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AIM AND SCOPE

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EDITORIAL

Defensive medicine: It is time to finally slow down an epidemic

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Abstract

Defensive medicine is widespread and practiced the world over, with serious consequences for patients, doctors, and healthcare costs. Even students and residents are exposed to defensive medicine practices and taught to take malpractice liability into consideration when making clinical decisions. Defensive medicine is generally thought to stem from physicians' perception that they can easily be sued by patients or their relatives who seek compensation for presumed medical errors. However, in our view the growth of defensive medicine should be seen in the context of larger changes in the conception of medicine that have taken place in the last few decades, undermining the patient–physician trust, which has traditionally been the main source of professional satisfaction for physicians. These changes include the following: time directly spent with patients has been overtaken by time devoted to electronic health records and desk work; family doctors have played a progressively less central role; clinical reasoning is being replaced by guidelines and algorithms; the public at large and a number of young physicians tend to believe that medicine is a perfect science rather than an imperfect art, as it continues to be; and modern societies do not tolerate the inevitable morbidity and mortality. To finally reduce the increasing defensive behavior of doctors around the world, the decriminalization of medical errors and the assurance that they can be dealt with in civil courts or by medical organizations in all countries could help but it would not suffice. Physicians and surgeons should be allowed to spend the time they need with their patients and should give clinical reasoning the importance it deserves. The institutions should support the doctors who have experienced adverse patient events, and the media should stop reporting with excessive evidence presumed medical errors and subject physicians to "public trials" before they are eventually judged in court.

Key words: Adverse event; Clinical reasoning; Defensive medicine; Doctor-patient relationship; Healthcare cost; Medical education; Medical error

Core tip: The widespread practice of defensive medicine has negative consequences for patients, doctors, and healthcare costs. The growth of defensive medicine must be seen in the context of the changes in the conception of medicine, which have occurred in the last few decades and have undermined the patient–physician trust. To reduce the practice of defensive medicine, decriminalization of medical errors, increased time directly spent with patients, reaffirmation of the importance of clinical reasoning, and institutional support to doctors who have experienced adverse patient events are essential.

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INTRODUCTION

Defensive medicine has been practiced for decades[1] and spread to countries the world over to become an epidemic[2-5], causing unnecessary hospitalizations, tests, invasive procedures, drug prescriptions, consultations with other physicians, avoidance of high risk patients, and congested waiting lists. This can cause serious consequences. For example, in a patient with an infection a physician practicing defensive medicine may prolong antibiotic duration, prescribe unnecessary broad-spectrum antibiotics or combinations of agents, or prescribe unnecessary antibiotic treatments[6,7], which may contribute to the alarming spread of antibiotic resistance. Even students and residents frequently encounter defensive medicine practices and are in various instances taught to take malpractice liability into consideration when making clinical decisions[8]. Medicolegal systems tend to censure alleged errors of omission much more often than any other type of fault[9], thus incentivizing a continuously increasing and excessive number of diagnostic investigations as a strategy for reducing legal risk[10]. Indeed it was observed that the United States had created the "perfect storm" for overutilization of healthcare[11], and that there is "an unjustified enthusiasm for treatment on the part of both doctors and patients"[12,13]. Hence, the financial burden related to defensive medicine is considerable. The United States medical liability system costs $55.6 billion annually, and the contribution of defensive medicine is over 82% ($45 billion)[14], and in Italy the cost of defensive medicine has been estimated to be around 10–12 billion euro/year[15]. Indeed, higher resource use by physicians is associated with fewer malpractice claims[16].

Does defensive medicine solely derive from physicians' perception that they can easily be sued either by patients or their relatives seeking compensation for presumed medical errors, or is there more to it? We argue that a "defensive" attitude is part of a huge change in the conception of medicine that has taken place in the last decades and needs to be acted upon if we wish young people to continue to have an interest in, and the society at large to have trust in, the profession.

Clinical medicine has always been based on patient–physician trust, and this has traditionally been by far the main source of professional satisfaction for physicians. Indeed, factors such as prestige of medicine, intellectual stimulation, interaction with colleagues, and financial rewards are much less important[17].

Unfortunately, this fundamental trust has been progressively eroded by lack of patient face-time, increasing lack of clinical autonomy, and liability concerns. A national Survey of America's Physicians (completed by 17236 physicians; 10170 of whom wrote additional comments) gave a dismaying picture of the medical profession: just 14% of physicians surveyed have the necessary time to provide the highest levels of care, 60% have been detracted from patient interaction by electronic health records, 54% have a negative morale, 49% suffer from feelings of burn-out, 49% would not recommend medicine as a profession to their children, 48% intend to reduce hours, retire, get a non-clinical job, or limit patient access to their practices, and only 37% have positive feelings about the future of the medical profession[17]. This is not a picture limited to one country. On the contrary, these feelings are increasingly shared by doctors in many other countries.

Physicians cannot increasingly spend more time at inserting data into a computer than at directly caring for their patients (in the United States ambulatory practice, for each hour doctors give direct clinical face time to patients, approximately two further hours are spent on electronic health records and desk work in the clinic day)[18]. Caring is not only about examining patients, ordering tests and prescribing drugs. It is about spending time with patients, being at their side, talking to them without hurrying, showing a sincere interest in their condition and in its social implications, answering their questions, and addressing their concerns. If this relationship is lost or diminished to unacceptable levels, then defensive medicine is the logical consequence.

Medicine has moved from a family or personal doctor to a hospitalist/hospital employee model. Even in the USA, family doctors largely do not take care of their patients in a hospital close to home anymore. Patients feel that the doctors who have not spent enough time to talk to them could have missed or overlooked important aspects of their illness. The surgeons who have not had time to listen to their patients' fears and concerns will have acted superficially and may have made mistakes.

To fix these problems, doctors must be allowed to spend the necessary time with their patients, a privilege that even residents/registrars in the hospitals no longer have. In fact published studies have found that residents

spend more time using a computer than they do with patients[19-21]. Even though hiring more personnel and spending more funds will be necessary to allow doctors to spend more time with patients, this needs to be done. If defensive medicine is reduced, it may actually decrease health expenditures, not increase.

Another issue is the fact that patients, who are well informed and educate themselves *via* the internet, are ultimately in search of experienced physicians who they can trust and who will look after them and not only after an illness. Are patients looking for doctors who rigidly follow algorithms and guidelines? They aren't. Algorithms that transform patient care into a sequence of yes/no decisions do not consider the complexity of medicine and the reasoning inherent in clinical judgment. Young clinicians must abandon the idea that not adhering strictly to guidelines implies being sentenced in court, and should not think that guidelines are a magic bullet for all healthcare issues. The best evidence is helpful if used in the setting of a particular patient in a certain environment, interpreted and utilized on the basis of clinical experience. As much as a recipe book does not guarantee success in cooking, so clinical guidelines cannot guarantee success in diagnosis or treatment. In fact a standardized evidence-based practice, based on protocols and guidelines, is aimed at improving population rather than individual health.

Clinical reasoning is extremely important and dedicated time to learn this must be made in medical school curricula. Contrary to popular belief, mistakes are caused more often by errors in cognitive function (failure to elicit, synthesize, or act on available information) than lack of knowledge.

Medicine cannot be, and is not as black and white as protocols and checklists seem to imply. Physicians and surgeons must decide on the basis of imperfect data, and face unpredictable patient responses to treatment and outcomes that are not black and white[22]. It is time to stop disproportionate ordering of tests (carrying risks of false positive results or even iatrogenic harm)[23] in an attempt to achieve an unobtainable diagnostic certainty[24].

Hence the public and the physicians need to be educated that medicine is not a perfect science but rather an imperfect art, as it always has been. It is a huge mistake to expect perfection and totally predictable results that no one can guarantee even in the most technologically advanced environment. Complications are difficult to avoid and play an important role in medical malpractice suing. In one highly cited study in New York, adverse events were reported in 3.7% of all hospitalizations, and negligence was present in less than 30% of these cases[25]. The culture of discredit and culpability, which encourages physicians to hide and deny mistakes, has made any mistake or adverse outcome an intolerable failure[9]. Coupled with the modern society's lack of tolerance for inevitable morbidity and mortality (whereby even death is no longer considered a possible consequence of a disease but rather a preventable complication), a poor outcome is then presumed to indicate a wrong process[9]. A medical treatment that does not lead to the anticipated, positive outcome is regarded by the patient or relatives as a mistake, while it may just be unachievable even in the most advanced healthcare setting.

A defensive attitude is one of the contributing factors in the impressive reduction in the number of autopsies worldwide over the last few decades[26,27]. Some doctors fear that they could be sued should the findings prove a wrong diagnosis or a clinically missed pathology[28].

A vicious cycle starts when doctors are involved in an unexpected adverse event, mistake, and/or patient related harm; then are sued by the patient or relatives; next the (sometimes huge) trauma related to the event leads to physical, cognitive, and behavioral symptoms, including the practice of defensive medicine[29,30]. Support obtained by these physicians in their institutions is poor and inefficient[31]. Adequate support is necessary to help interrupt this negative series of events.

In conclusion, defensive medicine is the consequence of a deep crisis in the relationship between doctors and society, which has led people to consider modern medicine as able to treat any disease, and doctors to behave opportunistically rather than doing what they think is really in the best interest of their patients. The increasing pressure to examine more and more patients in a short period of time, and to get patients out of the hospital faster and faster needs to be stopped. Doctors would then be able to consider their patients' clinical and psychosocial history and no longer instantly order tests and prescribe drugs to diminish their legal responsibility should they be charged with imprudence, inexperience, or negligence. While decriminalizing medical mistakes and handling them in civil courts or by medical organizations in all countries can help, this must also be associated with changes in the health systems from a punitive attitude to one that favors identification and correction of structural errors. Physicians must of course know the best and most current evidence in their fields but always consider the evidence in the context of their experience and of the individual case they have in front of them. Finally, continuing efforts must be made to educate the public that information acquired from online sources outside of an appropriate clinical context is generally inappropriate. Also, the media should realize the extremely damaging nature of reporting presumed medical errors and subjecting physicians to public trials through newspapers, radios, television or websites before they are eventually judged in court[32]. We exhort colleagues not to succumb to pressure deriving from the system, the patients, and their peers[33], and we urge healthcare administrators, policymakers, patients' organizations and journalists to cooperate and make healthcare systems better and safer.

