daily). In this study, 14 of 39 children with quadriplegic cerebral palsy presented less than 3 evacuations per week[36]. In addition to the dietary factors, cerebral palsy is associated with intestinal motility disorders characterized by increased transit time in the transverse colon and rectum[7,38], as well as with changes in recto-anal function that result in a longer duration of the anal inhibitory reflex[5].

Gasrtointestinal sympoms of gastroesophageal reflux disease were found in 40.0% of the participants in this study. The children with evidence of gastroesophageal reflux exhibited lower height-for-age in addition to greater fluid intake, most likely because softer foods are less uncomfortable to swallow (TABLE 3).

The sample size might represent a limitation of this study. However, the frequency of gastrointestinal symptoms and the dietary data obtained were so clinically relevant that the number of patients was sufficient to obtain statistically significant differences in several parameters. A second limitation might be the decision to evaluate study only gastrointestinal symptoms. However, the Rome III diagnostic criteria for gastrointestinal functional disorders in patients without neurologic abnormalities recommends that the diagnosis should be established taking into account the gastrointestinal symptoms[39]. In addition, we considered unethical to perform diagnostic tests for dysphagia, gastrointestinal reflux disease/esophagitis (exposing the participants to radiation and sedation) specifically for this study.

CONCLUSION

This study found a wide variation in the results of the dietary survey. Whereas the protein intake was excessive in a large number of cases. The participants exhibited significant anthropometric deficits, which may be partially related to dietary factors. However, it is important to note that the nutritional deficit exhibited by patients with cerebral palsy is multifactorial. Gastrointestinal symptoms are a frequent occurrence among individuals with cerebral palsy. The patients with constipation exhibit lower fluid and dietary fiber intake; energy consumption is reduced in the patients with symptoms of dysphagia; and the individuals with symptoms of gastroesophageal reflux consume greater amounts of fluids. As a conclusion, within the context of multi-professional care provided to patients with cerebral palsy, nutritional interventions should be individualized as a function of the particular needs of each patient.

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Authors' contribution

All the authors participated in the conception and study design, interpretation of results and the writing and revising the final version of the manuscript. Caramico Favero DCO was responsable for data collection. All authors read and approve the final manuscript.

Caramico-Favero DCO, Guedes ZCF, Morais MB. Ingestão alimentar, estado nutricional e sintomas gastrintestinais em crianças com paralisia cerebral. Arq Gastroenterol. 2018;55(4):352-7.

RESUMO – **Contexto** – Paralisia cerebral pode estar associada com comorbidades como desnutrição, déficit de crescimento e sintomas gastrintestinais. Os problemas alimentares na paralisia cerebral podem ser secundários a anormalidades anatômicas e funcionais que interferem no processo de alimentação. **Objetivo** – O objetivo deste estudo foi avaliar a associação entre ingestão alimentar, estado nutricional e sintomas gastrintestinais em crianças com paralisia cerebral. **Métodos** – Estudo transversal que incluiu 40 crianças com paralisia cerebral (35 com tetraparesia espástica e 5 com coreoatetose não-espástica) com idade entre 4 e 10 anos. Todos os pacientes permaneciam exclusivamente na cama ou dependiam de cadeiras de rodas. Foi utilizado o inquérito dos alimentos consumidos habitualmente em casa que foi respondido pelos pais. Foram mensurados os dados antropométricos. Sintomas gastrintestinais associados com distúrbios da deglutição, refluxo gastroesofágico e constipação intestinal crônica foram obtidos. **Resultados** – A mediana do escore Z da estatura para idade (-4,05) foi menor (P<0,05) do que a mediana de peso-idade (-3,29) e peso-estatura (-0,94). Não se observou diferença entre os escores Z de peso-idade e peso-estatura. Três pacientes com paralisia cerebral (7,5%) apresentavam anemia leve com valor normal de ferritina. Sintomas de disfagia, refluxo gastroesofágico e constipação intestinal ocorreram, respectivamente, em 82,5% (n=33), 40,0% (n=16) e 60,0% (n=24) dos pacientes estudados. Os pacientes com sintomas de disfagia apresentaram menor ingestão energética diária (1280,2±454,8 Kcal vs 1890,3±847,1 Kcal; P=0,009), de carboidratos (mediana: 170,9 g vs 234,5 g; P=0,023) e de líquidos (483,1±294.9 mL vs 992,9±292,2 mL; P=0,001). Os pacientes com sintomas de refluxo gastroesofágico apresentaram maior ingestão diária de líquidos (720,0±362,9 mL) em relação aos pacientes sem este tipo de manifestação clínica (483,7±320,0 mL; P=0.042) além de maior déficit de estatura-idade (escore Z: -4,9±1,7 vs 3,7±1,5; P=0,033). Os pacientes com sintomas de constipação intestinal apresentaram menor ingestão diária de fibra alimentar (9,2±4,3 g vs 12,3±4,3 g; P=0.031) e líquidos (456,5±283,1 mL vs 741,1± 379,2 mL; P=0,013). **Conclusão** – Crianças com paralisia cerebral apresentam uma grande variabilidade na ingestão alimentar que pode, pelo menos em parte, constituir um fator de agravo para o déficit de crescimento. Sintoma de disfagia, refluxo gastroesofágico e constipação intestinal associaram-se com diferentes padrões de ingestão alimentar. Portanto, a intervenção nutricional deve ser individualizada levando em consideração os sintomas gastrintestinais e o estado nutricional.

DESCRITORES – Paralisia cerebral. Gastroenteropatias. Estado nutricional. Transtornos de deglutição. Refluxo gastroesofágico. Constipação intestinal.

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ORIGINAL ARTICLE

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Best polypectomy technique for small and diminutive colorectal polyps: a systematic review and meta-analysis

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ABSTRACT – **Background** – Polypectomy of colorectal polyps is the mainstay of colorectal cancer prevention. Identification of the best polypectomy technique is imperative. **Objective** – This review aims at comparing efficacy of nine different resection methods for small colorectal polyps (<10 mm). **Methods** – We searched and selected only randomized controlled trials. Primary outcome was complete resection rates of small polyps by histological eradication. Secondary outcomes were: adverse events, retrieval tissue failures rates and duration of procedure. **Results** – Eighteen trials including 3215 patients and 5223 polyps were analysed. Overall, cold polypectomy had a significantly shorter time of procedure than hot polypectomy (RD -5.92, 95%CI -9.90 to -1.94, *P*<0.05), with no statistical difference on complete histological eradication (RD 0.08, 95%CI -0.03 to 0.19, *P*>0.05). Regarding cold polypectomy techniques, cold snare was found superior to cold forceps on complete and en-bloc resection rates and less time consuming. When comparing endoscopic mucosal resection (EMR) with hot-snare and cold-snare, the latter showed no-inferiority on histological eradication, adverse events or retrieval tissue failure rates. **Conclusion** – Cold polypectomy is the best technique for resection of small colorectal polyps. Among cold methods, dedicated cold snare was found superior on histological eradication. Cold snare endoscopic mucosal resection might be considered an option for polyps from 5 to 9 mm.

HEADINGS – Colonic polyps, surgery. Endoscopic mucosal resection. Follow-up studies.

INTRODUCTION

Colorectal cancer is the third most common cancer and the fourth most common cause of death worldwide[1]. The development of such neoplasms occurs as a consequence of multi-step genetic mutations from normal colonic epithelium to a pre-malignant lesion (adenoma) and adenocarcinoma ultimately[2,3]. Resection of pre-malignant lesions, generally found as polyps, is considered the mainstay of the colorectal cancer prevention[4].

The endoscopic polypectomy is a minimally invasive procedure for removal of colorectal polyps. Currently, there are many techniques and, usually, endoscopists choose based on personal preferences and polyp size. Small polyps, defined as those smaller than 10 mm, are the most common findings on screening colonoscopy[5,6]. Consequently, the employment of an effective and safe polypectomy technique specific for small lesions is imperative.

Polyps smaller than 3 mm are usually resected with a biopsy forceps; for polyps from 4 mm to 9 mm, the endoscopist normally opts for hot or cold snare[7]. However, delimitation and snaring of flat and depressed lesions may be challenging[8]. Such cases render the endoscopic mucosal resection (EMR) a useful option.

Previous meta-analyses only compared diminutive polyps or no more than four polypectomy techniques[9-11]. Therefore, the literature lacks an updated and high-quality study regarding outcomes of resection methods for small polyps. This systematic review and meta-analysis aims at comparing all available techniques reported on randomized clinical trials to determine the best therapeutic option for this subgroup of polyps.

METHODS

Protocol and registration

This protocol was outlined and registered prior to the beginning of the study. We specified eligibility criteria and methods of analysis on the International Prospective Register of Systematic Reviews –University of York (PROSPERO) (http://www.crd.york.ac.uk/PROSPER) under registration number CRD42017068726)[12]. Also, it was approved by our institution's Internal Review Board (IRB number 293/17). Finally, we conducted this study in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) recommendations[13].

Eligibility criteria

• Participants

All participants were adults (≥18yo). There were no restrictions as to gender or number of polyps per patient. Studies with patients diagnosed with familial polyposis syndrome, inflammatory bowel disease or incomplete colonoscopy were excluded.

Declared conflict of interest of all authors: none
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• Intervention

Different endoscopic polypectomy methods for colorectal polyps smaller than 10mm. We included studies comparing two or more different techniques in spite of the outcomes assessed. Techniques compared were: cold snare polypectomy (CSP), cold forceps polypectomy (CFP), standard forceps polypectomy (SFP), hot snare polypectomy (HSP), hot forceps polypectomy (HFP), suction pseudopolyp technique (SPT), jumbo forceps polypectomy (JFP), endoscopic mucosal resection (EMR), cold snare EMR (CS-EMR) and hot snare EMR (HS-EMR).

Hot procedures were those which electrical current was used to resect the polyp, cold procedures were those performed without it and EMR were those in which a submucosal injection was made before polypectomy.

• Outcomes

- histological complete resection rates (complete resection confirmed by pathologist by the specimen examination);
- *en-bloc* resection rates (visual polyp eradication judged by endoscopist's experience and discretion);
- early and delayed bleeding (intraprocedural bleeding which required hemostatic treatment and bleeding within 2 weeks after polypectomy requiring endoscopic intervention, respectively);
- perforation;
- retrieval tissue failure rates (failure to retrieve a polyp after its resection);
- duration of the procedure (time for polypectomy only).

• Studies

This systematic review included only randomized controlled trials providing outcomes of diverse colorectal polypectomy techniques for polyps smaller than 10 mm. Non-comparative studies and abstracts were excluded. There were no restrictions regarding language or publication date.

Sources of information

We searched Medline/PubMed, Cochrane Central Register of Randomized Controlled Trials/CENTRAL, LILACS, and EMBASE from inception to November 1st, 2017.

Our search strategies were:

- Medline / PubMed: (adenomatous OR adenoma OR adenomatosis OR polyps OR polyp) AND (colon OR colorectal OR colonic OR rectal OR rectum OR colorectum OR intestinal OR intestine) AND (surgery OR snare OR forceps OR resection OR surgical instruments OR polypectomy) AND random*;
- Lilacs: (adenoma OR polyp) AND (endoscopy OR endoscopic) AND polypectomy;
- Cochrane/CENTRAL: (adenoma OR polyp) AND (endoscopy OR endoscopic);
- Embase: (adenomatous OR adenoma OR adenomatosis OR polyps OR polyp) AND (colon OR colorectal OR colonic OR rectal OR rectum OR colorectum OR intestinal OR intestine) AND (surgery OR snare OR forceps OR resection OR surgical instruments OR polypectomy) – only randomized controlled trials

Study selection

Two reviewers (TCV and BWM) independently searched titles and abstracts to assess eligibility. Then, a full-text evaluation confirmed that studies fulfilled all eligibility criteria. Results from individual assessment were then confronted and any disagreement was resolved by consensus with a third researcher (MEGH).

Data collection process and data items

The main author extracted the absolute numbers from the eligible full-text articles. The second researcher (BWM) verified the extracted data. These results were then stratified by polypectomy technique and by outcome. Data collected from the studies included: patients' demographics, endoscopic procedures, number of lesions, lesion size and location, incomplete resection rates, retrieval tissue failure rates, immediate or delayed bleeding and perforation; procedure duration time.

Risk of bias in individual studies

The risk of bias of the studies was assessed with The Cochrane Risk of Bias tool[14] following pre-determined parameters: adequacy of random sequence generation; allocation concealment; double-blinding; incomplete outcome data and selective outcome reporting.

Summary measures and planned methods of analysis

The analyses were carried out using the Review Manager 5.3 software (RevMan 5.3 − Cochrane Informatics & Knowledge Management Department)[15]. We employed risk differences for dichotomous variables using a fixed-effects model to provide forest and funnel plots for each comparison. Data on risk difference and 95 % confidence interval (CI) for each outcome were calculated using the Mantel−Haenszel test, and inconsistency (heterogeneity) was assessed using the Chi-square (Chi^2) and the Higgins method (I^2)[16]. We calculated the number needed to treat or to harm (NNT or NNH) if the difference achieved statistical superiority.

Risk of bias across studies and additional analyses

We assessed publication bias using a funnel plot analysis. Asymmetry may result from the non-publication of small trials with negative results (supporting the null hypothesis) or from missing data in the published studies (selective reporting bias). If the heterogeneity (I^2) was higher than 50%, we considered reports outside the funnel plot as outliers and excluded them from the analysis. Then, we performed another meta-analysis and reassessed heterogeneity. We considered true heterogeneity if I^2 was higher than 50% and outliers could not be detected. We acknowledge that other factors might produce asymmetry in funnel plots leading to a high heterogeneity (true study heterogeneity), such as differences in trial quality or differences in the population studied. In these cases, we changed the effect from fixed to random.

Forest plots exhibit the risk differences and their confidence intervals for each comparison group. Specific forest and funnel plots assessed each outcome. An additional forest plot was designed if high heterogeneity demanded exclusion of outliers.

RESULTS

Study selection

The initial search identified 1470 studies that were screened through title and abstract evaluation. Among them, twenty-five articles were selected for full-text assessment. Subsequently, we excluded seven studies that either compared obsolete cauterization techniques or were meta-analyses/abstract-only papers. Finally, eighteen studies were selected for this meta-analysis. (FIGURE 1).

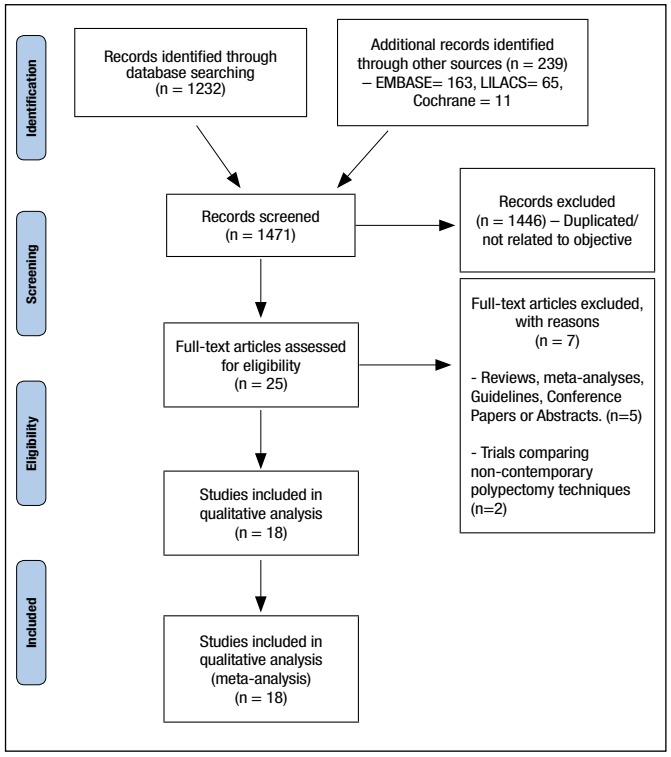


FIGURE 1. Study selection flowchart. Adapted from references 13 and 36.

Study characteristics

All eighteen studies were randomized controlled trials published in English. A total of 3215 patients accounted for 5223 polypectomies. All patients were adults diagnosed with polyps smaller than 10 mm. The exclusion criteria of the RCTs were similar (familial polyposis syndrome, inflammatory bowel disease and/or incomplete colonoscopy). One study included patients specifically under anticoagulation therapy[17].

Eleven direct comparisons were analysed. If a trial used three arms in the study (e.g. cold snare vs hot snare vs cold forceps polypectomy) we extracted individual data and analysed three separate comparisons.

Most studies assessed our primary and secondary outcomes: complete resection rate, adverse events, retrieval tissue failure rate and duration of procedure; however, the definition of latter differed significantly among studies: some trials started the stopwatch when they initiated the exam; others started during the withdraw; yet, a few timed only the polypectomy procedure.

A summary of the characteristics of the included trials is shown in TABLE 1.

Risk of bias within studies

FIGURE 2 summarizes the risk of bias within the studies according to the Cochrane Risk of Bias tool. There was no double-blind trial, which is classified as a potential source of bias. Most trials randomized each polyp separately. Yet, some studies used only one polypectomy technique in the same patient, independent of number and size of polyps removed.

TABLE 1. Characteristics of the included trials.

Author, Year	Polyp size criteria (mm)	Mean polyp size (mm)	Males (%)	Age (yr)	Polypectomy method comparisons	No. of polyps	Histology – adenoma (%)
Papastergiou V, 2017[33]	6–9 mm	8.2	58.7	63.6	CS-EMR X HS-EMR	164	72.6
Zhang Q, 2017[32]	6–9 mm	7.6	55.0	64.9	CSP X EMR	525	69.3
Komeda Y, 2017[23]	3–5 mm	4.0	69.0	69.0	CSP X HFP	283	93.6
Kawamura T, 2017[18]	4–9 mm	5.4	68.2	66.0	CSP X HSP	687	87.2
Park SK, 2016[24]	<5 mm	NA	73.3	56.0	CSP X CFP	231	79.6
Kim H-S, 2016[31]	5–9 mm	6.3	61.3	64.1	HSP X EMR	353	89.8
Horiuchi A, 2015[28]	< 10 mm	6.4	87.4	67.7	CSP X DCSP	210	70.9
Din S, 2015[27]	3–7 mm	4.0	65.2	63.5	CSP X DCSP	161	67.6
Aslan F, 2015[29]	3–5 mm	4.4	64.2	60.6	SFP X JFP	263	68.4
Kim JS, 2015[8]	≤ 7 mm	4.4	81.0	62.0	CSP X CFP	145	88.3
Gomez V, 2015[19]	< 6 mm	3.6	57.0	60.4	CSP X HSP X CFP	62	60.0
Din S, 2015[26]	3–7 mm	4.0	67.9	63.7	CSP X SPT	148	67.5
Horiuchi A, 2014[17]	< 10 mm	6.3	70.0	67.2	CSP X HSP	159	91.8
Aslan F, 2014[20]	5–10 mm	8.7	70.1	58.9	CSP X HSP	149	81.7
Lee CK, 2013[25]	< 5 mm	3.7	53.7	57.2	CSP X CFP	117	69.9
Draganov PV, 2012[30]	≤ 6 mm	NA	45.7	60.0	SFP X JFP	305	39.3
Paspatis GA, 2011[21]	3–8 mm	5.5	56.0	60.4	CSP X HSP	1083	80.7
Ichise Y, 2011[22]	< 8 mm	5.6	66.0	65.3	CSP X HSP	205	91.2

EMR: endoscopic mucosal resection; CS-EMR: cold snare EMR; HS-EMR: hot snare EMR; CSP: cold snare polypectomy; HFP: hot forceps polypectomy; HSP: hot snare polypectomy; CFP: cold forceps polypectomy; DCSP: dedicated cold snare polypectomy; SFP: standard forceps poly-pectomy; SPT: suction pseudopolyp technique; JFP: jumbo forceps polypectomy.

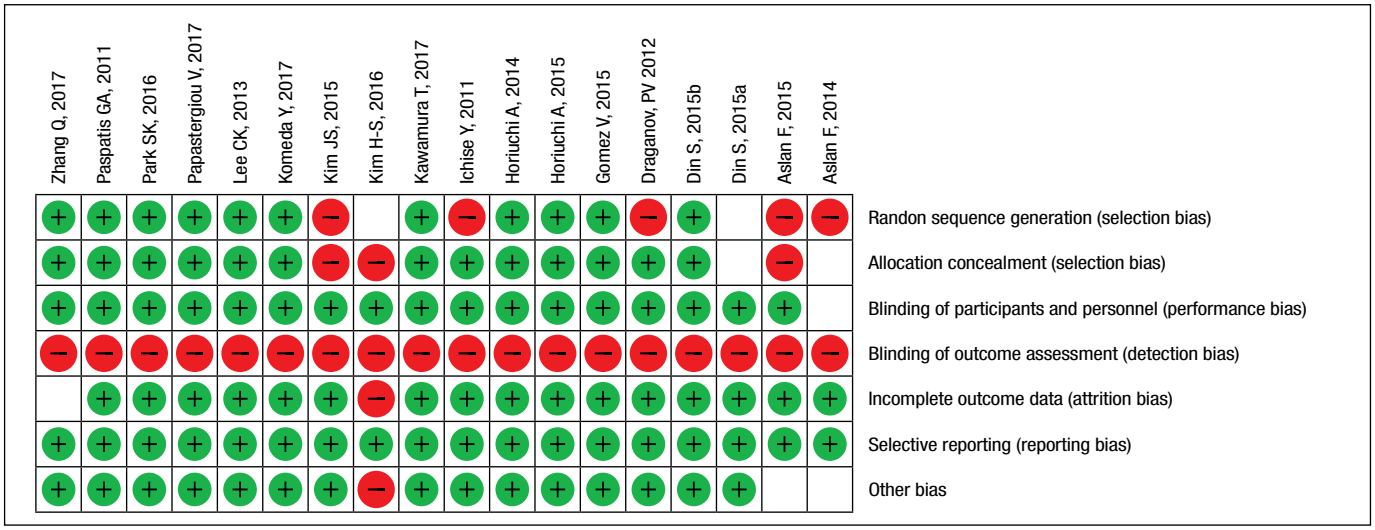


FIGURE 2. Summary of risk of bias appraisal for individual studies.

Synthesis of results and risk of bias across studies

Among eighteen analysed trials, 3215 patients and 5223 polypectomies were assessed. In summary, our analyses entailed ten different comparisons. Additionally, we created two more groups that were confronted: all cold versus all hot procedures.

The comparisons are outlined next:

1. cold snare versus hot snare
2. cold snare versus hot forceps
3. cold forceps versus hot snare
4. cold snare versus cold forceps
5. cold snare versus suction pseudopolyp technique
6. cold snare versus dedicated cold snare
7. standard forceps versus jumbo forceps
8. EMR versus hot snare
9. EMR versus cold snare
10. EMR + cold snare versus EMR + hot snare
11. hot polypectomy versus cold polypectomy

We summarized all favourable results of these comparisons in TABLE 2.

1. Cold snare vs hot snare

Three studies compared complete resection rates between cold and hot snare polypectomy[18–20] cold snare, and cold biopsy forceps. Kawamura et al.[18] and Gómez et al.[19] confirmed complete resection by obtaining biopsies from the resection margins after polypectomy, whereas Aslan et al.[20] only confirmed it from the polyp examination. The mean risk difference [RD] was 0.01 (95% CI, -0.02 to 0.03) with I^2=0%, meaning absolute homogeneity. Hence, this analysis showed equivalence of methods (FIGURE 3A).

Although all four studies[17,18,21,22] assessed bleeding, only Horiuchi A et al.[17] and Kawamura T et al.[18] observed this adverse event (AE). Both used the same methodology. Seven patients presented delayed bleeding amongst 437 allocated for the hot snare group while none of the 376 in the cold snare group did. However, this finding did not achieve statistical difference rendering these methods equally safe. The I^2 values for immediate and delayed bleeding and total AEs were higher than 50%, that is, highly heterogeneous. Since this comparison entailed only two trials, an outlier exclusion was inappropriate. Therefore, this analysis considered true heterogeneity and demanded adoption of the random effect model. None of the trials reported perforations. (FIGURE 3B).

TABLE 2. Summary of favourable results.

	Complete resection	En-bloc resection	Retrieval tissue failure	Adverse events	Duration of procedure
CSP x HSP	=	O	=	=	CSP
CSP x HFP*	CSP	CSP	=	O	=
CSP x CFP	CSP	CSP	CFP	O	CSP
CSP x SPT*	=	=	=	O	O
CSP x DCSP	DCSP	DCSP	=	O	=
CFP x HSP*	=	O	O	O	O
SFP x JFP*	JFP	JFP	O	=	JFP
EMR x HSP*	=	=	=	EMR	=
EMR x CSP*	EMR	EMR	O	=	CSP
CS-EMR x HS-EMR*	=	O	=	=	O

CSP: cold snare polypectomy; HSP: hot snare polypectomy; HFP: hot forceps polypectomy; CFP: cold forceps polypectomy; SPT: suction pseudopolyp technique; DCSP: dedicated cold snare polypectomy; SFP: standard forceps poly-pectomy; JFP: jumbo forceps polypectomy; EMR: endoscopic mucosal resection; CS-EMR: cold snare EMR; HS-EMR: hot snare EMR. O: not analysed. = No statistical difference. * Only one trial analysed.

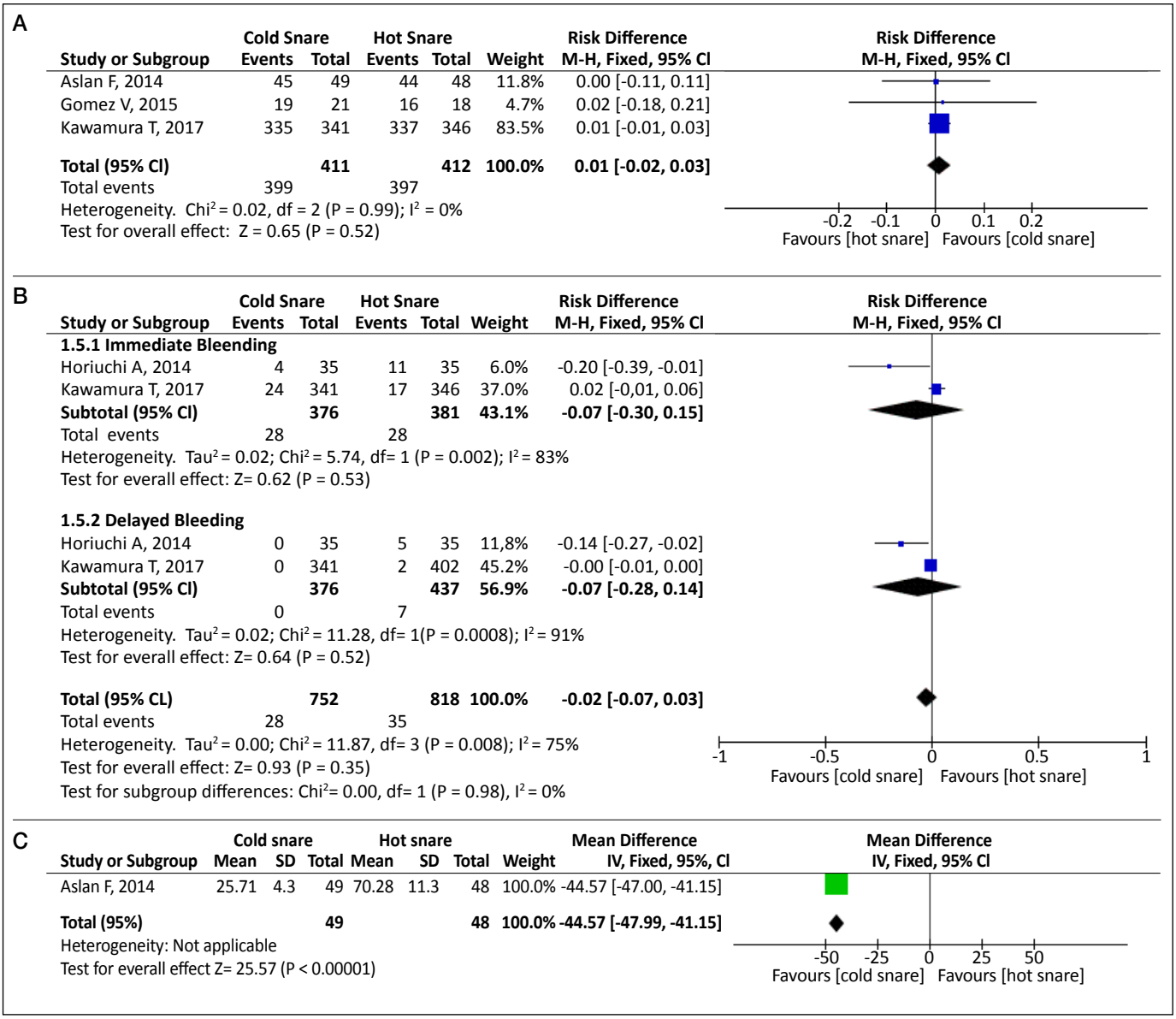


FIGURE 3. Cold snare vs Hot Snare forest plots. **(A)** Complete histological eradication. **(B)** Adverse events. **(C)** Duration of procedure.

Only Aslan et al.[20] assessed duration of procedure by the time of polypectomy itself. In this trial, cold snare polypectomy was significantly faster than hot. Accordingly, the risk difference for duration of procedure was -44.57 (95% CI, -47.99 to -41.15). (FIGURE 3C).

Four studies compared failure on retrieving the specimen[17,18,21,22]. None of them showed difference between methods. The pooled mean risk difference was 0.00 (95 % CI, -0.03 to 0.03), with the I^2=0%.

2. Cold snare vs hot forceps

Cold snare polypectomy was significantly superior to hot forceps regarding complete and *en-bloc* resection rates (RD 0.33, 95%CI 0.22 to 0.44 and RD 0.19, 95%CI 0.12 to 0.26, respectively) as compared on the single study performed by Komeda et al.[23]. The NNT for complete resection was 3. That is, for every three cold snare polypectomies, one would have been incomplete if performed with hot forceps. On the other hand, there was no statistical difference between those techniques regarding negatives outcomes (retrieval tissue failure rate: RD 0.03, 95%CI, -0.01 to 0.07 and adverse events: RD 0.01, 95%CI, -0.06 to 0.07).

3. Cold forceps vs hot snare

On this subgroup of a single study[19], we analysed cold forceps versus hot snare. Gomez et al.[19] assessed only complete resection rate and found no statistical difference between the afore mentioned methods (RD -0.06, 95%CI, -0.28 to 0.17).

4. Cold snare vs cold forceps

Four studies assessed complete resection rates, with no difference between methods, to compare cold snare and cold forceps[8,19,24,25]. The cold snare was significantly superior (*P*=0.0007)

with an NNT of 11. The risk difference was 0.09 (95%CI, 0.04 to 0.14) and the I^2=49% (FIGURE 4A). Only Lee CK et al.[25] assessed *en-bloc* resection rate and also found cold snare to be significantly better than cold forceps (NNT 4, RD 0.23, 95% CI, 0.09 to 0.36) (FIGURE 4A).

Three authors compared the retrieval tissue failure rates[8,24,25]. Despite lacking statistical difference on the first two studies, the pooled analysis favoured the cold forceps (RD 0.06, 95% CI, 0.03 to 0.10). There was no heterogeneity according to the Higgins test for this comparison group (FIGURE 4B).

The analysis of duration of procedure was also highly homogenous and found statistical difference favouring the cold snare technique (RD -0.70, 95% CI, -1.16 to -0.24) (FIGURE 4C).

5. Cold snare vs suction pseudopolyp technique

The pseudopolyp suction technique (SPT) consists in aspirating the polyp into the suction channel and rapidly excising the lesion with a cold snare before it restores the original shape.

A single trial performed by Din S et al.[26] compared cold snare to SPT and found equivalence between methods regarding complete and en-bloc resection rates and retrieval tissue failure rate. Complete resection rate: RD -0.12, 95%CI -0.29 to 0.04; En-bloc resection rate: RD: -0.06, %95CI: -0.13 to 0.01; Retrieval tissue failure rate: RD 0.01, 95%CI: -0.09 to 0.11.

6. Cold snare vs dedicated cold snare

Din S et al. and Horiuchi A et al.[27,28] compared two types of snares for cold polypectomy. The standard one versus another specially designed for cold resection. The so-called dedicated cold snare has a thinner braided wire and is smaller than the traditional snare. Also, it is not insulated.

The pooled analysis concerning complete resection rates significantly favoured the DCS (*P*=0.02, RD 0.10, 95%CI, 0.02 to 0.19) with I^2=0%. The calculated NNT was 10 (FIGURE 5). Furthermore, based on Horiuchi A et al. data[28], it was possible to sub-classify the polyps according to their size. Then, histological eradication was found to be significantly higher only when polyps were larger than 8 mm (RD 0.38, 95%CI, 0.11 - 0.65) (FIGURE 5). Both confirmed complete resection by absence of residual tissue at resection margin.

A single trial assessed *en-bloc* resection and the results favoured the dedicated cold snare polipectomy (DCSP) (NNT=4.5, RD 0.22, 95%CI 0.08 to 0.36)[28].

Concerning adverse events and duration of procedure, there was no difference between techniques (RD= -0.02, 95%CI -0.12 to 0.09 and RD -1.00, 95% CI -4.82 to 2.82, respectively). Although, the duration of procedure was measured as the whole procedure, not only the polypectomy itself.

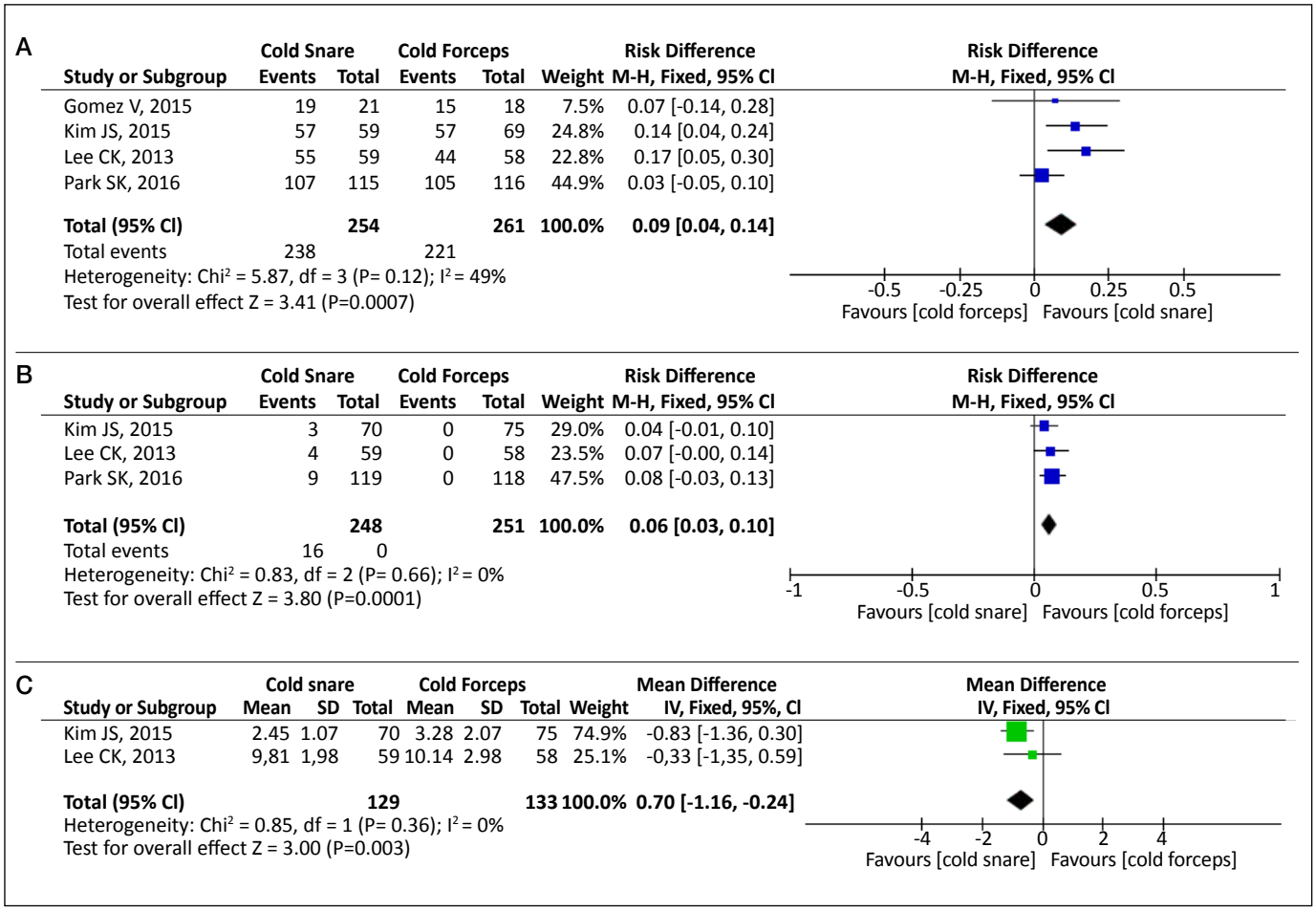


FIGURE 4. Cold Snare vs Cold Forceps forest plots. (**A**) Complete resection rates. (**B**) Retrieval tissue rate failure. (**C**) Duration of procedure.

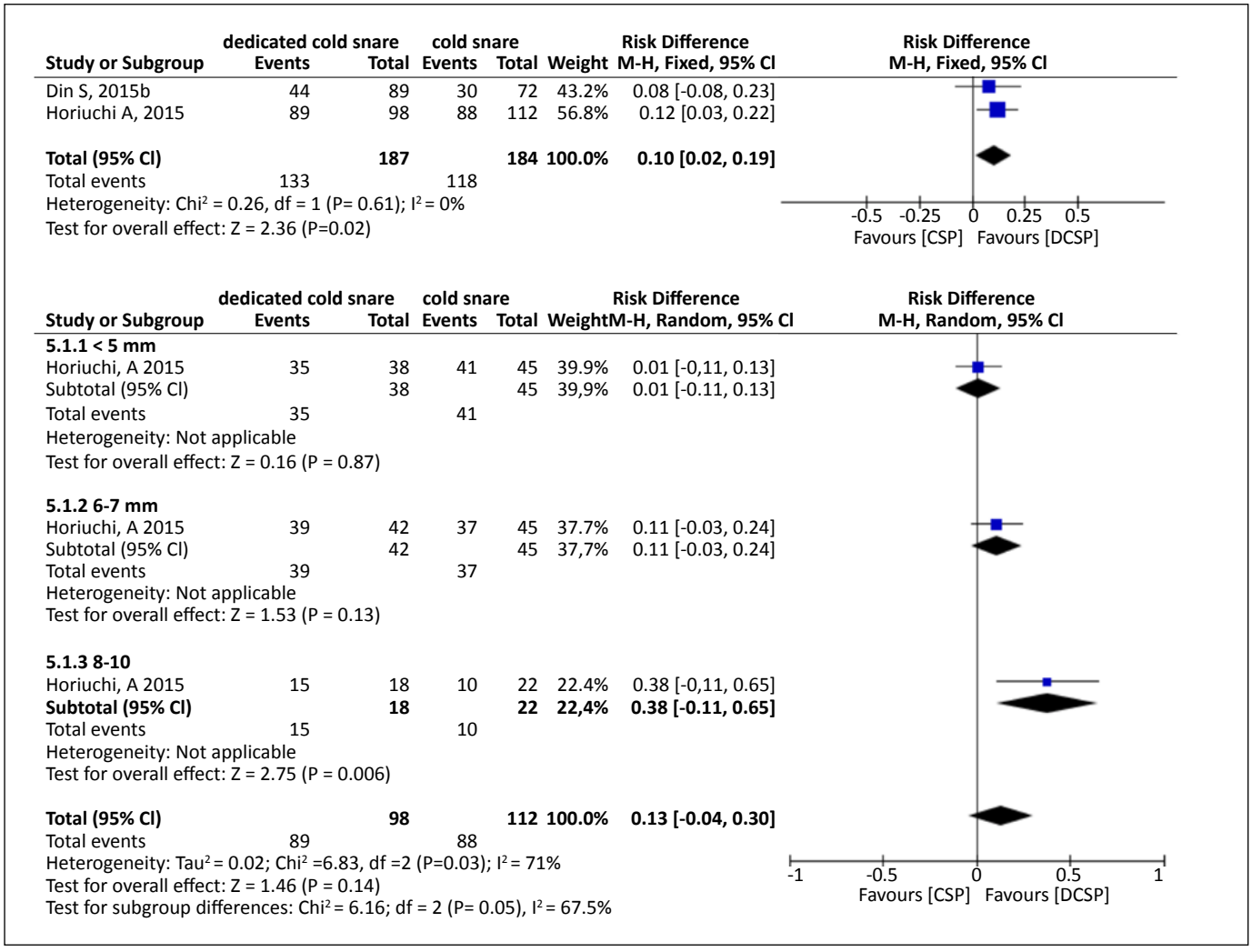


FIGURE 5. Forest plot analyses comparing standard and dedicated cold snare for complete histological eradication.

Again, only Din S et al.[27] evaluated retrieve tissue failure rates and showed the equivalence of methods (RD -0.05, 95%CI -0.17 to 0.07).

7. Standard forceps vs jumbo forceps

The risk difference regarding complete resection rate of jumbo forceps versus standard forceps was 0.09 (95% CI 0.04 to 0.15). The number needed to treat was 11, with I^2=0% (FIGURE 6).

Aslan et al.[29] confirmed the complete resection with biopsies of the resected margins and Draganov et al.[30] by the specimen analyses.

Only Draganov et al.[30] compared *en-bloc* resection rates between these methods and showed statistical difference favouring the jumbo forceps with an NNT of 3.5 (RD -0.28, 95% CI -0.38 to -0.18).

Concerning adverse events, we found equivalence of methods despite high heterogeneity (RD=0.01, 95% CI -0.05 to 0.07; I^2=59%).

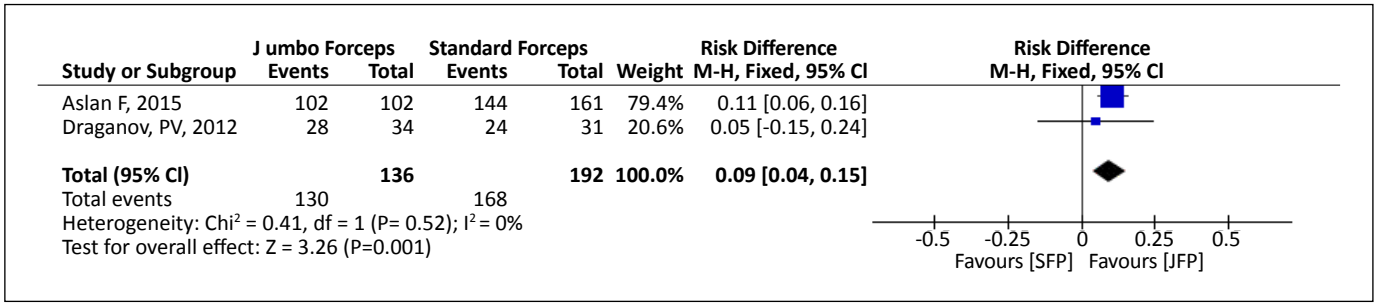


FIGURE 6. Forest plot comparing jumbo forceps and standard forceps for complete resection rate.

8. EMR vs hot snare

Only Kim H-S et al.[31] compared endoscopic mucosal resection and hot snare polypectomy. There were no differences regarding complete resection rate and *en-bloc* resetion (RD=0.04, 95% CI -0.02 to 0.11 and RD -0.01, 95%CI -0.08 to 00.05, respectively) but EMR was superior in terms of adverse events with an NNH of 20 (RD= -0.05, 95% CI -0.08 to -0.01).

9. EMR vs cold snare

Only Zhang Q et al.[32] compared endoscopic mucosal resection and cold snare polypectomy. Considering complete and *en-bloc* resection rates, EMR was superior to CSP both with a NNT of 14 (RD 0.07, 95 % CI 0.03 to 0.11 and RD 0.07, 95% CI 0.03 to 0.12, respectively). There was no difference in adverse event rates between groups (RD= -0.01, 95% CI -0.04 to 0.02). Regarding duration of procedure, CSP was faster than EMR (RD 0.8, 95% CI 0.28 to 1.32).

10. CS-EMR vs HS-EMR

Papastergiou V et al.[33] compared cold (CS-EMR) versus hot endoscopic mucosal resection (HS-EMR) for small polyps in a non-inferiority trial. The risk difference was -0.04 (95% CI, -0.11 to 0.04) concerning complete histological eradication. Accordingly, adverse events and retrieval tissue rate failures were similar between methods (RD 0.02, 95% CI -0.02 to 0.07 and RD 0.02, 95% CI -0.05 to 0.10, respectively).

11. Hot polypectomy vs cold polypectomy

We created this subgroup analysis comprising six studies to compare all cold polypectomy techniques versus all hot procedures[18-20,23,32,33].

Concerning complete resection rates, our meta-analysis showed no statistical difference between the two techniques (RD 0.08, 95% CI -0.03 to 0.19). Also, there was no change in results when diminutive polyps were analysed separately from small polyps (RD=0.20, 95% CI -0.08 to 0.48). Nevertheless, this analysis found true high heterogeneity (I^2>90%). (FIGURE 7A).

Only two trials compared *en*-bloc resection rate[23,32]. This analysis also found high heterogeneity (I^2=97%) and no statistical difference between cold and hot polypectomy (RD=0.06, 95% CI -0.20 to 0.32) (FIGURE 7B).

Four trials evaluated adverse events[17,18,23,32]. The initial result found high heterogeneity (I^2=80%) (FIGURE 7C). The sensitivity analysis through a funnel plot identified an outlier study (FIGURE 7D)[18]. After removing the outlier, we found absolute homogeneity and no statistical difference between groups (RD 0.02, 95 % CI -0.00 to 0.04) (FIGURE 7E).

Six trials assessed retrieval tissue failure rate[17,18,21-23,33]. Our meta-analysis showed equivalence of methods (RD 0.01, 95% CI -0.01 to 0.02) with I^2=0% (FIGURE 7F).

Finally, cold polypectomy was statistically faster than hot procedures. Concerning the duration of procedure, the risk difference was -44.60 (95% CI -48.00 to -41.19) with no heterogeneity (FIGURE 7G).

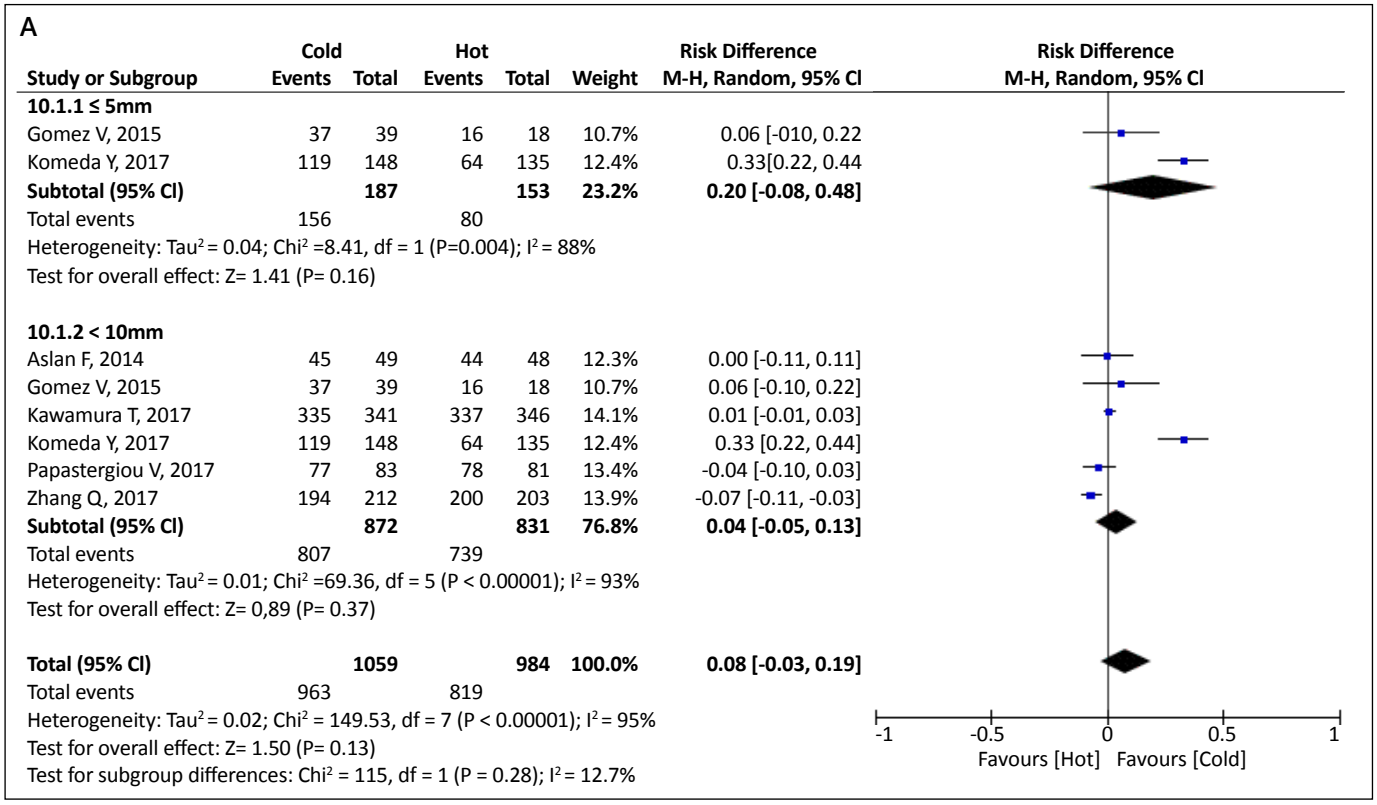
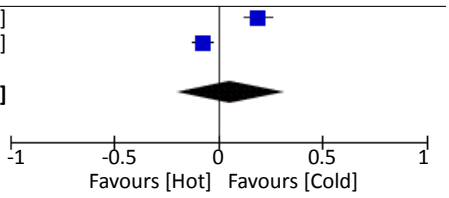


FIGURE 7. Pooled analyses of cold polypectomy techniques vs hot methods. **(A)** Forrest plot analysis for complete histological eradication rate. **(B)** Forrest plot analysis for *en-bloc* resection rate. **(C)** Forrest plot analysis for adverse events rate. **(D)** Funnel plot analysis for adverse events. **(E)** Forrest plot for adverse event after excluding the outlier. **(F)** Forrest plot for retrieval tissue rate failure rate. **(G)** Forrest plot for duration of procedure.

B

Study or Subgroup	Cold Events	Cold Total	Hot Events	Hot Total	Weight	Risk Difference M-H, Random, 95% CI
Komeda Y, 2017	147	148	108	135	49.6%	0.19 [0.12, 0.26]
Zhang Q, 2017	234	267	245	258	50.4%	-0.07 [-0.12, -0.03]
Total (95% CI)		**415**		**393**	**100.0%**	**0.06 [-020, 0.32]**
Total events	381		353			

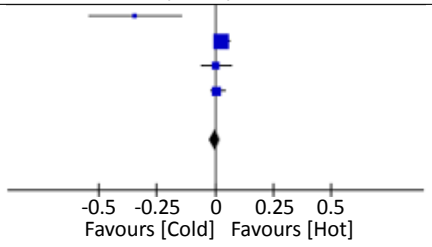
Heterogeneity: Tau² = 0.03; Chi² = 39.09, df = 1 (P < 0.00001); I² = 97%
Test for overall effect: Z= 0.44 (P= 0.66)



C

Study or Subgroup	Cold Events	Cold Total	Hot Events	Hot Total	Weight	Risk Difference M-H, Fixed, 95% CI
Horiuchi A, 2014	4	35	16	35	4.8%	-0.34 [-0.54, -0.15]
Kawamura T, 2017	24	341	17	402	51.0%	0.03 [-0.01, 0.06]
Komeda Y, 2017	13	148	11	135	19.5%	0.01 [-0.06, 0.07]
Zhang Q, 2017	5	179	3	179	24.7%	0.01 [-0.02, 0.04]
Total (95% CI)		**703**		**751**	**100.0%**	**0.00 [-0.02, 0.03]**
Total events	46		47			

Heterogeneity: Chi² = 14.65, df = 3 (P = 0.002); I² = 80%
Test for overall effect: Z= 0.14 (P= 0.89)



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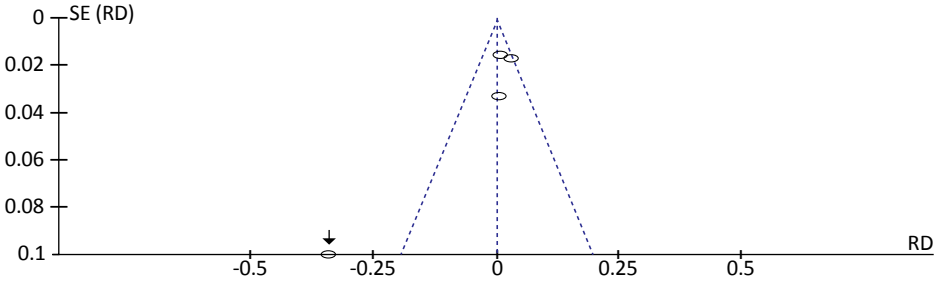
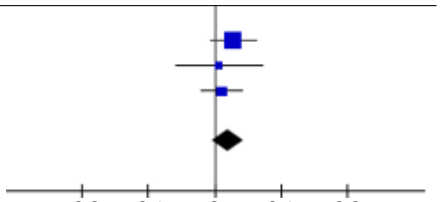


E

Study or Subgroup	Cold Events	Cold Total	Hot Events	Hot Total	Weight	Risk Difference M-H, Fixed, 95% CI
Horiuchi A, 2014	4	35	16	35	0.0%	-0.34 [-0.54, -0.15]
Kawamura T, 2017	24	341	17	402	53.5%	0.03 [-0.01, 0.06]
Komeda Y, 2017	13	148	11	135	20.5%	0.01 [-0.06, 0.07]
Zhang Q, 2017	5	179	3	179	26.0%	0.01 [-0.02, 0.04]
Total (95% CI)		**668**		**716**	**100.0%**	**0.02 [-0.00, 0.04]**
Total events	42		31			

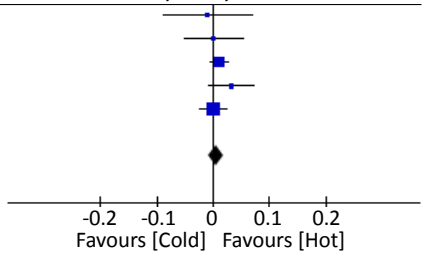
Heterogeneity: Chi² = 0,69.65, df = 2 (P = 0.71); I² = 0%
Test for overall effect: Z= 1.59 (P= 0.11)



F

Study or Subgroup	Cold Events	Cold Total	Hot Events	Hot Total	Weight	Risk Difference M-H, Fixed, 95% CI
Horiuchi A, 2014	5	78	6	81	6.3%	- 0.01 [-0.09, 0.07]
Ichise Y, 2011	4	101	4	104	8.1%	0.00 [-0.05, 0.05]
Kawamura T, 2017	7	394	3	402	31.5%	0.01 [-0.01, 0.03]
Komeda Y, 2017	7	148	2	135	11.2%	0.03 [-0.01, 0.07]
Paspatis GA, 2011	21	530	22	553	42.9%	- 0.00 [-0.02, 0.02]
Total (95% CI)		**1251**		**1251**	**100.0%**	**0.01 [-0.01, 0.02]**
Total events	44		37			

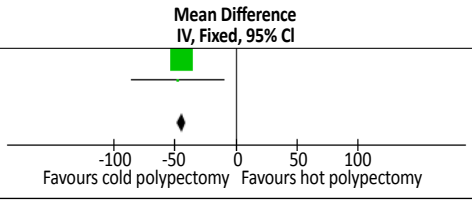
Heterogeneity: Chi² = 2.42, df = 4 (P = 0.66); I² = 0%
Test for overall effect: Z= 0.90 (P = 0.37)



G

Study or Subgroup	Cold Polypectomy Mean	Cold SD	Cold Total	Hot Polypectomy Mean	Hot SD	Hot Total	Weight	Mean Difference IV, Fixed, 95% CI
Aslan F, 2014	25.71	4.3	49	70.28	11.3	48	99.2%	-44.57 [-47.99, -41.15]
Zhang Q, 2017	282	204	179	330	162	179	0.8%	-48.00 [-86.16, -9.84]
Total (95% CI)			**228**			**227**	**100.0%**	**-44.60 [-48.00, -41.19]**

Heterogeneity: Chi² = 0.03, df = 1 (P = 0.86); I² = 0%
Test for overall effect: Z= 25.69 (P < 0.00001)



DISCUSSION

This meta-analysis is the largest regarding treatment of the most common kind of colonic polyps. The strength of this review is the use of only RCTs including more than 5000 polypectomies. Also, our study presents the greatest number of comparisons currently available in the literature.

Comparing methods with electrocautery (hot polypectomy) versus without it (cold polypectomy) we found no statistical difference in complete resection, adverse event or retrieval tissue rates. However, cold polypectomy was time-saving compared to diathermy, which may favour cold techniques. Yet, our results are in accordance with several independent articles suggesting that cold polypectomy presents at least the same curability rates as hot polypectomy whereas the same risk of adverse events[9,23,32,33]. Thus, complete resection rates among diminutive polyps (<5 mm) and small polyps (<10 mm) are similar, showing that using electrocautery does not improve the resection rate but carries the hypothetical drawback of increased perforation and bleeding risks.

Among cold polypectomy techniques, the cold snare was superior to forceps in terms of complete and *en-bloc* resection (9% vs 23% incomplete resection rates, respectively), possibly because it may resect 2 mm to 3 mm of normal mucosa around the base of the polyp leading to greater complete resection rates[34]. Furthermore, it is faster than forceps[8,25]. Despite the absence of statistical difference in failure to recover resected polyp in Lee CK et al.[25] and Kim JS et al.[8], the pooled analysis favoured forceps technique. Even though some experts with large experience in NBI advocate for selective 'resect and discard' strategy for diminutive polyps, most guidelines recommend retrieval of all resected polyps[10,35]. Therefore, the higher failure to retrieve rate of cold snare may be considered its major drawback.

The comparison of cold snare versus pseudopolyp suction technique in a single trial showed no difference regarding complete resection, *en-bloc* resection or retrieval tissue failure rates[26]. Duration of procedure and costs were not assessed but the latter demands employment of a dedicated cap which inevitably renders SPT more time consuming and more expensive.

Another comparison group evaluated the two types of snare: the standard one (with or without electrocautery) versus a dedicated cold snare which has no input port for electrocautery. Two articles[27,28] were included and demonstrated that the (DCSP) was significantly superior in terms of complete and *en bloc* resection rates. A subgroup analysis showed statistical significance exclusively for polyps larger than 8mm in Horiuchi's et al.[28] trial. Nevertheless, these techniques presented similar adverse event rates and duration of procedure. Jung et al.[11] suggested that the superiority of the DCSP could be due to the thinner wire and its shield shape. On the other hand, they also suggested that using a limited cold snare would probably increase the overall polypectomy cost since the examiner would need to change devices if a larger or pedunculated polyp was detected. Since few studies adequately evaluated this device, further randomized trials are needed to correctly assess cost-effectiveness and convenience of DCSP.

The pooled analysis enrolling results from Aslan et al.[29] and Draganov et al.[30] compared jumbo to standard forceps. The first was significantly superior to the latter regarding curability rate while carrying the same risk of adverse events. Duration of procedure could not be analysed because Draganov et al.[30] measured the exam duration and the whole procedure, not the time of polypectomy itself. As discussed by Raad D et al.[10], JFP has a wider opening diameter and, therefore, needs fewer bites to achieve complete resection.

Although no trial confronted JFP to CSP head-to-head, Jung YS et al.[11] performed a network meta-analysis to indirectly compare them and showed no statistical difference on histological eradication.

Three trials evaluated the EMR technique for polyps from 5 to 9mm: Kim et al., Zhang et al., and Papastergious et al.[31-33]. However, each trial had a distinct control group. Kim H-S et al.[31] compared EMR to hot snare alone and found no statistical difference on complete resection rates, although the latter presented a higher risk of adverse events. Zhang et al.[32] compared EMR to cold snare alone and showed that EMR is superior in terms of histological and endoscopic complete resection regardless of the same adverse event rate. The major drawback was the extended duration of the procedure. Finally, Papastergiou et al.[33] compared injection in submucosa followed by cold snaring or by hot snaring and showed that these methods are both effective and safe.

Our study is not free of limitations. Firstly, despite the great number of randomized controlled trials included, no comparison group had more than six studies at the same analysis and some of the groups had only one study analysed. Secondly, most studies had a considerable variety of polyp sizes but only a few categorized by size; that fact precluded a strong statistical analysis for polyps <5 mm separately from 5 to 10 mm. Thirdly, there were no follow-up colonoscopies to assess recurrence rates. Finally, one-quarter of the articles had low methodological quality, which might somehow impair reliability.

Despite these limitations, we were able to reach important conclusions. Also, this is the largest and most updated meta-analysis available in the literature to support the daily practice of endoscopists.

CONCLUSION

This meta-analysis shows that cold polypectomy techniques have equivalent curability rate and is as safe as hot polypectomy techniques for resection of polyps smaller than 10mm. Cold snaring is the best option for resection of such polyps. The use of electrocautery seems to be unnecessary. Further studies are needed to compare cold snare alone versus EMR.

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Authors' contributions

Tranquillini CV and Bernardo WM: search, data collection, writing of the manuscript. Brunaldi VO: writing and correction of the manuscript. Moura ET, Marques SB, Moura EGH: review and correction of the manuscript.

Tranquillini CV, Bernardo WM, Brunaldi VO, Moura ET, Marques SB, Moura EGH. Revisão sistemática e meta-análise sobre técnicas de polipectomia de pólipos colorretais pequenos e diminutos. Arq Gastroenterol. 2018;55(4):358-68.

RESUMO – **Contexto** – A polipectomia de pólipos colorretais é a base da prevenção do câncer colorretal. A identificação da melhor técnica de polipectomia é imperativa. **Objetivo** – Esta revisão tem como objetivo comparar a eficácia de nove diferentes métodos de ressecção para pólipos colorretais pequenos (<10 mm). **Métodos** – Pesquisamos e selecionamos apenas ensaios clínicos randomizados. O desfecho primário foi taxas de ressecção completa de pólipos pequenos por confirmação histológica. Os desfechos secundários foram: eventos adversos, taxas de falha de recuperação do espécime e duração do procedimento. **Resultados** – Dezoito estudos, incluindo 3215 pacientes e 5223 pólipos foram analisados. No geral, a polipectomia a frio teve um tempo de procedimento significativamente menor do que a polipectomia a quente (RD -5,92; IC 95% -9,90 a -1,94; *P*<0,05), sem diferença estatística na erradicação histológica (RD 0,08; IC 95% -0,03 a 0,19; *P*>0,05). Em relação às técnicas de polipectomia a frio, a alça fria foi considerada superior ao uso de pinça fria nas taxas de ressecção completa e em bloco, além de um menor tempo de procedimento. Ao comparar a ressecção endoscópica da mucosa utilizando alça quente ou alça fria, esta última mostrou não-inferioridade na erradicação histológica, eventos adversos ou taxas de falha do tecido de recuperação. **Conclusão** – A polipectomia a frio mostrou ser a melhor técnica para ressecção de pequenos pólipos colorretais. Entre os métodos frios, a alça fria dedicada foi considerada superior na erradicação histológica. ressecção endoscópica da mucosa com alça fria pode ser considerado uma opção para pólipos de 5 a 9 mm.

DESCRITORES – Pólipos do colo, cirurgia. Ressecção endoscópica de mucosa. Seguimentos.

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ORIGINAL ARTICLE

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Digestive diseases in elderly and factors associated with length of stay in the Hepatology and Gastroenterology Unit of the Campus Teaching Hospital of Lome (Togo)

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ABSTRACT – **Background** – The digestive pathologies are frequent in the elderly and often have a latent and atypical symptomatology. **Objective** – To assess the epidemiological and evolutionary current data on digestive diseases in the elderly, and look for factors associated with length of hospital stay. **Methods** – Retrospective study of 10 years, including patients aged 60 and over hospitalized for digestive diseases in the Gastroenterology Department of the Campus Teaching Hospital of Lome, Togo. **Results** – Of 5933 hospitalized patients, there were 1054 patients (17.8%) aged 60 years and over with a digestive pathology (526 men and 528 women). The average age was 69.5 years ±7.9 ranging from 60 to 105 years. The average length of hospital stay was 7.45 days ±6.2 ranging from 1 to 44 days. HIV prevalence was 2.4%. In order of decreasing frequency, there were hepatobiliary pathologies (54.3%) with a predominance of cirrhosis and liver cancer, eso-gastroduodenal pathologies (23.1%) with predominance of ulcers, gastric cancer and esophageal cancer, intestinal pathologies (8.7%) with a predominance of food poisoning, pancreatic pathologies (4.2%) with a predominance of pancreatic cancer and peritoneal pathologies (1.4%). Gastric cancer was the second digestive cancer found after liver cancer. Pancreatic head cancer was the second disease after gastric cancer which need a transfer in a surgical ward (P=0.031). There were 204 deaths (19.4%). The longest duration of hospitalization was due to gastric cancer (9.16 days). **Conclusion** – Hepatobiliary diseases were the most frequent and associated with a high death rate and a long hospital stay.

HEADINGS – Aged. Digestive system diseases. Hospitalization. Togo.

INTRODUCTION

The digestive pathologies are frequent in the elderly and often have a latent and atypical symptomatology[1]. Age greater than 60 represents a vulnerable area, given the structural alterations that occur and the loss of many functional reserves[2]. In sub-Saharan Africa, the scarcity of scientific work on the pathologies of the elderly and the lack of geriatric-type medical structures make it difficult to take care of the entire population[3]. In Togo[4] where the proportion of people aged 65 and over represents 3.32% of the population in 2015, there are no geriatric medical structures and rare are the publications on digestive pathologies in this age group[5,6]. This study was therefore conducted to clarify the current epidemiological and evolutionary data on the digestive pathologies of the elderly and to look for the factors associated with the length of stay of the elderly patient hospitalized in a unit of Hepatology and Gastroenterology.