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MINIREVIEWS

Web-based learning in inflammatory bowel diseases: General truths and current specifics

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Abstract

In a field rapidly evolving over the past few years, the management of inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, is becoming increasingly complex, demanding and challenging. In the recent years, IBD quality measures aiming to improve patients' care have been developed, multiple new medical therapies have been approved, new treatment goals have been set with the "treat-to-target" concept and drug monitoring has been implemented into IBD clinical management. Moreover, patients are increasingly using Internet resources to obtain information about their health conditions. The healthcare professional with an interest in treating IBD patients should deal with all these challenges in everyday practice by establishing, enhancing and maintaining a strong core of knowledge and skills related to IBD. This is an ongoing process and traditionally these needs are covered with additional reading of textbook or journal articles, attendance at meetings or conferences, or at local rounds. Web-based learning resources expand the options for knowledge acquisition and save time and costs as well. In the new era of communications technology, web-based resources can cover the educational needs of both patients and healthcare professionals and can contribute to improvement of disease management and patient care. Healthcare professionals can individually visit and navigate regularly relevant websites and tailor choices for educational activities according to their existing needs. They can also provide their patients with a few certified suitable internet resources. In this review, we explored the Internet using PubMed and Startpage (Google), for web-based IBD-related educational resources aiming to provide a guide for those interested in obtaining certified knowledge in this subject.

Key Words: Inflammatory bowel diseases; Ulcerative colitis; Crohn's disease; Technology-enhanced learning; E-learning; Web-based learning; Continuing medical education

Core tip: In a field that is rapidly evolving, healthcare professionals face new challenges in inflammatory bowel diseases (IBD) management that includes new drugs, new treatment goals and new quality measures in patient care. In an era of advanced communications technology, web-based resources offer diverse, substantial, very efficient, and easily accessible accredited educational opportunities. These can save time and cost compared to the more traditional modes of knowledge acquisition. In this review, we provide a guide of web-based IBD-related educational resources for practitioners to allow acquisition or maintenance of certified skills and knowledge in IBD management.

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INTRODUCTION

The Inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are an area of gastroenterology which has rapidly evolved over the past few years with vast scientific and medical advances. Additionally, the incidence and prevalence of IBD is rising worldwide[1]. In recent years, multiple new medical therapies have been approved, new treatment goals have been set with the "treat-to-target" concept and drug monitoring has been implemented into IBD clinical management[2,3]. Moreover, IBD quality measures aiming to improve patients' care have been developed[4,5].

The management of IBD is becoming increasingly complex, challenging and demanding; this requires additional education for practicing gastroenterologists, both in the hospital and the ambulatory care setting. As described in their seminal review of e-learning medical education, Ellaway and Masters[6] note that traditional continuing medical education (CME) includes face–to–face courses, conferences, seminars, grand rounds, or it may be informal with reading of journal articles or texts. The authors mention that there are barriers to formal traditional CME including increased professional workload, family commitments, distances to travel to conferences, and costs of attending courses. Informal traditional CME have also barriers including lack of time, isolation and lack of access to professional colleagues, lack of libraries and library services, delayed delivery of documents, lack of access to technology and information technology problems, as well as cost.

At the trainee level, it is essential to improve the experience of gastroenterology (GI) residents with management of IBD by providing additional education and by enhancing their exposure in IBD patients within their core training program. Furthermore, advanced IBD fellowships in high-volume academic centers, provide the opportunity for a more extended training in the field for those who want to follow a career focused on IBD. Other options for trainees include mentorship in IBD programs, electives in IBD, or courses with intensive training in IBD, like IBD Xcel[7-9].

Technology has occupied every aspect of our lives and is widely used both in education and in medicine. While it is known that most learners benefit from and value the one-on-one interaction with instructors and that many small and seemingly less significant interactions are lost in the streamed and streamlined online environment, there are on line technologies that exist to try to replicate and employ these important features[10]. The internet is used by patients and physicians to obtain information and knowledge for various health conditions. In a recent survey of adult GI program directors and trainees in the United States, Cohen *et al*[11] reported that only one third of the trainees were satisfied with their level of IBD exposure, while more than half were uncomfortable dealing with IBD special situations including the management of pouch, stoma, pregnancy or postoperative patients. Web-based resources were the first choice, more than any other, as an information aid for IBD clinical care and were selected by almost half of the trainees[11]. Another recent survey of 223 gastroenterologists in the United States, found that 82% of them used Internet-based resources including the UpToDate, PubMed and the Crohn's and Colitis Foundation of America (CCFA) websites to obtain information about management strategies for their IBD patients[12].

Evidently, both GI trainees and specialists use on-line resources to cover their educational and clinical practice needs in IBD management. It is known that learning from these types of resources is likely equivalent to that obtained from more traditional methods of instruction[13]. In this review, we explored and described existing web-based IBD learning resources for physicians and patients, with the aim to provide a guide to those who are interested in maintaining up-to-date knowledge and skills in IBD. Some of these resources also had interactive components that allow learners to dialogue with experts and peers[10].

LITERATURE SEARCH

We conducted literature searches in PubMed to identify peer-reviewed articles related to eb-based educational material for IBD. We also used two Web search engines, Google.ca and Startpage (http://www.startpage.com),

to identify websites containing educational material related to IBD. Search results were generated using the following search strategy: ("inflammatory bowel diseases" OR "ulcerative colitis" OR "Crohn's disease") AND ("online") AND ("resources" OR "CME" OR "educational"). Furthermore, we performed targeted searches by browsing websites of known IBD-related national or international organizations or societies.

We performed hand-searching to assess and review the contents of each web site. We did not use any specific medical website tool to assess the quality of the educational web sites. Journal articles published in PubMed the last 10 years were considered for review. Website inclusion criteria consisted of active websites that were in the English language, related to IBD education including CME courses, and targeted to patients, undergraduate medical education students, postgraduate medical education and healthcare professionals.

We downloaded or manually entered references from all sources into the online Endnote reference manager (http://www.myendnoteweb.com) database recording each website's name/organization, year, access date, and the URL.

INTERNATIONAL AND NATIONAL SOCIETIES WEB SITES

European Crohn's and Colitis Organization

The European Crohn's and Colitis Organization (ECCO) has contributed substantially to the education of gastroenterologists interested in IBD[14]. On the ECCO web-site there is free access to a list of previously published *ECCO Guidelines* and *the ECCO e-Guide,* a toolkit harboring a collection of algorithms based on the ECCO guidelines, disease information, disease activity calculators and other useful resources[15].

In 2013, ECCO launched e-CCO[16], an online learning platform aimed at improving the care of IBD patients by providing a comprehensive educational package for health care professionals involved in IBD management. The e-CCO platform is subdivided to *IBD basics, e-Library*, *e-Courses*, *Advanced Topics* and the *ECCO IBD Curriculum*, all of which are accessible only with membership. The *e-library* is made up of abstracts, presentations (slides or videos) and webcasts from the ECCO congresses, created and delivered by experts in IBD. The current e-CCO learning portfolio contains 24 extensive *e-Courses* based on the ECCO guidelines, and over 40 original videos and podcasts on basic IBD topics and current controversies in IBD treatment. The *e-courses* are accredited, and a certificate is available when participants successfully pass the post-test. Feedback is also available during the course and after the post-test.

Finally, ECCO created the *ECCO IBD Curriculum* which is a framework for all of ECCO's educational activities and operates as its foundational educational core: the guide for the gastroenterologists interested in IBD, the index of the entire on-line ECCO content and the tool to be used by national or by individual physicians for educational purposes. The *ECCO IBD Curriculum* is organized in 16 broad topics, ranging from a general understanding of disease and treatment, to more specific situations in the management of IBD patients. Each domain within the curriculum is constantly enriched and updated with new material incorporated from ECCO educational and scientific activities. The purpose of the *ECCO IBD Curriculum* is to provide the knowledge and skills necessary for a gastroenterologist to become an IBD expert.

The Crohn's and Colitis Foundation of America

The Virtual Preceptorship program: The CCFA website offers a domain with information about IBD for patients and physicians[15]. In the Programs and Materials section, CCFA provides the *Virtual Preceptorship* program[17], to enrich physician training in the diagnosis, treatment and management of IBD with 5 online interactive and accredited activities. In the same section, there are also available, for physicians and patients, free educational brochures and fact sheets providing current information and treatment options about IBD.

The Rising Educators, Academicians and Clinicians Helping IBD group: This group has been founded under the auspices of the CCFA and its mission is to cover the educational needs of trainees and junior faculty members interested in IBD. It facilitates mentorship with established experts, fosters collaborative research between junior investigators, advises on career development and trajectory, and develops best practices for patient care, through educational and career development seminars, mentoring programs, networking events, research collaborations, participation activities within the CCFA, and trainee educational modules[18].

In August 2016, the Rising Educators, Academicians and Clinicians Helping IBD (REACH-IBD) group and the University of Nebraska Medical Center jointly launched the *IBD Clinical Practice Video Series*[19], which was a year-long accredited on-line activity. Based on identified knowledge gaps among trainees, this program covered 4 topics with videos and quizzes about the latest information on IBD treatments and on management of special IBD situations including postoperative recurrence in Crohn's disease, pouch endoscopy, pregnancy in IBD, and advanced treatment approaches for IBD care and recognizing complications. The modules were free, highly educational, and accessible; and included a pre-presentation quiz. Unfortunately, the pre- and post-learning assessments, the evaluations and the request for credit are no longer available.

Canadian Association of Gastroenterology

The Canadian Association of Gastroenterology (CAG)

has developed and launched the *ePortal* in the Education section of CAG's web-site[20]. The *ePortal* is an accredited program dedicated to maintain members' up-to-date knowledge in various topics in Gastroenterology and to contribute to their Maintenance of Certification requirements. The material in *ePortal* is a collection of presentations and videos related to gastroenterology practice coming primarily from previous national (Canadian Digestive Disease Week) or local meetings. The site is organized into *ePortal course categories* and the IBD section includes 97 courses which are listed only in chronological order and not by subject. The *ePortal* automatically saves each member's individual educational activities which can be reviewed and printed anytime.

UNIVERSITY WEBSITES

"IBD LIVE" Webcast Program

In 2009, Dr. M. D. Regueiro at the University of Pittsburgh initiated the *"IBD LIVE" webcast program*[21,22], an interinstitutional and interdisciplinary videoconference educational activity, where sites can remotely join in live IBD case discussions on Thursday mornings from 7:00 AM to 8:00 AM EST. Two cases per conference are selected, prepared and discussed. Currently, over 25 East Coast academic IBD Centers participate in this interactive live conference of difficult cases, and the participants can hold discussions with IBD experts in an active learning environment. Admission to the program is free through an easy registration process and the webcast participants may view the discussion and submit questions or comments *via* a chat feature. Previous webcasts have been archived and are also available. The IBD LIVE is accredited by the UPMC Center for CME in the Health Sciences.

This program is a significant educational initiative which allows participants to remain in their home working environment and participate in a CME-approved multidisciplinary conference which provides them with the opportunity to promote their knowledge, exchange opinions and ideas with other colleagues and IBD experts, start collaborations with other centers and finally improve their patients' care.

IBD GROUPS WEBSITES

The IBD Working Group

The IBD Working Group (IBDWG) provides an educational forum for healthcare professionals interested in IBD and aims to improve the quality-of-care of patients with IBD[23]. The web-site contains high-quality and clinically-oriented educational resources focused on IBD, which have been prepared in collaboration with top experts in IBD from Europe and Northern America. The content of the site is available after free subscription and is organized in sections where the presentations (slides with or without audio) and other educational material are listed. Post activity tests with feedback are available, but unfortunately CME credit is no longer available for the activities and the content has not been updated since 2016.

IBD Dialogues and E-mentoring in IBD

Mentoring in IBD (MIIBD) is an innovative and successful annual national symposium for Canadian gastroenterologists (*The Master Class*) that takes place at Toronto[24]. *Mentoring in IBD* also operates regional satellite meetings, a website, a newsletters and regular emails focused on clinical questions with new research supported by Canadian and International experts in IBD.

IBD Dialogue was launched in 2004, is a quarterly published newsletter delivered *via* e-mail and is based on hot topics presented at the annual national *Mentoring in IBD: The Master Class* symposium each year[25]. *IBD Dialogue* reports new advances in management of IBD and open case-based discussions with experts and peers.

E-mentoring in IBD was launched in 2008 and is an interactive scientific e-bulletin on state-of-the-art issues in IBD management published twice per month and is delivered to the subscribers electronically *via* email[26]. *E-Mentoring in IBD* provides with short comments on the results papers from the current IBD literature, together with level of evidence and hyperlinks.

The Mentoring in IBD website has free access and users can find accredited educational material, browse or download publications including previous *IBD dialogue* and *E-mentoring* letters and watch videos presented by experts in IBD. The subscription to receive the newsletters and the bulletin is easy to obtain and is free of charge[24].

INDUSTRY SPONSORED WEBSITES

SEEMLI: Standardizing the Endoscopic Evaluation of Mucosal Lesions in IBD

SEEMLI is a CAG accredited program supported by AbbVie and its purpose is to increase gastroenterologists' proficiency in performing endoscopies in IBD patients and to enhance their experience and skills in using different endoscopic scoring methods[27]. The program focuses on the most common endoscopic scores used in clinical practice: the Simple Endoscopic Score for Crohn's Disease, the Ulcerative *Colitis* Endoscopic Index of Severity, the Mayo Endoscopic Score for ulcerative colitis, and the Rutgeerts score for post-ileocolic resection of Crohn's disease. The program provides information on how to use each of these methods, discusses of some of the pros and cons of each method, and provides practice opportunities using endoscopic videos. Subscription is free, and a user's dashboard is created to show the courses taken and their progress.

IBD Talks and IBD Points Educational programs

These online modules were co-developed by the Canadian Association of Gastroenterology and AbbVie

through an educational grant[28]. IBD Talks and IBD Points are educational programs developed to meet the learning needs of practicing IBD experts on how Motivational Communication could be useful in clinical practice. They were developed by a multidisciplinary faculty including gastroenterologists, IBD nurses, and a motivational communication expert.

The activity is an accredited self-assessment program as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada and was approved by CAG on 23/03/2016. The program expires in March 2019. Each module is eligible for 2 hour-credits after the completion of all of its stages: Pre-test, Learning Module, Post-test, Self-Assessment Evaluation and Program Evaluation. Upon completion of a free subscription, a dashboard is created which shows the progress of each module.

INDEPENDENT RESOURCES WEBSITES

Imedex E-learning Center

Imedex® is an industry leader in providing certified, independent continuing medical education for health care professionals, with high quality scientific activities in multiple specialties which aims to improve disease management and patient care. The e-learning activities include video and audio material from interviews, debates and panel discussions with world-renowned experts that translate the latest research into clinically relevant information, in various areas in medicine including gastroenterology and IBD[29]. The subscription is free, the activities are accredited and are organized in lists for each specialty. Subscribers can sign up for email updates and alerts for all types of educational activities.

You and IBD

This website is designed to provide patients and health care practitioners with updated information about IBD[30]. Learners can find excellent animations, slideshows, downloads, quizzes, and a library of visual tools. Topics cover a wide range of topics related to IBD management including causes, diagnosis, medications, operations and lifestyle choices. The site has free access and with a free registration the user can download free educational resources, receive updates and future notifications from the website.

MyCME

Haymarket Medical Education, a medical education company, has developed *MyCME* offering independent continuing education programs for physicians, physician assistants, nurse practitioners, pharmacists, nurses, and other healthcare professionals[31]. In MyCME website, with a free subscription, the user can have access to CME activities listed under specialty categories. To receive the certificate, participants must read the learning objectives and disclosure statements, complete a pre-test, study or watch the educational activity, and finally complete the post-test and activity evaluation form. The online certificate can be saved on myCME within the user's Profile/CME History, which can be accessed any time. *MyCME application* is also available and free to download.

CME outfitters

CME Outfitters (CMEO) is an independent resource of accredited, evidence-based medical educational activities operating since 2002, aiming to improve patients' care by improving clinical competence of the health care professionals including physicians and other healthcare providers[32]. In the CMEO webpages the user can find continuing medical education activities organized by date, credit type, specialty and topic (*i.e.*, Gastroenterology: IBD, Crohn's disease, or ulcerative colitis). Information is presented in multiple formats designed to satisfy diverse learning preferences, including nationally televised satellite broadcasts (live and recorded), internet webcasts podcasts, symposia at major medical meetings or conferences. Subscription is free and for each CMEO activity there is knowledge evaluation during the credit request process with a pre-test and a scored post-test joined with performance feedback and evaluation of the activity.

GastroCE

GastroCE is another independent source of accredited evidence-based medical educational activities which contains articles, lectures, videos, case studies, and CME modules covering various topics in IBD[33]. Subscription is free and for each CME activity there is a pre-test knowledge evaluation and a scored post-test. Self-assessment tests are also available. Participant activities are stored in history pages for each account. Resources for patients are also included in this website.

Medscape

Medscape is a well-known online global website for physicians and healthcare professionals, which offers, with free subscription, up-to-date medical news and expert perspectives, essential drug and disease information, and relevant professional educational activities including accredited CME. Medscape also offers the *Medscape*, *MedPulse News* and the *CME and Education applications*.

In the section of *Medscape Gastroenterology*, the user can find the latest news about different diseases and conditions including IBD[34]. Under the CME and Education List the *Inflammatory Bowel Disease CME Learning Center* is available with up-to-date accredited CME activities[35].

OTHER WEBSITES

The websites containing educational material for IBD comprise a long list and they cannot be covered completely with this review. A few more interesting on-line sources or websites include the *IBD module*

Table 1 Inflammatory bowel disease educational resources for healthcare professionals and patients

	Website		
	Name	**Address**	**Educational activity type**
Societies			
ECCO	e-CCO	https://e-learning.ecco-ibd.eu/	IBD curriculum, archived videos, webcasts, CME, *etc.*
CCFA	Virtual Preceptorship	http://www.crohnscolitisfoundation.org/science-and-professionals/programs-materials/virtual-preceptorship.html	Videos, brochures, CME
	REACH-IBD	http://programs.rmei.com/IBDKnowledgegap	Videos
CAG	ePortal	https://www.cag-acg.org/education/eportal	Videos, slide shows, CME, MOC
ACG	ACG Education Universe	http://universe.gi.org	Accredited courses, CME, MOC
AGA	AGA Education	http://www.gastro.org/education	Accredited courses, CME, MOC
Universities			
University of Pittsburgh	IBD LIVE	https://services.choruscall.com/links/UPMC/ibd/	Live-webcasts, archived webcasts
IBD groups			
The IBD working group	IBDWG	http://www.ibdwg.org	Videos, slide shows
Mentoring in IBD	IBD dialogue	http://www.mentoringinibd.com/category/ibd-dialogue/classic-edition/	Bulletin *via* email
	e-mentoring IBD	http://www.mentoringinibd.com/category/e-mentoring/	Newsletter *via* email
Industry sponsored			
	SEEMLI	https://www.seemli.ca/Dashboard - /MyCourses	Videos, power point presentations, CME
	IBD Talks and Points	https://www.ibdtalkspoints.ca/login/index.php	Videos, CME
Independent resources			
Imedex	Imedex E-learning Center	http://elc.imedex.com/	Videos, CME
You and IBD	You and IBD	http://www.youandibd.com/en-ibd/home	Animations, slide shows, quiz
Haymarket Medical Education	MyCME	http://www.mycme.com/	Videos, CME
CME outfitters	CME outfitters	https://www.cmeoutfitters.com/	Videos, CME
GastroCE	GastroCE	https://cme.healio.com/gastroce/	Articles, lectures, videos, case studies, CME
Medscape	IBD CME Learning Center	https://www.medscape.org/resource/ibd/cme	News, videos, CME
MEDPAGE TODAY	Gastroenterology	https://www.medpagetoday.com/gastroenterology	News, videos
AMEDEO	Literature Guide in IBD	http://amedeo.com/medicine/ibd.htm	Journal scan email
PubMed	My NCBI	https://www.ncbi.nlm.nih.gov/sites/myncbi/searches/	Journal scan email

ECCO: The European Crohn's and Colitis Organization; IBD: Inflammatory bowel diseases; CME: Continuing medical education; CCFA: The Crohn's and Colitis Foundation of America; REACH-IBD: The Rising Educators, Academicians and Clinicians Helping IBD; CAG: The Canadian Association of Gastroenterology; ACG: The American College of Gastroenterology; AGA: The American Gastroenterological Association; IBDWG: The IBD Working Group; SEEMLI: Standardizing the Endoscopic Evaluation of Mucosal Lesions in IBD; MOC: Maintenance of certification.

"IBD: Key Concepts and Treatment Paradigms" at the American College of Physicians website[36], the Cleveland Clinic Center for continuing education[37], the MEDPAGE today[38], the American Society of Colon and Rectal Surgeons website[39], and the Inflammatory Bowel Disease Toolkit[40].

More IBD-related accredited educational material can be found online in the American College of Gastroenterology (ACG; *ACG Education Universe*)[41] and the American Gastroenterological Association (AGA; *GI self-assessment modules SAM, Digestive Diseases Self-Education Program, DDSEP*)[42] websites, which cover various topics in GI disorders including IBD.