METHODS

This was a retrospective study that focused on the files of patients aged 60 and over hospitalized in the Hepatology and Gastroenterology Unit of the Campus Teaching Hospital of Lome (Togo) from January 1st, 2005 to December 31st, 2014. Included were all patients whose exit diagnosis included at least one digestive pathology. Incomplete files and / or patients with digestive diseases of a surgical nature from the outset and/or extra-digestive conditions were not included in the study. The studied parameters included the socio-demographic data (age, gender, profession, category of room of hospitalization), the frequency of the various digestive pathologies, their clinical manifestations as well as the mode of evolution of each pathology, the length of stay and the hospitalization period of the patient in relation to the effective start date of the health insurance of the officials of Togo (March 1st, 2012). There were three categories of hospitalization rooms: category 1 (air-conditioned room with internal shower); category 2 (air-conditioned or ventilated room with external shower) and category 3 (common room). Data entry was done with Excel 2013; the statistical analysis was done with the STATA software version 2012. The different parameters were compared using the Pearson Chi2 test or using the Fisher test and Mann-Whitney test, the significance level was P<0.05. For the average stay, the average comparison test (normal law) was used when the explanatory variable is binary. For the explanatory variables of more than two modalities the Anova test was carried out after verification of the conditions of validity of this test (homoscedasticity, normality, independence).

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RESULTS

Of the 5933 patients hospitalized during the study period, there were 1304 patients aged 60 years and more, of whom 1054 (17.8%) had a digestive pathology (526 men and 528 women).

General characteristics of the study population

The mean age was 69.5 years ±7.9 (Extremes: 60 and 105 years). The most represented age group was 60 to 70 years old (53.9%) as shown in FIGURE 1. On the socio-professional level, 255 patients (24.2%) were active; 386 patients (36.6%) were retired and 929 (88.1%) patients were hospitalized in a Category 3 room. The human immunodeficiency virus (HIV) was found in 25 cases (11 men and 14 women) or 2.4% and the age group of 60 to 70 years was the most affected with 23 cases. Data on Comorbidities (diabetes, high blood pressure), lifestyle (alcohol and tobacco consumption) and patients' eating habits were not specified in the records.

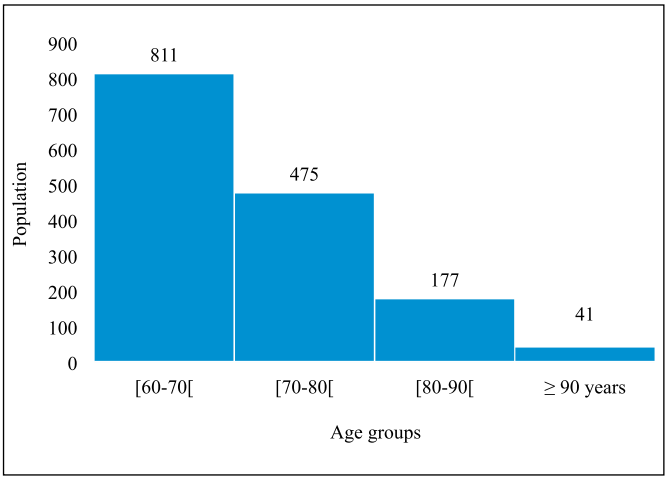

FIGURE 1. Distribution of 1504 patients by age groups.

General characteristics of digestive diseases

Hepatobiliary pathologies represented 54.3% followed by eso-gastroduodenal pathologies (23.1%) as shown in TABLE 1.

Special characteristics of digestive pathologies

• Eso-gastroduodenal pathologies

Ulcers were found in 7.3% of cases; 6.8% of patients had an inflammatory condition. Gastric cancer was found in 5.8% of patients; esophageal cancer represented 1.2% of cases (TABLE 1). The sex ratio for oesophageal cancer was 5.5 in favor of men, and the 60 to 70 age group was the most affected with 8 cases. Gastric cancer was the second cancer found after liver cancer.

• Intestinal and anal pathologies

Diarrhea was associated with HIV in six cases. The sex ratio for colorectal cancer was 2.75 in favor of men; the 60 to 70 age group was the most affected with 11 cases. A case of rectal caustic necrosis occurred in a 70-year-old woman following an enema to treat constipation.

• Hepatobiliary pathologies

Cirrhosis was observed in 27.5% of cases; 24.6% of patients had liver cancer. Liver cancer was divided between 238 cases of hepatocarcinoma (HCC) and 21 cases of hepatic metastases whose primary tumor could not be specified (TABLE 1). Liver cancer was the first digestive cancer with a sex ratio of 1.1. Cirrhosis was found significantly in men (P=0.035).

TABLE 1. Main digestive pathologies and their general evolution (n=1504).

	Population (n)	Percentage (%)
Hepatobiliary pathologies		
Cirrhosis	290	27.5
Hepatocarcinoma	238	22.5
Liver metastases	21	1.9
Cholangiocarcinoma	7	0.7
Toxic hepatitis	7	0.7
Hepatic abscess	5	0.5
Hépatonephritis	5	0.5
Eso-gastroduodenal pathologies		
Esophageal cancer	13	1.2
Gastroesophageal reflux	11	1.0
Esophagitis	9	0.9
Mallory-Weiss syndrome	4	0.4
Gastric cancer	61	5.8
Gastritis	43	4.1
Gastric ulcer	38	3.6
Gastric polyp	2	0.2
Cardiac cancer	1	0.1
Bulbar ulcer	39	3.7
Bulbo-duodenitis	19	1.8
Bulb lymphoma	3	0.3
Intestinal and anal pathologies		
Food poisoning	41	3.9
Colorectal cancer	15	1.4
Functional colopathy	13	1.2
Diverticulosis of the colon	6	0.6
Colitis	2	0.2
Rectal necrosis	1	0.1
Hemorrhoids	12	1.1
Anal cancer	2	0.2
Pancreatic pathologies		
Pancreatic cancer	40	3.8
Chronic pancreatitis	2	0.2
Acute pancreatitis	2	0.2
Pathologies of the peritoneum		
Peritoneal tuberculosis	15	1.4
Other pathologies		
General evolution of digestive pathologies	87	8.3
Death	204	19.4
Discharge	12	1.1
Exit	788	74.8
Transfer	50	4.7

• Pancreatic pathologies

Pancreatic head cancer represented 3.8% of cases as shown in TABLE 2; the sex ratio was 1.82 for men.

• Pathologies of the peritoneum

Peritoneal tuberculosis (n=15 or 1.4%) was observed in the 60 to 70 age group with 13 cases and was associated with HIV in 6 cases.

The evolution of different digestive pathologies and the length of stay

The average duration of hospital stay was 7.45 days ±6.2 (Extreme 1 and 44 days). There were 204 deaths (19.4%) (TABLE 1). The evolution of different digestive pathologies was not related to age (P=0.3228) as shown in TABLE 2, nor to sex (P=0.070). The death rate was 37.7% for HCC and 36.8% for cirrhosis (P<0.0001); pancreatic cancer was the second pathology after gastric cancer requiring surgical transfer (P=0.031) (TABLE 2). Food poisoning had a residence time of 3.22 days and the period of stay for gastric cancer was 9.16 days (P <0.0001) (TABLE 3). The length of stay did not present any significant variation from one age class to another (TABLE 3). Hospitalization in a category 1 room was associated with an increase in length of stay (P=0.012); category 3 was associated with a decrease in length of stay (P=0.0064). The average length of stay before and after March 1, 2012 was 7.31 days and 7.41 days, respectively (P=0.139).

DISCUSSION

The digestive pathologies of the elderly

In order of decreasing frequency, there were hepatobiliary pathologies with a predominance of cirrhosis and liver cancer, eso-gastroduodenal pathologies with predominance of ulcers,

TABLE 3. Average length of hospital stay according to age group, pathological groups and main digestive diseases.

	n	Average length (day)	*P* value
Age groups (years)			0.2080
[60-70[	569	7.77	
[70-80[	333	7.30	
[80-90[	124	6.71	
90 years and over	28	6.18	
Pathological groups			0.0049
Hepatobiliary pathologies	574	8.03	
Intestinal pathologies	136	6.01	
Eso-gastroduodenal pathologies	279	6.95	
Pancreatic pathologies	44	8.27	
Pathologies of the peritoneum	8	8.25	
Main digestives pathologies			<0.0001
Hepatocarcinoma	238	6.92	
Cirrhosis	290	8.96	
Gastritis	43	6.28	
Gastric cancer	61	9.16	
Food poisoning	41	3.22	
Cancer of the head of the pancreas	37	7.92	
Bulbar ulcer	39	7.51	
Gastric ulcer	38	6.68	

TABLE 2. Evolution at the end of hospitalization according to digestive pathologies and age groups (n=1504).

	Death		Discharge		Exit		Transfer		*P* value
	n	%	n	%	n	%	n	%	
Age groups									0.3228+
[60-70[	108	52.9	7	58.4	430	54.5	24	48.0	
[70-80[	64	31.4	3	25.0	251	31.9	15	30.0	
[80-90[	30	14.8	1	8.3	83	10.5	10	20.0	
90 years and over	2	0.9	1	8.3	24	3.1	1	2.0	
Total	204	100	12	100	788	100	50	100	
Digestives pathologies									<0.0001+
Hepatocarcinoma	77	37.7	2	16.7	158	20.1	1	2	
Cirrhosis	75	36.8	4	33.3	206	26.1	5	10	
Gastritis	1	0.5	0	0.0	42	5.3	0	0.0	
Gastric cancer	6	2.9	0	0.0	47	5.9	8	16	
Food poisining	0	0.0	0	0.0	40	5.1	1	2	
Cancer of the head of the pancreas	9	4.4	0	0.0	22	2.8	6	12	
Bulbar ulcer	2	0.9	1	8.3	36	4.6	0	0.0	
Gastric ulcer	1	0.5	0	0.0	36	4.6	1	2	
Other pathologies	33	16.2	5	41.7	197	25	28	56	
Total	204	100	12	100	788	100	50	100	

+Fisher's exact test.

gastric cancer and esophageal cancer, intestinal pathologies with a predominance of food poisoning, pancreatic pathologies with a predominance of pancreatic cancer and peritoneal pathologies. Djibril et al.[5] noted in order of frequency of intestinal pathologies with a predominance of food poisoning, hepatobiliaries with a predominance of decompensated cirrhosis, eso-gastroduodenal with a predominance of ulcers, anorectal, peritoneal and pancreatic. Gastrointestinal disorders represent the third cause of consultations by general practitioners among subjects older than 65 years in Western countries[7]. The data on esophageal cancer in our study were not different from those of Peghini et al.[8] who had noted 16 cases of esophageal cancer including 6 cases in patients aged 60 and over with a predominance in the age group of 60 and 70 and that it affected men much more. This is in agreement with literature data that states that esophageal cancer in Africa is common from the fifth decade[8-10]. Zeng et al.[11] in a comparative study of esophageal cancer in the elderly and the young subject had found a sex ratio 5 times the normal in favor of men in subjects over 70 years; the authors noted that, female gender was an independent favorable prognostic factor for esophageal cancer, which was similar to previously reported findings in United States[12]. However, the prognostic impact of sex differed between elderly patients and younger patients because gender was not an independent negative prognostic factor in patients 70 years of age or older[11]. These findings may be explained by the hypothesis that the endocrine milieu in pre- and perimenopausal females functions as a protective factor against esophageal cancer, while older postmenopausal females lose this estrogen exposure[13]. In addition, males showed a higher incidence of drinking and smoking, which are also risk factors for inducing esophageal cancer at an earlier age[14]. Gastric cancer is one of the most common cancers of the digestive tract in the general population in Africa according to several authors[6,15]. Peghini et al.[8] noted 72 cases of gastric cancer of which 34 (47.2%) cases in patients aged 60 years and more. The frequency of gastric cancer found in the series of Djibril et al.[5] is lower than that found in our series. Our study population consisted of patients aged 60 and more, unlike the study population of Djibril et al.[5] who recruited patients aged 65 and more; which could explain the low frequency of gastric cancer found by Djibril et al. Bouglouga et al.[16] noted 32 cases of gastric cancer, 14 cases (43.7%) in patients aged 60 years and more over a period of 8 years. However, the frequency of gastric cancer in our study remains lower than that observed in the series of Kadende et al. (37.5%)[15], Diarra et al. (48.54%)[17]. This disparity could be explained by the difficulty of our patients to honor the complementary examinations especially the digestive fibroscopy. In Brazil, gastric cancer was in 2014, the fourth most common cancer in men and the sixth in women; deaths from gastric cancer occurred in the majority of cases in subjects over 66 years of age[18]. The association is mainly attributed to low socioeconomic status, which increases the likelihood of transmission and reinfection of *Helicobacter pylori* in household clusters with large families, poor sanitation, and less frequent use of antibiotics[18]. The retrospective nature of our present study did not allow us to analyze the factors associated with death from gastric cancer. Cirrhosis and liver cancer overcame hepatobiliary pathologies as in the Djibril et al. series[5]. Liver cancer was the first digestive cancer in our series. Bouglouga et al.[19] noted that the main etiologies of Cirrhosis in Togo were hepatitis B virus (57.2%), C virus (25%) and alcohol (18.7%). Viral hepatitis is a provider of cirrhosis and HCC, hence the interest of screening and vaccinating the Togolese population against viral hepatitis. HCC is the most common complication of liver cirrhosis affecting elderly people[20]. The rate of HCC increases with age in cirrhotic patients[21]. HCV and HBV coinfection is a condition particularly prone to malignant transformation[22]. Another emerging condition that contributes to the development of HCC in old age is the non-alcoholic fatty liver disease (NAFLD). The hypothesis that obesity and diabetes mellitus are important risk factors for cryptogenic chronic liver disease in patients with HCC is supported by the analysis of surgically-treated patients[23]: in a series of 18 patients who underwent liver resection for HCC developing on cryptogenic cirrhosis, 12 patients were >65 years of age. These observations have prompted the increasing interest in surveillance programs for cirrhotic patients, aiming to detect any HCC development as early as possible. Our present study did not allow us to specify the impact of NAFLD on the occurrence of HCC in the elderly. Pancreatic pathologies were dominated by cancer of the pancreatic head, whose care was done in a surgical environment; its frequency in our study is higher than that noted by Djibril et al.[5] (three cases in eight years in patients aged 65 and more). Surgical resection is the only potentially curative treatment for pancreatic cancer[24,25]. Unfortunately, only 15% to 20% patients are candidates for pancreatectomy due to the late presentation of symptoms and/or detection of the disease[26,27]. Furthermore, the rate of resectability diminishes with age. Likewise, some authors reported that 40% of patients between the ages of 66-70 years are candidates for a pancreatectomy, but by the age of 85 years, only 7% are eligible candidates[28,29]. Mortality due to pancreatic cancer also increases proportionally with age: 6.7% of patients aged 65-69 years, 9.3% of patients aged 70-79 years, and 15.5% of patients aged 80 years or older[24]. The frequency of peritoneal tuberculosis in the study by Djibril et al.[5] (3%) was higher than that found in our study. In Mali, Dembélé et al.[30], in two years, found 26 cases of peritoneal tuberculosis (associated with HIV) of which only one case (3.85%) in a patient aged over 65 years. Peritoneal tuberculosis is much more common in young people who are immunocompromised by HIV, as noted by Bouglouga et al.[31]. Infectious pathologies are more common after age 60 because of age-related immune changes and iron deficiency[32]; the immunodepression with HIV found in these patients is a factor favoring the occurrence of this affection in this age group which constitutes a fragile ground. Food poisoning was the most common intestinal pathology followed by colorectal cancer; their frequency was lower in our study than that of Djibril et al.[5]. Fayomi et al.[33] reported 29 cases of food poisoning, including one case in a male patient aged 60 years and more. We noted a frequency of 1.4% of colorectal cancers in agreement with the literature; colorectal cancers are typically rare in Africa, whose average age of diagnosis is around 50 years[34]. The colon carcinoma is one of the most frequent lethal causes in the western countries; 90 per cent of the cases of colon carcinoma are found in patients older than 50 years of age[35].

The evolution of different digestive pathologies and the length of stay

Cirrhosis was associated with a significant increase in the period of stay; this could be due to the impact of the complications of cirrhosis whose management in hospital could lead to a

long hospital stay. The death was observed in case of cirrhosis in a significant way probably in connection with the complications of cirrhosis (ascitic decompensation, HCC, infections, digestive bleeding) occurring on a fragile ground that the subject is 60 years old and more. Health insurance for public agents does not significantly change the hospital stay period. This health insurance which covers the cost of hospitalization of patients could explain the long stay of patients in category 1 room. Lawson-Ananissoh et al.[36] in 2013 noted that health insurance for public agents, by covering a portion of patient expenses, has significantly reduced the direct financial cost of hospital care of cirrhosis. Overall, serious pathologies outside HCC had led to a long hospital stay and benign pathologies aside the bulbar ulcer had led to a short hospital stay; the very reserved prognosis of HCC could explain the short hospital stay of patients hospitalized for HCC unlike other cancers (pancreas, stomach); the hemorrhagic complication of bulbar ulcer could explain the long hospital stay unlike other benign pathologies; hemorrhagic complication is more common in bulbar ulcers than in gastric ulcers[37]. The delay in performing digestive fibroscopy due to financial difficulties or lack of technical support could contribute to increase the length of stay related to gastric cancer.

CONCLUSION

About one-fifth of the patients hospitalized in the Hepatology and Gastroenterology department of the Campus Teaching Hospital of Lome (Togo) were aged 60 and more and had a digestive pathology. Hepatobiliary pathologies were the most common, associated with a high rate of death and a long period of stay. Gastric cancer and cirrhosis were the main pathologies that led to a long hospital stay. Food poisoning was associated with a short hospital stay.

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Authors' contribution

Lawson-Ananissoh LM is the principal initiator of this study. He participated in the data collection, statistical analysis and writing of this article. Bouglouga O, Bagny A, El-Hadji Yakoubou R participated in statistical analysis, data collection and bibliographic research. Kaaga L, Redah D participated in the reading and the final correction of this article.

Lawson-Ananissoh LM, Bouglouga O, Bagny A, El-Hadji Yakoubou R, Kaaga L, Redah D. Doenças digestivas em idosos e fatores associados à duração da permanência na unidade Hepatologia e Gastroenterologia do Hospital Universitário de Ensino de Lomé (Togo). Arq Gastroenterol. 2018;55(4):369-74.

RESUMO – **Contexto** – As patologias digestivas são frequentes no idoso e têm geralmente uma sintomatologia latente e atípica. **Objetivo** – Avaliar os dados epidemiológicos e de evolução sobre as doenças digestivas nos idosos, e procurar fatores associados ao período de permanência hospitalar. **Métodos** – Estudo retrospectivo de 10 anos, incluindo pacientes com idades de 60 ou mais, hospitalizados para doenças digestivas no Departamento de Gastroenterologia do Hospital Universitário de Ensino de Lomé, Togo. **Resultados** – De 5933 pacientes hospitalizados, havia 1054 pacientes (17,8%) com idade de 60 anos ou mais com uma patologia digestiva (526 homens e 528 mulheres). A idade média foi de 69,5 anos ± 7,9 variando de 60 a 105 anos. A duração média da estadia hospitalar foi de 7,45 dias ±6,2 variando de 1 a 44 dias. A prevalência do HIV foi de 2,4%. Em ordem de diminuição da frequência, houve patologias hepatobiliares (54,3%) com predominância de cirrose e câncer hepático, patologias do esôfago-gastroduodenal (23,1%) com predominância de úlceras, câncer gástrico e câncer esofágico, patologias intestinais (8,7%) com predominância de intoxicação alimentar, patologias pancreáticas (4,2%) com predominância de câncer pancreático e patologia peritoneal (1,4%). O câncer gástrico foi o segundo câncer digestivo encontrado após o câncer de fígado. Câncer de cabeça pancreática foi a segunda doença após o câncer gástrico, que necessitou transferência para a enfermaria cirúrgica (P=0,31). Houve 204 mortes (19,4%). A maior duração da internação foi devido ao câncer gástrico (9,16 dias). **Conclusão** – As doenças hepatobiliares foram as mais frequentes e associadas a uma elevada taxa de mortalidade e a uma longa estadia hospitalar.

DESCRITORES – Idoso. Doenças do sistema digestório. Hospitalização. Togo.

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ORIGINAL ARTICLE

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The old one technique in a new style: developing procedural skills in paracentesis in a low cost simulator model

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ABSTRACT – **Background** – Paracentesis is a routine medical procedure quite relevant in clinical practice. There are risks of complications related to paracentesis, so it is essential a proper trainee for the younger practicer. **Objective** – The article describes the construction and the application of a low cost paracentesis simulator for undergraduate medical students and it also describes the perception of students about the simulator as well. **Methods** – A low-cost model was developed by the Program of Tutorial Education for training medical students during three editions of an undergraduate theoretical-practical course of bedside invasive procedures. The authors constructed a model from very low-cost and easily accessible materials, such as commercial dummy plus wooden and plastic supports to represent the abdomen, synthetic leather fabric for the skin, upholstered sponge coated with plastic film to represent the abdominal wall and procedure gloves with water mixed with paint to simulate the ascitic fluid and other abdominal structures. One semi-structured form with quantitative and qualitative questions was applied for medical specialists and students in order to evaluate the paracentesis simulator. **Results** – The paracentesis model has an initial cost of US$22.00 / R$70.00 for 30 simulations and US$16.00 / R$50.00 for every 30 additional simulations. It was tested by eight medical doctors, including clinical medicine, general surgeons and gastroenterologists, and all of them fully agreed that the procedure should be performed on the manikin before in the actual patient, and they all approved the model for undergraduate education. A total of 87 undergraduate medical students (56% male) individually performed the procedure in our simulator. Regarding the steps of the procedure, 80.5% identified the appropriate place for needle puncture and 75.9% proceeded with the Z or traction technique. An amount of 80.5% of the students were able to aspire the fluid and another 80.5% of students correctly performed the bandage at the end of the procedure. All the students fully agreed that simulated paracentesis training should be performed prior to performing the procedure on a real patient. **Conclusion** – The elaboration of a teaching model in paracentesis provided unique experience to authors and participants, allowing a visible correlation of the human anatomy with synthetic materials, deepening knowledge of this basic science and developing creative skills, which enhances clinical practice. There are no data on the use of paracentesis simulation models in Brazilian universities. However, the procedure is quite accomplished in health services and needs to be trained. The model described above was presented as qualified with low cost and easily reproducible.

HEADINGS – Medical education. Simulation. Paracentesis.

INTRODUCTION

Paracentesis is a routine medical procedure and quite relevant in clinical practice, which consists of a needle puncture under local anesthesia of the abdominal cavity to collect ascitic fluid for therapeutic purpose or for laboratory diagnostic analysis. The accomplishment of this procedure is associated to lower mortality of hospitalized patients with ascites without a determined diagnosis, as well as the reduction of health costs[1,2]. However, like all invasive procedures, it presents complications, which can be minor ones (9%) or large ones (approximately 1%). In addition, a considerable percentage of complications (6%) are related to problems with the technique used by the health professional[3].

Due to its importance, high frequency in daily medical, and risk of complications, paracentesis training is essential in recognized medical curricula, such as The American Board of Internal Medicine (ABIM) and the Accreditation Council for Graduate Medical Education[4]. In this context, the use of simulators to develop meaningful learning of procedural ability by undergraduate students proves to be effective. The great challenge is to make low-cost models, which present a good correlation with reality, to acquire the technique with repeated training[5-7].

Thus, we demonstrate the construction and application of a simulated model of low cost paracentesis for the training of medical students in order to present an alternative way for teaching and training of the paracentesis technique.

METHODS

The first step of our study was the construction of four equal training models of paracentesis that were made in 2014, with the same characteristics of tested simulator in this study. The material

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for one model was a plastic mannequin, gloves filled with water, gummed tape, sponges and synthetic fabric leather. These materials were chosen due to easy access and handling, low-cost and reproducibility.

Using the plastic manikin as model patient, we made a circular incision on the whole abdominal from both costal borders and pelvic bones to simulate the abdominal cavity. We took out this part of model in such way that we proper filled the interior of the cavity with old newspapers in order to facilitate the attachment of a plastic compartment. The latter was filled with a geriatric diaper and the gloves.

Two gloves were positioned at the left iliac fossa of model and they had been previously filled with water to simulate the ascitic fluid which should be withdrawn from peritoneal cavity. Two other gloves had been filled with red and yellow liquid (colored by gouache paint) in order to simulate blood from abdominal vessels (red one) and enteric content (yellow one). These latter ones were proper positioned in the remaining space of the model cavity.

The disposal of the gloves in the model cavity had been chosen because our simulator could be able to allow the student perform a correct paracentesis with ascitic fluid (clear water) and a disaster paracentesis as well due to a visceral or vessel accidental punction.

The subcutaneous tissue was resembled by a 0.5 cm thick rectangular sponged used to cover the abdominal cavity. In the lower base of the sponge, in contact with the abdominal cavity, two layers of gummed tape were fixed, the first in the horizontal direction and the second in the perpendicular direction, representing the parietal peritoneum. These layers, when punctured with the needle, simulated a resistance as occurs in performing the real procedure. Lastly, the abdominal cavity was covered externally with the synthetic fabric leather tissue, to represent the skin (FIGURE 1).

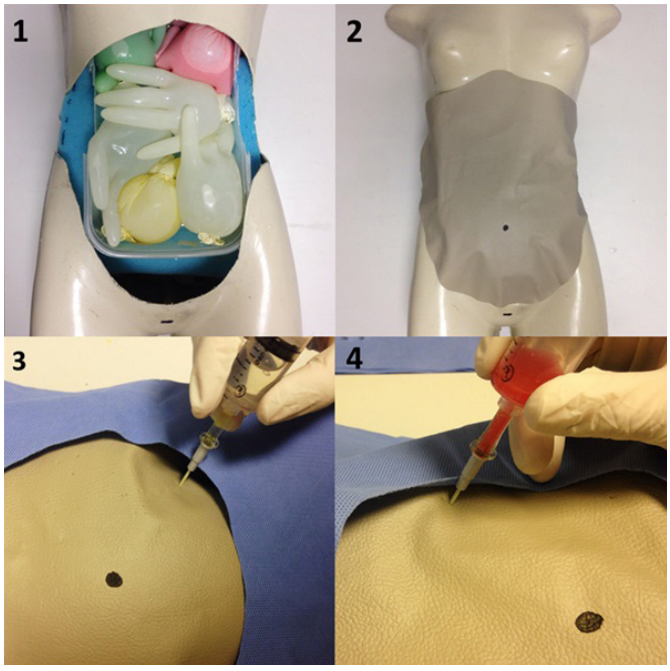

FIGURE 1. Simulator Model of Paracentesis: from different views. Materials from inside the manikin; 2. Manikin ready for use; 3. Correct puncture view; 4. Incorrect puncture view.

After the model was finalized, it was presented to medical specialists to train and approve the use of the teaching model. Thus, it was tested with eight physicians, who subsequently answered a questionnaire of semi-structured perception about quantitative and qualitative aspects of the model.

Primarily, the model was made to support the training with 10 students without needing to replace materials, especially the gloves. After that, the second step was the application of our model during three editions of an undergraduate theoretical-practical course of bedside invasive procedures during the period of 2015-2016.

This course was ministered by peer tutors previously trained in several procedures. The participants were instructed through a 10 minutes theoretical class, followed by a practical demonstration on the paracentesis simulated model, lasting around 10 minutes. Then, students performed the procedure individually, supervised by peer instructors. At this time, a checklist evaluation was used concerning procedure's performance. This checklist was elaborated by the researchers based on the literature[8,9,10] (FIGURE 2).

Stages of the procedure	Hit	Incomplete	Mistake
1. Explained the procedure and obtained the consent of the patient or legal guardian.			
2. Has made sure that the bladder is empty (request voiding, use a Foley or a relief tube).			
3. Positioned the patient (horizontal dorsal decubitus with discreetly elevated head).			
4. Delimited the puncture site (2 cm below the umbilical scar, in the midline or lower quadrants, from the anterior-superior iliac spine, measured 2-4 cm up and to the center of the abdomen).			
5. Confirmed the presence of fluid at the puncture site with percussion.			
6. Performed local antisepsis.			
7. Positioned sterile field.			
8. Performed local anesthesia with 5 mL of 2% lidocaine (anesthetic button – 1mL + deep tissue anesthesia – 4 mL).			
9. Used a Z technique or needle insertion with skin traction (the student aspired at each advance of approximately 3 mm and ceased when he/she aspirated ascitic fluid or when he/she felt a sudden decrease in resistance).			
10. Student removed 20-60 mL of liquid (he/she sent liquid for laboratory tests to diagnose or connected the vial with vacuum as a treatment).			
11. Removed the needle.			
12. Made sterile occlusive dressing.			
13. Kept the patient under observation for about 60 minutes.			

FIGURE 2. Checklist of paracentesis.

The performance was considered satisfactory if a score greater than 70% was achieved. Each student took about 6 to 10 minutes to practice the procedure. After each training, the instructor provided individual feedback based on the checklist.

Focusing on evaluate the perception of the students about the model, a semi-structured questionnaire with quantitative and qualitative aspects was applied. The data collected were analyzed through the SPSS v. 22 using descriptive statistics.

The Kolmogorov Smirnov test was used to verify the normality of the sample. Values with $P<0.05$ were considered statistically significant.

This project was approved by the Research Ethics Committee of the University of Fortaleza under CAAE number: 30948814.2.0000.5052.

RESULTS

Low cost materials were used for the elaboration of the manikin (TABLE 1). This final formatting was the result of numerous tests with different materials, as exemplified below. Initially, a diaper was used to avoid wetting the simulator. Throughout the training, it was found that by filling one glove and covering it with two, the liquid did not overflow even after 30 repetitions of the technique. This change reduced costs with material replenishment, making the model even more reproducible at low cost.

TABLE 1. Materials of the Manikin.

Simulated Structure	Material	Cost (US$/R$)
Abdomen	Commercial dummy plus wooden and plastic supports	6.00/19.00
Skin	Synthetic leather fabric	7.50/23.77*
Abdominal wall	Upholstered sponge coated with plastic film	2.80/8.87*
Ascitic fluid and other structures abdominal	Procedure gloves with water mixed with paint characteristic of each structure	5.40/17.11*

*Cost for each 30 simulation.

It was also performed the inclusion of dyes to simulate different body fluids, allowing a greater similarity of the procedure to reality. To the simulator, gummed tape mass was added to the topography of the pubic symphysis to simulate this anatomical structure and serve as a reference point for the students.

Next, the model was tested and approved by eight professors from a university in Fortaleza, specialized in Gastroenterology (four), General Surgery (three) and Clinical Medicine (one). All reported having performed and assisted paracentesis in patients. The professionals answered a questionnaire of perception about the manikin, whose data are present in TABLE 2.

After the authors got the final version of the model, with a total cost of US$22.00 / R$70.00 initially for 30 simulations and US$16.00 / R$50.00 for each 30 further simulation, they used it in three editions of the course of bedside invasive procedures, when a total of 87 undergraduate medical students (56% male) of varying ages and belonging to five distinct teaching institutions, individually performed the procedure. Of these, 90.8% were in the first three years of medical school. The step-by-step of the checklist, as well as correctness and errors in performing the paracentesis procedure in the proposed simulator, is summarized in TABLE 3.

TABLE 2. Results of the professionals' perception questionnaire about the paracentesis model.

Question	TA	PA	IN	PD	TD
The model can be used for undergraduate teaching	100%	0%	0%	0%	0%
Before performing paracentesis in humans, mannequin training is required	100%	0%	0%	0%	0%
The model is realistic	50%	50%	0%	0%	0%
The model is easy to reproduce	100%	0%	0%	0%	0%

Subtitle. Likert Scale. TA: I totally agree. PA: I partially agree. IN: indifferent. PD: partially disagree. TD: I totally disagree.

TABLE 3. Percentage of successful, incomplete or incorrect steps during the procedure.

Check list	Hit	Incomplete	Mistake	*P*
Selection of material	33.3%	6.9%	59.8%	<0.05
Positioning the patient	32.2%	20.7%	47.1%	<0.05
Asepsis and antisepsis	63.2%	31%	5.8%	<0.05
Location of puncture	80.5%	14.9%	4.6%	<0.05
Anesthesia	60.9%	32.2%	6.9%	<0.05
Puncture in Z technique or traction	75.9%	16.1%	8%	<0.05
Aspiration of ascitic content	80.5%	14.9%	4.6%	<0.05
Realization of the dressing	80.5%	0%	19.5%	<0.05

The 87 answers to the perception questionnaire that was applied with students after the training revealed that 97.7% agree that it is an easy reproducible simulator and 100% agree that the simulator should be used for training before performing the paracentesis on real patients.

The model consists of an unprecedented production in the context of medical education, with no reports of use of similars models in the Brazilian literature, with the potential to develop the teaching of the procedure in a simple and accessible way.

DISCUSSION

It was seen that 50% of physicians fully agreed and another 50% partially agreed that the model was realistic and had good anatomical correlation. Those who agreed partially suggested in the qualitative part of the questionnaire that there should be a change in the model to better reproduce the physical examination

that is performed before the procedure, putting more abdominal fluid into the cavity and leaving the abdominal wall more flexible, making it possible to reposition the umbilical scar according to the need and to hear the sounds of tympani and softness to the percussion of the abdomen.