Finally, with the *AMEDEO Medical Literature Guide* subscribers receive newsletters with an overview of new articles published on a pre-selected topic (*i.e.*, IBD) and personalized journal subset[43]. Similarly, PubMed in the *My NCBI homepage* offers the opportunity to receive regular emails with new results based in a previously saved set of search terms[44]. Both medical literature web search engines are available with free subscription.

CONCLUSION

The advent of new therapies in IBD, the shift of treatment goals from control of symptoms to endoscopic mucosal healing in the treat-to-target approach, the advances in imaging technology and in surgical techniques, and the change to a more patient-centered approach in IBD-related clinical practice and care have made the management of IBD very demanding and challenging. The healthcare professional with an interest in treating IBD patients should deal with all these challenges in his or her everyday practice by establishing, enhancing and maintaining a strong core of knowledge and skills related to IBD management. Moreover, patients are

increasingly using the Internet to obtain information about their health conditions and therefore the healthcare professional should be well informed with up-to-date knowledge during clinical discussions with them.

Traditionally the needs in acquiring or updating knowledge are covered with additional reading of relevant textbook or journals, and attendance of meetings, congresses or small group discussions. Technology-enhanced learning with the Internet as its major source expands the options for information and knowledge acquisition and may save on some of the time and costs of attending meetings or conferences.

In this review, we have explored internet learning resources for the Inflammatory Bowel Diseases. There are many resources with diverse characteristics that provide information about new drugs or strategies, improve or maintain knowledge, or enhance experience and management skills. We presented and listed websites that offer substantial and variable educational material related to IBD (Table 1), but of course there many more. Each healthcare professional should individually visit and regularly navigate the relevant websites that tailor educational activities to their existing needs. It would also be very wise to be familiar with and have available a few certified internet resources with material suitable for patients. Particularly important and useful are the *ECCO IBD Curriculum*[13], the *CME outfitters*[32], the *GastroCE*[33], the *"IBD LIVE" webcast program*[19], the *You and IBD*[17], and the *E-mentoring in IBD* websites[23], which are among the most impressive, novel and authoritative educational activities related to IBD.

In the new era of communications technology, Internet-based resources can cover the educational needs of both patients and healthcare professionals who treat IBD and can contribute to the improvement of disease management, patient care and patient outcomes. Aiming at improving the online educational resources, future studies should investigate the quality and the utility of these websites.

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MINIREVIEWS

Dual HER2 inhibition strategies in the management of treatment-refractory metastatic colorectal cancer: History and status

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Abstract

Human epidermal growth factor receptor 2 (HER2) signaling pathway activation has been identified as a contributor to de novo or acquired resistance to epidermal growth factor receptor (EGFR) inhibitors in a small subset of patients with metastatic colorectal cancer (mCRC). Dual anti-HER2-targeted treatment exhibits strong antitumor activity in preclinical models of HER2-positive mCRC, supporting its testing in clinical trials. The HERACLES trial at four Italian academic cancer centers has confirmed the effectiveness of dual blockage of HER2 with trastuzumab plus lapatinib in patients with heavily pretreated HER2-positive mCRC, refractory to the anti-EGFR antibodies cetuximab or panitumumab. Here, we reviewed the preclinical studies exploring the role of HER2 signaling in the development of anti-EGFR therapy resistance and discussed the status of clinical trials assessing the activity of HER2 inhibitors in this setting.

Key words: Epidermal growth factor receptor; Cetuximab; Panitumumab; Human epidermal growth factor receptor 2; Anti-epidermal growth factor receptor resistance; Trastuzumab; Dual inhibition

Core tip: We reviewed the preclinical studies exploring the role of human epidermal growth factor receptor 2 (HER2) signaling in the development of anti-epidermal growth factor receptor therapy resistance in metastatic colorectal cancer and discussed the status of clinical trials assessing the activity of HER2 inhibitors in this setting.

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INTRODUCTION

The occurrence of distant metastases is an unfortunate but common event during the clinical course of colorectal cancer (CRC). Approximately three-quarters of patients with CRC present with unresectable stage Ⅳ disease at initial diagnosis or at follow up[1]. These patients usually benefit from modern systemic therapies, including chemotherapy alone or in combination with targeted therapy. However, in the treatment decision-making process, clinicians should consider various patient (age, performance status, comorbidity, and life expectancy) and tumor characteristics [location (*i.e.*, right-sided *vs* left-sided), mutation profile (*i.e.*, RAS mutated *vs* RAS wild-type), disease extent, and possibility of secondary resection] that may influence the treatment effectiveness and morbidity outcomes.

Patients with metastatic CRC (mCRC) who have poor performance status and very extensive disease are mostly managed by a palliative care approach. Expectedly, the administration of chemotherapy may create tolerability issues in elderly patients. Therefore, single-agent chemotherapy (fluoropyrimidine or irinotecan) is generally preferred to classical combination regimens in elderly patients. Otherwise, all physically fit patients with mCRC, particularly those who have a greater chance for salvage surgical resection following systemic therapy, should be aggressively treated to obtain better clinical outcomes. In the modern clinical practice, epidermal growth factor receptor (EGFR, also known as HER1) pathway inhibition in CRC cells using EGFR-targeting monoclonal antibodies (cetuximab and panitumumab) is an important component of this aggressive approach to treatment[2].

Because of their mechanism of action, anti-EGFR antibodies should be administered only in patients with CRC whose tumors do not contain activating mutations in one of their *RAS* genes (K-, N-, and H-RAS)[3,4]. Briefly, these drugs specifically bind to the extracellular portion of EGFRs in cancer cells to prevent triggering their activation by endogenous ligands, such as epidermal growth factor and transforming growth factor alpha[5] (Figure 1). Therefore, anti-EGFR antibodies successfully inhibit ligand-induced dimerization of EGFR with itself and with another HER family member (HER2, HER3, and HER4). This causes deactivation of intracellular mitogenic signaling pathways including the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR cascades, leading to G1 phase cell cycle arrest and apoptosis in cancer cells[5,6].

Conversely, in tumors harboring *RAS* mutations, the RAS-RAF-MEK-ERK pathway remains consecutively active, independent of the canonical EGFR signaling[7]. In this case, anti-EGFR antibodies are completely inactive and sometimes detrimental[8].

EGFR inhibitors are preferentially administered together with oxaliplatin-based (*i.e.*, 5-fluorouracil, leucovorin, and oxaliplatin) and irinotecan-based (*i.e.*, 5-fluorouracil, leucovorin, and irinotecan) doublet chemotherapy regimens, or intensified chemotherapy regimens such as FOLFOXIRI (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan). Recent retrospective evidence revealed the relatively impaired antitumor activity of cetuximab in the frontline treatment of patients with mCRC whose tumors arise from the right side of the colon. Despite this finding, EGFR inhibitors are still important in both chemo-naïve and carefully selected chemo-refractory cases[9-13]. Notably, a recent phase 2 study comparing panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) with the antiangiogenic drug bevacizumab plus mFOLFOX6 in patients with previously untreated RAS wild-type mCRC reported a median survival time exceeding 40 mo for patients receiving panitumumab[14].

Wild-type RAS status does not guarantee a response to anti-EGFR drugs, and these drugs cannot induce any tumor shrinkage in a significant proportion of patients (30%–50%) with RAS wild-type mCRC. Numerous studies have elucidated the underlying mechanisms of anti-EGFR treatment refractoriness (*de novo* or primary resistance) in these patients. These studies consistently revealed that the presence of other genetic alterations in tumor cells potentiating the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR signaling, such as BRAF (V600E) mutation, PI3KCA (exon 20) mutation, and PTEN loss, can at least partially account for unresponsiveness[15-19]. In patients with these mutations, the use of angiogenesis inhibitors instead of EGFR inhibitors or the administration of intensified chemotherapy backbone such as FOLFOXIRI along with anti-EGFR agents are reasonable treatment strategies[20].

Additionally, almost all patients with mCRC who initially respond to EGFR inhibitors become resistant to the treatment over time (secondary or acquired resistance). The identification of compensatory cellular mechanisms leading to treatment failure is crucial to determine effective salvage pharmacological interventions that can re-induce tumor regression.

Over the last few years, studies have shown that despite its rarity, HER2 signaling pathway activation in cancer cells, primarily due to HER2 overexpression and gene amplification may play an important role in the development of primary and secondary resistance to anti-EGFR therapies in patients with mCRC[21,22].

HER2-POSITIVE COLORECTAL CANCER AS A NEW CLINICAL ENTITY

In contrast to other proteins in the HER family, HER2 has no endogenous ligand and is considered an example of an orphan receptor that is functionally incomplete[23]. It has

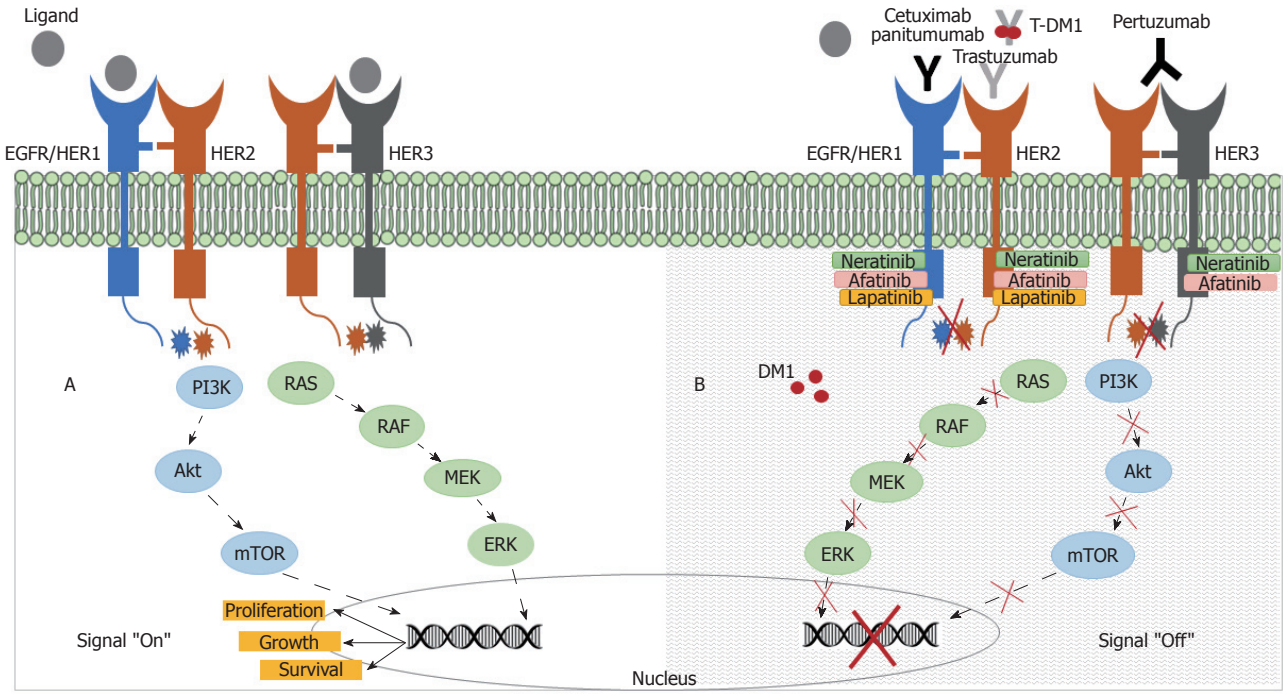

Figure 1 Epidermal growth factor receptor-related signaling pathways and anti-epidermal growth factor receptor and anti-human epidermal growth factor receptor 2 targeted drugs in colorectal cancer. A: Following ligand binding, the epidermal growth factor receptor (EGFR) (HER1) forms active homo- or heterodimers, resulting in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors. This triggers the RAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathways that transmit mitogenic signals to the nucleus; B: Dimerization of the receptors can be inhibited by EGFR-targeted (cetuximab or panitumumab) or HER2-targeted antibodies (trastuzumab or pertuzumab). Small molecule tyrosine kinase inhibitors (neratinib, afatinib, or lapatinib) can block EGFR and HER2 signaling by preventing adenosine triphosphate binding to the catalytic domain of protein kinases.

the strongest catalytic tyrosine kinase activity; therefore, it is a preferable dimerization partner, particularly for EGFR and HER3[23,24]. HER2 overexpression leads to increased EGFR membrane expression and activity[25]. HER2 overexpressing cells have significantly prolonged the activation of mitogen-activated protein kinase (originally called extracellular signal-regulated kinase, ERK) and c-Jun N-terminal kinase downstream signaling pathways following stimulation with EGFR or HER3 ligands compared with HER2-low expressing cells[26]. HER3 is considered an obligate dimerization partner in HER2-induced tumor cell proliferation[27,28]. HER2 overexpression is associated with enhanced HER3 phosphorylation and increased PI3K/Akt pathway activation[27,28].

The clinical and biological significance of HER2 signal activation in CRC has become an important research topic after the identification of *HER* gene amplification as a potential mechanism of anti-EGFR treatment resistance in patient-derived xenograft models and cell lines[21,22]. Bertotti *et al*[21] produced a large patient-derived xenograft platform using tumor samples from patients with CRC undergoing liver metastasectomy. They found that only a small portion (2%–3%) of genetically unselected xenopatients showed *HER2* gene amplification. However, in xenopatients whose tumors were KRAS wild-type and cetuximab-resistant, the frequency of *HER2* gene amplification increased to 13.6%. Furthermore, in a subset of xenopatients with cetuximab-refractory KRAS/NRAS/BRAF/PIK3CA wild-type CRC, its frequency increased to 36%. This suggested that HER2 amplification could be a key driver of anti-EGFR resistance in CRC, and anti-HER2 therapy could be an option in selected patients. Therefore, the effects of anti-EGFR and anti-HER2 therapies in cetuximab-resistant, HER2-amplified mCRC xenopatients were investigated. Dual EGFR/HER2 inhibition with pertuzumab (an anti-HER2 monoclonal antibody that blocks HER2/HER3 dimerization) plus lapatinib (a small molecule dual inhibitor of EGFR and HER2 receptor tyrosine kinases) caused significant tumor regression. A combination of lapatinib and cetuximab also significantly reduced tumor volume, but to a lesser extent than pertuzumab plus lapatinib.

Yonesaka *et al*[22] found that the activation of HER2 signaling either by *HER2* gene amplification or HER3-activating heregulin ligand overproduction led to de novo or acquired resistance to cetuximab in human CRC cell lines by increasing activation of ERK 1/2 signal pathway. Treatment of these cetuximab-resistant cell lines with HER2 small interfering RNA (siRNA) and inhibition of HER2/HER3 dimerization using lapatinib and pertuzumab could restore cetuximab sensitivity both in vitro and in vivo. These preclinical findings were further confirmed by the authors in a cohort of patients with mCRC exhibiting de novo or acquired resistance to cetuximab-based therapy. In these patients, *HER2* gene amplification in tumor specimens or high levels of circulating heregulin in patient plasma samples was detected.

Using HER2-amplified patient-derived tumor grafts,

Leto *et al*[29] confirmed the necessity of dual HER2 inhibition to induce effective tumor shrinkage in patients with CRC. They indicated that trastuzumab plus lapatinib or irreversible pan-HER inhibitor afatinib alone have higher antitumor activity than lapatinib monotherapy in HER2-amplified patient-derived CRC and gastric cancer cell-line xenografts. Delayed reactivation of HER3 and EGFR during lapatinib treatment has been proposed as a reason for its reduced effectiveness.

Kavuri *et al*[30] revealed that HER2 somatic mutations (S310F, L755S, V77L, V842I, and L866M) can activate the HER2 signaling pathway and cause panitumumab and cetuximab resistance in CRC cell lines, irrespective of the presence of HER2 amplification or overexpression. In addition, the *HER2* gene was sequenced in 48 CRC PDX samples that were cetuximab-resistant and wild-type for KRAS, NRAS, BRAF, and PIK3CA. Only four (8.3%) PDXs were found to have HER2-activating mutations. Treatment of mice carrying these HER2 mutant xenografts with dual HER2-targeted therapy with either trastuzumab plus neratinib (an irreversible pan-HER tyrosine kinase inhibitor) or trastuzumab plus lapatinib led to sustained tumor regression. These data suggest that a small number of patients with anti-EGFR therapy-refractory mCRC can have HER2 activating mutations, and these patients may benefit from dual HER2 blockage.

CLINICAL RELEVANCE OF HER2 EXPRESSION IN METASTATIC COLORECTAL CANCER

Studies have shown that HER2 overexpression seems to have no prognostic value in CRC. Richman *et al*[31] investigated the relationship between HER2 overexpression and survival in 1342 patients with mCRC who were previously enrolled in the FOCUS and PICCOLO cancer therapy trials. Among them, HER2 overexpression by fluorescence in situ hybridization (FISH) and/or immunohistochemistry (IHC) was identified in 29 (2.2%) patients but was not predictive of disease-free and overall survival (OS). Seo *et al*[32] found that *HER2* gene amplification was associated with tumor location and was more frequently detected in tumors originating in the rectum than those originating in the right and left colon. However, they did not see a relationship between HER2 overexpression and several aggressive clinicopathological features of CRC, including infiltrative tumor border, invasion depth, perineural invasion, lymph node metastasis, and distant metastasis.

Tu *et al*[33] reported HER2 overexpression in 102 (11.6%) of 878 Chinese patients with CRC. HER2 overexpression was more frequent in patients with early-stage CRC compared to patients with advanced stage CRC. HER2 overexpression was associated with gender, age, histological type, tumor location, and other prognostic indicators such as tumor grade, depth of invasion, lymph node metastases, and distant metastases. Again, it was not a significant predictor of survival. All these findings were confirmed by a meta-analysis of 18 studies comprised 2867 patients with CRC[34].

Conversely, several studies found that HER2 overexpression or amplification was predictive of resistance to EGFR inhibitors in patients with mCRC. Jeong *et al*[35] identified HER2 amplification in seven (4.9%) of 142 patients with mCRC with RAS and BRAF wild-type tumors. These 142 patients were treated with cetuximab after failure of oxaliplatin, irinotecan, and fluoropyrimidine. The patients with HER amplification had significantly shorter progression-free survival (PFS) than did those without HER2 amplification [median, 3.1 mo *vs* 5.6 mo; hazard ratio (HR) 2.73, $P = 0.019$]. In addition, there was a trend for poor OS in patients with HER2-amplified tumors (10.1 mo *vs* 13.5 mo, HR 1.31; $P = 0.488$).

Martin *et al*[36] evaluated the *HER2* gene status by FISH in 170 patients with KRAS wild-type mCRC receiving cetuximab or panitumumab alone or in combination with chemotherapy for first- or second-line treatment. Among these patients, seven (4%) had *HER2* gene amplification in 90% of tumor cells and were classified as HER2-all-A patients. Sixty-one percent of the patients had HER2 overexpression due to polysomy or gene amplification in minor clones (HER2-FISH+ cases), and 35% of patients had slight or no HER2 gain (HER2-FISH- cases). Patients who were classified as HER-all-A had worse outcomes than those designated as HER2-FISH+ and HER2-FISH- in terms of response rate ($P = 0.0006$), PFS ($P < 0.0001$), and OS ($P < 0.0001$). These findings suggest that that tumor HER2 copy numbers may predict the response to anti-EGFR treatment in patients with KRAS wild-type mCRC.

CLINICAL TRIALS USING ANTI-HER2 AGENTS IN METASTATIC COLORECTAL CANCER

Early studies that investigated the effectiveness of using the anti-HER2 antibody trastuzumab in combination with irinotecan- and oxaliplatin-based chemotherapy in previously treated patients with mCRC revealed promising antitumor activity[37,38]. Since these studies were conducted in unselected patients, they did not provide useful information on the clinical activity of this therapeutic approach.

Some studies investigated whether HER2 inhibition could restore sensitivity to EGFR inhibitors in unselected patients with mCRC (Table 1). In a phase Ⅰ/Ⅱ trial, Rubinson *et al*[39] evaluated the efficacy and tolerability of a combination of pertuzumab and cetuximab in patients with cetuximab-refractory KRAS wild-type metastatic CRC. The study was terminated early following the enrollment of 13 patients due to intolerable side effects such as diarrhea, skin rash, and mucositis. Only seven patients were evaluable for response, with one

Table 1 Summary of completed and ongoing clinical trials of anti- human epidermal growth factor receptor 2 agents in metastatic colorectal cancer

Study	Phase	Treatment	Number of patients	Patient population	RR	mPFS	mOS
Rubinson[39]	Ⅰ / Ⅱ	Cetuximab + pertuzumab	7	Chemo- and cetuximab-refractory	14%	2.1 mo	3.7 mo
Sartore-Bianchi (HERACLES)[40]	Ⅱ	Trastuzumab + lapatinib	27	Chemo- and cetuximab/ panitumumab-refractory	30%	21 wk	46 wk
Hainsworth (MyPathway)[44]	Ⅱ	Trastuzumab + pertuzumab	34	Chemo-refractory	35%	NR	NR
Siena (HERACLES-RESCUE)[42]	Ⅱ	Trastuzumab-emtansine	Recruiting	Chemo- and cetuximab/ panitumumab and trastuzumab plus lapatinib-refractory			
NCT03457896	Ⅱ	Neratinib + trastuzumab or cetuximab	Recruiting	Cetuximab and/or chemo-refractory			
MOUNTAINEER[45]	Ⅱ	Tucatinib + trastuzumab	Recruiting	Chemo- and bevacizumab-refractory			

RR: Response rate; mPFS: Median progression-free survival; mOS; Median overall survival; Chemo: Chemotherapy; NR: Not reported.