Nevertheless, all the physicians who tested the model agreed that the model is easy to reproduce and that it can be used for undergraduate education, corroborating with data in the literature that says that to teach an efficient paracentesis, models must be updated and reproducible, and, above all, at an affordable cost, so that they can be used not only by universities, but also by extracurricular practical courses[11,12].

Regarding the steps to perform the procedure, 59.8% of the students made an error when selecting the material, and only 32.2% correctly positioned the patient to start the abdominal fluid collection. Simple care such as checking equipment can make the difference between success and failure of a procedure. This simple conference can prevent the onset of various complications for the patients[13].

At the step of determining the localization for the puncture, 80.5% of the students identified the appropriate place and 75.9% proceeded with the Z technique or traction technique. Of the total, 60.9% had the concern of minimizing the patient's pain by using anesthesia. The risk of errors such as these can be reduced through standardization of behaviors and implementation of strict protocols, such as the safety checklist of the surgical procedure proposed by the World Health Organization[14].

In the end, in one of the last stages of the procedure, 80.5% of the students were able to aspire the ascitic fluid to send for analysis, with 80.5% performing the bandage to finish the procedure.

In agreement with the proposal of the authors, the result of an international multicenter evaluation showed that the use of checklist almost doubled the chance of patients receiving surgical treatment with adequate standards of care, reducing morbidity and mortality[14].

It was interesting to notice that most of the students and all professionals fully agreed that simulated paracentesis training should be performed prior to performing the procedure on a real patient. This is in agreement with many studies that state that the practice in synthetic models is efficient for the learning process, for the acquisition of skills and for students to gain confidence, in comparison to teaching based only on the observation of physicians performing the procedures[15,16]. As a consequence, training in simulated mannequins, such as paracentesis, contributes to improve safety in performing this important procedure in the real patient[10,14].

In this way, our model is reproducible in undergraduate students and professionals opinion, as seen in the results of the perception questionnaire, corroborating the hypothesis that the model can be used for large-scale teaching in other courses or within the medical curriculum itself to improve students confidence and learning of paracentesis.

Furthermore, it is important to highlight that commercial models for simulation of the paracentesis procedure vary the cost between US$1000-2000 / R$3000-6000. These models require logistical planning for storage and transportation in different sectors of the institution.

Also, the lack of a model in the country that brings all these benefits reinforces the relevance of propagating the use of this simulator, expanding the studies in other schools.

CONCLUSION

It was observed that, with low-cost and easily accessible materials, it was possible to create a paracentesis training model for undergraduate students, being well evaluated by the study population. We highlight the significant anatomy learning that the construction and visualization of the finalized model provided to authors and participants.

There are no data on the use of paracentesis simulation models in Brazilian universities. However, the procedure is performed in the health services and needs to be trained. Further studies are needed to demonstrate the efficacy of this method, with more varied samples and more experienced professionals to test the model.

Authors' contribution

Mesquita DAK: collection of the data, literature review, data interpretation and text translation. Queiroz EF: structuring methodology, data interpretation and text translation. Oliveira MA: collection of the data, checked the results, data interpretation and text translation. Cunha CMQ: quality control and statistical analyses and data interpretation. Maia FM and Correa RV were the chiefs investigators and revised the data and critically revised the manuscript. All authors revised and approved the final report.

Mesquita DAK, Queiroz EF, Oliveira MA, Cunha CMQ, Maia FM, Correa RV. A antiga técnica em um novo estilo: desenvolvendo habilidades procedimentais em paracentese em simulador de baixo custo. Arq Gastroenterol. 2018;55(4):375-9.

RESUMO – **Contexto** – A paracentese é um procedimento médico de rotina bastante relevante na prática clínica. Devido à sua importância na assistência médica diária e seus riscos de complicações, o treino do procedimento é essencial em currículos médicos reconhecidos. **Objetivo** – Descrever a construção de um simulador de paracentese de baixo custo, destacando a percepção de estudantes sobre o seu uso para treinamento na graduação em Medicina. **Métodos** – Um modelo de baixo custo foi desenvolvido pelo Programa de Educação Tutorial para treinamento de estudantes de Medicina durante três edições de um curso teórico-prático de procedimentos invasivos à beira do leito. Os autores construíram um modelo a partir de materiais comuns e de fácil acesso, como manequim comercial e suportes de madeira e plástico para representar o abdômen, tecido de couro sintético para a pele, esponja revestida com filme plástico para representar a parede abdominal e luvas de procedimento com água misturada com tinta para simular o líquido ascítico e outras estruturas abdominais. Para avaliar o modelo, aplicou-se um questionário semiestruturado com aspectos quantitativos e qualitativos para médicos especialistas e estudantes. **Resultados** – O modelo para paracentese tem orçamento inicial de US$22.00 / R$70,00 para 30 simulações e US$16.00 / R$50,00 para cada 30 simulações adicionais. Foi testado por oito especialistas (clínico geral, cirurgião geral e gastroenterologista), dos quais quatro são gastroenterologistas, e todos concordaram plenamente que o procedimento deve ser realizado no manequim antes de ser feito no paciente real, e todos eles aprovaram o modelo para o ensino de graduação. Durante as edições do curso, um total de 87 estudantes de graduação em Medicina (56% homens) realizaram individualmente o procedimento. Em relação às etapas do procedimento, do total de alunos avaliados, 80,5% identificaram o local apropriado para a punção e 75,9% procederam com a técnica Z ou tração. Ao final, 80,5% dos alunos conseguiram aspirar ao conteúdo ascítico, com 80,5% realizando o curativo e finalizando o procedimento. Todos os alunos concordaram plenamente que o treinamento com paracentese simulada deve ser feito antes de se realizar o procedimento em um paciente real. **Conclusão** – A elaboração de um modelo de ensino em paracentese proporcionou experiência única a autores e participantes, permitindo uma visível correlação da anatomia humana com materiais sintéticos, aprofundando o conhecimento desta ciência básica e desenvolvendo habilidades criativas, o que potencializa a prática clínica. Não há dados sobre o uso de modelos de simulação de paracentese em universidades brasileiras. No entanto, o procedimento é bastante realizado nos serviços de saúde e precisa ser treinado. O modelo descrito acima foi apresentado como de qualidade, baixo custo e de fácil reprodutibilidade, sendo inédito no cenário da educação médica nacional, mostrando-se uma ferramenta complementar de ensino na graduação e preparando os alunos para o procedimento in vivo.

DESCRITORES – Educação médica. Simulação. Paracentese.

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ORIGINAL ARTICLE

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Gastric fundic gland polyps: can histology be useful to predict proton pump inhibitors use?

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ABSTRACT – **Background** – Fundic gland polyps allegedly increased in frequency in recent decades, and had attracted great attention due to possible association with prolonged proton pump inhibitor therapy. Prolonged use of this drug could cause parietal cell hyperplasia, obstruction of glandular lumen and cystic dilation of the gland. **Objective** – This study aims to analyze clinical and pathological features of fundic gland polyps in patients with and without proton pump inhibitor therapy in a selected population from Brazil. **Methods** – It was selected a sample of 101 Brazilian patients (78 females and 23 males), from a five years retrospective search of the files from a private pathology laboratory. The patients had an average age of 57 years and we included patients with a histological diagnosis of fundic gland polyp. The clinical data were obtained from their files and all histological slides were reviewed and examined with hematoxylin and eosin (HE) and Giemsa. **Results** – Information about the use or non-use of proton pump inhibitors (PPI) was obtained in 84 patient files. In 17 cases we could not determine if PPI were used or not. Among those in which the information was available, a positive history of anti-acid therapy was observed in 63 (75.0%) patients. Parietal cell hypertrophy/hyperplasia and parietal cell protrusions were detected in most slides. Histological findings were identical in PPI users and PPI negative patients. *Helicobacter pylori* infection was detected in just two samples. Epithelial dysplasia or adenocarcinoma were not observed in our cases. Histopathological analysis of fundic gland polyps could not distinguish between PPI and non-PPI related cases. Parietal cell cytoplasmic protrusions, an alleged marker of prolonged acid suppression therapy, was detected in both groups. **Conclusion** – Histological features could not discriminate anti-acid therapy related fundic glands polyps in our patients.

HEADINGS – Gastric mucosa. Polyps, pathology. Proton pump inhibitors.

INTRODUCTION

Fundic gland polyps (FGP) are small exophytic and usually asymptomatic lesions of fundic and upper body gastric mucosa. At histology, dilated cystic glands lined with parietal cells, chief cells and occasionally with mucous foveolar cells characterize them. These polyps can occur in hereditary or non-hereditary context. Hereditary cases are mainly associated with the Familial Adenomatous Polyposis Syndrome (FAP) and frequently multiple lesions can be found, such as Fundic Gland Polyposis[1-2].

Sporadic fundic gland polyps are reported as the most common type of gastric polyp[1]. It can be single or multiple and usually is an incidental finding in patients submitted to upper endoscopy or on chronic treatment with proton pump inhibitor (PPI) medication[3-8]. Prolonged use of this drug could cause parietal cell hyperplasia, obstruction of glandular lumen and cystic dilatation of the gland[5-8]. In fact, it seems that growing incidence of fundic gland polyps parallels increasing medical use of proton pump inhibitors[7,9-11]. A negative association with *Helicobacter pylori* (*H. pylori*) infection and prevalence among female patients has also been recorded[2-3,8].

The aim of this study is to analyze clinical and pathological features of fundic gland polyps in patients with and without proton pump inhibitor therapy in a selected population from Brazil.

METHODS

This is an observational, retrospective and descriptive study based on morphological analyses and primary clinical data collection from patients with gastric fundic gland polyps. We performed a five years retrospective search of the pathology files from a private Anatomic Pathology Laboratory. All cases with a final diagnosis of FGP were selected. A total of 101 patients with a histological diagnosis of FGP were selected from an Anatomic Pathology Laboratory files.

Representative microscopic slides of these cases were retrieved from files and reviewed by a pathologist from this study. All biopsies were previously fixed in 40g/L formaldehyde and included in paraffin. Histological sections were stained with hematoxylin and eosin (HE) and Giemsa. Number and size of tissue fragments submitted to histopathological analysis were obtained from gross description in pathology reports. Histological features analyzed in all polyps consisted of: type of cyst epithelial lining (parietal cell, chief cell, mucous cell); parietal cell and superficial foveolar epithelium morphology (parietal cell hyperplasia, parietal cell protrusions, foveolar hyperplasia); presence or not of intraglandular secretion, glandular atrophy, intestinal metaplasia, epithelial dysplasia, chronic inflammatory infiltrate in lamina propria, neutrophilic infiltrate and *H. pylori* infection. Parietal cell protrusions were defined as tongue-

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like cytoplasmic protrusions into dilated glandular lumen. All microscopic sections in each slide were scrutinized for the parameters above. These features were reported as positive or negative. Parietal cell protrusions were also classified as focal (limited area in a polyp) or diffuse (detected in most polyp glands). Gastric mucosa from other sites were also studied whenever provided. *H. pylori* search was performed in HE and Giemsa stains at high power microscopy (40x). Histological analysis was blinded for patients' clinical and medication intake history. Clinical information were collected from medical files from each patient in a private Gastroenterology clinic.

Statystical analysis were performed with Prism Software for MacIntosh 4.0.®, using the Chi-square test and the Student t test. The significance level was set at $P<0.05$.

The study was approved by the ethical committee of Federal University of Sergipe (UFS) and followed the precepts of the declaration of Helsinki and the resolution 466/2012 of the National Health Council.

RESULTS

From the 101 patients sample, 23 (23%) were male and 78 (77%) were female. Mean age was 57 years, which ranged from 21 to 98 years. History of PPI treatment was retrieved from the medical files of 84 patients (83%). Clinical data are depicted in TABLE 1.

The number of fragments obtained by endoscopy and submitted to histological analysis ranged from one (7.9%), between two and ten (73.3%) and over ten (18.8%). The majority of polyps (79.2%) measured between 2 and 5 mm. Histological analysis disclosed a mixed cell population, which consisted of parietal, chief and mucous cells lining the cysts in most polyps (78.2%). These mixed type polyps showed some hybrid cysts lined by both mucous and oxyntic cells (FIGURE 1). A minority of polyps (21.8%) consisted exclusively of cysts lined by parietal or chief cells (oxyntic type), mainly the former (FIGURE 2). Morphological description and comparison between groups was summarized in TABLE 2.

TABLE 1. Patients clinical characteristics.

Number of patients	N	%
	101	100
Gender		
Male	23	23%
Female	78	77%
Age		
0-30	5	5%
31-60	58	57%
>60	38	38%
PPI use		
Unknown	17	17%
No use	19	19%
Use	65	64%
< 01 year	16	16%
01-05 years	38	38%
> 05 years	11	11%

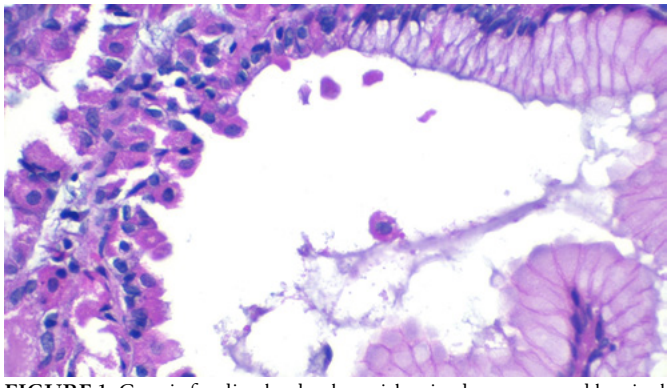

FIGURE 1. Gastric fundic gland polyp with mixed type cyst and luminal secretion.

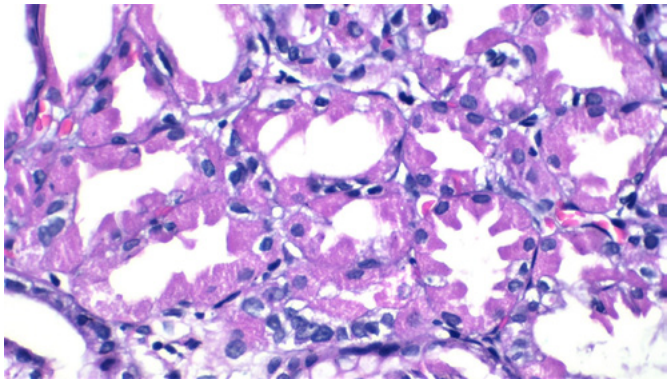

FIGURE 2. Oxyntic type cyst with apical cytoplasmic protrusions of parietal cells.

Mild antral gastritis was observed in 27 cases and limited areas of antral intestinal metaplasia were detected in only three cases. Dysplasia was not observed in fundic or antral mucosa in any case. Histological findings were identical in PPI users and PPI negative patients, with no statistically significant finding ($P>0.05$).

DISCUSSION

Fundic gland polyps has attracted great attention due to possible association with PPI prolonged therapy and some alleged increase in frequency in recent decades[12]. Indeed, they are frequently reported as the most common gastric polyps in Western population[1,13]. Nevertheless, in a study from Brazil, FGP is the second in frequency (16%), much less than hyperplastic polyps (71%)[14]. Similar results were described in a Spanish population based study[15].

In our study, most polyps occurred in adult females with a positive history of PPI treatment. Female prevalence is a well-known characteristic of FGP[2,8,11-12]. It could reflect a direct gender related influence in fundic gastric mucosa proliferation or a higher prevalence of PPI use among female patients.

Most lesions, in our study, measured more than 2.0 mm and were endoscopically removed as gastric polyps suspected to be a FGP. Actually, a diagnosis of FGP can be achieved by endoscopy with a high degree of accuracy because they detach completely at their base by forceps biopsy[16].

A high frequency of PPI use was also found in our cases favoring a contributory role of acid suppression therapy in FGP

TABLE 2. Histological characteristics and comparison between histological findings found in PPI user and PPI negative patients.

Pathological features	A (n=84)		B (n=17)	Total N = 101 (100%)	*P*-value
	PPI positive (n=63)	PPI negative (N=21)	PPI use unknown		
Cyst lining					
Mixed	49	17	13	79 (78.2%)	1.00*
Oxintic	14	4	4	22 (21.8%)	
Parietal cell hyperplasia					
Positive	61	21	16	98 (97%)	1.00*
Negative	2	0	1	3 (3%)	
Parietal cell protrusions					
Positive focal	19	8	11	38 (37.6%)	0.3051*
Positive diffuse	28	11	3	42 (41.6%)	
Negative	16	2	3	21 (20.8%)	
Mucous plug					
Positive	28	11	11	50 (49.5%)	0.6165**
Negative	35	10	6	51 (50.5%)	
Foveolar hyperplasia					
Positive	18	6	3	27 (26.7%)	1.00*
Negative	45	15	14	74 (73.3%)	
H. pylori infection					
Positive	1	1	0	2 (2%)	0.4398*
Negative	62	20	17	99 (98%)	

A: clinical information regarding PPI use available; B: clinical information regarding PPI use not available. PPI: proton pump inhibitor. *Fisher's exact test. ** Chi-square test.

development in gastric mucosa, which is in accordance with many reports in literature. Actually, FGP genesis seems to be related to prolonged PPI use[4,6-8,11].

Cats et al.[5], showed parietal cell proliferation, development of cytoplasmic protrusions and glandular cystic dilatation in patients on chronic PPI therapy. Histological changes in oxyntic mucosa were observed in 18% of early treatment patients and in 86% of patients over one year of treatment. Similar results were obtained by Jalving et al.[8] who also describes the development of FGP in 18% of the group with less than one-year-treatment-patients and in 57% of the patients after one year of therapy. However, the notion of PPI therapy as a risk factor for FGP development is declined by some researchers[17,18].

PPI treatment in mices can induce morphological changes in epithelial gastric cells, with enlargement of parietal cells and decreased number of chief cells[19]. Hypergastrinemia, secondary to PPI chronic use can lead to parietal cell proliferation and hyperplasia[5,8-9]. Prolonged acid suppression therapy also induces obstruction of parietal cells canaliculi with hydrochloric acid, cytoplasmic hypertrophy and apical cell protrusions. Increased intraglandular pressure results in cystic dilatation[5,19-20]. FGP can results from a dual mechanism of cellular proliferation and obstruction of glandular secretion flow, both related to PPI therapy[20].

Parietal cell hyperplasia and parietal cell protrusions were detected in most cases of FGP in this study in patients with and without PPI therapy. Parietal cell protrusions were only focally observed in many polyps and could be undetected without careful histologic analysis.

Cysts were lined by a mixed cell population in 77% of cases, which includes parietal, chief and mucous foveolar-type cells. These findings are the classical histological picture of FGP, with disorganized glands, which substantiated the classification of these lesions as hamartomatous in the past[1,21]. Mucous lined cysts probably is a consequence of secretion flow obstruction and pits dilatation, since foveolar hyperplasia was only observed in 27% of cases. In fact, true proliferative changes of foveolar epithelium are more related to chronic active gastritis and *H. pylori* infection. The presence of intraglandular mucous plugs and exfoliated cells in almost half (47.5%) of our sample reinforces the glandular flow obstruction hypothesis for FGP development.

A minority of polyps (22%) consisted of cysts lined by parietal/chief cells only. Some of these cases showed tiny cysts in a background of hyperplastic and hypertrophied oxyntic mucosa with parietal cell protrusions, a histological change frequently described in non-polypoid gastric mucosa of chronic PPI users[3,5,8]. Nevertheless, we could not find a specific morphologic marker of PPI related-FGP in our study, since these findings were also detected in patients without use of PPI drugs. Recently, these morphologic changes in oxyntic mucosa have also been linked to *H. pylori* chronic gastritis[22]. Indeed, the precise role of anti-acid therapy in oxyntic cell changes is still a debatable issue by some authors[23].

A very low frequency of *H. pylori* infection (2%) was detected in our study which corroborates the well-known inverse association of FGP and this bacteria[2,3,8]. Genta et al. describes a 0.5% frequency of *H. pylori* infection in 6081 patients with FGP[24]. Cats et al.[5] speculates that the degradation of gastric mucus by

H. pylori proteases acts as a protection factor for cystic dilatation by improving glandular secretion. In fact, acquisition of *H. pylori* infection can cause regression of FGP[25].

Mutations in β-catenin have been described in many cases of sporadic FGP[13,26-27]. Mutations in APC/β-catenin genes could induces parietal cell proliferation and deregulate cell membrane function, leading to glandular obstruction and cystification[20]. According to Abraham[28], they are neoplastic growths that have very limited potential for malignant transformation. Actually, low grade dysplasia is detected in only 1% of sporadic FGPs[28-30].

Dysplasia or gastric adenocarcinoma were not identified in any case in 101 fundic gland polyps examined in the present study. Our findings supports the established concept of a benign proliferative lesion, no matter it discloses some neoplastic-like features at molecular level.

CONCLUSION

Fundic gland polyps were more prevalent in middle-aged women, frequently with a positive history of proton pump inhibitors therapy and a very low frequency of *H. pylori* infection. Parietal cell protrusions were equally detected in PPI and non-PPI related polyps. Histological features could not discriminate anti-acid therapy related fundic glands polyps in our patients.

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Authors' contribution

Brito HLF: conception and design, analysis and interpretation, writing the article, critical revision of the article, data collection, provision of materials, patients and resources, statistical expertise, literature search, logistic support, analysis of the slides. Barros C: analysis and interpretation, writing the article, data collection, literature search. Freire MV: analysis and interpretation, writing the article, statistical expertise, literature search. Silva Filho MN: analysis and interpretation, provision of materials, patients and resources, logistic support. Nascimento TV: analysis and interpretation, critical revision of the article, provision of materials, patients and resources, logistic support.

Brito HLF, Barros C, Freire MV, Silva Filho MN, Nascimento TV. Pólipos de glândulas fúndicas: a histologia pode ser utilizada para prever uso de inibidores de bomba de próton? Arq Gastroenterol. 2018;55(4):380-4.

RESUMO – **Contexto** – Os pólipos das glândulas fúndicas do estômago supostamente aumentaram em frequência nas últimas décadas e atraíram grande atenção devido à possível associação com a terapia prolongada com inibidores da bomba de prótons. O uso prolongado deste fármaco pode causar hiperplasia das células parietais, obstrução do lúmen glandular e dilatação cística da glândula. **Objetivo** – Este estudo tem como objetivo analisar os aspectos clínicos e patológicos dos pólipos das glândulas fúndicas em pacientes com e sem terapia com inibidores da bomba de prótons em uma população selecionada do Brasil. **Métodos** – Foi selecionada uma amostra de 101 pacientes brasileiros (78 do sexo feminino e 23 do sexo masculino), a partir de uma pesquisa retrospectiva de cinco anos dos arquivos de um laboratório privado de patologia. Os pacientes tinham uma idade média de 57 anos e foram incluídos pacientes com diagnóstico histológico de pólipo das glândulas fúndicas. Os dados clínicos foram obtidos a partir de seus prontuários e todas as lâminas histológicas foram revisadas e examinadas com hematoxilina e eosina (HE) e Giemsa. **Resultados** – Informações sobre o uso ou não uso de inibidores da bomba de próton (IBP) foram obtidas em 84 prontuários de pacientes. Em 17 casos, não foi possível determinar se o IBP foi usado ou não. Entre aqueles em que a informação estava disponível, observou-se uma história positiva de terapia com IBP em 63 (75,0%) pacientes. A hipertrofia das células parietais/hiperplasia e protrusões das células parietais foram detectadas na maioria das lâminas. Os achados histológicos foram idênticos em usuários de IBP e pacientes não usuários. A infecção por *Helicobacter pylori* foi detectada em apenas duas amostras. A displasia epitelial ou o adenocarcinoma não foram observados em nossos casos. A análise histopatológica dos pólipos das glândulas fúndicas não pôde distinguir entre os casos IBP e não relacionados ao IBP. As protuberâncias citoplasmáticas das células parietais, um suposto marcador de terapia prolongada de supressão de ácido, foram detectadas em ambos os grupos. **Conclusão** – Características histológicas não podem discriminar os pólipos das glândulas fúndicas relacionados à terapia anti-secretora em nossos pacientes.

DESCRITORES – Mucosa gástrica. Pólipos, patologia. Inibidores da bomba de prótons.

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ORIGINAL ARTICLE

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Serum cytokine of IL-2, IL-10 and IL-12 levels in patients with stomach adenocarcinoma

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ABSTRACT – **Background** – Gastric adenocarcinoma is the fourth most common cause of cancer-associated death worldwide. **Objective** – We evaluated the immunological status of patients with gastric cancer before surgery and circulating cytokines as potential diagnostic biomarkers for gastric cancer. **Methods** – We included 90 healthy controls and 95 patients with distal Gastric adenocarcinoma in Mazandaran, Sari, Iran. We measured serum IL-2, IL-10 and IL-12 Levels by a sandwich enzyme-linked immunosorbent assay using the IBL international GMBH kit. **Results** – The serum IL-10 levels in the patients with Gastric adenocarcinoma were significantly higher than those of the healthy controls (P=0.02). There were no significant differences in serum IL-2 and IL-12 levels between patients with gastric cancer and healthy controls. **Conclusion** – Increased levels of IL-10 might be useful as diagnostic biomarkers for Gastric adenocarcinoma; however, this needs to be confirmed with larger number of patients and with control groups other than blood donors, properly age paired. These results suggest that positive expression of IL-10 may be useful as a molecular marker to distinguish stage of gastric cancers which can be more readily controlled.

HEADINGS – Stomach neoplasms. Adenocarcinoma. Cytokines.

INTRODUCTION

Gastric cancer (GC) is the third type of cancer to its malignity and the second most common cause of death by cancer worldwide; approximately it is two thirds of new cases per year occur in the developing countries[1,2]. Mortality rates are higher in Asian and Latin American countries, where cases are usually diagnosed at later stages, leading to very low survival rates. It arises more in men, than women (2:1), 95% of cases are adenocarcinomas which is the most common malignant tumor regardless of age, race or inclining factors presented by a patient[3,4].

It has been found that the immune microenvironment in tumor tissues is highly organized in a molecular and cellular level[5]. It is complex of many diverse kinds of cells: such as endothelial cells, fibroblasts, lymphocytes and macrophages. It also contains numerous soluble molecules: such as growth factors, cytokines, chemokines which may have protumoral or anti-tumoral possessions that depend on the situation of the immune response[6-8]. It has been shown that through cytokine production, may promote tumor angiogenesis, metastasis and induce to T cell differentiation and activation. In different tumors, a propensity is detected on the expression of anti-inflammatory cytokines and a decreased expression of proinflammatory cytokines; this change in expression could ease tumor progression by subversion of the mechanisms of cell immunosurveillance[9-11].

Interleukin 2 (IL-2) is generated in an immune response Th1 cytokine, and interleukin 4 and 10 (IL4, IL10) are an immune response Th2 cytokines. These cytokines are crucial mediators of the Th1/ Th2 stability and they are involved in the process of inflammation-mediated carcinogenesis in human organs, including the gastrointestinal tract[3,12].

Interleukin-10 (IL-10) is a pleiotropic cytokine produced by macrophages, T-helper 2 (Th2) cells, and B lymphocytes and both can stimulate and suppress the immune response[13]. IL-10 production and secretion may be rationally presumed to be up-regulated in cancer patients. Actually, increased serum levels of IL-10 have been established in patients with diverse histotypes of solid and hematopoietic tumors and these levels have been shown to associate with level of disease[14,15]. In addition, it has been proposed that IL-10 may be released not only by immune cells but directly by tumor cells because serum levels of this cytokine often associate with tumor load, while surgical excision of neoplasia may be followed by a reduction in IL-10 serum levels[14,16,17].

Interleukin-12 (IL-12) was initially recognized as a natural killer (NK) cell stimulatory factor, being a disulfide-linked heterodimeric cytokine composed of 35 and 40 KDa subunits. Secreted mainly by antigen presenting cells (APC), such as macrophages, some B cells, and dendritic cells, IL-12 activates NK cells and T cells to produce interferon-γ (INF-γ), and expands their cytotoxic activity and proliferation[18]. Interleukin-12 was newly found to induce antitumor effects against a different types of tumors in vivo. Besides, it is an immunoregulatory cytokine, which may provide a vital connec-

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tion between nonspecific immune mechanisms and the expansion of a specific T cell-mediated immune response[19-21]. A primary in vitro study proposed that the administration of IL-12 produced immunomodulatory activity and generated noticeable antitumor activity. Although some detectives have inspected the effects of intravenous IL-12 on patients with metastatic renal cell cancer or malignant melanoma, there are very few reports on serum IL-12 levels in cancer-bearing patients[20,22].

The inflammatory mediators produced nearby in the gastric mucosa may spread on the blood circulation and be found in plasma samples. In this study, we tested the hypothesis that circulating levels of inflammatory cytokines could work as indirect indicators of tissue damage, and that their measurement might be a useful biomarker for the early detection of GC, resulting in a better long-term prognosis. Therefore, we measured the serum IL-2, IL-10 and IL-12 levels of patients with gastric cancer before surgery, to evaluate the preoperative immunological status of these patients.

METHODS

Study population and blood samples

We examined 95 patients aged from 22 to 90 years, admitted to hospital for surgical treatment. As a control for normal serum, IL-2, IL-10 and IL-12 concentrations, 90 healthy clinical personnel volunteered. Written informed consent was obtained from all patients. Blood samples were collected before surgery and specimens were stored at -80°C until later analysis.

Cytokine assays

The concentration of IL-2, IL-10 and IL-12 in plasma samples was measured by ELISA using commercially available kits, (IBL international IBL GMBH, Germany) via captur-sandwich assay according to the manufacturer's instructions. The concentration of cytokines was calculated based on standard curves provided with the kits, and results were expressed in ng/ml. For ELISA all samples were tested in duplicate and the average values were used in the analysis[4].

Statistical analysis

A statistical descriptive analysis was performed using Prism statistical software Results are expressed as mean ±SD. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc tests was used to compare the results of all assays. Value of $P<0.05$ was considered to be significant[8].

RESULTS

TABLE 1 shows the demographics of the 60 male and 35 female enrolled patients and healthy controls.

TABLE 2 shows the Serum IL-2, IL-10 and IL-12 levels in healthy subjects and patients with gastric adenocarcinoma.

FIGURE 1 shows the serum levels of Serum IL-2, IL-10 and IL-12 levels in patients and healthy controls. We found significantly increased IL-10 serum levels in GC patients, in comparison to healthy controls ($P=0.02$). Circulating levels of all IL-2 and IL-12

TABLE1. Characteristics of patients and healthy controls.

Sample	Number	Age (mean)	Gender		Tumor type		Tumor stage		
			Male	Female	Adenocarcinoma	SCC	I	II	III
Patient	95	62	60	35	69	26	40	30	25
Normal	90	51	50	40	–	–		–	

TABLE 2. The Serum IL-2, IL-10 and IL-12 levels in healthy subjects and patients with gastric adenocarcinoma.

IL	Mean ± SEM of cancer	Mean ± SEM of control	Difference between means	95% confidence interval	R squared	*P* value
IL2	0.152 ± 0.045	0.152 ± 0.045	0.152 ± 0.045	0.152 ± 0.045	0.152 ± 0.045	0.152 ± 0.045
IL10	0.02752 ± 0.002	0.02752 ± 0.002	0.02752 ± 0.002	0.02752 ± 0.002	0.02752 ± 0.002	0.02752 ± 0.002
IL12	0.3483 ± 0.028	0.3483 ± 0.028	0.3483 ± 0.028	0.3483 ± 0.028	0.3483 ± 0.028	0.3483 ± 0.028

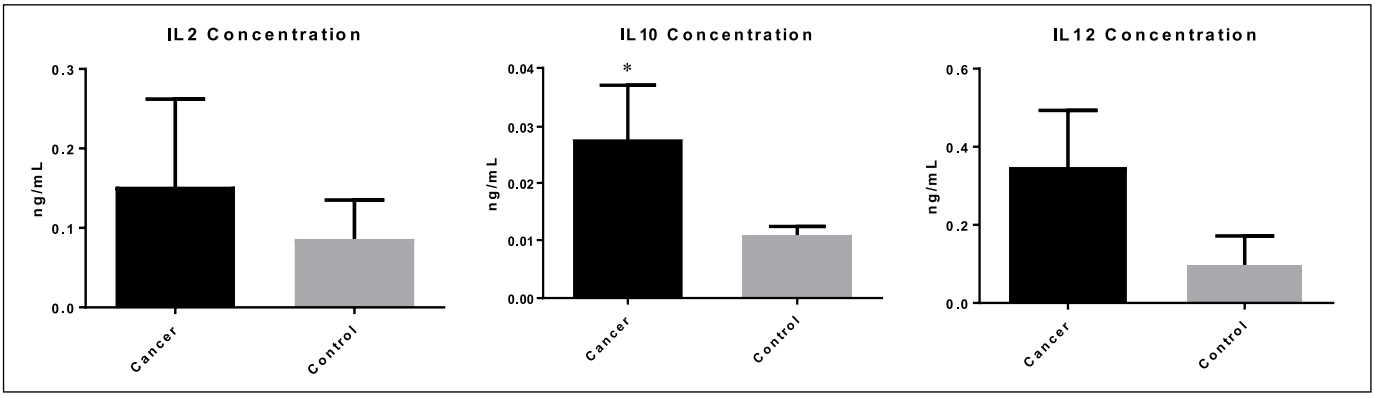

FIGURE 1. The serum levels of Serum IL-2, IL-10 and IL-12 levels in patients and healthy controls. The sign (*) show significantly ($P<0.05$) increased compared to the control group.

were higher in GC patients than in the healthy control group but there were no significant differences among them.