(14%) patient showing a partial response lasting more than six months, and two (29%) patients achieving stable disease. These results suggested that the use of dual HER2 inhibitors with minimally overlapping toxicities could be a promising option to overcome cetuximab resistance in mCRC.

The seminal HERACLES (HER2 Amplification for Colorectal Cancer Enhanced Stratification) phase 2 trial conducted by Italian researchers tested the activity of dual-targeted trastuzumab and lapatinib therapy in patients with treatment-refractory, KRAS codon 12/13 wild-type and HER2-positive mCRC[40]. The rationale for this therapeutic approach was primarily based on the above-mentioned preclinical data suggesting promising activity for dual anti-HER2 blockade in this setting. Before patient enrollment, the authors screened 914 patients with KRAS exon 2 (codons 12 and 13) wild-type mCRC and identified 48 (5%) patients who had HER-positive tumors according to the HERACLES Diagnostic Criteria for colorectal cancer (tumors with 3+ HER2 score in more than 50% of cells by IHC or with 2+ HER2 score and a HER2:CEP17 ratio higher than 2.0 in more than 50% of cells by FISH)[41]. Of these 48 patients, 27 were eligible for the study. Twenty (74%) patients had previously received at least four treatment regimens, including the anti-angiogenesis drugs bevacizumab, regorafenib, or aflibercept, and all patients had been previously treated with the anti-EGFR antibodies cetuximab or panitumumab. Trastuzumab was given intravenously (initial loading dose 4 mg/kg followed by 2 mg/kg weekly), and lapatinib was given orally (1000 mg/d). The treatment was continued until disease progression or until withdrawal of treatment because of an adverse event. The primary endpoint was objective response rate (complete plus partial response). The secondary endpoints were PFS and safety. All 27 patients were evaluable for response. One had a complete response, and seven had a partial response with an overall objective response rate of 30%. Twelve (44%) patients achieved disease stabilization longer than 16 wk. Median PFS was 21 wk (95%CI: 16-32), and 12 (45%) patients were alive at one year. Treatment was mostly well tolerated. Six of 27 patients (22%) experienced grade 3 adverse events consisting of fatigue, skin rash, and increased bilirubin concentration. The study authors also investigated the molecular determinants of response, and they found that patients with a high *HER2* gene copy number (> 9.45 copies/cell) had significantly longer PFS compared with patients whose tumors had a lower gene copy number (median, 29 wk *vs* 16 wk, $P = 0.0001$). Patients who had a gene copy number higher than 9.45 were also more likely than patients with a gene copy number lower than 9.45 (44% *vs* 0%, $P = 0.02$) to have a response to treatment. These results showed that the combination of trastuzumab and lapatinib is safe and effective in treating patients with HER2-positive mCRC resistant to chemotherapy and anti-EGFR agents.

The HERACLES-RESCUE clinical study is currently investigating the activity of trastuzumab-emtansine (T-DM1), an antibody-drug conjugate consisting of trastuzumab linked to the cytotoxic agent emtansine, in patients with HER2-positive mCRC progressing after trastuzumab plus lapatinib[42]. The rationale for the selection of T-DM1 in this study resulted from testing in patient-derived xenograft models of CRC generated from patients with acquired resistance to trastuzumab and lapatinib in the HERACLES study. These models were found to have high levels of HER2 expression, and treatment with T-DM1 resulted in significant tumor regression, whereas no response was observed in animals treated with pertuzumab alone. Another relevant study, the HERACLES cohort B trial is evaluating the clinical activity of lapatinib or pertuzumab in combination with T-DM1 in patients who are HER2–therapy-naïve and have HER2-positive mCRC[43].

The MyPathway phase Ⅱ trial is investigating the efficacy and safety of pertuzumab plus trastuzumab in patients with treatment-refractory mCRC showing

overexpression or amplification of HER2 by gene sequencing and/or by FISH or IHC[44]. The interim efficacy data reflects initial results from 34 patients. Twelve patients have achieved partial response, and three have achieved stable disease for longer than four months. The median duration of response is 11.1 mo.

Another interesting phase Ⅱ trial (NCT03457896) is examining the efficacy of pan-HER inhibitor neratinib plus trastuzumab or neratinib plus cetuximab in patients with quadruple wild-type (KRAS/NRAS/BRAF/PIK3CA wild-type) HER2-amplified, HER2-nonamplified (wild-type), or HER2-mutated mCRC. In this trial, patients with HER2-amplified CRC with prior anti-EGFR therapy and/or HER2-mutated CRC with or without prior anti-EGFR therapy will be treated with trastuzumab plus neratinib until disease progression. Patients with HER2 wild-type or HER2-amplified CRC with no prior anti-EGFR therapy will receive cetuximab plus neratinib until disease progression.

The MOUNTAINEER study will test the combination of tucatinib and trastuzumab in patients with HER2 positive, anti-HER2 targeting therapy-naïve, and RAS wild-type mCRC who have been previously treated with chemotherapy and an antiangiogenic drug[45]. Tucatinib is a very potent and highly selective small molecule inhibitor of HER2 receptor. In HER2 positive xenograft models of CRC, it has shown substantial antitumor activity[46].

CONCLUSION

Extensive preclinical efforts have identified HER2 amplification or overexpression as a distinct and druggable molecular target in patients with mCRC who exhibit poor sensitivity to anti-EGFR. The ever-expanding clinical experience reveals that dual HER2 blockade may be an effective therapeutic strategy to overcome or reverse tumor resistance in this setting. Moreover, some case examples suggest that sequential HER2 blockade may provide long-term clinical benefit without causing significant class-specific adverse effects in patients with molecularly selected and treatment-refractory mCRC[47]. The initial results of the HERACLES-RESCUE study will most likely clarify this issue.

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ORIGINAL ARTICLE

Basic Study

Isolation and characterization of a new candidate human inactivated rotavirus vaccine strain from hospitalized children in Yunnan, China: 2010-2013

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Author contributions: Wu JY performed the majority of experiments and analyzed the data; Zhou Y, Zhang GM, Yi S, Mu GF, Yin N, Xie YP, and Lin XC performed the molecular investigations; Li HJ and Sun MS designed and coordinated the research; Wu JY and Zhou Y wrote the paper.

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Abstract

AIM

To determine the distribution of rotavirus *VP7* gene in

hospitalized children in Yunnan, China.

METHODS

A total of 366 stool specimens were collected from hospitalized children in hospitals in Yunnan Province from September 2010 to December 2013. The genomic RNA electropherotypes and the G genotypes of the rotaviruses were determined. A phylogenetic analysis of the *VP7* gene was performed. Rotavirus isolation was performed, and characterized by plaque, minimum essential medium, and all genes sequence analysis. Quantification of antibodies for inactivated vaccine prepared with ZTR-68 was examined by enzyme-linked immunosorbent assay and microneutralization assay.

RESULTS

Group A human rotavirus was detected in 177 of 366 (48.4%) stool samples using a colloidal gold device assay. The temporal distribution of rotavirus cases showed significant correlation with the mean air temperature. Rotaviruses were isolated from 13% of the rotavirus-positive samples. The predominant genotype was G1 (43.5%), followed by G3 (21.7%), G9 (17.4%), G2 (4.3%), G4 (8.7%), and mixed (4.3%) among a total of 23 rotavirus isolates. A rotavirus strain was isolated from a rotavirus-positive stool sample of a 4-month-old child in The First People's Hospital of Zhaotong (2010) for use as a candidate human inactivated rotavirus vaccine strain and for further research, and was designated ZTR-68. The genotype of 11 gene segments of strain ZTR-68 (RVA/Human-wt/CHN/ZTR-68/2010/G1P[8]) was characterized. The genotype constellation of strain ZTR-68 was identified as G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1. The *VP7* and *VP4* genotypes of strain ZTR-68 were similar to Wa-like strains.

CONCLUSIONS

A high prevalence of the G1, G2, and G3 genotypes was detected from 2010 to 2012. However, a dominant prevalence of the G9 genotype was identified as the cause of gastroenteritis in children in Yunnan, China, in 2013. A candidate human inactivated rotavirus vaccine strain, designated ZTR-68 was isolated, characterized, and showed immunogenicity. Our data will be useful for the future formulation and development of a vaccine in China.

Key words: Rotavirus; Genotype G; G1P[8]; Inactivated rotavirus vaccine; Genotype characterization; Rapid antigen detection kit; Phylogenetic analysis

Core tip: A high prevalence of the G1, G2, and G3 genotypes was detected from 2010 to 2012. However, a dominant prevalence of the G9 genotype was identified as the cause of gastroenteritis in children in Yunnan, China, in 2013. A candidate human inactivated rotavirus vaccine strain, designated ZTR-68 was isolated, characterized, and showed immunogenicity. Our data will be useful for the future formulation and development of a new inactivated rotavirus vaccine in China.

Wu JY, Zhou Y, Zhang GM, Mu GF, Yi S, Yin N, Xie YP, Lin XC, Li HJ, Sun MS. Isolation and characterization of a new candidate human inactivated rotavirus vaccine strain from hospitalized children in Yunnan, China: 2010-2013. *World J Clin Cases* 2018; 6(11): 426-440 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i11/426.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i11.426

INTRODUCTION

Rotavirus is a major cause of diarrhea in children aged below five years, and causes approximately 600000 deaths in both developed and developing countries each year[1,2]. It is estimated that approximately 35000 children die per year in China from rotavirus, which is the second largest number of rotavirus deaths in the world[3,4]. Rotavirus is a non-enveloped RNA virus, and the viral genome contains 11 segments of dsRNA. The surface structural proteins *VP7* and *VP4* define the virus' G and P genotypes, respectively[5]. Globally, human infections have been mainly caused by five G types, which are G1-G4 and G9[6,7]. Together, the genome codes for six structural proteins and five nonstructural proteins. Rotaviruses are now classified into G-genotypes based on the relatedness of the genes encoding *VP7*[8,9]. Molecular sequencing of rotaviruses has also led to the development of a classification system. In this system, each internal gene is assigned a particular genotype based on established nucleotide identity cut-off percentages. Now, the acronym Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx is used to classify the *VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6*-encoding segments[10].