DISCUSSION

Over the last few years, a number of findings, ranging from the molecular characterization of tumor antigens to the recognition of costimulatory molecules, have provided critcal visions into our knowledge of tumor immunology[23,24]. The immune system is capable to respond to cancer, because activated mononuclear cells can be established both peripherally and at the tumor site; however, failure by lymphocytic infiltrates to contain tumor growth proposes an insuffisient immune response to neoplasms[25]. Indications proofs the idea that the type of T-helper (Th) response may be relevant to the expansion of an effective immune response because of the communally conflicting effects of the cytokines produced. In fact, although Th1-type cytokines (i.e., IL-2 and interferon-γ) have been shown to increase the antitumor activity of cytotoxic T-cells in vitro, Th2-type cytokines, and IL-10 in particular, have been confirmed to apply reverse effects[26-28]. IL-10 inhibits the Th1-type pathway activation, averts APC from procurement access to tumor antigens, and down-regulates surface expression of costimulatory molecules CD80 or CD86 on tumor cell. As it is known that IL-10 is Th2- cytokine, which increases antibody synthesis, indorses the humoral immune response and suppresses the antitumor immunity, established by Stanilov et al.[29,30]. who showed that Functional antagonist of IL-12p70 is the IL-10 which was of high level in the serum of a 48 colorectal cancer patients.IL-10 appears to be more of a pro-tumor than anti-tumor properties. The pro-tumor properties of IL-10 can be clarified by the inhibitory effect on the Th1-cytokine production, in particular IL-12p70, its inhibitory effect by involving apoptosis and stimulation of cell proliferation[31,32]. Also, there is confirmation that the tumor infiltrated lymphocytes inside the tumor mass are not effective because some tumor cells secrete IL-10. IL-10 secretion is one of the mechanisms with which the tumor cells "prevent" the immunological surveillance which at the end will also associate to increase the IL-10 serum level which can elucidate the important increased level of serum IL-10 in our study[33,34]. The essential roles of different cytokines in regulating antimicrobial immunity and inflammation make them attractive candidates for being genetic host markers in assessing individual susceptibility to Gastric Cancer progress[35].

In this investigation we have studied the levels of inflammation-associated cytokines such as IL-2, IL 10 and IL-12 in the sera of gastric cancer patients. Since the levels of IL-2, IL-10 and IL-12 may be produced by normal cells, it was important to establish the levels of these cytokines in benign conditions. As expected based on its in vitro properties, in the current study a positive correlation between the presence of gastrointestinal tumors and high IL-10 concentrations was found. Elevated serum levels of IL-10 in fact were observed in patients with advanced gastrointestinal malignancies when compared with healthy controls; moreover, IL-10 serum levels were demonstrated to be higher in patients with metastatic disease compared with patients with disseminated disease. In our study the significant increase of the IL10 serum level may be because the association of IL-10 genotypes (single nucleotide polymorphism) with Gastric Cancers appears to be biologically and clinically important. IL-10 is a key immunosuppressive cytokine that gears the immune response towards a Th2 cell response. Such IL-10 haplotypes are related to susceptibility and severity of Gastric Cancers. The finding that there was an increased risk of Gastric Cancers in high IL-10 producer haplotype was in agreement with the concept that Th2 cytokines including IL-10 are highly expressed in patients with Gastric Cancers as was shown in our study results and This idea could partially be clarified by reported findings that increased expression of mRNA and raised serum levels of IL-10 are correlated with the progression of Gastric Cancers[15,36-37]. Similar results recently have been reported in patients with different histotypes of solid and hematopoietic tumors suggesting that IL-10 overproduction may be a communal survival strategy of several types of human malignancies[38]. Also keeping in mind that inflammatory cells may be less frequent within metastases than in primary lesions, it is likely that the main source of IL-10 may be the tumor itself rather than the inflammatory infiltrates[39].

Certainly, a number of studies have uttered on IL-10 gene activation and IL-10 protein production in some tumor specimens and cell lines[40-43]. In addition, IL-10 serum levels showed an advance significant increase in nonresponder patients, whereas these levels were shown to be unmodified in responder patients at the end of the follow-up period. These results suggest that positive discovery of IL-10 expression may be used as a molecular marker for characterizing of gastric. Xiong-Fei in his paper said that since IL-10 can both reduce and enhance anti-cancer possessions, it may be significant to discover the role of IL10 polymorphisms in the development of Gastric Cancer in different clinical stages, or Gastric Cancer of different subsites[44]. Results of another study demonstrated that the intraperitoneal with IL-10 treatment was able suppressed peritoneal dissemination of gastric cancer cells and reduce peritoneal metastasis and increase survival rate, in the inoculated mice[3]. Relative to IL-10, Jing Liang, found increased expression of IL 10 in patients with stages III and IV with low level differentiations possessed significantly higher positive detection ratios than patients with moderate or high-level differentiation in the Chinese population[3,45,46]. Whence is necessary and very important, to study a larger population of patients with cancer, to understand the role of the IL-2, IL-10 and IL-12 cytokines in the immune response suppression induced by tumor cell in gastric cancer, to be used as molecular markers to distinguish different stages of cancer, offering the patient a better quality of life and a longer survival rate.

In conclusion, the increased IL10 serum level in gastric cancer may be due to the Functional antagonism of IL-10 toward IL-12p70 which will cause more IL10 secretion and may be the secretion of this cytokine by the tumor cell itself to modulate the Immune system towards Th2 rather than Th1. While in gastric cancers the association of IL-10 genotypes with Gastric Cancers specifically the single nucleotide polymorphism of the IL10 promoter region may be the cause of such serum elevation. If this hypothesis is true, the inhibition of IL-10 production or the administration of anti-IL-10 agents could become a new therapeutic tools for treating patients with GC. The results of the current study show that measurement of basal levels of serum IL-10 is of independent prognostic utility in patients with advanced gastrointestinal carcinoma and may be useful for the detection of disease progression.

Shokrzadeh M, Mohammadpour A, Hoseini V, Abediankenari S, Ghassemi-Barghi N, Tabari YS. Níveis séricos de citocinas IL-2, IL-10 e IL-12 em pacientes com adenocarcinoma do estômago. Arq Gastroenterol. 2018;55(4):385-9.

RESUMO – **Contexto** – O adenocarcinoma gástrico é a quarta causa mais comum de morte relacionada ao câncer em todo o mundo. **Objetivo** – Avaliar o status imunológico dos pacientes com câncer gástrico antes da cirurgia e as citocinas circulantes como potenciais biomarcadores diagnósticos para câncer gástrico. **Métodos** – Incluímos 90 indivíduos controles saudáveis e 95 pacientes com adenocarcinoma gástrico distal em Mazandaran, Sari, Iran. Os níveis de soro Il-2, IL-10 e Il-12 foram medidos por um ensaio de imunoabsorção enzimática pela técnica de sanduíche usando o kit IBL International GmbH. **Resultados** – Os níveis séricos IL-10 nos pacientes com adenocarcinoma gástrico foram significativamente superiores aos dos controles saudáveis (*P*=0,2). Não houve diferenças significativas nos níveis de soro IL-2 e IL-12 entre pacientes com câncer gástrico e controles saudáveis. **Conclusão** – Níveis aumentados de IL-10 podem ser úteis como biomarcadores diagnósticos para adenocarcinoma gástrico; no entanto, isso precisa ser confirmado com maior número de pacientes e com grupos de controle que não sejam doadores de sangue, adequadamente emparelhado por idade. Estes resultados sugerem que a expressão positiva do IL-10 pode ser útil como um marcador molecular para distinguir a fase de câncer gástrico que pode ser mais facilmente controlada.

DESCRITORES – Neoplasias gástricas Adenocarcinoma. Citocinas.

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ORIGINAL ARTICLE

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Characterization of enteroaggregative *Escherichia coli* among diarrheal children in Western Brazilian Amazon

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ABSTRACT – **Background** – Enteroaggregative *Escherichia coli* (EAEC) is one of the main acute and chronic diarrhea causes both in children and adults, mainly in developing countries. **Objective** – The aim of the present study is to characterize EAEC strains isolated from faecal samples and to identify genes potentially contributing to virulence, biofilm production and antimicrobial resistance in children admitted to a pediatric hospital in Porto Velho, Rondônia State. **Methods** – The total of 1,625 *E. coli* specimens were isolated from 591 children in the age group 6 years or younger who were hospitalized in Cosme and Damião Children Hospital in Porto Velho, between February 2010 and February 2012, with acute gastroenteritis. Colonies suggestive of *E. coli* were subjected to polymerase chain reaction testing in order to identify the virulence factors. The in vitro adhesion assays using HEp-2 adherence were tests. Biofilm detection through spectrophotometry and antimicrobial susceptibility tests were conducted in the disk diffusion method. **Results** – The mentioned study examined 591 stool samples from children with diarrhea. Diarrheogenic *E. coli* was found in 27.4% (162/591) of the children. EAEC was the diarreagenic *E. coli* most frequently associated with diarrhea 52.4% (85/162), which was followed by enteropathogenic *E. coli* 43.8% (71/162), enterotoxigenic *E. coli* 2.4% (4/162), and enterohemorrhagic *E. coli* 1.2% (2/162). The *aggR* gene was detected in 63.5% (54/85) of EAEC isolates; moreover, statistically significant correlation was observed among typical EAEC (*aggR*) and *aatA* (P<0.0001), *irp2* (P=0.0357) and *shf* (P=0.0328). It was recorded that 69% (59/85) of the 85 analyzed EAEC strains were biofilm producers; 73% (43/59) of the biofilm producers carried the *aggR* gene versus 42.3% (11/26) of non-producers (P=0.0135). In addition, there was association between the *aatA* gene and biofilm production; 61% (36/59) of the samples presented producer strains, versus 19.2% (5/26) of non-producers (P<0.0004). Antibiotic sensitivity test evidenced that most EAEC were ampicillin 70.6% (60/85), sulfamethoxazole 60% (51/85), tetracycline 44.7% (38/85) and cefotaxime 22.4% (19/85) resistant. **Conclusion** – As far as it is known, the present study is pioneer in Northern Brazil to investigate EAEC virulence factors and to show the antimicrobial susceptibility of EAEC strains isolated from children with diarrhea.

HEADINGS – *Escherichia coli*, classification. Infantile diarrhea. Child. Virulence, genetics.

INTRODUCTION

According to the World Health Organization (WHO), diarrhea is the second leading morbidity and mortality cause among children in the age group 5 years or younger. Diarrhea resulted in 530,000 deaths in 2015, or in approximately 1,400 deaths per day, worldwide[1]. The main diarrheal-syndrome causes in children include inappropriate hygiene, insufficient water and food sanitation, besides lack of adequate health infrastructure[2]. Several viral, bacterial and parasitic agents are associated with diarrhea, among them bacteria belonging to species *Escherichia coli* (which is a natural species composing the intestinal flora). This species is strongly associated with diarrhea cases in children due to the acquisition of specific virulence factors contributing to its pathogenicity, the so-called diarreagenic *E. coli* (DEC)[3].

Diarreagenic *E. coli* is divided in six pathotypes depending on the pathogenicity mechanism, namely: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli*, enteroaggrevative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC) and diffusely adherent *E. coli*[4]. The EAEC is associated with chronic and persistent diarrhea cases in developing countries, besides being one of the most relevant opportunistic pathogens affecting HIV patients. However, despite the several advances concerning the understanding about this bacterium species, its infection mechanism is not entirely understood because the heterogeneity of the pathogen impairs a proper diagnosis, as well as the understanding about its pathogenicity[5].

Previous studies have described the three-stage model applied to EAEC pathogenesis: 1- initial adherence to mucosal surface, 2- biofilm formation, and 3- inflammatory response induction and

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toxin release. Several virulence factors affect the infection process; however, they can be used as pathogenesis identification markers[6].

The EAEC strains are defined in HEp-2 cells through their aggregative adherence (AA) or "stacked brick" phenotype, which is the gold standard method to identify such pathogen. However, the adherence test is not able to distinguish pathogenic from nonpathogenic EAEC strains. In addition, molecular biology assays based on virulence factor detection have been widely used to identify and characterize DEC isolates[7]. The use of molecular techniques, and of epidemiological studies on this isolates, to investigate virulence markers in EAEC is important. Studies have been demonstrating that EAEC strains are heterogeneous and complex groups associated with many virulence factors. Different gene targets have been used to detect EAEC by using polymerase chain reaction (PCR). The knowledge about this genes and their function has been making the development of diagnostic methods and the understanding about the pathogenicity of EAEC possible[8].

Some studies adopted the PCR technique to conduct EAEC molecular identification of DEC categories or to assess the presence of specific virulence factors associated with genes such as aggregative adherence fimbriae (AAFs), transcriptional activator (*aggR*)[9], plasmid-encoded toxin (*pet*)[10], Shigella enterotoxin 1 (ShET1)[11], EAEC heat-stable enterotoxin (EAST1)[12], mucinase activity (Pic)[11], secreted proteins (aap)[13], dispersin transporter (aatA)[14], and yersiniabactin system (irp2)[15] associated with genes in EAEC. The *aggR* gene is important for the pathogenesis and adherence properties of EAEC; moreover, the presence or absence of *aggR* is used to classify EAEC as typical or atypical, respectively.

The biofilm formation has been linked to several human diseases. Biofilms are highly organized communities of microorganisms structured within an array of exopolysaccharides (EPS)[16]. The bacterial arranged in biofilms tend to be more resistant to antimicrobial therapy, and the ability to biofilm formation in combination with the heterogeneity of virulence genes have been evidenced in EAEC strains[17].

In addition to mechanisms directly related to virulence, the antimicrobial resistance has been identified through clinical and non-clinical EAEC sources. The antimicrobial resistance has been seen as one of the most important factors to help assessing the impact this pathogen has on public health, and EAEC associated with multidrug resistant (MDR) was reported from different parts of the world[19]. MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories[19].

Since EAEC is one of the main agents causing diarrhea, mainly in developing countries, it is worth conducting characterization and epidemiological surveillance studies, mainly in Northern Brazil, where public and environmental health is directly associated with poverty. Porto Velho is the capital of Rondônia State (Western Brazilian Amazon); the city is among the worst capitals when it comes to basic sanitation. Such poor sanitation condition has strong impact on local health indicators, which depict the epidemiological scenario in the region, as well as the high incidence of diarrheal diseases and infant mortality rates. Given the need of setting the profile of aetiological agents causing acute gastroenteritis, the aim of the present study was to characterize EAEC strains isolated from faecal samples and to identify genes potentially contributing to virulence, biofilm production and antimicrobial resistance in children admitted to a pediatric hospital in Porto Velho, Rondônia.

METHODS

Study site and patients

The total of 1,625 *E. coli* specimens were isolated from 591 children in the age group 6 years or younger who were hospitalized in Cosme and Damião Children Hospital in Porto Velho, between February 2010 and February 2012, with acute gastroenteritis. Cases were defined as acute gastroenteritis when patients presented liquid or semi-liquid stools, and three or more evacuations within 24-h periods. Sample collection was carried out three times a week, for two consecutive years. One fecal sample was collected from each child participating in the experiment. A sterile universal collector was used during the collection procedure. The samples were registered, labeled and stored at −80ºC. The experiment was approved by the Ethical Committee of Rondônia Tropical Medicine Research Centre (protocol N. 0113/2010).

Bacteriology

E. coli strains were selected from MacConkey, Salmonella-Shigella and xylose lysine deoxycholate selective agar provided by HiMedia U.S.A selective agar. All colonies were processed through routine microbiological and biochemical tests purchased at bioMérieux France (API20E system). Five colonies suggestive of *E. coli* were subjected to PCR testing in order to identify the virulence factors.

HEp-2 adherence test

All *E. coli* isolates were subjected to HEp-2 adherence tests[20]. The EAEC 042 was used as aggregative adherence positive control.

Analysis of *E. coli* virulence factors through multiplex polymerase chain reaction (PCR)

The EAEC virulence factors *aggR, astA*, and *pic* were identified through PCR by using specific primers as previously described by Müller et al.[18]. The *aatA, shet1A, shf, irp2*, and *pet* virulence factors were identified according to the method described by Mohamed et al.[17].The EAEC 042 was used as positive control. Non-pathogenic *E. coli* strain HB101 was used as negative control, as well as to monitor PCR contamination.

Biofilm detection through spectrophotometry

The 96-well polystyrene microtiter plates were used to detect biofilms on polystyrene, according to previously described procedures[21]. The following strains were used to assure the quality of the biofilm assay: *Pseudomonas aeruginosa* PAOI, EAEC 042 – which is a strong biofilm producer, and the non-pathogenic *E. coli* strain HB101 (negative control).

Antimicrobial sensitivity test

Antimicrobial susceptibility tests were conducted in Mueller-Hinton agar (HiMedia U.S.A) through the disk diffusion method, according to Clinical and Laboratory Standards Institute guidelines[22]. Gentamicin (GEN 10 µg), imipenem (IMP 10 µg), piperacillin/tazobactam (TZP 10 µg), tetracycline (TET 30 µg), trimethoprim/sulfamethoxazole (SXT 25 µg), amoxicillin/clavulanic acid (AMC 30 µg), amikacin (AMI 30 µg), ampicillin (AMP 10 µg), cefotaxime (CTX 30 µg), and ceftazidime (CFZ 30 µg) antibiotic disks (Sensifar-cefar®, Brazil) were used. The *E. coli* strain ATCC 25922 was used for quality control in all tests.

Statistical analysis

Data were analyzed through Fisher's exact test conducted in GraphPad Prism 5.0. Results were significant at *P*-values < 0.05.

RESULTS

Pathogens associated with diarrhea

Pathogenic agent isolates analyzed in the study were taken from a study conducted from February 2010 to February 2012 in Porto Velho, Rondonia, Brazil. The mentioned study examined 591 stool samples from children with diarrhea. Diarrheogenic *E. coli* was found in 27.4% (162/591) of the children. EAEC was the DEC most frequently associated with diarrhea – 52.4% (85/162), which was followed by EPEC 43.8% (71/162), ETEC 2.4% (4/162), and EHEC 1.2% (2/162). Among other enteropathogenic bacteria, *Salmonella* sp was found in 7.1% (42/591) cases and *Shigella* species were recorded in 2.1% (13/591) of the cases.

The mean age of the patients was 17.2 months; nonetheless, this pathotype incidence was higher in children under 2 years old, although there was not statistically significant difference.

The total of 85 EAEC isolates (all of them presenting aggregative adherence pattern) from 51 patients were identified through cell assays in order to characterize cellular adhesion. All EAEC strains were subjected to the PCR technique in order to find the virulence factors. Infection caused by EAEC was detected in both male 60.8% (31/51) and female 39.2% (20/51) patients. Typical EAEC (*aggR*-positive) was found in 63,5 % (54/85) and atypical EAEC (*aggR*-negative) in 36.5% (31/85) of the strains (TABLE1).

TABLE 1. Distribution of epidemiological factors among children infected by EAEC.

	Age (in months)				
	0-6 (n = 134)	7-12 (n = 162)	13-24 (n = 194)	>25 (n = 101)	*P*
Gender					
Male	75 (56)	92 (56.8)	102 (52.6)	56 (55.4)	0.6475
Female	59 (44)	70 (43.2)	92 (47.4)	45 (44.6)	
EAEC	17 (12.7)	23 (14.2)	36 (18.6)	9 (8.9)	
Typical	12 (70.6)	17 (73.9)	20 (55.6)	5 (55.6)	0.4452
Atypical	5 (29.4)	6 (26.1)	16 (44.4)	4 (44.4)	
Other enteropathogens					
EPEC	14 (10.4)	18 (11.1)	26 (13.4)	13 (12.9)	0.8351
EHEC	1 (0.7)	0 (0)	1 (0.5)	0 (0)	-
ETEC	1 (0.7)	0 (0)	2 (1)	1 (1)	-
Salmonella spp	2 (1.5)	4 (2.5)	6 (3.1)	1 (1)	0.621
Shigella spp	7 (5.2)	12 (7.4)	16 (8.2)	7 (6.9)	0.7701

EAEC: enteroaggrevative *E. coli*; EPEC: enteropathogenic *E. coli*; EHEC: enterohemorrhagic *E. coli*; ETEC: enterotoxigenic *E. coli*.

All children participating in the study presented typical enteropathogen infection symptoms including diarrhea, vomiting and fever. Bloody diarrhea was recorded in 23.5% (12/51) of the cases and EAEC was the only pathogen detected in 58.3% (7/12) of these cases.

Prevalence of virulence genes

One or more virulence marker genes were detected in all EAEC isolates. The *irp2* gene was the most commonly identified one, since it was recorded in 75.3% (64/85) of the isolates; it was followed by *astA*, *aggR*, and *aatA,* which were found in 64.7%, (55/85), 63.5% (54/85), and 48.2% (41/85) of the isolates, respectively. In addition, many other genes were involved in EAEC pathogenesis, including *set1A, afa1, shf, pic*, and *pet,* these genes were more often found in typical EAEC strains than in atypical EAEC strains. There was statistically significant correlation between typical EAEC and the presence of *aatA* (*P*<0.0001), *irp2* (*P*=0.0357) or *shf* (*P*=0.0328) genes (TABLE 2).

TABLE 2. Incidence of virulence genes in typical and atypical enteroaggrevative *E. coli* strains.

Virulence factors	Typical n (%)	Atypical n (%)	*P*-value	OR (IC 95%)
set1A	19 (35.2)	14 (45.2)	0.4883	0.66 (0.24–1.8)
astA	37 (68.5)	18 (58.1)	0.3548	1.56 (0.57–4.31)
afa1	19 (35.2)	15 (48.4)	0.2574	0.58 (0.21–1.57)
aatA	36 (66.7)	5 (16.1)	<0.0001	10.08 (3.13–39.43)
Pet	7 (13)	1 (3.2)	0.2483	4.41 (0.52–207.77)
irp2	45 (83.3)	19 (61.3)	0.0357	3.11 (1.01–9.94)
Shf	24 (44.4)	6 (19.4)	0.0328	3.29 (1.08–11.42)
Pic	15 (27.8)	7 (22.6)	0.7975	1.31 (0.43–4.39)
biofilm	43 (79.6)	16 (51.6)	0.0134	3,603 (1.253–10.789)

Association with biofilm production

Of the 85 analyzed EAEC strains, 69.4% (59/85) were biofilm producers; therefore, there was statistically significant relation between biofilm production and the presence of *aggR* gene (TABLE 2). It is worth highlighting that 73% (43/59) of the biofilm producers had the *aggR*, whereas this gene frequency in non-producer strains was 42.3% (11/26; *P*=0.0135 [OR=3.665; IC95%=1.393 to 9.639]). Similarly, there was association between the presence of *aatA* gene and biofilm producers (61% [36/59]), versus 19.2% (5/26) association in non-producers; *P*=0.0004 [OR=6.574; IC95% 2.173 to 19.89]. No correlation was observed between biofilm production and the presence of other virulence genes such as *set1A*, *afa1*, *irp2*, *pic, astA, pet*, and *shf* (FIGURE 1).

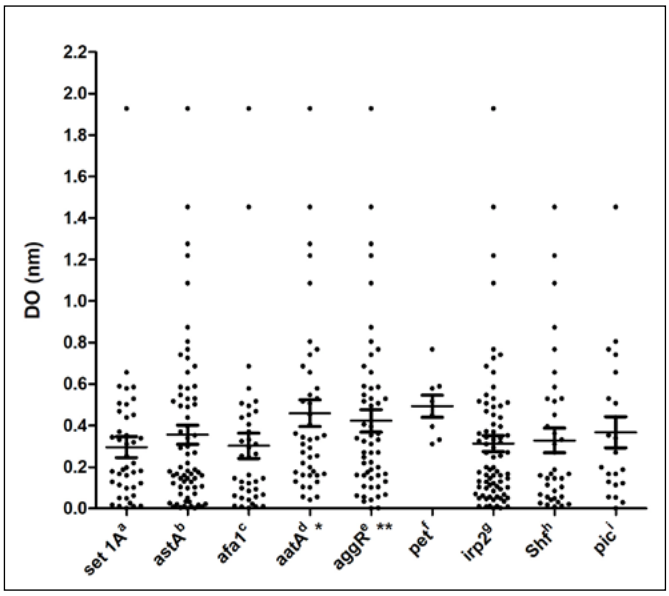

FIGURE 1. Biofilm formation and presence of virulence genes in EAEC isolates among diarrheal children. [a] shigella enterotoxin 1; [b] enteroaggregative heat-stable enterotoxin 1; [c] aggregative adherence; factor; [d] dispersin transporter; [e] transcriptional activator; [f] plasmid encoded toxins; [g] yersiniabactin biosynthesis; [h] cryptic open reading frame; [i] mucinase activity genes. *P* values represent correlation between biofilm production and virulence genes in EAEC: **P*=0.0004; ***P*=0.0135 (Fisher's exact test).

Antibiotic sensitivity in EAEC isolates

All 85 EAEC isolates were tested against 10 different antibiotics; the susceptibility profiles are shown in TABLE 3. At least one strain resistant to each of the tested antibiotics was identified. Results evidenced that most EAEC isolates were resistant to AMP 70.6% (60/85), SXT 60% (51/85), TET 44.7% (38/85), and CTX 22.3% (19/85). Multidrug resistance (MDR) was detected in 36.5% (31/85) of the tested strains. The MDR strains were resistant to antibiotics belonging to the most common chemotherapy classes, including AMP 96.8% (30/31), SXT 96.8% (30/31), TET 61.3% (19/31), CTX 54.8% (17/31).

TABLE 3. Antimicrobial profiles of EAEC: enteroaggrevative *E. coli* isolates.

Antibiotic	Sensitive n (%)	Resistant n (%)
Amoxicilin/clavulanic acid (AMC)	61 (71.8)	24 (28.2)
Amikacin (AMI)	83 (97.6)	2 (2.4)
Ampicillin (AMP)	25 (29.4)	60 (70.6)
Cefotaxime (CTX)	66 (77.6)	19 (22.3)
Ceftazime (CRX)	70 (82.4)	15 (17.6)
Gentamicin (GEN)	72 (84.7)	13 (15.3)
Imipenem (IPM)	80 (94.1)	5 (5.9)
Piperacillin/tazobactam (PPT)	75 (88.2)	10 (11.8)
Tetracycline (TET)	47 (55.3)	38 (44.7)
Sulfamethoxazole (STX)	34 (40)	51 (60)

DISCUSSION

Studies about the interaction and importance of different pathogenic *E. coli* strains associated with acute diarrhea in Brazil have highlighted EPEC as the major etiologic agent of infant diarrhea[23,24]. However, studies have been reporting EAEC as an emerging enteric pathogen that causes persistent diarrhea and malnutrition in children living in developed countries[25].

With regard to the current study, 8.6% (51/591) of childhood gastrointestinal singular infection or co-infection by other enteropathogens could be attributed to EAEC. Studies conducted abroad reported EAEC infection prevalence from 2% to 24%[26,27]. Previous studies carried out in Brazil showed that 0.5% to 41% of acute gastroenteritis cases requiring hospitalization were caused by this pathotype[28-30]. The high incidence of diarrhea cases in developing countries can be attributed to standard fecal-oral contamination routes resulting from deficient sanitary infrastructure, low education level, nutritional deficiency and inappropriate personal and food-related hygiene practices. Such incidence results in high economic burden for national public health systems[31,32]. It is worth emphasizing that patients in the current study presented critical social patterns related to low per capita income and untreated water intake.

Diarrheagenic *E. coli* infections in the current study most affected children in the age group 2 years or younger. Besides diarrhea, the major clinical symptoms associated with DEC among the assessed children were vomiting and fever. This outcome is consistent with reports from India[33], Tanzania[34], Libya[35], Cambodia[36], Panama[37], and Israel[38].

There are just few reports about bloody diarrhea cases associated with EAEC and they rarely concern children[5,33]. With regard to the current study, bloody diarrhea was observed in 23.5% (12/51) of the assessed cases and EAEC was identified as the only pathogen in 58.3% (7/12) of them; however, other studies have reported conflicting results. A study conducted in Nigeria did not identify bloody stool as symptom of childhood EAEC infection[26]. The prevalent virulence genes in the herein analyzed isolates were *irp2*, in 75.3% (64/85) of the cases; *astA*, in 64.7% (55/85); and *aggR*, in 63.5% (54/85). Previous studies have reported *irp*2 genes in EAEC samples but it was rarely found in EPEC, enteroinvasive *E. coli* and ETEC, besides being absent in EHEC, *Shigella* and *Salmonella enterica*[17,39]. The *irp2* gene is part of the High-pathogenicity island found in the chromosome of Yersinia species (*Y. pestis*, *Y. pseudotuberculosis* sorotype O1 and *Y.enterocolitica* biotype 1B) involved in iron uptake, which is mediated by the siderophore yersiniabactin and found in EAEC 042.

Elias et al., 2002, used the EAEC probe and found that *irp2* was the second most prevalent marker (91.4%) among the 70 EAEC probe + strains[40]. There was statistically significant association between the typical EAEC *aatA* (*P*<0.0001) and *irp2* (*P*=0.0357) genes. Patzi-Vargas conducted a study about DEC carrying supplementary virulence genes; he showed that the *aatA* genes are significantly more common in EAEC isolates than in non-DEC strains[25]. Tokuda et al[41] showed that *aatA* and *irp2* genes are more prevalent in typical EAEC isolates than in atypical ones. Results in the present study evidenced a whole variety of virulence genes combinations in typical EAECs, fact that was not so common in atypical EAECs.

Some researchers have assumed that several virulence gene combinations can be directly associated with diarrhea or with strain virulence[35,42].

Pathogenic implications of *aggR* gene have been assessed in a number of studies worldwide[17,41]. Interestingly, current results showed that children over one month old presented greater risk of being infected by typical than by atypical EAEC (P=0.0155). These findings suggest that children have greater probability of being exposed to the most virulent strains as they age.

Andrade and collaborators showed that EAEC strains found in small intestine and colon mucosa produce large amounts of biofilm and cause changes in the epithelia, fact that possibly explains the long duration of diarrheal episodes[43]. Biofilm production, adhesiveness and other EAEC virulence factors are directly associated with certain genes, including the regulatory gene *aggR*. The *aggR* is one of the most studied genes since it controls the expression of other genes related to EAEC pathogenesis. The presence of *aggR* in the present study was indeed statistically correlated with biofilm production (P=0.0135); this finding corroborates findings reported in studies conducted in Mexico and Mongolia, which have also indicated that the presence of *aggR* gene is associated with biofilm formation[44,45]. Although it was possible recording significant association between the presence of *aatA* gene and biofilm production (P=0.0004), the *shf* gene and biofilm production were not correlated to each other. This results were different from those evidenced in a previous study that correlated *shf* with biofilm formation, even when it was associated with *aggR*[46].

Antimicrobial resistance, in EAEC populations, mainly MDR, has been reported in several studies[47,48]. Results in the present study evidenced greater EAEC resistance to AMP, SXT, TET and CTX than to other antibiotics. The resistance to these antibiotics was expected since they are the low-cost chemotherapeutic drugs of choice in many studies[49]. Antibiotics, mainly β-lactams, are often used to treat DEC infections, mainly in persistent diarrhea cases when the infectious agent is not identified, although these infections are self-limiting and only require oral-rehydration therapy. Most of the herein tested EAECs showed MDR phenotype, and this result is consistent with other studies. The most common MDR phenotypes recorded in the current study included resistance to β-lactams, TET, aminoglycosides, SXT, and GEN. There was no association between antimicrobial resistance and biofilm production, although this correlation was previously described by other researchers[50].

As far as it is known, the present study is pioneer in showing the pathogenic potential and heterogeneity of virulence EAEC genes and co-infections caused by other enteric pathogens in children with gastroenteritis living in Rondônia. Data presented in the current study may contribute to the better understanding about the role played by EAEC in children with diarrheal illnesses living in Porto Velho. The results may help developing strategic plans to control antimicrobial resistance in these poor regions.

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Authors' contribution

Taborda RLM, Silva LA, and Rodrigues RS: collected data for the study, and carrying out the cell adhesion assays and biofilm formation. Batista FS, and Orlandi PP: reviewers of the current article. Matos NB: design and execution of the project.

Taborda RLM, Silva LA, Orlandi PP, Batista FS, Rodrigues RS, Matos NB. Caracterização de *Escherichia coli* enteroagregativa entre crianças com diarreia na Amazônia ocidental brasileira. Arq Gastroenterol. 2018;55(4):390-6.