In China, 70% of children deaths caused by rotavirus occur in rural areas each year, mostly among children aged below five years[11]. Many changes in China, such as economic development, improved access to medical care, improved sanitation, and the one child family rule, may all contribute to this decline in overall diarrheal deaths and in the number of rotavirus deaths as well. In western China, urbanization is ongoing, and the economy is still poor and developing. City Zhaotong, County Xiangyun and XishuangBanna are relatively impoverished districts in Yunnan province, located in southwestern China. In this study, stool samples from hospitalized children admitted for diarrhea were collected to determine the distribution of rotavirus. All hospitalized children were under five years old. The prevalence of rotavirus infection peaks during the winter season, when the temperature is low. We wanted to isolate and characterize a human virus for use as a

candidate human inactivated rotavirus vaccine strain and for further research. A rotavirus strain was isolated from a rotavirus-positive stool sample of a 4-month-old child in The First People's Hospital of Zhaotong (2010), which was designated ZTR-68.

MATERIALS AND METHODS

Ethics statement

Collection and use of human stool specimens was approved by the Ethics Committee of the Institute of Medical Biology (YISHENGLUNZI [2016] 3), and was provided following children stool samples with written informed consent.

Collection of stool specimens

Stool samples were collected from hospitalized children in Yunnan, China. Group A rotavirus antigens were detected using an enzyme immunoassay (colloidal gold device assay, Rotavirus Group A Diagnostic assay, Colloidal gold device, Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.) according to the manufacturer's instructions. These samples were collected from September 2010 through December 2013.

Rotavirus isolation

An extract of child stool sample was agitated in 20% (wt/vol) PBS, pH 7.0 ± 0.2. The suspension sample was centrifuged for clarification at 8000 *g* for 20 min. The extract was treated with trypsin (10 μg/mL) for 60 min and inoculated onto a monolayer of MA104 cells for 90 min. After washing, the monolayer was maintained in serum-free minimum essential medium (MEM, Institute of Medical Biology, IMBCAMS, Kunming, China) supplemented with trypsin (1.5 μg/mL) and streptomycin (50 μg/mL) for 4 d. A viral lysate was treated with the freeze-thawing procedure three times and centrifuged at 7700 *g* for 30 min before infected in MA104 cells. The rotavirus strain ZTR-68 was isolated from a 4-month-old child hospitalized in The First People's Hospital of Zhaotong, Yunnan Province, China. This patient had acute diarrhea associated with a positive result of fecal rotavirus antigen detected by Rotavirus Group A Diagnostic assay (Colloidal gold device, Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). The virus isolation procedure was performed by previously described methods[12,13].

Extraction and electropherotyping of viral RNA

Rotavirus dsRNA was extracted from stools and infected cell cultures by using the MiniBEST Viral RNA Extraction Kit (TaKaRa Biotechnology, Dalian, China) in accordance with the manufacturer's instructions. Rotavirus dsRNA was analyzed by 10% polyacrylamide gel electrophoresis (PAGE) analysis. For the analysis, 15 μL of RNA was electrophoresed, and the gels were stained with silver nitrate and photographed.

Table 1 Primer sequences used in reverse transcription-polymerase chain reaction

Primer	Sequence	References
GeneralF	5′- AATGTATGGTATTGAATATACCAC -3′	This study
GeneralR	5′- TAATGATCTTGACCTTTTGGACA -3′	This study
Beg9	5′- GAGAGAATTTCCGTTTGG -3′	[14,15]
End9	5′-GGTCACATCATACAATTCTAACCTAAG -3′	[14,15]

Polymerase chain reaction amplification and sequence analysis of VP7

The rotavirus dsRNA was used as a template for reverse transcription polymerase chain reaction (RT-PCR) using the Prime Script® One Step RT-PCR Kit (TaKaRa Biotechnology, Dalian, China) in accordance with the manufacturer's instructions. The rotavirus *VP7* general primers GeneralF/GeneralR designed in this study and previously described primers, Beg9/End9[14,15] (Table 1) were used. RT-PCR amplification was carried out in a 50 μL reaction volume containing PrimeScript 1 Step Enzyme Mix, 2 × 1 Step Buffer, 10 μmol/L primers, and template RNA (less than 1 μg). Thermocycling was performed for 30 min at 50 ℃, 2 min denaturation step at 94 ℃, and 30 cycles of 30 s denaturation step at 94 ℃, 30 s annealing step at 55 ℃-60 ℃, and a 1 min extension step at 72 ℃ for each assay. The RT-PCR products were analyzed by electrophoresis on a 1% agarose gel (Invitrogen, Spain) in Tris-borate buffer containing ethidium bromide and visualized under ultraviolet light. The 2000 bp DNA ladder marker (Fermentase) was used as a size marker to estimate the lengths of the products. The PCR amplicons were purified using a column-based purification kit (OMEGA, the United States) and sequenced with an automated DNA sequencer (ABI 3730XL, the United States).

Phylogenetic analysis of nucleotide and amino acid sequences

The *VP7* nucleotide and amino acid sequences were analyzed. The selected sequences were aligned with ClustalX[16]. The phylogenetic tree (neighbor-joining, maximum parsimony, maximum likelihood) was constructed using the MEGA 4 from dissimilar distances and pairwise comparisons with the Kimura 2-parameter model[17].

Plaque assay

The virus was plaque assessed by previously described methods with modifications[12]. Virus stocks were activated with trypsin (10 μg/mL) for 60 min at 37 ℃. The virus was diluted in MEM and 300 μL/well were plated on 6-well plates (Corning, the United States) with a monolayer of MA104. Then the inoculums were covered with 4 mL per well of MEM with 3.5% agarose. The

agar was natural solidification, and then the plates were incubated at 37 ℃ for 4 d. Visualized plaques could be read after adding 2% neutral red in 1 mL MEM.

Structural genes and nonstructural genes RT-PCR and nucleotide sequencing

RT-PCR was performed for amplification of all structural and nonstructural genes using the primers listed in the supplementary data. Briefly, the extracted RNA genome of rotavirus was denatured at 96 ℃ for 5 min, and RT-PCR was carried out by using a Prime Script® One Step RT-PCR Kit (TAKARA, China). This involved an initial reverse transcription step of 30 min at 50 ℃, 2 min denaturation step at 94 ℃, and 30 cycles of 30 s denaturation step at 94 ℃, 30 s annealing step at 55 ℃-60 ℃ and 1 min extension step at 72 ℃ for each assay. PCR products were electrophoresed in 2% agarose gels containing ethidium bromide and visualized under UV. PCR amplicons were purified by a column-based purification kit (OMEGA, the United States) and sequenced with an automated DNA sequencer (ABI 3730XL, Uthe United States). Phylogenetic tree (neighbor-joining, maximum parsimony, maximum likelihood) was constructed using the MEGA 4 from dissimilar distances and pairwise comparisons with 1000 bootstrap replicates and the Kimura 2-parameter model. The genotype of 11 gene segments of strain ZTR-68 (RVA/Human-wt/CHN/ZTR-68/2010/G1P[8]) was characterized. The genotype constellation of strain ZTR-68 was identified as G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1.

Electron microscopy

The morphology of the rotavirus strain ZTR-68 stained by phosphotungstic acid negative staining was checked by electron microscopy (HITACHI H-600, Japan) to ensure that the virus contained triple-layered particles (TLP).

Vaccine preparation and vaccination of mice

Rotavirus strain ZTR-68 was cultivated in MA104 cells and the virus was purified by isopycnic CsCl gradient centrifugation as described previously[18], Triple-layered particles and double-layered particles were collected separately. Both purified viruses were dialyzed in 10 mmol/L PBS pH 7.0 to remove CsCl. TLPs were inactivated with formalin at 400 μg/mL concentration at 37 ℃ for 120 h. The inactivated vaccine was stored at 4 ℃ before use. The vaccine was quantified for proteins by Lowry method[19] and rotavirus antigen was quantified by enzyme-linked immunosorbent assay (ELISA). Balb/c mice (18-22 g) were purchased from Institute of Medical Biology, CAMS (Kunming, China). Mice were separated in groups of 20, and vaccinated with 0 μg, 10 μg or 20 μg inactivated rotavirus vaccine (IRV) formulated with alum by injection into the abdominal cavity. Pre-bleed was taken before injection. Serums were collected 2 wk after injection. All experiments were approved by the Institutional Animal Care and Use Committee of Institute of Medical Biology, CAMS (Kunming, China) and conducted in accordance with the ethical guidelines for animal experiments and safety guidelines.

Fluorescent cross-rotavirus antibody test

MA104 cell line was maintained in MEM with 10% fetal calf serum (GIBCO, the United States) in 6-well microtiter plates (Corning, the United States). The cell monolayers were incubated at 37 ℃ in an incubator containing 50 mL/L CO_2 under humid conditions, and infected with different viruses (strain Wa, SA11, S2, ZTR-68) with virus titer in 10^4-10^5 PFU/mL, and incubated for 16 h. The plates were fixed in chilled acetone (-20 ℃) for 15 min and air dried, then washed with 0.01 mol/L PBS buffer and blocked with 3% BSA for 1 h at 37 ℃. Mouse anti rotavirus strain ZTR-68 20 μg inactivated vaccine antibody (1:500 dilution, 0.5 mL volume) was added to microtitre plate wells and incubated at 37 ℃ for 1 h. After washing with 0.01 mol/L PBS, goat anti mouse FITC conjugate (MILLIPORE, 1:1000) was added and incubated for 1 h. The slides were examined under fluorescent microscope (Nikon, Japan) indicative of rotavirus.