RESUMO – **Contexto** – A *Escherichia coli* enteroagregativa (EAEC) é um dos principais agentes causadores de diarreia aguda e crônica em crianças e adultos, principalmente em países em desenvolvimento. **Objetivo** – Caracterizar cepas de EAEC isoladas de amostras fecais e identificar genes que potencialmente contribuem para a virulência, produção de biofilme e resistência antimicrobiana em crianças internadas em um hospital pediátrico em Porto Velho, Rondônia. **Métodos** – Um total de 1.625 cepas de *E. coli* foram isolados de 591 crianças com gastroenterite aguda na faixa etária de 6 anos que foram internadas no Hospital Infantil Cosme e Damião na cidade de Porto Velho, entre fevereiro de 2010 e fevereiro de 2012. Colônias sugestivas de *E. coli* foram submetidas a reação em cadeia da polimerase para identificação de fatores de virulência. O ensaio de adesão *in vitro* foi desenvolvido com célula HEp-2. A detecção de biofilme foi realizada através do teste de espectrofotometria e os testes de susceptibilidade aos antimicrobiana foram realizados através do método de difusão em disco. **Resultados** – A *E. coli* diarreiogênica foi encontrada em 27,4% (162/591) das crianças e a EAEC foi a *E. coli* diarreiogênica mais frequentemente associada à diarreia com 52,4% (85/162), seguida pela *E. coli* enteropatogênica 43,8% (71/162), *E. coli* enterotoxigênica 2,4% (4/162) e *E. coli* enterohemorrágica 1,2% (2/162). O gene *aggR* foi detectado em 63,5% (54/85) dos isolados de EAEC com correlação estatisticamente significante entre esse gene com os genes *aatA* (P<0,0001), *irp2* (P=0,0357) e *shf* (P=0,0328). Neste estudo 69% (59/85) das cepas de EAEC eram produtoras de biofilme, destas 73% (43/59) possuíam o gene *aggR*, ao passo que entre as não produtoras 42,3% (11/26) possuíam o gene (P=0,0135). Essa associação também foi observada com o gene *aatA*, presente em 61% (36/59) das cepas produtoras e em 19,2% (5/26) das não produtoras (P<0,0004). O teste de sensibilidade aos antibimicrobianos evidenciou que a maioria das EAEC eram resistentes a ampicilina 70,6% (60/85), ao sulfametoxazol 60% (51/85), a tetraciclina 44,7% (38/85) e a cefotaxima 22,4% (19/85). **Conclusão** – Este é o primeiro estudo no Norte do Brasil sobre a investigação dos fatores de virulência de EAEC mostrando a susceptibilidade antimicrobiana de cepas de EAEC isoladas de crianças com diarreia.

DESCRITORES – *Escherichia coli*, classificação. Diarreia infantil. Criança. Virulência, genética.

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ORIGINAL ARTICLE

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Malnutrition and clinical outcomes in surgical patients with colorectal disease

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ABSTRACT – **Background** – Malnutrition is a frequent condition among hospitalized patients and a factor of increased risk of postoperative complication. **Objective** – This study aimed to evaluate the impact of malnutrition on phase angle (PA), body water distribution and clinical outcomes in surgical patients with colorectal disease. **Methods** – This retrospective study was performed in a tertiary hospital with 40 patients admitted electively. In the preoperative evaluation, global subjective assessment and bioelectrical impedance analysis were performed to determine nutritional status, PA, extracellular water (ECW), intracellular water (ICW) and total body water (TBW). In postoperative evaluation, the length of hospital stay and severe complications, according to Clavien-Dindo classification, were determined. The optimal PA cutoff for malnutrition screening was determined by ROC curve analysis. **Results** – Seventeen (42.5%) patients were diagnosed as malnourished and 23 (57.5%) as well-nourished according to global subjective assessment. Twelve (30.0%) patients developed severe complications. The malnourished group presented lower values of serum albumin (P=0.012), hematocrit (P=0.026) and PA (P=0.002); meanwhile, ECW/ICW (P=0.019) and ECW/TBW (P=0.047) were higher. Furthermore, 58.8% of malnourished patients developed severe postoperative complications compared to 8.7% of well-nourished. Malnutrition was independent predictor of severe postoperative complications (OR=15.00, IC: 2.63-85.68, P=0.002). The optimal PA cutoff obtained was 6.0º (AUC=0.82, P=0.001), yielding sensitivity, specificity, positive predictive value and negative predictive value of 76.5%, 87.0%, 81.3% and 83.4%, respectively. **Conclusion** – Malnutrition was an independent predictive factor for severe complications in patients underwent to elective major coloproctological surgery. Besides that, malnutrition was associated with lower PA values and greater ratio of ECW. The PA provided great accuracy in nutritional screening, implying a useful marker of malnutrition.

HEADINGS – Malnutrition. Electric impedance. Nutrition Assessment. Colorectal Surgery.

INTRODUCTION

Malnutrition is one of the main comorbidities of surgical patient. According to a systematic review published in 2017 by Correia et al.[1], the prevalence of hospital malnutrition in Latin America ranged from 2.6% to 73.2%. High rates of malnutrition were observed in patients who underwent gastrointestinal surgery on admission. Surgical trauma itself triggers a sequence of inflammatory events related to the metabolic response to trauma[2]. Consequently, pre-existing malnutrition associated with trauma sets negative factors in postoperative evolution, predisposing patients to greater morbidity and mortality rates[3]. Several studies have shown worse clinical outcomes in malnourished patients who underwent surgical procedure[4-6].

Despite the variety of instruments for assessing nutritional status, there is no gold standard method. However, American Society for Parenteral and Enteral Nutrition (ASPEN) and Brazilian Nutrition Association (ABRAN) recommend using Global Subjective Assessment (SGA) as a nutritional screening tool, due to its good reproducibility, easy execution and high correlation with anthropometric and biochemical parameters[7-9].

Bioelectrical impedance analysis (BIA) is a safe technique that estimates indirectly body composition by applying painless electric current. BIA-derived phase angle (PA) is an important parameter obtained from the result of the arctangent of reactance by resistance. Some authors have affirmed that PA reflects nutritional status and acts as indicator of prognosis in clinical practice, because PA is influenced by the degree of hydration and amount of intact cell membrane[10-12]. Therefore, both malnutrition and inflammatory processes interfere in final PA value – the lower PA values, the greater the severity of the underlying disease[13,14].

In this context, this study aims to evaluate the impact of malnutrition on PA values, body water distribution, length of hospital stay and severe postoperative complications in patients with colorectal disease who underwent to elective major surgery at tertiary university hospital.

METHODS

Setting and patients

This retrospective and analytical cross-sectional study was approved by the Research Ethics Committee and was performed with

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40 patients admitted to tertiary university hospital (Base Hospital of São José do Rio Preto – SP – Brazil) during 2016 to 2018. All patients signed informed consent form beforehand. The inclusion criteria consisted of patients older than 18 years and younger than 80 years, hospitalized for elective abdominal major surgery via laparotomy or videolaparoscopy and who presented status I, II or III in the American Society of Anesthesiologist (ASA) system. The exclusion criteria were age less than 18 years or older than 80 years, patients with emergency operation indication or minor surgery, presence of limb amputation, impossibility to remain in the supine position or to communicate.

Preoperative evaluation

Patients were evaluated within 24 hours of hospital admission. The nutritional assessment was performed by a trained nutritionist. Initially, anthropometric data (weight and height) were obtained, followed by SGA application, which included weight changes in the last six months, functional capacity, gastrointestinal symptoms and clinical signs of malnutrition assessed by physical examination. From this data, patients were classified as well-nourished (A), mildly or moderately malnourished (B) and severely malnourished (C). For statistical purposes, patients were grouped into well-nourished (SGA: A) and malnourished (SGA: B + C).

BIA was performed with a portable analyzer (Biodynamics Model 450), which applies a painless electrical current of 800μA with a single frequency of 50 kHz. Patients were previously informed about the preparations for performing BIA according to all manufacturer's recommendations. The BIA-derived intracellular water (ICW), extracellular water (ECW) and total body water (TBW) were obtained from regression equations performed by the analyzer.

Biochemical data (hematocrit and serum albumin) were collected from electronic medical records according to the protocol established by the medical group.

Postoperative evaluation

The length of hospital stay was counted from admission to discharge. Postoperative outcomes were assessed according to Clavien-Dindo classification (CDC) of surgical complications. It is based on the type of therapy needed to correct the complication. All patients with grades III to V in the CDC were considered to have severe complication[15,16]. Grade III includes patients requiring surgical, endoscopic or radiological intervention; grade IV includes presence of one or more organic dysfunctions, requiring intensive care; and grade V includes deaths. The organic dysfunctions were evaluated by SOFA-score that is a tool commonly used in our service and was considered only new organic dysfunctions when SOFA-score ≥2[17].

Statistical analysis

Variables were expressed as mean (SD), number (%) or median (interquartile range) where appropriate. Inferential analyzes and binary logistic regression were performed using *SPSS IBM 14*. The Shapiro-Wilk test for normality was used and Student's t-test was applied to compare two parametric continuous variables, while Mann-Whitney test was performed in non-parametric samples. To determine the relationship between malnutrition and postoperative complications, Fisher's exact test was used for two qualitative variables. To obtain optimal PA cutoff value for malnutrition screening, ROC curve was performed using *BioEstat 5.0* software. For all analyzes, $P<0.05$ was considered significant.

RESULTS

The study included 40 patients with a mean age of 59.4±12.3 years of which 52.5% of patients were female. In the sample, 33 (82.5%) patients were diagnosed with colorectal cancer, 03 (7.5%) with Crohn's disease, 03 (7.5%) with diverticular disease and 01 (2.5%) with familial adenomatous polyposis (TABLE 1).

TABLE 1. Patient characteristics (n = 40).

Variable	M ± SD or N (%) or m (IQR: Q3–Q1)
Age (years)	59.4 ± 12.3
Gender	
Male	19 (47.5)
Female	21 (52.5)
Diagnosis	
Neoplasm	33 (82.5)
Crohn's disease	03 (7.5)
Diverticular disease	03 (7.5)
Familial adenomatous polyposis	01 (2.5)
SGA	
A	23 (57.5)
B or C	17 (42.5)
Clinical outcomes	
Length of hospital stay (days)	04 (4 - 6)
Severe complications*	12 (30.0)
Death	02 (5.0)

M: mean; SD: standard deviation; N: number; m: median; IQR: interquartile range; Q1: first quartile; Q3: third quartile; SGA: Subjective Global Assessment. *Degree III to V of Clavien-Dindo classification.

According to the SGA, 17 (42.5%) patients were diagnosed as moderately (SGA: B) or severely (SGA: C) malnourished and 23 (57.5%) as well-nourished (SGA: A) during preoperative period. In the postoperative course, 12 (30.0%) individuals developed severe complications (grade III, IV or V) that caused the death of 02 (5.0%) of them (TABLE 1).

In TABLE 2, patients were categorized into two groups according to the nutritional diagnosis by SGA. Malnourished group presented lower values of serum albumin (P=0.012), hematocrit (P=0.026) and PA (P=0.002); meanwhile, ECW/ICW (P=0.019) and ECW/TBW (P=0.047) were higher. Although malnourished patients showed higher median length of hospital stay than well-nourished group, there was no statistical difference between these two nutritional status (P=0.051).

TABLE 3 shows that 58.8% of malnourished patients developed severe postoperative complications compared to 8.7% of well-nourished individuals. The chance of severe complication in malnourished patients was at least 160% higher than in the well-nourished group (P=0.001). Furthermore, using logistic regression model, malnutrition diagnosed by SGA was an independent predictor of severe postoperative complications (P=0.002) (TABLE 4).

TABLE 2. Anthropometric, biochemical, body composition data and length of hospital stay between different nutritional status groups according to the Subjective Global Assessment.

Variable	Well-nourished A (n=23)	Malnourished B + C (n=17)	*P* value
	M ± DP or m (IQR: Q3–Q1)		
Weight (kg)	74.7 ± 12.2	65.4 ± 13.3	0.077
BMI (kg/m^2)	28.0 ± 3.7	26.1 ± 5.0	0.328
Albumin (g/dl) #	4.2 (4.6–4.0)	3.9 (3.6–4.2)	0.012*
Hematocrit (%)	37.7 ± 4.8	34.7 ± 4.1	0.026*
PA (º)	6.5 ± 0.7	5.5 ± 0.9	0.002*
Resistance (ohm)	512.9 ± 82.6	550.8 ± 97.4	0.213
Reactance (ohm)	58.1 ± 9.7	52.7 ± 11.9	0.177
Fat free mass (%)	32.3 ± 3.9	29.8 ± 3.8	0.092
ECW/TBW (%)	46.3 ± 3.5	49.3 ± 3.8	0.047*
ECW/ICW #	0.8 (0.8–1.0)	1.0 (0.9–1.0)	0.019*
Length of hospital stay (days) #	4.0 (3.0–5.0)	5.0 (4.0–10.0)	0.051

M: mean; SD: standard deviation; N: number; m: median; IQR: interquartile range: Q1: first quartile; Q3: third quartile. #Non-parametric data. PA: phase angle; ECW: extracellular water; TBW: total body water; ICW: intracellular water. *Significant difference (P<0.05).

TABLE 3. Frequency of severe postoperative complications among well-nourished and malnourished patients.

	Severe postoperative complications N (%)		Total	OR (95% CI)
	Yes	No		
Malnourished	10 (58.8)	07 (41.2)	17 (100)	15.0 (2.6–86.7)
Well-nourished	02 (8.7)	21 (91.3)	23 (100)	P=0.001*
Total	12 (30.0)	28 (70.0)	40 (100)	

OR: odds ratio; CI: confidence interval. *Significant result (P<0.05) according to Fisher's exact test.

TABLE 4. Logistic regression analysis for severe postoperative complications.

Variable	B (coefficient)	Exp (B)	95% CI for Exp(B)	*P* value
Malnourished (SGA)	2.708	15.000	2.626–85.681	0.002
Constant	-2.351	0.095		0.001

SGA = Subjective Global Assessment. CI = confidence interval.

The ROC curve-derived optimal PA cutoff value obtained was 6.0º, yielding a sensitivity, specificity, positive predictive value and negative predictive value of 76.5%, 87.0%, 81.3% and 83.4%, respectively. The area under the curve still showed great accuracy in discriminating between well-nourished and malnourished patients (AUC=0.82, P=0.001) (TABLE 5).

TABLE 5. ROC curve-derived optimum phase angle value for malnutrition screening.

	AUC (95% CI)	*Cut-off*	S (%)	E (%)	PPV (%)	NPV (%)	*P* value
Phase angle	0.82 (0.65–0.95)	6.0º	76.5	87.0	81.3	83.4	0.001

AUC: area under curve; S: sensibility; E: specificity; PPV: positive predictive value; NPV: negative predictive value.

DISCUSSION

Malnutrition and postoperative complications

In the present study, 42.5% of patients presented malnutrition on admission. Most of the hospitalized patients had colorectal cancer, justifying the high prevalence of malnutrition and the need for nutritional screening on hospital admission[2,4,18]. Correia et al.[1] identified nine Brazilian studies that performed SGA to determine the prevalence of hospital malnutrition in surgical patients between 1994 and 2014. The authors found 17.6% to 66.0% of malnutrition prevalence mainly in patients who underwent gastrointestinal resection or colon surgeries. This wide prevalence is related to multiple factors involved in the development of malnutrition, such as, previous socioeconomic status, comorbidities, disease progression, previous chemotherapy or radiotherapy treatment and degree of hospital complexity.

In our study, malnourished patients presented lower values of albumin and hematocrit in comparison with well-nourished group, resulting in less physiological reserve to metabolic response to trauma and worse clinical evolution than malnourished group[19,20]. Some authors reported that serum albumin level less than 4.0 to 4.5 g/dL is associated with increase in number of complication cases, length of hospital stay and postoperative mortality after intestinal resections due to dysfunction in collagen synthesis and formation of granulation tissue that impair the tissue healing[21,22]. The median level of albumin was less than 4.0 g/dL in the malnourished group. This result warned us about the impairment healing process and postoperative recovery in this nutritional status.

Several studies have already shown that malnutrition increases the risk of postoperative complications, length of hospital stay, hospital costs and mortality[1,23,24]. In this context, our results showed high prevalence of severe postoperative complications (30.0%), predominantly in the malnourished group. Maurício et al.[25] evaluated patients who underwent elective surgery for colorectal cancer and identified similar rate of postoperative complications (33.3%), but their results are overestimated because the authors considered complication grades II to V in the CDC. However, using the same criteria for severe postoperative complications as our study, Mosquera et al.[4] found 15.0% of complications in patients who have underwent major elective gastrointestinal surgery. Whereas, Härter et al.[14] obtained 18.7% of outcomes after oncologic surgery. These two studies identified higher frequency and severity of complications in malnourished patients than in well-nourished, as observed in our study, which the odds was at least 1.6 times greater to develop severe postoperative complications compared to well-nourished individuals.

Furthermore, according to logistic regression, it was verified that malnutrition was an independent predictor factor for the development of severe postoperative complications, as well as observed by other authors[4,10,25]. Although the malnourished group did not show a longer hospital stay compared to the well-nourished group, the worst clinical outcome occurred in two malnourished patients who died during hospitalization.

Bioelectrical impedance analysis: phase angle and body water

The resistance and reactance were possible using BIA. These two variables were directly detected by the analyzer without performing regression equations[26]. Higher amount of intact cell membrane indicates greater cellularity and so higher reactance and PA values. Therefore, PA is an indirect marker of membrane integrity and cellular vitality. Certain conditions such as malnutrition and inflammatory processes alter the electrical properties of cells and result in decrease of PA[10]. As our sample consisted of patients living with consumptive or chronic inflammatory diseases, it was expected reduction in PA in comparison to healthy individuals, especially because cellular catabolism and inflammatory process are intensified in these clinical conditions[27]. These metabolic alterations trigger higher consumption of body cell mass and, consequently, lead to decrease in cellularity. In our study, there was decrease in the mean PA among malnourished patients compared to well-nourished group. These results are in accordance with other authors[5,24], including Gupta et al.[28] that followed hospitalized individuals with malnutrition and colorectal cancer.

In the literature, there is no well-established PA cutoff value to determine the nutritional status. The cutoff varies according to ethnic factors, gender, age, weight, length and morbid conditions[10]. In order to use PA as a marker of malnutrition, the characteristics of the ROC curve were analyzed and an optimum cut-off was estimated (PA=6.0º). Therefore, PA <6.0º showed an accuracy of 82.5% to identify malnourished patients. (FIGURE 1). This great PA accuracy contributes towards malnutrition screening marker in clinical practice since BIA is a non-invasive tool that is easy and quick to perform. Gupta et al.[28] performed SGA in patients with colorectal cancer and found PA cut-off of 6.0º, yielding 82.2% of sensitivity and 54.5% of specificity. Lukaski et al.[10] found only two studies between 2012 to 2017 that evaluated the optimum PA cutoff to screening malnutrition by SGA. The first study was performed by Kyle et al.[29] who obtained different cutoff for men (5.0º) and women (4.6º), with lower sensitivity and specificity values and accuracy (83%) similar to those found in our study. In the second study[30], sensitivity and specificity were 80.0% and 56.7%, respectively, using PA cutoff of 4.7º. Therefore, although there are no well-established cutoff values, it is expected that more studies will define cutoff values adjusted for each particular clinical scenario.

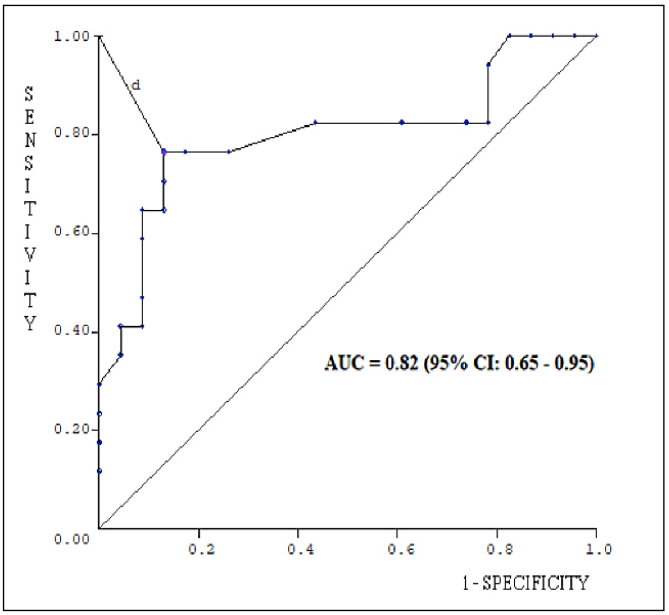

FIGURE 1. The ROC curve of phase angle showing accuracy of 82% for diagnosis of malnutrition in screening nutritional assessment.

In addition, as PA reduction represents loss of membrane integrity, it is possible to infer that cells also become unable to maintain adequate ratios of ICW. This fact allows to excessive displacement of intracellular fluid to the extracellular space and to increase the proportion of ECW/ICW, justifying the highest ECW/ICW value in malnourished group. Changes in cell size, cell permeability and fluid distribution in tissues determine PA values[11]. Although the increase in ECW/ICW and ECW/TBW ratio did not impact the postoperative complications in our study, some authors related increasing ECW to worse outcomes. For example, Ohashi et al.[31] associated increasing ECW/ICW with unfavorable clinical outcomes in malnourished elderly patients with chronic kidney disease[32]. In Korea, Lee et al.[33] followed severely malnourished patients at intensive care unit and found significant fluid imbalance characterized by higher proportion of ECW/TBW compared to well-nourished individuals; and the increase in this ratio was higher in patients who died.

CONCLUSION

In conclusion, the present study showed high prevalence of malnutrition prior to major surgical procedure and severe postoperative complications, of which malnutrition was independent predictive factor for its occurrence. Our results confirmed that malnutrition is associated with lower values of PA and greater distribution of water in the extracellular compartment. We also revealed that PA presented high accuracy in nutritional screening, implying a useful malnutritional marker.

The limitations of this study include the evaluation of patients from only a single medical center; limited sample size; and heterogeneity of group that consisted of patients with colorectal cancer at different stages of treatment.

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Authors' contribuition

Nishiyama VKG: data collection, survey execution, interpretation of the data and writing of text. Albertini SM: data collection and interpretation of the data. Moraes CMZG: data collection, survey execution and interpretation of the data. Godoy MF: statistical analysis. Netinho JG: conception, design of the research and interpretation of the data.

Nishiyama VKG, Albertini SM, Moraes CMZG, Godoy MF, Netinho JG. Desnutrição e complicações clínicas em pacientes cirúrgicos com doença colorretal. Arq Gastroenterol. 2018;55(4):397-402.

RESUMO – **Contexto** – A desnutrição é uma condição frequente entre pacientes hospitalizados e é um fator de risco para complicações pós-operatórias. **Objetivo** – Este estudo tem como objetivo avaliar o impacto da desnutrição sobre o ângulo de fase (AF), a distribuição de água corporal e complicações clínicas em pacientes cirúrgicos com doença colorretal. **Métodos** – Trata-se de um estudo retrospectivo realizado em um hospital universitário terciário com 40 pacientes admitidos eletivamente. Na avaliação pré-operatória, foram realizadas a avaliação subjetiva global e análise de bioimpedância elétrica com a finalidade de determinarem o estado nutricional, AF, água extracelular (AEC), água intracelular (AIC) e água corporal total (ACT). Na avaliação pós-operatória, o tempo de internação hospitalar e a presença de complicações graves, segundo a classificação de Clavien-Dindo, foram determinados. O melhor ponto de corte do AF para o rastreamento de desnutrição foi obtido a partir da análise da curva ROC. **Resultados** – Dezessete (42,5%) pacientes foram diagnosticados como desnutridos e 23 (57,5%), como bem nutridos de acordo com a avaliação subjetiva global. Doze (30,0%) pacientes desenvolveram complicações pós-operatórias graves. O grupo desnutrido apresentou menores valores de albumina sérica (P=0,012), hematócrito (P=0,026) e AF (P=0,002); enquanto que as relações de AEC/AIC (P=0,019) e AEC/ACT (P=0,047) estiveram elevadas. Além disso, 58,8% dos pacientes desnutridos desenvolveram complicações pós-operatórias graves em comparação a 8,7% dos pacientes bem nutridos. A desnutrição foi fator preditivo independente para o desenvolvimento de complicações pós-operatórias graves (OR=15,00, IC: 2,63-85,68; P=0,002). O melhor ponto de corte do AF obtido foi 6.0º (AUC=0,82; P=0,001) com sensibilidade, especificidade, valor preditivo positivo e valor preditivo negativo de 76,5%, 87,0%, 81,3% e 83,4%, respectivamente. **Conclusão** – A desnutrição foi fator preditivo para o desenvolvimento de complicações graves em pacientes submetidos à cirurgia eletiva coloproctológica de grande porte. Além disso, a desnutrição foi associada a menores valores de AF e maior proporção de AEC. O AF forneceu boa acurácia no rastreamento da desnutrição, sugerindo seu uso como potencial marcador de desnutrição.

DESCRITORES – Desnutrição. Impedância elétrica. Avaliação nutricional. Cirurgia colorretal.

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ORIGINAL ARTICLE

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Profile of patients with chronic hepatitis C in a public health program in Southern Brazil

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ABSTRACT – **Background** – Chronic hepatitis C (CHC) can progress to cirrhosis and its complications as hepatocellular carcinoma, leading to morbidity and mortality. To know the profile of patients with CHC virus is fundamental to optimize management. **Objective** – To describe the profile of patients with CHC in a public health program in Southern Brazil. **Methods** – A retrospective study was carried out in patients with CHC who underwent treatment against hepatitis C virus in a dispensation and pharmaceutical assistance center of the Public Health Department of the State of Rio Grande do Sul, South Brazil. All medical records of patients attended between December/2015 and December/2016 were evaluated. **Results** – A total of 1,431 records of patients with CHC were evaluated. Males were the most prevalent (802; 56%) patients. The mean age was 58.6±9.9 years, ranging from 18 to 89 years. Genotype 1 was the most frequent (866;60.5%) of the patients. Ninety (6.3%) patients were transplanted from a solid organ, and of these, 73 (5.1%) were transplanted from the liver. The fibrosis evaluation was performed in 1,300 (90.8%) patients. Of these, 566 (39.6%) were evaluated through liver biopsy. Regarding the degree of fibrosis, 779 (54.4%) presented fibrosis grade 4 (cirrhosis). The genotype 3 was the most associated with fibrosis grade 4, and genotype 1 was associated with high viral load. **Conclusion** – The present study made possible the evaluation of the characteristics of patients with CHC in a public health program in South Brazil. There was a predominance of CHC in males, and the mean age was 59 years. They presented a predominance of genotype 1, higher viral load in patients with genotype 1 and greater degree of fibrosis in patients with genotype 3.

HEADINGS – Chronic hepatitis C. Epidemiology. Sustained virologic response.

INTRODUCTION

It is estimated that about 115 million people worldwide have anti-HCV positive antibody, corresponding to a world prevalence of 1.6%. The prevalence of truly viraemic people is estimated at 1.1%, equivalent to about 80 million people in the world with chronic hepatitis C (CHC) infection[1].

The CHC is considered to be of low prevalence in many countries as the United Kingdom, Scandinavia, North America, Western Europe, Australia and South Africa (0.2% to 0.5%). On the other hand, the regions most affected are those in the Mediterranean and Eastern Europe, with a prevalence of 2.3% and 1.5%, respectively[1].

In Brazil, approximately 10,000 cases are notified each year. A population-based study on hepatitis A, B and C virus infections in Brazilian capitals found an anti-HCV prevalence of 2.1% in the Northern Region; 0.7% in the Northeast; 1.3% in the Midwest; 1.3% in the Southeast; 1.2% in the Southern Region; and 0.8% in the Federal District[2].

Hepatitis C vírus (HCV) is primarily transmitted parenterally, more often through the sharing of injection material among illicit drug users, due to the reuse or inadequate sterilization of medical equipment in health services, blood transfusions or blood products without analysis[3].

In general, acute infection is asymptomatic and is rarely associated with a potentially fatal disease. Approximately 15% to 40% of infected people acutely eliminate the virus spontaneously within six months, without the need for any treatment. The remaining 60% to 85% will develop chronic infection if not treated, with a risk of developing cirrhosis in 15% to 30% of cases[4].

The treatment of chronic hepatitis C has undergone rapid evolution. Treatment with direct-acting antiviral drugs (DAA) can cure most people infected with HCV in a short-course (usually 12 or 24 weeks), with sustained virological response rates above 90% in most cases[5-7].

In this context, the objective of this study is to describe the characteristics of patients with HCV in a public reference service in Southern Brazil.

METHODS

A descriptive, retrospective study was carried out in all the CHC patients attended at the Hospital Sanatorio Partenon, a dispensing center for medications used to treat CHC in the Public Health system between December 2015 and December 2016, totaling 1,431 individuals. All the patients who accepted to participate in the research voluntarily and signed the Free and Informed Consent Term were included.

The necessary information for the development of the study were collected from patients' charts after approval by the local Research Ethics Committee under number 1,783,047 (October/2016).

The sex, age, HCV genotypes, viral load, different degrees of fibrosis, and liver or kidney transplantation were evaluated.

Declared conflict of interest of all authors: none
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This paper is in accordance with the Declaration of Helsinki, the Universal Declaration on Bioethics and Human Rights, and Resolution 466/12 of the National Health Council of Brazil, approved by the local Research Ethics Committees of the Reference Institutions, respecting the ethical and legal aspects, that regulate the research in Brazil.

The data collected were analyzed in the statistical program Statistical Package for Social Sciences (SPSS®) version 18.0 (SPSS Inc., Chicago, IL). Quantitative variables were presented by mean and standard deviation or median and variation when they were not normally distributed, and were analyzed by Student's t-test. Qualitative variables were presented by frequency and percentage and were analyzed by Pearson's Chi-square test (c^2). The level of significance assumed was 5%.

RESULTS

A total of 1,431 records of patients with CHC were evaluated. Males were the most prevalente (802; 56%) patients. The mean age of the patients was 58.6±9.9 years, ranging from 18 to 89 years. Genotype 1 was the most frequent (866; 60.5%) of the patients. Genotype 2 was observed in 81 (5.7%) patients and genotype 3 in 484 (33.8%) patients (TABLE 1).

TABLE 1. Characteristics of the population (n = 1,431).

Characteristics	
Male gender; n (%)	802 (56.0)
Age years; mean ± SD	58.6 ± 9.9
Genotypes; n (%)	
1	866 (60.5)
2	81 (5.7)
3	484 (33.8)
Transplanted; n (%)	
Liver	73 (5.1)
Kidney	18 (1.2)
Evaluation of fibrosis; n (%)	
Hepatic biopsy	566 (39.6)
Transient hepatic elastography	261 (18.2)
Clinical / laboratory diagnosis	459 (32.1)
Fibrotest	14 (1.0)
NA	131 (9.2)
Degree of fibrosis; n (%)	
2	180 (12.6)
3	341 (23.8)
4	779 (54.4)
NA	131 (9.2)

N: number; m: mean; SD: standard deviation; NA: non available.

Among the patients evaluated, 90 (6.3%) were previously transplanted from a solid organ, of which 73 (5.1%) were liver transplanted, and 18 (1.2%) transplanted from the kidney. Among these, one patient was submmited to liver and kidney transplantation (TABLE 1).

The fibrosis evaluation was performed in 1,300 (90.8%) patients. Of these, 566 (39.6%) were evaluated by liver biopsy, 262 (18.2%) by transient hepatic elastography, and 459 (32.1%) by clinical-laboratory evaluation. Only 14 (1.0%) of the patients had fibrosis evaluated by Fibrotest® and 131 (9.2%) did not evaluate the degree of fibrosis (TABLE 1).

Regarding the degree of fibrosis, 180 (12.6%) of the patients presented grade 2 fibrosis for more than 3 years; 341 (23.8%) had grade 3 fibrosis; and 779 (54.4%) had grade 4 fibrosis (cirrhosis) (TABLE 1).

When the distribution of the different genotypes was evaluated in relation to sex, there was no statistically significant difference (P=0.726; TABLE 2).

Genotypes were analyzed for different degrees of fibrosis, and fibrosis grade 2 fibrosis was associated with genotype 1, grade 3 was associated with genotypes 1 and 2, and grade 4 with genotype 3 (TABLE 2).

TABLE 2. Distribution of genotypes according to sex, different degrees of hepatic fibrosis and viral load.

	Genotype 1 N (%)	Genotype 2 N (%)	Genotype 3 N (%)	*P*
Sex (n=1,431)				0.726
Female	377 (43.5)	39 (48.1)	213 (44.0)	
Male	489 (56.5)	42 (51.9)	271 (56.0)	
Fibrosis (n=1,300)				< 0.001
F2	117 (65.0)*	7 (3.9)	56 (31.1)	
F3	216 (63.3)**	26 (7.6)**	99 (29.0)	
F4	425 (54.6)	39 (5.0)	315 (40.4)***	
Viral load (n=1,227)				0.020
< 600.000	304 (54.3)	30 (5.4)	226 (40.4)§	
≥ 600.000	410 (61.5)#	39 (5.8)	218 (32.7)	

*F2 is associated with Genotype 1; **F3 is associated with Genotype 1 and 2; ***F4 is associated with Genotype 3; # High viral load is associated with Genotype 1; § Low viral load is associated with Genotype 3.

The pre-treatment viral load (PTVL) were evaluated in 1,227 individuals, of which 560 had PTVL <600,000 IU/mL and 667 patients had PTVL > 600,000 IU/mL. Regarding the distribution of genotypes according to viral load, a significant difference was observed in the distribution of genotypes when PTVL was high (≥ 600,000 IU/mL), observing that genotype 1 is associated with high PTVL and genotype 3 was associated with low PTVL (TABLE 2).

On the other hand, there was no difference between the degrees of fibrosis and the high viral load (P=0.559). Of the patients with low PTVL, 41.9% had grade 2 fibrosis, 46% grade 3 fibrosis and 46.4% had grade 4 fibrosis. Of the patients with high PTVL, 58.1% had grade 2 fibrosis, 54% grade 3 fibrosis, and 53.6% had grade 4 fibrosis.

In relation to patients previously submitted to liver transplantation when compared to those not transplanted, mean age was higher (62.1±5.8 vs 58.4±9.9 years respectively; P<0.001), male gender (55.3% vs 69.9% respectively; P=0.009) was less prevalent as well as genotype 1 (45.2% vs 61.2% respectively; P=0.008). There was no difference regarding the PTVL (P=0.162). Advanced fibrosis was observed more frequently in the liver transplanted patients (P=0.011).

DISCUSSION

Hepatitis C virus infection is currently one of the leading causes of chronic liver disease worldwide. Knowing the characteristics of HCV patients may be of interest for the definition of public policy strategies aimed at diagnosis, prevention and treatment.

In the present study, there was a predominance of CHC in the male gender, representing 56.0% of the patients. According to the epidemiological information of the Brazilian Health Department, about 60% of cases of hepatitis C between 1999 and 2011 are male[8]. These results are consistent with the literature, which shows a significant predominance of males in patients with HCV[9-13]. Some authors suggest that the higher prevalence of HCV infection in males can be explained by the fact that men present higher risk sexual behavior in relation to women[13].

Regarding age, the patients included in the present study were between 18 and 89 years old, and the mean age was 58.6±9.9 years. According to Cruz et al.[14] in a study carried out in a Brazilian public health unit on the epidemiological profile of HCV, it was shown that the group most affected was between 40 to 49 years, while in the study by Amaral et al.[12], the highest prevalence was between 46 to 56 years (24.92%).

Regarding the genotypes, several studies have shown that genotype 1 is the most prevalent in the world, followed by genotype 3 and genotype 2 being less frequent[1,15-19]. According to the epidemiological information of the Brazilian Health Department, there is a predominance in the notification of hepatitis C infection of genotype 1 (67.7%), followed by genotypes 3 (25.9%) and 2 (5.7%)[8], similar to the present study, which showed a predominance of genotype 1 followed by 3, genotype 2 being less frequent. However, the results of this study corroborate data from the study that evaluated the distribution of genotypes in Brazil, showing a higher prevalence of genotype 3 in the South region[20].

It was also verified that there was no statistically significant association between sex and genotype distribution in individuals with CHC (P=0.726), in agreement with other authors[9,12,20-22].

Regarding the degree of fibrosis, about 1/3 of the patients did not evaluate fibrosis because they had enough clinical or laboratory evidence for the diagnosis of cirrhosis, or because it was not mandatory to obtain treatment for CHC. Hepatic biopsy was used about twice as much as the hepatic elastography for the evaluation of fibrosis (39.6% vs 18.2%). The majority of patients treated were cirrhotic.

The genotypes were evaluated in relation to the degree of fibrosis, and we observed that advanced fibrosis (F3 and F4) was present in most individuals. Genotype 1 was associated with grade 2 and 3 fibrosis, genotype 2 with grade 3 fibrosis and genotype 3 with grade 4 fibrosis, which has been demonstrated in some studies[23,24].

In the present study, a significant difference was observed in the distribution of genotypes when there was a high viral load, observing that genotype 1 is associated with high viral load and genotype 3 was associated with low viral load. On the other hand, there was no difference between the degrees of fibrosis and the high viral load (P=0.559).

Patients in the present study who had undergone liver transplantation were older patients with advanced fibrosis and less frequently male, with genotype 1 being less present than in the subgroup of non-transplanted patients.

As possible limitations of the study, we emphasize the fact that it is a retrospective study, with its potential methodological limitations. However, the large number of patients included allows to know the characteristics of individuals who had access to CHC treatment in the first year of the Public Protocol implementation using the new DAA, possibly representing patients with more severe liver disease.

In conclusion, the present study made it possible to evaluate the characteristics of patients with CHC in a public health program in South Brazil, who received the DAA recently incorporated by the Brazilian Protocol of the public health system. This research evidenced a predominance of CHC in males, aged between 18 and 89 years, with a predominance of genotype 1, higher viral load in patients with genotype 1 and more advanced degree of fibrosis in patients with genotype 3.

Authors' contribution

Minme R: data collection, writing of text, statistical analysis. Holzmann I: data collection, writing of text. Tovo CV: writing of text, statistical analysis. Almeida PRL: conception of the study, writing of text. All of the authors contributed with the revision and approval of the final version of the manuscript.

Minme R, Holzmann I, Tovo CV, Almeida PRL. Perfil dos pacientes com hepatite C crônica em um programa de saúde pública do sul do Brasil. Arq Gastroenterol. 2018;55(4):403-6.

RESUMO – **Contexto** – A hepatite crônica C (HCC) pode evoluir para cirrose e suas complicações como carcinoma hepatocelular, acarretando morbimortalidade. Conhecer o perfil dos pacientes portadores do vírus da HCC é fundamental para o melhor manejo do tratamento. **Objetivo** – Descrever o perfil dos pacientes portadores de HCC em um programa de saúde pública do sul do Brasil. **Métodos** – Foi realizado um estudo retrospectivo onde foram incluídos os pacientes com HCC que realizaram o tratamento contra o vírus C em um polo de dispensação e assistência farmacêutica da Secretaria Estadual da Saúde do Estado do Rio Grande do Sul, Brasil. Foram avaliados todos os prontuários dos pacientes tratados entre dezembro/2015 e dezembro/2016. **Resultados** – Foram avaliados 1.431 registros de pacientes portadores de HCC. O sexo masculino foi o mais prevalente (802; 56%) pacientes. A idade média dos pacientes foi de 58,6±9,9 anos, com variação de 18 a 89 anos. O genótipo 1 foi o mais frequente, em 866 (60,5%) dos pacientes. Noventa (6,3%) pacientes eram transplantados de órgão sólido, sendo que 73 (5,1%) eram transplantados de fígado. A avaliação de fibrose foi realizada em 1.300 (90,8%) pacientes. Dentre estes, 566 (39,6%) foram avaliados através de biópsia hepática. Em relação ao grau de fibrose, 779 (54,4%) apresentavam fibrose grau 4 (cirrose). Os genótipos foram analisados em relação aos diferentes graus de fibrose, sendo observado que o genótipo 3 está associado com o grau 4 de fibrose. O genótipo 1 está associado com alta carga viral. **Conclusão** – O presente estudo possibilitou a avaliação do perfil dos pacientes portadores de HCC em um programa de saúde pública do Brasil. Houve uma predominância de HCC no sexo masculino, e a média de idade foi de 59 anos. Apresentam um predomínio do genótipo 1, maior carga viral nos pacientes portadores do genótipo 1 e maior grau de fibrose nos portadores de genótipo 3.

DESCRITORES – Hepatite C crônica. Epidemiologia. Resposta viral sustentada.

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ORIGINAL ARTICLE

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Potential preventive effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in patients with polyps or colorectal cancer

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ABSTRACT – **Background** – Colorectal cancer is one of the major causes of death worldwide. Many studies have been done on the biology of its formation as well as its treatment in recent years. One of the factors involved in the formation or treatment of this malignancy can be attributed to the microbial flora in the intestine. **Objective** – This study investigate the potential preventive effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in patients with polyps or colorectal cancer (CRC). **Methods** – A total of 77 samples were selected in the form of three groups including individuals suffering from CRC, polyps and healthy subjects. Genomic DNA of fecal specimens and standard strains were extracted and amplified employing primers targeting of the 16S rRNA gene for initial detection. Absolute Real Time PCR quantification was used to determine the copy of the bacterial expression per gram of feces. **Results** – No significant difference were observed between age and gender in the mentioned groups (P=0.06). The average copy number of *Lactobacillus acidophilus* shows Significant difference between the healthy group and those with polyps (P<0.0001), the healthy group and those with colorectal cancer (P<0.0001), as well as those with polyps and the colorectal cancer patients (P<0.0001). **Conclusion** – These results may indicate that taking *Lactobacillus acidophilus* in people with a family history of CRC and people with polyps may be a way of preventing, treating or reducing the severity of CRC.

HEADINGS – Colorectal neoplasms. Polyps. Probiotics. *Lactobacillus acidophilus*, *Lactobacillus plantarum*.

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of mortality in the world, which has grown significantly in recent years, may cause to malignancy[1].

The presence of polyps in the inner wall of the colon is a high-risk complication for the development of CRC[2]. Adenoma is the most common and important type of colon polyps, and it is the basis for the creation of tumors in the colon. The prevalence of this disorder is directly related to aging, and in countries with high prevalence, such as United States, fifty to sixty five percent of the population over 65 years old have Adenoma[3-5].

The incidence of CRC in Iran is lower than that of western countries. It is the fifth most common diagnostic cancer in Iranian men and women population respectively[6]. Statistics show that the incidence of illness in our country has increased over the past 25 years[7]. Recent studies in Iran have also shown that about 43 percent of patients with CRC are under the 50 years old[8].

Effective factors in CRC include genetic and environmental factors. Environmental factors in this disease are more important than genetic factors. The environmental factors including inappropriate diets such as high fat, low fiber, and low carbohydrates[9,10]. Direct statistical correlation between germs and cancer has been identified[11], for example, *Fusobacterium* and *Escherichia coli* are now recognized as a primary cause of peptic ulcers and gastric cancers[12,13].

Intestinal microbiota in human consists of two major phyla of Bacteroides and Firmicutes[14]. In the intestinal flora there are two kinds of beneficial and harmful types of bacteria. Intestinal microbiota has different roles, including intestinal health, immune system modification, presence of drug metabolism, decomposition of carcinogenic factors, vitamin production, fermentation, electrolyte absorption, and epithelial cell growth, preventing the accumulation of pathogenic bacteria such as *Escherichia coli* and *Clostridium* in the intestines and preventing allergies[15].

Probiotics are living organisms that, have an effect on the health of the host by affecting the microbial flora. Probiotics often belong to the human intestinal microbial flora. There is a belief in probiotics that the microbial flora of the intestine has a protective effect against disease. The protective role of probiotics is effective when treated as a microbial flora in the Intestine[16]. The *Lactobacillaceae* family is one of the most important probiotics. This family consists of gram positive, catalase-negative and non-sporulated rods. Although there are many bacteria that have probiotic prop-

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erties[17], but *Lactobacillus* and *Bifidium* can be mentioned as the most common probiotics in digestive tract and play an important role in the treatment of the digestive disease[18].

This study, for the first time aimed to determine the mean population of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in patients with polyps or CRC in comparison of healthy peoples to investigate the potential preventive effect of these bacteria on polyps or development of colorectal cancer.

METHODS

Sampling

Healthy peoples, individuals with polyps or CRC were diagnosed by a gastroenterologist using colonoscopy. CRC positive cases were confirmed by histopathology test. A total of 77 samples were selected in the form of three groups of 25 people including individuals suffering from CRC, 28 polyps and 24 healthy subjects, respectively. Exclusion criteria were a personal history of CRC, IBD or IBS and regular use of non-steroidal anti-inflammatory drugs (NSAIDS), statins, or probiotics. Patients did not receive antibiotics for one month before surgery. Fecal specimens were transported to the laboratory on ice and frozen at −80°C.

Preparation of standard strain

Standard strains of *Lactobacillus acidophilus* (DSM20079) and *Lactobacillus plantarum* (DSM20174) were prepared from the Iranian Biological Resource Center (IBRC). These strains were cultured in MRS agar and MRS broth media for 48 hours, then a suspension cultures of bacteria was prepared for isolation of genomic DNA.

Genomic DNA extraction

Genomic DNA of fecal specimens and standard strains were extracted using Qia amp DNA stool mini kit (Qiagen) and the tissue genomic DNA extraction mini kit (Favorgen Biotech Corp), According to the manufacturer's instructions, respectively. Integrity size of DNA were checked by 0.1% agarose gel electrophoresis. DNA concentrations were determined with the Nano Drop 2000 (Thermo. Scientific) and stored at −20°C prior to amplification steps.

Polymerase chain reaction (PCR)

Extracted DNA was amplified employing primers targeting of the 16S rRNA gene. Forward and reverse primers for *Lactobacillus acidophilus* and *Lactobacillus plantarum* were 5′- AATTCTCTTCTCGGTCGCTCTA-3'; 5′-CCTTTCTAAGGAAGCGAAGGAT-3', and 5′-TTACCTAACGGTAAATGCGA-3'; 5′-GCCGCCTAAGGTGGGACAGAT-3' respectively. The specificity of the primers was confirmed by PCR in 25 µL reaction mixtures containing 2.5 µL reaction buffer, 2 µL of template, 1 µL (each) primer, 1 µL DNTP (mix), 0.5 µL Taq polymerase, 2 µL DNA- template, 1 µL Mgcl2. PCR was performed with an initial denaturation step of 94°C for 5 min, followed by 30 cycles of 94°C for 1min, and 55°C for 30s.

10 µL of the PCR was subjected to electrophoresis on a 2% agarose gel containing Gelred, and the DNA bands were visualized by UV illumination to confirm the generation of 176-bp (*Lactobacillus acidophilus*), 166-bp (*Lactobacillus plantarum*) amplicons.

Real-time quantification PCR of total *Lactobacillus acidophilus* and *Lactobacillus plantarum* load

Quantitative RT-PCRs were performed in a reaction volume of 20 µL containing 10 µL SYBR Green PCR Master Mix, 1 µL each of the forward and reverse primers, 2 µL rox and 2 µL of DNA extracted from the fecal samples. The amount of DNA in the 77 fecal samples was determined in duplicate, and the mean values were calculated. Amplification and detection of DNA were performed with the LightCycler® 96 Real-Time PCR System - Roche Life Science. The reaction conditions were 95°C for 2 min and 95°C for 5s, followed by 40 cycles of 95°C for 15 s and 55°C for 10s. Data analysis was conducted with sequence detection software lightcycler 96. Purified genomic DNA in the range 1 ng of *Lactobacillus acidophilus* and *Lactobacillus plantarum* were used as the standard for determining the amount of *Lactobacillus acidophilus* and *Lactobacillus plantarum* DNA by real-time PCR (FIGURE 1).

Absolute quantification was used to determine the copy of the bacterial expression per gram of feces using LightCycler® 96. In the sample editor section, at least three concentrations were

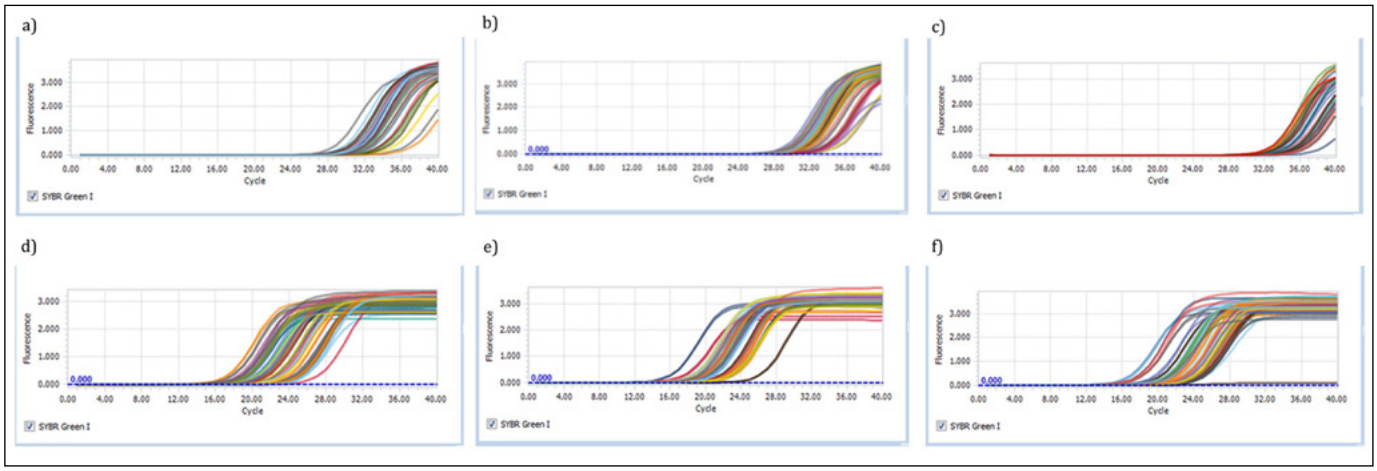

FIGURE 1. Real-time quantification PCR graphs of total bacterial loada. a) *Lactobacillus acidophilus*-healthy control, b) *Lactobacillus acidophilus*-polyps, c) *Lactobacillus acidophilus*- colorectal cancer, d) *Lactobacillus plantarum*-healthy control, e) *Lactobacillus plantarum*- polyps, f) *Lactobacillus plantarum*-colorectal cancer.

selected from the standard strains, and the copy number of the bacteria per gram of feces were determined in each sample based on the standard curves.

Statistical analysis

Descriptive data were analyzed with the Statistical Package for the Social Sciences 21 (SPSS 21). Chi- square, Mann-Whitney Test and Kruskal-Wallis Test were applied for data analysis. *P* values of <0.05 were considered significant.

RESULTS

Sampling and demographic analysis

Using ANOVA statistical test no significant difference was found between the ages of the studied groups (P=0.06). In addition no significant difference were observed between gender in the mentioned groups (P=0.06). The age range of 55-65 years included the highest number of polyps and CRC, in addition the results of statistical analysis showed that the frequency of O blood group is higher in patients with polyps and CRC (P<0.05). There were no significant differences in the other variables (TABLE 1).

TABLE 1. Demographic data recorded for each study participant included age, blood group, and smoking.

Variables	Healthy N (%)	Polyps N (%)	Cancer N (%)	*P*-value
Smoking	5 (17.9)	9 (14.3)	8 (28.6)	0.68
Age	52 ± 8	57 ± 9	58 ± 9	0.06
A blood group	5 (17.9)	6 (21.4)	6 (21.4)	0.12
B blood group	10 (35.7)	4 (14.3)	4 (14.3)	0.14
AB blood group	6 (21.4)	4 (14.3)	4 (14.3)	0.07
O blood group	7 (25)	14 (50)	14 (50)	0.00
Fat-rich diet	11 (39.3)	9 (32.1)	16 (57.1)	0.15

Specimens and DNA isolation

To better demonstrate the distribution of bacterial DNA in samples taken from healthy peoples and those with polyps or CRC, the percentage of those carrying bacterial DNA was measured. All samples of healthy people and those with polyps contained DNA of *Lactobacillus acidophilus*. However, 86% of the subjects with CRC had DNA of *Lactobacillus acidophilus*. 100% of samples had *Lactobacillus plantarum* DNA.

Real-time quantification PCR of total *Lactobacillus acidophilus* and *Lactobacillus plantarum* load

The average copy number of *Lactobacillus acidophilus* was calculated in three groups studied (P<0.0001). A statistically significant difference was found between the healthy group and those with polyps, the healthy group and those with colorectal cancer, as well as those with polyps and the colorectal cancer patients (TABLE 2).

Average copy number of *Lactobacillus plantarum* was calculated in three groups and There was no significant difference between the three groups (P>0.05) (TABLE 3).

TABLE 2. Average copy number of *Lactobacillus acidophilus* in each groups.

Studied groups	Average (copy number per gram feces ± SD)	Total *P*-value		*P*-value
Healthy	2.2501×10^{10} ± 1.2591×10^{7}	< 0.0001	Healthy / polyps	< 0.0001
Polyp	8.7742×10^{8} ± 2.3760×10^{8}		Healthy/ câncer	< 0.0001
Cancer	2.4323×10^{6} ± 1.2591×10^{6}		Polyps / câncer	< 0.0001

TABLE 3. Average copy number of *Lactobacillus plantarum* in each groups.

Studied groups	Average (copy number per gram feces ± SD)	Total *P*-value
Healthy	3.2×10^{11} ± 17×10^{9}	0.133
Polyps	3.0×10^{10} ± 12×10^{9}	
Cancer	5.0×10^{10} ± 10×10^{9}	

DISCUSSION

The number of bacteria estimated by the molecular techniques is greater than the total bacterial load detected by culture methods, demonstrating the utility of real-time PCR in the analysis of the bacterial load[19].

So far, no study has been done to determine the population of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in patients with CRC and polyps. Therefore, the present study was conducted to determine the copy number of *Lactobacillus acidophilus* and *Lactobacillus plantarum* from fecal specimens of patients with polyps and CRC in comparison with control group. In this study, for the first time, Cyber Green method was used to check the copy of these two bacteria per gram of feces. According to the results of this study, The mean copy number of *Lactobacillus acidophilus* decreased in people with CRC compared to healthy subjects and people with polyps, Also, The mean of copy number of this bacterium is lower in people with polyps in comparison of healthy people.

Significant difference in the mean copy number of *Lactobacillus plantarum* in three groups was not observed. There are a variety of reasons, for example *Lactobacillus acidophilus* is one of the most important and most frequent colon flora and it may change faster than the changes in bowel conditions affected by polyps and CRC.

Studies indicate that the population of intestinal bacteria is different between healthy subjects and people with CRC[20]. For example, researches have shown that in patients with CRC, the population of some bacteria such as *Escherichia*, *Citrobacter*, *Shigella Flavobacterium*, *Acinetobacter* and *Chryseobacterium* decreased[21].

Intestinal bacteria play different roles in inducing disease or protecting individuals. Some species exacerbate the formation of a tumor and cause cancer, while others contribute to the health of the intestine. For example, *Eubacterium rectal* and *Eubacterium eligens* are firmicutes, which have a significant correlation with CRC. While the intestinal proteobacteria population declines in patients with CRC[22].

Although most cases of CRC occur individually, the role of inherited genetic factors in the development of CRC is 35%. The first-degree relatives of CRC patients are at increased risk of developing CRC, with a relative risk of 2.2. This risk is strongly correlated with the number of affected family members, For example, in a family with two or more people with colorectal cancer, the relative risk for the other family members increases to 4. In

addition the relative risk of developing adenoma or CRC in people with a history of adenoma in their family increases to 2 folds[23].

There is evidence that treatment with probiotics may modulate gastrointestinal function and reduce digestive disorders[24]. Fermented products such as short chain fatty acids (SCFA) are produced by the consumption of probiotics[25]. Probiotics in the mouse colon have been shown to induce a protective glutathione transferases II enzyme. These factors reduce the burden of the genotoxic substances in the intestine and also increases the production of factors that disable toxic compounds, for example Butyrate is one of these protective factors that slows the propagation of cancer cells[26]. It was also observed that abundance of Bacteroides and Bifidobacteria was associated with a reduction in the risk of colon polyps[27].

Considering the results obtained in this study as well as using the results of previous studies, it can be concluded that after the diagnosis of adenomatous polyps, doctors can prescribe probiotics to treat or prevent CRC. Older people can probably prevent the development of polyps or their progression to malignancy by including probiotics in their diet.

In this study, there is a significant difference in blood groups, so that in the blood group O, the number of people with polyps and CRC is more than other groups. It can be argued that colon cancer is more likely to occur in those who have a blood type O and they can prevent the disease by using a proper diet and taking probiotics. Periodic screening can be helpful to assess, diagnose and treat the disease at the early stages. Significant difference between smoking and CRC was not observed.

CONCLUSION

CRC is one of the leading causes of mortality in the world, which has grown significantly in recent years, and may cause to malignancy. In this study, there was a significant difference in population of *Lactobacillus acidophilus* bacteria in patients with polyps, CRC and healthy subjects. However, no significant difference was found in the three groups in the study of *Lactobacillus plantarum* population. These results may indicate the Potential preventive effect of *Lactobacillus acidophilus* in people with a family history of CRC. In addition, this bacterium may be used as a supplement to patients with intestinal polyps in the future. Further studies are needed to confirm the preventive or therapeutic role of this bacterium.

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Authors' contribution

Zinatizadeh N; laboratory expriments, data analysis and writing of the manuscript. Khalili F; study design, clinical diagnosis and sampling. Fallah P; laboratory expriments and data analysis. Farid M; data analysis. Geravand M; laboratory expriments. Yaslianifard S; study design, laboratory expriments, data analysis and writing of the manuscript.

Zinatizadeh N, Khalili F, Fallah P, Farid M, Geravand M, Yaslianifard S. Arq Gastroenterol. Potencial efeito preventivo do *Lactobacillus acidophilus* e *Lactobacillus plantarum* em pacientes com pólipos ou câncer colorretal. Arq Gastroenterol. 2018;55(4):407-11.

RESUMO – **Contexto** – O câncer colorretal é uma das principais causas de morte em todo o mundo. Muitos estudos têm sido feitos sobre a biologia de sua formação, bem como o seu tratamento nos últimos anos. Um dos fatores envolvidos na formação ou no tratamento desta malignidade pode ser atribuído à flora microbiana no intestino. **Objetivo** – Este estudo investigou o potencial efeito preventivo de *Lactobacillus acidophilus* e *Lactobacillus plantarum* em pacientes com pólipos ou câncer colorretal (CCR). **Métodos** – Um total de 77 amostras foram selecionadas e três grupos foram formados, a saber, indivíduos portadores de CCR, pólipos e indivíduos saudáveis. O DNA genomico de espécimes fecais e de amostras padrão foi extraído e amplificado empregando primers que focalizaram o gene do rRNA 16S para a deteção inicial. A quantificação do PCR em tempo real absoluto foi utilizada para determinar a cópia da expressão bacteriana por grama de fezes. **Resultados** – Não foram observadas diferenças significativas entre idade e sexo nos grupos citados (P=0,06). O número médio de cópias de Lactobacillus acidophilus mostra diferença significativa entre o grupo saudável e aqueles com pólipos (P<0,0001), o grupo saudável e aqueles com câncer colorretal (P<0,0001), bem como aqueles com pólipos e câncer colorretal pacientes (P<0,0001). **Conclusão** – Estes resultados podem indicar que a ingestão de Lactobacillus acidophilus em pessoas com antecedentes familiares de CCR e pessoas com pólipos pode ser uma forma de prevenir, tratar ou reduzir a gravidade da CCR.

DESCRITORES – Neoplasias colorretais. Pólipos. Probióticos. *Lactobacillus acidophilus*. *Lactobacillus plantarum*.

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ORIGINAL ARTICLE

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Surgical management of cystic lesions of the pancreas: a single-centre experience

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ABSTRACT – **Background** – Cystic lesions of the pancreas represent a group of pancreatic diseases with great histological heterogeneity, varying from benign lesions, some of them with malignant potential, to overt malignant lesions. **Objective** – To describe the cases of cystic lesions of the pancreas which underwent surgical intervention at a tertiary university hospital. **Methods** – This is a retrospective population-based study (historical cohort) which was carried out enrolling individuals attended at the Outpatient service of Pancreas Surgery of the *Hospital de Clínicas* of Unicamp. The individuals underwent surgical procedures performed from January 2012 through December 2016. **Results** – In the period evaluated, 39 cases of cystic lesions of the pancreas which underwent surgery were identified, 26 (66.6%) of which were female. The average age at diagnosis was 47.4±16.4 years (range, 18-73). In regards to symptoms, 35 (89.7%) were symptomatic. The average length of hospital stay was 10 days (range 4-76). Surgeries performed to treat the lesions depended on the localization and type of the lesions: cystojejunostomy (41%), distal pancreatectomy (36%), pancreaticoduodenectomy (15.4%), drainage of ruptured and/or infected pseudocyst (5.2%) and central pancreatectomy (2.6%). **Conclusion** – Cystic lesions of the pancreas are a group of lesions with a highly varying presentation and diagnostic approach and may require an also highly variable surgical treatment. An appropriate preoperative imaging diagnosis is essential for their management.

HEADINGS – Neoplasms, cystic, mucinous, and serous. Serous cystadenoma. Mucinous cystadenoma. Pancreatic pseudocyst. Pancreatic neoplasms.

INTRODUCTION

Cystic lesions of the pancreas (CLPs) represent a group of pancreatic diseases with great histological heterogeneity, varying from benign lesions, some of them with malignant potential, to overt malignant lesions. These lesions can be classified into three major groups: pseudocysts, non-neoplastic cysts and cystic neoplasms of the pancreas. With the exception of the pancreatic pseudocyst, cystic neoplasms of the pancreas occur more frequently than their non-neoplastic counterparts. The incidence of these lesions has increased over recent decades, a fact apparently related to the progress of imaging methods; it is estimated that they affect approximately 0.7% to 24.3% of the population[1,2].

Pancreatic pseudocysts are the most frequent CLPs, corresponding to 80% of them. This type of lesion may be one of the complications of acute pancreatitis, chronic pancreatitis and pancreatic trauma. Simple pancreatic cysts are considered rare, usually asymptomatic and incidentally diagnosed. The cystic neoplasms of the pancreas correspond to a group of diseases with peculiar characteristics and are classified as serous cystic neoplasm, mucinous cystic neoplasm (with significant malignant potential), and intraductal papillary mucinous neoplasm (IPMN) types I (main duct), II (branch duct) and III (mixed), that are considered pre-malignant lesions as well. Solid pseudopapillary pancreatic neoplasm, also known as Frantz's tumor, is characterized by pancreatic lesions that may also contain cystic spaces or regions of cystic degeneration[1-5].

The symptoms of CLPs vary according to the type and localization of the lesion, ranging from asymptomatic, secondary manifestations to extrinsic compression until the presence of abdominal pain, weight loss, low back pain, nausea, constipation, diarrhea, abdominal distension, among others. The diagnosis of a pancreatic cystic lesion can be poorly made by means of clinical assessment and fundamentally requires imaging methods. Computed tomography (CT) is the most commonly used imaging test for the diagnosis of cystic lesions, as it allows the identification and characterization of the lesion. Magnetic resonance imaging (MRI) is another alternative with the advantage of better evaluating the ductal anatomy of the pancreas and not requiring the use of iodinated contrast. More recently, the use of endoscopic ultrasound scan (EUS) has considerably improved the etiological diagnosis of these lesions and allows fine-needle aspiration of the cystic fluid content for assessment of cytological and biochemical examinations, especially tumor markers such as carcinoembryonic antigen (CEA) and CA 19-9. The diagnosis and classification of the type of pancreatic cystic lesion is very important to evaluate the degree of malignancy of the lesion and to define the best therapeutic option and best prognosis for these patients[2,3,5].

The treatment of CLPs is directly related to the type of lesion. Some of them, such as incidentally found asymptomatic cystic serous neoplasms, only require careful clinical and radiologic following. Bypasses by endoscopy or surgery may be indicated for pseudocysts depending on the symptoms produced by them.

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Conventional segmental or even total pancreatic resections are indicated in the case of cystic lesions depending on the characterization of the lesion[1-5].

In Brazil, recent publications on CLPs are limited to literature reviews, case series and reports[6,7]. This study aims to describe the cases of CLPs which underwent surgical intervention at a tertiary university hospital.

METHODS

This is a retrospective population-based study (historical cohort) which was carried out enrolling individuals attended at the Outpatient service of Pancreas Surgery of the *Hospital de Clínicas* of Unicamp. The research protocol was evaluated by the local Research Ethics Committee and approved under the reference number 2.318.647/CAAE: 72405517.5.0000.5404/Unicamp.

The individuals underwent surgical procedures performed from January 2012 through December 2016. Data were obtained through medical records and outpatient worksheets. Individuals were identified according to the electronic database of surgical procedures of the hospital and histopathological examinations were checked to confirm the cystic nature of the lesions. Included in this study were individuals over 18 years old which underwent surgical treatment for cystic lesions of the pancreas, with histopathological diagnosis confirmed according to World Health Organization (WHO) classification criteria[8] and of both genders. All individuals which underwent cystojejunostomy or drainage of infected/ruptured pseudocysts had a small portion of the cyst's capsule resected and sent to biopsy to exclude the possibility of a neoplasm. Individuals included in vulnerable groups (those with mental disabilities, institutionalized) and those with incomplete data in the health records were excluded from the sample.

The variables evaluated in this study were: age (expressed in full years at the time of treatment), gender (expressed in male and female), histopathological type, symptoms at diagnosis, preoperative imaging, type of surgery, surgical complications, and surgical mortality.

Statistical analysis

Data were expressed as means ± standard deviation. For the execution of descriptive statistical analysis, it was used Microsoft Excel® 2016.

RESULTS

In the period evaluated, 39 cases of CLPs which underwent surgery were identified, 26 (66.6%) of which were female. The average age at diagnosis was 47.4 years (range, 18-73). In regards to symptoms, 35 (89.7%) were symptomatic, presenting abdominal pain (77%), weight loss (41%), nausea and vomiting (38.5%), and abdominal distension (30.8%) as the commonest symptoms. TABLE 1 details the main symptoms observed.

The most prevalent localization of the CLPs was the body-tail transition of the pancreas, accounting for 35.9% of the cases, whereas pancreatic head lesions were found in 20.5% of the cases. FIGURE 1 details the localization of the lesions. The preoperative diagnosis was made mainly through ultrasound, computed tomography, nuclear magnetic resonance and/or magnetic resonance as described in TABLE 2.

TABLE 1. Symptomatology of the individuals who underwent surgery for cystic lesions of the pancreas.

	N	%
Asymptomatic	4	10.3
Abdominal Pain	30	77
Weight loss	16	41
Nausea and vomiting	15	38.5
Jaundice	5	12.8
Choluria	4	10.2
Fecal acholia	3	7.7
Itch	2	5.1
Inappetence	4	10.2
Abdominal distension	12	30.8
Dyspepsia	3	7.7
Diarrhea	3	7.7
Constipation	4	10.2

N: number of individuals.

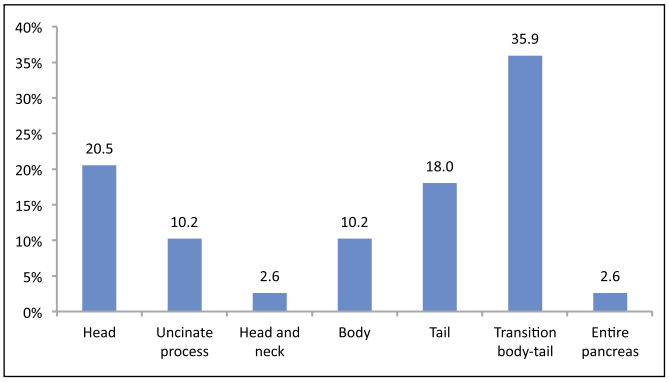

FIGURE 1. Frequency of cystic lesions of the pancreas according to localization.

TABLE 2. Imaging methods used to diagnose cystic lesions of the pancreas.

Method	N (%)
US	22 (56.4)
EUS	18 (46.1)
CT	32 (82)
MRI	21 (54)

US: ultrasound scan; EUS: endoscopic ultrasound scan; CT: computed tomography; MRI: magnetic resonance imaging; N: number of individuals.

The average length of hospital stay was 10 days (range 4-76). Surgeries performed to treat the lesions depended on the localization and type of the lesions. The surgeries performed were cystojejunostomy (41%), distal pancreatectomy (36%), pancreaticoduodenectomy (15.4%), drainage of ruptured and/or infected pseudocyst (5.2%) and central pancreatectomy (2.6%). Surgical complications were seen in 17 (43.6%) patients; pancreatic fistula (10.2%), intestinal subocclusion (7.7%) were the most common. The surgical procedure associated with a higher morbidity was pancreaticoduodenectomy (50%). Overall surgical mortality was 5.1%. TABLE 3 details the morbidity observed.

TABLE 3. Complications after surgical resection of cystic lesions of the pancreas.

	N	%
Hospital-acquired infection	3	7.7
Incisional hernia	3	7.7
Wound infection	4	10.2
Pancreatic leak	4	10.2
Intestinal obstruction	3	7.7
Septic shock	1	2.6
Hypovolemic shock	4	10.2
Acute renal failure	1	2.6
Pulmonary thromboembolism	1	2.6

N: number of individuals.

In regards to the histopathological evaluation, there were 18 cases of pancreatic pseudocyst (46.1%). TABLE 4 describes the histopathological findings of the entire sample. Three patients (50%) presented invasive carcinoma associated with IPMN; the three individuals presented the same histology (ductal adenocarcinoma) and TNM staging (T1N0); there was no late cancer-related mortality after a mean follow-up of 28.7±9 months. None of the individuals with mucinous neoplasms presented an invasive carcinoma at surgery and during the follow-up (31.5±13.1 months). The complete demographic profile of the patients according to the histopathological findings is detailed in TABLE 5.

TABLE 4. Histopathological analysis of cystic lesions of the pancreas.

Subtype	N	%
Pseudocyst	18	46.1
IPMN	6	15.4
Serous neoplasm	6	15.4
Mucinous neoplasm	5	12.8
Frantz's tumor	4	10.2

IPMN: intraductal papillary mucinous neoplasm; N: number of individuals.

DISCUSSION

CLPs have a varied prevalence in the population. Considering only pseudocysts, the overall prevalence is significant due to its relationship with pancreatitis, which is a disease that presents a somewhat common occurrence. However, a much lower prevalence is found when analyzing only pancreatic neoplastic cystic lesions (1% to 10% of pancreatic neoplasms). These, despite their rarity, account for about 30% of pancreatic resections[2,3,5-9]. The incidence of CLPs is increasing (2), mainly due to advances in imaging technology and increased access to computed tomography (CT) and magnetic resonance imaging (MRI).

The predominance of the diseases occurred in females (66.6%) and the most common histopathological diagnosis was pseudocyst; these epidemiological and histological profiles are compatible with those of the existing literature, since cystic neoplastic lesions are more frequent in the female population. In summary, the majority of the neoplastic cystic lesions (serous neoplasms, mucinous neoplasms and Frantz's tumors) were mostly observed in females, whereas pseudocysts presented no predominance and IPMNs were more common among men; Frantz's tumor predominated in young adults, whereas the others were more frequent among middle-aged to elderly individuals. Usually, the Frantz's tumor is 10 times more frequent in women[9]. Regarding the clinical presentation of patients at diagnosis, the majority (89.7%) were symptomatic, differently from what has been reported in other series with 40% to 75% of cases being asymptomatic[2-4,7,9]; it is very likely that this was due to the fact that all individuals of this series underwent surgery and thus the surgical indication biased the results. Therefore, the majority of the individuals in the current series due presented large-volume lesions diagnosed after causing mass effect and consequently abdominal pain, nausea, vomiting, weight loss and abdominal distension. These more prevalent symptoms are consistent with those cases that have already been described, especially in the presence of extensive and palpable mass in the physical examination and previous pancreatitis associated with pseudocyst formation[10]. US, CT, MRI, and EUS were performed for the imaging diagnosis. US is useful for distinguishing solid from cystic lesions, presents low cost and is highly accessible; however, it is operator-dependent, does not provide further information on the nature of the lesion and may be ineffective when there is interposition of intestinal loops on the pancreas[2-5]. EUS is a diagnostic method with a high

TABLE 5. Epidemiologic profile of the patients according to histopathological subtype.

	Pseudocyst	Frantz's tumor	Serous neoplasm	Mucinous neoplasm	IPMN
N	18	4	6	5	6
Sex					
Male	50%	–	–	–	66.6%
Female	50%	100%	100%	100%	33.3%
Age (years)	50.2±15.4 (range, 18-76)	19.2±2.1 (range, 18-22)	50±4.1 (range, 45-60)	40±12.1 (range, 22-57)	62.3±7.4 (range, 51-72)
Symptoms					
Asymptomatic	–	25%	20%	20%	16.6%
Symptomatic	100%	75%	83.3%	80%	83.3%

IPMN: intraductal papillary mucinous neoplasm.

sensitivity for cystic lesions with at least 2 high-risk factors, such as size greater than 3 cm, dilation of the main pancreatic duct or the presence of an associated solid component; it allows fine-needle aspiration of the cystic fluid and subsequent analysis that may aid in the diagnosis of these lesions[6]; however, it was scarcely used in this series, which reflects the low availability of this method in our country and especially in the public health system. According to the guideline of the American Gastroenterology Association (AGA 2015), patients with no relevant results in the EUS and fine needle puncture should perform MRI after one year to guarantee no chance of malignancy of the lesion[11,12]. Hence, it may be pointed that US is useful for gross detection and/or screening of lesions, whereas CT and/or MRI are enough to diagnose and indicate the appropriate therapy for the majority of the cases of CLPs, albeit EUS may provide relevant and essential information for those cases whose characterization was not appropriate by means of those more available methods.

The CLPs of the present study were mostly in the body-tail transition (35.9%), followed by head (20.5%) and tail (18%). A French study by Gaujoux et al. presented the pancreatic head (47%-48%) as the most prevalent, followed by body involvement (26%-28%); however, this study only divided the location of the cysts into pancreatic head, body and tail[10].

According to the 2015 AGA guideline, the diagnosis and management of cystic pancreatic lesions, asymptomatic patients must undergo surgical excision of the lesion when they present a solid component and dilation of the pancreatic duct and/or risk factors in EUS and needle puncture[11,12]. Allen emphasizes that surgical resection of the lesion should be reserved for cases of presumed precancerous cysts due to their size, solid component and/or dilation of the main duct, when there is concern about the development of malignancy[4,13]. To appropriately identify and propose the adequate therapy for CLPs is relevant for both general physicians and specialists. Given their heterogeneity, CLPs may represent from innocuous findings at imaging examinations that only require cautious following to more aggressive diseases that lead to complex surgeries. Different guidelines are available but so far no optimal diagnostic or therapeutic algorithm exists. In general, mucinous neoplasms and main-duct IPMNs are always considered for surgery due to its high malignant potential. Frantz's tumors are also considered for resection for the majority of the cases, since it is considered a neoplasm of uncertain behavior, as well as it usually reaches large volumes and causes symptoms due to extrinsic compression of adjacent organs. In contrast, serous neoplasms are only considered for resection whenever they become highly symptomatic due to mass effect, which is unlikely to happen. In regards to branch-duct IPMNs, the surgical indication is warranted in an individual basis, since their behavior is not as clearly aggressive as the main-duct type. Pseudocysts usually need surgical intervention when they reach high volumes and lead to clinical symptoms due to the compression of near organs. In view of this, all of the patients in the current series were surgically treated, due to symptoms, risk of cancer and/or a high degree of suspicion for malignancy[14-17].

The surgical mortality rate in this study was 5.1% (two patients); both deaths were caused by postoperative sepsis related to surgical complications, one after a cystojejunostomy due to a pseudocyst, another one after a pancreaticoduodenectomy due to IPMN-related invasive cancer. Pancreatic fistula and wound infection were the most common complications among our patients, which is comparable to other series. The mortality and surgical complication rates of the current study were comparable with those of Reames et al.[9]; however, they were higher than those of the study carried out in 2011 by Gaujoux[10]. Both morbidity and mortality were more associated with complications of the surgical procedures and/or the clinical status of the patients than with the nature of the lesions[17].

This study presents some limitations that need to be addressed. Firstly, its retrospective design may lead to data of poorer quality. Moreover, due to the nature of the series, enrolling only surgical patients, it suffers from selection bias, since a number of patients who presented non-surgical cystic lesions were not included. Nevertheless, given the scarcity of local data in regards to this group of diseases, the results observed are significant and provide relevant evidence in regards to the approach of CLPs, since they present a series from a high-volume public hospital. The strength of this casuistry is to show the distribution of a group of diseases that is difficult to appropriately diagnose and even more difficult to define the adequate treatment in a high-volume university hospital. This casuistry is more recent than other previously published and was collected after the consolidation of the imaging diagnosis of intraductal papillary mucinous neoplasms and a more deep understanding of their risk.

CONCLUSION

CLPs are a group of lesions with a highly varying presentation and diagnostic approach and may require an also highly variable surgical treatment. An appropriate preoperative imaging diagnosis is essential for their management.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Statement of human and animal rights

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors' contribution

Sia GB collected the data and wrote the first draft of the study. Soares PFC provided clinical assistance for the individuals enrolled in the study. Gestic MA, Chaim EA, and Callejas-Neto F contributed relevant intellectual inserts and provided critical revision. Cazzo E designed the study and wrote substantial portions of the study.

Sia GB, Soares PFC, Gestic MA, Chaim EA, Callejas-Neto F, Cazzo E. Tratamento cirúrgico de lesões císticas do pâncreas: uma experiência unicêntrica. Arq Gastroenterol. 2018;55(4):412-6.

RESUMO – **Contexto** – As lesões císticas do pâncreas representam um grupo de doenças pancreáticas com grande heterogeneidade histológica, variando desde lesões benignas, algumas com potencial pré-maligno, até outras degeneradas para formas malignas. **Objetivo** – Descrever os casos de LCPs submetidos à intervenção cirúrgica em um hospital universitário terciário. **Métodos** – Trata-se de um estudo retrospectivo populacional (coorte histórica) realizado com a participação de indivíduos atendidos no Ambulatório de Cirurgia do Pâncreas do Hospital de Clínicas da Unicamp. Os indivíduos foram submetidos a procedimentos cirúrgicos realizados no período de janeiro de 2012 a dezembro de 2016. **Resultados** – No período avaliado, foram identificados 39 casos de lesões císticas do pâncreas operados, sendo 26 (66,6%) do sexo feminino. A idade média no diagnóstico foi de 47,4±16,4 anos. Em relação aos sintomas, 35 (89,7%) eram sintomáticos. O tempo médio de internação foi de 10 dias (variação de 4-76). As cirurgias realizadas para o tratamento das lesões dependeram da localização e do tipo das lesões: derivação pseudocisto-jejunal (41%), pancreatectomia distal (36%), pancreaticoduodenectomia (15,4%), drenagem de pseudocistos rotos e/ou infectados (5,2%) e pancreatectomia central (2,6%). **Conclusão** – As lesões císticas do pâncreas são um grupo de lesões cuja apresentação e abordagem diagnóstica são altamente heterogêneas e que podem requerer um tratamento cirúrgico altamente complexo e variável. Um diagnóstico pré-operatório adequado é essencial para definir o seu tratamento.

DESCRITORES – Neoplasias císticas, mucinosas e serosas. Cistadenoma seroso. Cistadenoma mucinoso. Pseudocisto pancreático. Neoplasias pancreáticas.

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REVIEW

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Irritable bowel syndrome, food intolerance and non- celiac gluten sensitivity. A new clinical challenge

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ABSTRACT – Approximately 80% of irritable bowel syndrome (IBS) patients report that their symptoms are triggered after ingesting one or specific food groups. Gluten, wheat and related proteins (e.g., amylase-trypsin inhibitors, and fermentable oligo-di-mono-saccharides and polyols (FODMAPs) are the most relevant IBS symptom triggers, although the true 'culprit(s)' is/are still not well established. The concept of causal relationship between gluten intake and the occurrence of symptoms in the absence of celiac disease and wheat allergy was termed non-celiac gluten sensitivity (NCGS). The borderline between celiac disease, wheat allergy, IBS and NCGS is not always clearly distinguishable, and the frequency and clinical identity of NGCS are still unclear. An overlap between IBS and NCGS has been detected. The incomplete knowledge of the etiopathogenesis of these clinical conditions, lack of data on their real epidemiology, as well as the absence of a gold standard for their diagnosis, make the overall picture difficult to understand "It is crucial to well define the interaction between IBS, food intolerance and NGCS, since the role of diet in IBS and its dietary management is an essential tool in the treatment of a large number of these patients". The objective of the present review is to provide an overview highlighting the interaction between IBS, food intolerance and NCGS in order to unravel whether gluten/wheat/FODMAP sensitivity represents 'facts' and not 'fiction' in IBS symptoms.

HEADINGS – Irritable bowel syndrome. Food intolerance. Celiac disease. Wheat hypersensitivity.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder and one of the most commonly diagnosed gastrointestinal diseases with a global estimated prevalence of 10%-20%[1-5]. This percentage varies with the methodology used in the studies and with the geographic area evaluated. Characterized by recurrent symptoms, it has no biological markers available for its diagnosis and approximately 80% of IBS patients report that their symptoms are triggered after ingesting one or specific food groups. Today IBS diagnosis is based on Rome IV criteria[6,7]. Clinical criteria known as Roma criteria are those used for the diagnosis of functional digestive disease, including IBS[8-10]. Although they are criteria under construction and updated since its first edition in 1990, most gastroenterologists do not use them for daily clinical practice, and their use is often reserved for research projects. These data help us understand both the difficulties of homogenizing samples for clinical research and the universalization of the clinical-epidemiological and diagnostic-therapeutic aspects of patients with IBS. In addition, a large number of patients exhibit the mild form of the disease and never seek medical services, which also makes it difficult to study the natural history of the disease[1,9,11-13]. The pathogenesis of IBS is multifactorial[14-20]. However, the triggering factors of IBS symptoms may be present in different combinations for each patient. However, it is not clear how these factors act as triggers in the generation of symptoms associated with IBS[12,18-20]. The heterogeneous pathogenesis of IBS could lead to alterations in motility, visceral sensation, brain-intestinal interactions, microbiome, bile acid metabolism and intestinal permeability. In addition, an immune activation is probably involved in low-grade inflammation[14,15,18,19]. Given the complexity of its pathophysiology and the clinical subgroups resulting from this umbrella of options, IBS is considered to be a gastrointestinal-brain disorder and is clinically defined as a biopsychosocial disease[12,20]. A significant number of IBS patients report the onset of symptoms after ingesting one or specific food groups[1,9,11-13]. The most frequent ones are those that present lactose, fructose in excess of glucose, fructan, galactooligosaccharides and polyols[21-26]. In addition, it has recently been reported that a percentage of patients with a negative diagnosis for celiac disease reported that foods containing gluten triggered the symptoms of IBS. This association is included in the concept of IBS-like disorders[27-36]. A subset of patients diagnosed with IBS report worsening symptoms when they eat foods that contain gluten and improve with the withdrawal of these foods from the diet. However, most of these patients report intolerance and worsening of symptoms to other nutrients in their diet[37,38]. The new clinical entity still without specific clinical contour was denominated of non-celiac gluten sensitivity (NCGS), related to sensitivity to the wheat and the gluten, and has aroused so much the interest of the scientific community as of the population in general. Its clinical

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picture is similar to that of patients with IBS. The overlap of IBS with NCGS gave rise to a large number of pathophysiological theories that could influence the therapeutic management of patients with IBS who report food intolerance and the appearance of symptoms after eating foods containing gluten, improving with the withdrawal of these foods from the diet[39-42]. The overlap between IBS and NCGS gave rise to a large number of pathophysiological theories that influence the therapeutic management of patients with IBS who report food intolerance and the onset of symptoms after eating gluten-containing foods and improve with the withdrawal of these foods from the diet. Although it may be described as a new subgroup of patients with IBS, most of these patients report intolerance and worsening symptoms to other nutrients in their diet[12,43-45]. The objective of the present review is to provide an overview highlighting the interaction between IBS, food intolerance and NCGS in order to unravel whether gluten/wheat/fermentable oligo-di-mono-saccharides and polyols (FODMAPs) sensitivity represents 'facts' and not 'fiction' in IBS symptoms.

IBS and food intolerance

Approximately 80% of IBS patients report that their symptoms are triggered by at least one food item and they increasingly ask for dietary and behavioral counseling[6,12,13,17,21,22,28,29]. In recent years, (fermentable oligosaccharides, disaccharides, monosaccharides, polyols) and gluten/wheat have been increasingly recognized as a possible trigger for symptoms compatible with a diagnosis of IBS[38,39]. The mechanisms of food intolerance in IBS remain unknown[14,18,21,23,25,28]. These triggering foods do not reflect food allergies or even IgE-mediated classical food allergy seems to play an important role in IBS[21-23,30-33,46-48]. Wheat has been considered a frequent trigger in the genesis of IBS-associated symptoms. However, the component (s) of this cereal that is directly involved in generating the symptoms of IBS remains unknown. Gluten, other wheat proteins, for example, amylase-trypsin inhibitors and fructans (the latter belonging to FODMAPs) have been identified as possible factors for the generation and or exacerbation of IBS symptoms[27,28,37-39,40-43,48]. Symptoms related to FODMAPs share the same clinical characteristics associated with lactose intolerance and many foods rich in FODMAPs are also rich in lactose. Because of the high prevalence of lactose intolerance, it is not surprising that a diet that is poor in FODMAPs can reduce or even resolve gastrointestinal and extra-intestinal symptoms[49-51]. An important intersection exists between FODMAPs and NCGS. Thus, after a more detailed evaluation, symptoms associated with IBS could be triggered by FODMAPs and not by gluten itself. The same thing can be true for foods rich in Ni, very numerous in the FODMAPs family, such as pears, cabbage, garlic, onion and legumes. Multiple factors have been considered to contribute to food sensitivity in patients with IBS. Investigations have centered on food specific antibodies, carbohydrate malabsorption, and gluten sensitivity. Although some IBS patients related relief of symptoms on a gluten-free diet the specific relationship between gluten and increased intestinal permeability in IBS have not yet confirmed. We reported that IBS patients have difficulties with food in general and specific foods may not be involved in IBS pathogenesis. It is reasonable to assume that IBS causes food sensitivity, rather than vice versa[12,18,21,22,28,32,38,39,43,46]. The mechanisms involved in the pathophysiological alterations found in IBS seem to be multiple and are still uncertain. A unifying hypothesis for the generation of these symptoms would be the phenomenon of visceral hypersensitivity identified in most of the patients with IBS[21-23,30-33,46-48]. The phenomenon of visceral hypersensitivity may be related to an increased response of the neuroimmune circuits in the nervous system or gastrointestinal tract to external stimuli (for example environmental or psychosocial stimuli) or internal ones (tissue irritation, inflammation, infection). This increased response may result in abnormalities of digestive motility, inducing symptoms compatible with the clinical picture of IBS[12,14,15,18,21,23,33]. In synthesis, an abnormal neuroimmune interaction (genetic and psychosocial factors, food intolerance, and bacterial microflora) may contribute to the phenomenon of visceral hypersensitivity frequently observed in the patients with IBS. This finding suggests that patients with IBS symptoms have difficulties with foods in general. It is very probable that IBS causes food intolerance and not the opposite[12,15,16,31,41,43].

III- Gluten Related Disorders –NCGS and IBS

Although mankind has existed for more than 2.5 million years, only in the last 10000 years have we been exposed to wheat and increased its production exponentially. By the end of the twentieth century, wheat production increased fivefold[52]. This would be an explanation for the change in the epidemiology of celiac disease or gluten-sensitive enteropathy and the significant increase in the number of scientific publications regarding celiac disease (CD) and other related non celiac gluten sensitivity (NCGS)[43-45,47,50,51-,53]. The increase in the global prevalence of celiac disease may be true or associated with an increase in the number of diagnostic serological tests. In the case of NCGS, the increase in prevalence could be associated with an increase in global wheat consumption in the last decades[27,35,36,37,54]. The varied food forms of wheat contain more gluten than in the past and could be associated with digestive symptoms[52,53]. The concept of a causal relationship between the ingestion of gluten and the occurrence of symptoms in absence of CD and wheat allergy was first described in the late 1970s by Cooper and Ellis[55,56]. This clinical entity has been termed NCGS or NCWS. NCGS, the most famous of the GRDS and considered an adverse reaction to gluten, was recently "rediscovered" as a clinical entity without available diagnostic biomarkers. As defined by the Salerno Expert's Criteria NCGS is characterized by intestinal and extra-intestinal symptoms triggered by ingestion of gluten[57,58]. This association reported by some individuals has led to the spontaneous restriction of the consumption of foods containing gluten. Some authors report that NCGS has been described in 6%-10% of the population[53,57,58]. In contrast to allergy to wheat and celiac disease, its immunopathological process is not yet understood. Over 75% of these patients have HLA-DQ2 and / or HLA-DQ8.75% of these patients carry HLA-DQ2 and/ or HLA-DQ8[43]. NCGS can be defined to describe individuals who complain of intestinal and extra-intestinal symptoms related to gluten intake and report rapid improvement after withdrawal of these foods from the diet, and in which both the diagnosis of CD and wheat allergy are discarded[45,52,53,58,59-73]. This fact raises many unanswered questions. NCGS exist?, how it induces digestive symptoms this group of individuals is nonspecific. The most common symptoms are diarrhea, bloating and abdominal pain. The definition of NGCS has many similarities with IBS[43-45,52]. Historically, it has been reported that patients with undetected celiac disease (CD) may present with IBS type symptoms. An overlap between IBS and NCGS has been detected. However, incomplete knowledge of the etiopathogenesis of these clinical

conditions, lack of data on their real epidemiology, as well as the absence of a gold standard for their diagnosis, make the overall picture difficult to understand[22,27-28,34-37,44,45,57,58,61-63]. Gluten, wheat and related proteins (e.g., amylase-trypsin inhibitors, and fermentable oligo-di-mono-saccharides and polyols (FODMAPs) are the most relevant IBS symptom triggers, although the true 'culprit(s)' is/are still not well established[52,61,63,68,72,73]. In addition, Rome IV criteria seem unable to exclude an underlying possible IBS-like disorder. The lack of specific biomarkers hampers diagnosis of both conditions[6-10]. There is some evidence that the NCGS may exist, but probably only in a small number of people. In contrast to celiac disease, patients with self-reported NCGS are heterogeneous and suggestible by media advertising and food therapies without scientific evidence, which makes them a very difficult group of patients to study. Thus, efficient diagnostic criteria are necessary to make the differential diagnosis of a medical condition from the one in which the patients simply prefer to avoid gluten. It is unclear whether gluten triggers symptoms in patients with IBS and the mechanisms by which gluten or other wheat proteins trigger the symptoms in these patients are also not defined. Some patients improve with gluten withdrawal from the diet and return to symptoms after reintroduction. However, eliminating gluten from the diet alone does not seem to be enough to control the symptoms[27,37.44,45,53,58,60,62,66,68,69,71,72,74]. In addition, it is also difficult to diagnose overlap with other components of wheat. There is also evidence that IBS symptoms could be triggered by carbohydrate components of fructan and galactans, the FODMAPs. Including lactose, since many foods rich in FODMAPs are also rich in lactose. The FODMAPs are poorly absorbed short chain carbohydrates composed of small osmotically active molecules that can trigger IBS like symptoms by excessive accumulation of fluids and gases, inducing hyper visceral hypersensitivity, changes in intestinal microbiota, and alteration of enteric hormones and neurotransmitters that may explain the generation of symptoms[72-79]. In addition, α-amylase / trypsin inhibitors (ATIs) have been used in greater amounts to eliminate highly resistant pests and pests in wheat and cereals[75]. In view of their inflammatory and immunological potential, they also could be considered as possible inducers of intestinal and extra-intestinal manifestations in patients with CD or IBS[44-47].

In summary, wheat contains more than one potential inductor of IBS symptoms. The similarity of the epidemiological clinical picture of IBS-like disorders, the absence of biomarkers for the diagnosis of IBS and NCGS, combined with the discordant results of the double-blind placebo-controlled trials, hinder to define a culprit. These facts probably create many terms for the same clinical entity.

IV- Clinical and therapeutic aspects of IBS and NCGS overlap

After the steps of the difficult diagnosis of IBD-like disorders have been overcome, including a clinical evaluation and a rigorous anamnesis, the use of restrictive diets and frequent clinical follow-up are a therapeutic option. A statistically significant clinical improvement has been described in patients with IBS and food intolerance when using restrictive diets. Among the foods reported as being associated with the symptoms of IBS, those high in carbohydrates, gluten and wheat are common. Therefore, a better understanding of the dietary factors involved in IBS and the underlying mechanisms of gluten/wheat/FODMAPs sensitivity are crucial in determining the true benefit of the exclusion diet in IBS and its subsequent standardization. This effective evaluation could be translated into new and effective new dietary strategies for the management of patients with IBS[71-79].

Double-blind placebo-controlled trials with cross-over trials represent the current gold standard to confirm what would be the dietary factor (s) involved in generating functional symptoms associated with food intolerance in IBS patients and also in those diagnosed as IBS-like -disorder, due to the lack of specificity of the symptoms. Based on the different dietary factors associated with triggering symptoms, patients may be labeled as non-celiac or non-celiac gluten sensitive with sensitivity to wheat proteins or even sensitive to FODMAPs. Diagnostic investigation will be facilitated by both the awareness of these disorders and the careful analysis of the records and food anamnesis. It is important to emphasize that self-report of gluten sensitivity by the patient does not confirm the diagnosis of NCGS and that the prescription of a gluten-free diet for gastrointestinal and other symptoms may lead to underdiagnoses of CD[80-84]. Recently, Picarelli et al. developed an oral mucosal contact test for gluten (GOMPT), which seems to be a reliable and rapid tool to confirm the diagnosis of NCGS, although additional investigations are necessary since the population evaluated was small and the tests performed in a single diagnostic center[44]. A diet low in FODMAPs has been suggested as a strategy to improve symptoms in patients with IBS, regardless of the underlying cause. Although a small number of patients, many studies and randomized controlled trials have reported good control of IBS symptoms after a low diet in FODMAPs, with a general improvement in gastrointestinal symptoms in 68%-86% of patients with IBS. In addition, this diet appears to be superior to a gluten-free diet in patients diagnosed with NCGS[85-88]. However, identifying the most offensive FODMAPs in specific patients could attenuate dietary restrictions, such as lactose intolerance. In a retrospective case review, symptom improvement was observed in up to 85% of IBS patients with associated diagnosis of lactose malabsorption. However, prospective studies show that restriction to lactose alone is a trigger for IBS symptoms is not sufficient for the effective relief of symptoms in functional GI disease. Treatment of lactose intolerance should involve reduction of lactose intake rather than exclusion or even enzyme replacement for primary adult lactase deficiency, which has many available diagnostic tests characterized by different principles, availability, sensitivity, specificity, and cost[50,51,80,84,86,88]. Finally, in spite of the controversies, small Intestine Bacterial Overgrowth (SIBO) should always be considered as a differential diagnosis in patients with IBS, since the reported prevalence of SIBO in patients with IBS is generally high, varying from 4% to 64% and involving mainly patients with IBS-D. Some studies report that treatment with SIBO seems to be associated with improvement of symptoms in patients with IBS who associate them with food intolerance[80,83,85,86,88].

It is also necessary to emphasize that long-term restrictive diets probably have implications for intestinal homeostasis. There is evidence that intensive restriction of FODMAPs and wheat products could have long-term negative consequences, both from the nutritional point of view and the impact on the intestinal microbiota[85,86,88,89]. Thus, identifying more offensive FODMAPs in specific patients could mitigate food restrictions, preventing future clinical complications. In relation to the treatment of NCGS many questions remain unanswered and it needs to be verified whether the elimination of dietary gluten alone is sufficient for the control of symptoms, and to understand the overlap with other components

of wheat[90-93]. However, nutritional counseling requires additional financial resources and not all patients will benefit[77,78,79,83]. Dietrich et al.[80] analyzed the effect of a low FODMAPs versus a gluten-free diet (GFD) on clinical symptoms, psychological well-being, intestinal inflammation and intestinal integrity and microbiota in NGCS patients. Their reported that both diets caused microbial shifts in all participants, with a greater variability on genus level and metabolisms groups in NCGS patients. Their findings suggest a multifactorial etiology of NCGS due to a functional effect caused by FODMAPs, combined with a gluten-induced mild immune reaction, and an imbalance of the microbiota. Valeur et al.[38,93] have reported that the microbial composition of the intestine may be a tool to identify patients who are likely to respond to dietary restriction of FODMAPs in patients with IBS these findings provide additional information for the study of etiopathogenesis and treatment not only of CD, but also of gastrointestinal disturbances similar to IBS, such as NCGS. The need for better clinical understanding of the nature of NCGS including your trigger, diagnosis, treatment and risks, justify the recent increase in gluten-related diseases research[27,94-96].

CONCLUSION

The recent increase in scientific research on IBS disorders, such as the NCGS, is visible and justified. The borderline between CD, wheat allergy, IBS and NCGs is not always clearly distinguishable, and the frequency and clinical identity of the NGCS are still unclear. More careful planning of clinical trials will lead to a better understanding of the nature of NCGS and its association with IBS. These future findings may help to establish the magnitude of the problem including triggers, diagnosis, treatment, risks, and implications for human diseases, separating facts from myths.

Soares RLS. Síndrome do intestino irritável, intolerância alimentar e intolerância não celíaca ao glúten. Um novo desafio clínico. Arq Gastroenterol. 2018;55(4):417-22.

RESUMO – Cerca de 80% dos pacientes com síndrome do intestino irritável (SII) relatam que seus sintomas são desencadeados após a ingestão de um ou grupos específicos de alimentos. Nesse grupo, glúten, trigo e proteínas relacionadas (como inibidores de amilase-tripsina e oligo-di-mono-sacarídeos e polióis fermentáveis (FODMAPs) são os fatores desencadeantes de sintomas mais relevantes da SII, embora o verdadeiro 'culpado(s)' ainda não seja conhecido. O conceito de relação causal entre a ingestão de glúten e a ocorrência de sintomas na ausência de doença celíaca e alergia ao trigo foi denominado sensibilidade ao glúten não celíaca (SGNC). A fronteira clínica entre doença celíaca, alergia ao trigo, SII e SGNC não está claramente distinguível, apesar da sobreposição entre SII e SGNC ser frequentemente relatada na literatura. O conhecimento incompleto da etiopatogenia dessas condições clínicas, a falta de dados sobre sua epidemiologia real, bem como a ausência de um padrão ouro para seu diagnóstico da associação SII/SGNC, dificultam a compreensão dessa nova entidade. "É de suma importância definir com precisão a interação entre SII, intolerância alimentar e SGNC, já que o papel da dieta no tratamento da SII é uma ferramenta essencial no tratamento de um grande número desses pacientes". A presente revisão tem como objetivo apresentar dados atuais a respeito da interação entre SII, intolerância alimentar e SGNC. Além disso questiona-se, com os dados disponíveis, a sensibilidade ao glúten/trigo/FODMAPs, representa "fato" e não "ficção" na geração de sintomas associados a SII.

DESCRITORES – Síndrome do intestino irritável. Intolerância alimentar. Doença celíaca. Hipersensibilidade a trigo.

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Fortaleza, **23 a 26 de novembro 2019**

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