ELISA and microneutralization assay on sera

RV-specific IgG antibodies were detected in sera on days 0, 14, and 28. Goat anti rotavirus antibodies (Millipore, the United States) coated 96-well ELISA plates overnight at 4 ℃. The plates were washed with PBS pH 7.0 and blocked with 5% BSA in PBS. The plates were washed and incubated with supernatant of rotavirus Wa infected MA104 cells (~ 10^5 PFU/well) for 1 h at 37 ℃. Serially diluted mouse serum was added in each well and incubated for 1 h at 37 ℃, , then washed. HRP-conjugated rabbit anti mouse antibody (Millipore, the United States, 1:1000) was added. TMB (Tiangen, China) substrate was added for detection and was stopped with 2mol/L H_2SO_4. OD value was determined with an EIA reader (BioTek, the United States). Cutoff value was twofold of negative well value, and the antibody titer was defined as the highest dilution OD greater than the cutoff value. Geometric mean titer was given. Microneutralization assay was performed to measure rotavirus neutralizing activity as described previously[20]. Serial diluted mouse serum was added to 96-well plates and incubated with 1000 PFU of Wa per well for 1 h at 37 ℃. MA104 cells were prepared in 96-well plates as a monolayer and cultivated in MEM with 5 μg/mL trypsin (GIBCO, the United States) at 37 ℃ for 3 d. Then all 96-well plates of neutralization were transferred into 96-well plates MA104 cells grown in a monolayer and incubated at 37 ℃ for another 7 d. The plates were lysed by freeze/thaw three times. RV antigen was detected by ELISA method with HRP-labeled goat anti rotavirus antibody (Institute of Medical Biology, CAMS, Kunming, China). Cutoff value was twofold of cells well without virus value, neutralizing antibody titer was defined as the highest dilution that gave a lower than antigen cutoff value. Virus-only controls were performed to confirm the effectiveness of ELISA system. Graph analysis was performed using GraphPad Prism 5.02, and statistical

Table 2 Numbers of rotavirus positive samples out of total stool specimens tested in this study. Number within parenthesis represents the percentage of rotavirus positive sample *n* (%)

	During whole year		During September to February	
	Samples tested	Rotavirus positive	Samples tested	Rotavirus positive
September 2010 to August 2011	176	81 (46.0)	164	81 (49.4)
September 2011 to August 2012	102	48 (47.1)	95	48 (50.5)
September 2012 to December 2013	88	48 (54.5)	87	48 (55.2)
Total	366	177 (48.4)	346	177 (51.2)

analysis was performed using Excel 2016 with the *T* test (two tailed). *P* values lower than 0.05 were considered statistically significant.

GenBank Accession Numbers

The sequences were deposited in GenBank under the following accession numbers: KM247264-KM247286. ZTR-68 genomic sequences: JX509930-JX509940.

RESULTS

Epidemiologic features of rotavirus in hospitalized children

Group A human rotavirus was detected in 177 of 366 (48.4%) stool samples using the colloidal gold device assay (Table 2) and in 133 of 177 (75.14%) by dsRNA-PAGE between September 2010 and December 2013 (Figure 1A). On an average, the prevalence of rotavirus during each year was 48.4% and 51.2% during the peak season. The age of the 177 patients ranged from one and a half months to four and a half years old (Figure 2A), although the majority were less than 2 years old (n = 157, 88.69%). The temporal distribution of rotavirus cases showed a significant correlation with the mean air temperature. The number of rotavirus cases peaked during the fall and winter seasons when the temperature began to decrease (Figure 2B and 2C).

All 11 segments were visible in 133 samples, corresponding to 75.14% of the 177 rotavirus-positive samples and 36.33% of the total 366 stool samples. In total, 97 samples presented a long electropherotype, 36 presented a short electropherotype and none of the samples had a mixed pattern. The electropherotypes of the samples obtained from September 2011 to August 2012 presented a relatively higher level of diversity than the samples obtained from September 2010 to August 2011 and from September 2012 to December 2013. There was relative concordance between the electropherotypes and the distribution of G genotypes (Figure 1A and 3A).

Partial sequence analysis of VP7 gene

We determined the *VP7* nucleotide sequences for G1, G2, G3, G4, and G9 for the G genotype isolates obtained during the study period (Figure 1B, 3A). It was determined that phylogenetically, the G3 isolates clustered in one lineage, the G1 isolates clustered in two lineages, and the G2 and G4 isolates clustered in one lineage. Several of the G1 isolates maintained a similarity of 97.4% to 99.9% for 2010, 2011, and 2013 in cluster 1-2, and the others maintained a similarity of 98.5% to 99.2% for 2011 in cluster 1-3. The G3 isolates analyzed during this study showed 99.8% to 99.9% similarity for 2010 and 2011, and they composed one cluster with the strains RV3, YO, and AU-1. In the case of G9, one sequence in 2012 (12N-T107) was related to the rotavirus strains BJ-CR7440, BJ-Q794, and MRC-DPRU1102, which originated in China and Zimbabwe. Three sequences in 2013 showed high similarity (99.9%) with each other and were related to strains that originated in Spain (98.2%), Ecuador (97.9%-98.1%), Saudi Arabia (97.5%-97.9%), Belgium (98.6%-98.9%) and Thailand (98.3%-98.9%). One G2 isolate 11O-T65 was related to strains RMC/G66, TB-Chen, and DS-1, which originated in Ecuador. One mixed genotype isolate (12N-T66) was found, which contained a mixed G2 and G9 genotype and was phylogenetically located between the G2 cluster and the G9 cluster. In the case of G4, two 2011 sequences (11D-T100, 11D-T101) were related to the Hochi (85.5%) and Gottfried (86.5%-86.6%) strains. In the case of G1, seven isolates analyzed in one cluster, closed to Wa and KU cluster. One G1 isolate 10O-T68R was related to strains Wa and KU but not in one cluster. This isolate then cultured in MA104 cells and purified by plaque (Figure S3), and was designated as strain ZTR-68.

Rotavirus isolation and characterization

Positive rotavirus antigen was diagnosed in the diarrheal stool of a 4-month-old child who was infected naturally with rotavirus and developed severe, acute diarrhea sustained for four days and was hospitalized in The First People's Hospital of Zhaotong, Yunnan Province, China. PAGE analysis of the rotavirus dsRNA segments from stool specimen revealed a typical 4-2-3-2 pattern of rotavirus, specific to the group A rotaviruses. (Figure 3B) The presence of virus-like particles, almost 70 nm in diameter was confirmed in this specimen by electron microscopy. (Figure 3C, Figure S2) The rotaviruses from isolate extract 10O-T68R were adapted to infect MA104 cells and purified by plague technique. ZTR-68 showed a typical CPE of rotavirus and virus titers grew to over $10^{6.3}$ PFU/mL.

Genotype classification of ZTR-68

The ORF nucleotide sequences for the 11 genome

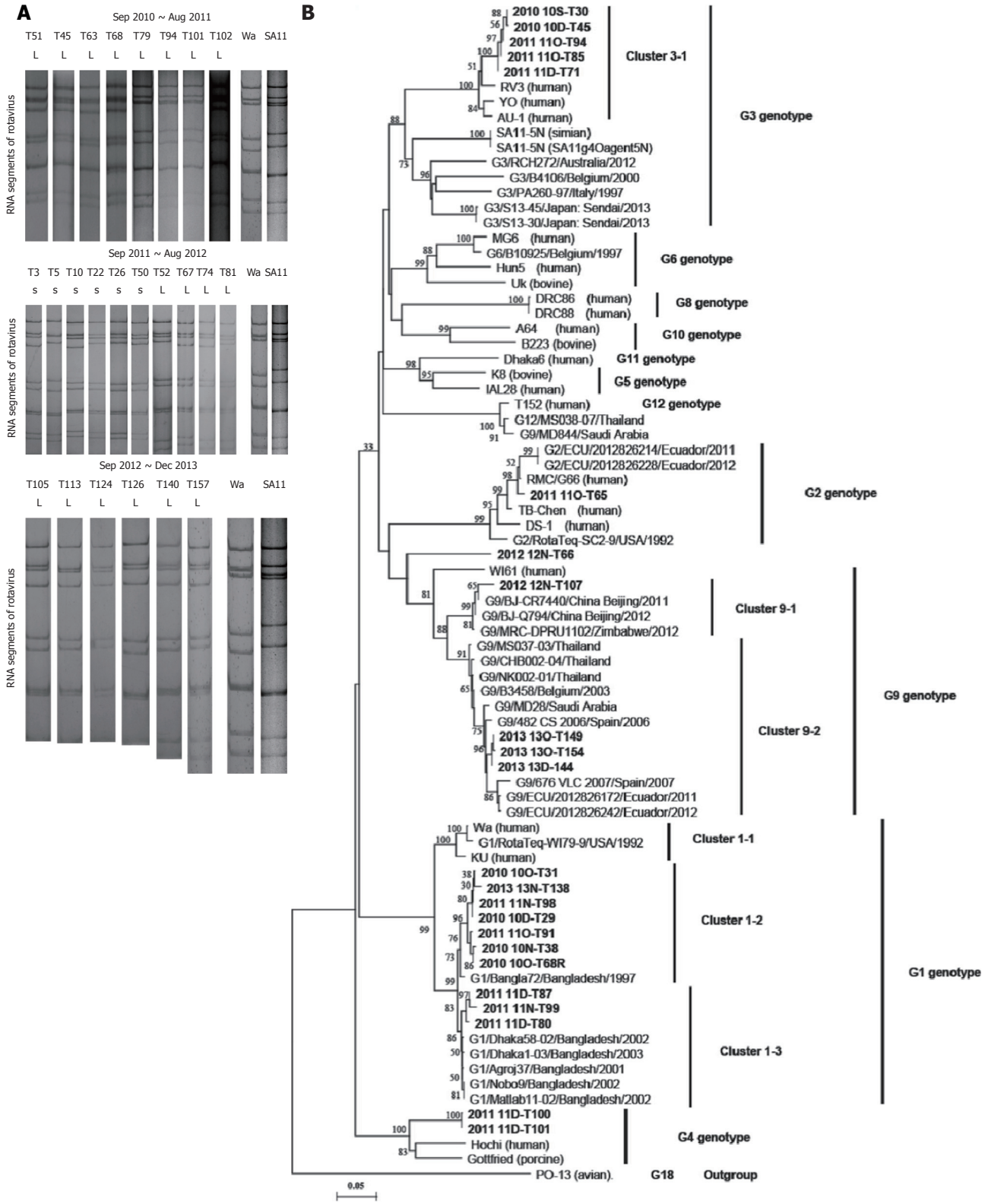


Figure 1 Polyacrylamide gel electrophoresis profiles and *VP7* gene sequences of rotavirus strains isolated from diarrhea stools collected in Yunnan, China. A: Electrophoretic migration pattern of RNA from 24 rotavirus-positive stool samples during September 2010 through December 2013. Rotavirus strains Wa and SA11 were used as the markers. The viral RNAs were analyzed by electrophoresis in a 10% polyacrylamide gel and visualized by staining with silver nitrate. L: long electropherotype; S: short electropherotype. Genes 10 and 11 of rotavirus RNA of some samples from September 2012 to December 2013 were not clear in this pattern; B: The partial sequences determined in this study are in bold. The most closely related sequences found in the GenBank database are also included. References for the sequences used in *VP7* gene comparisons marked with "G genotype/isolate/country/collected year". The scale bar represents 5% nucleotide sequence difference. Bootstrap values of > 50% (for 1000 iterations) are shown.

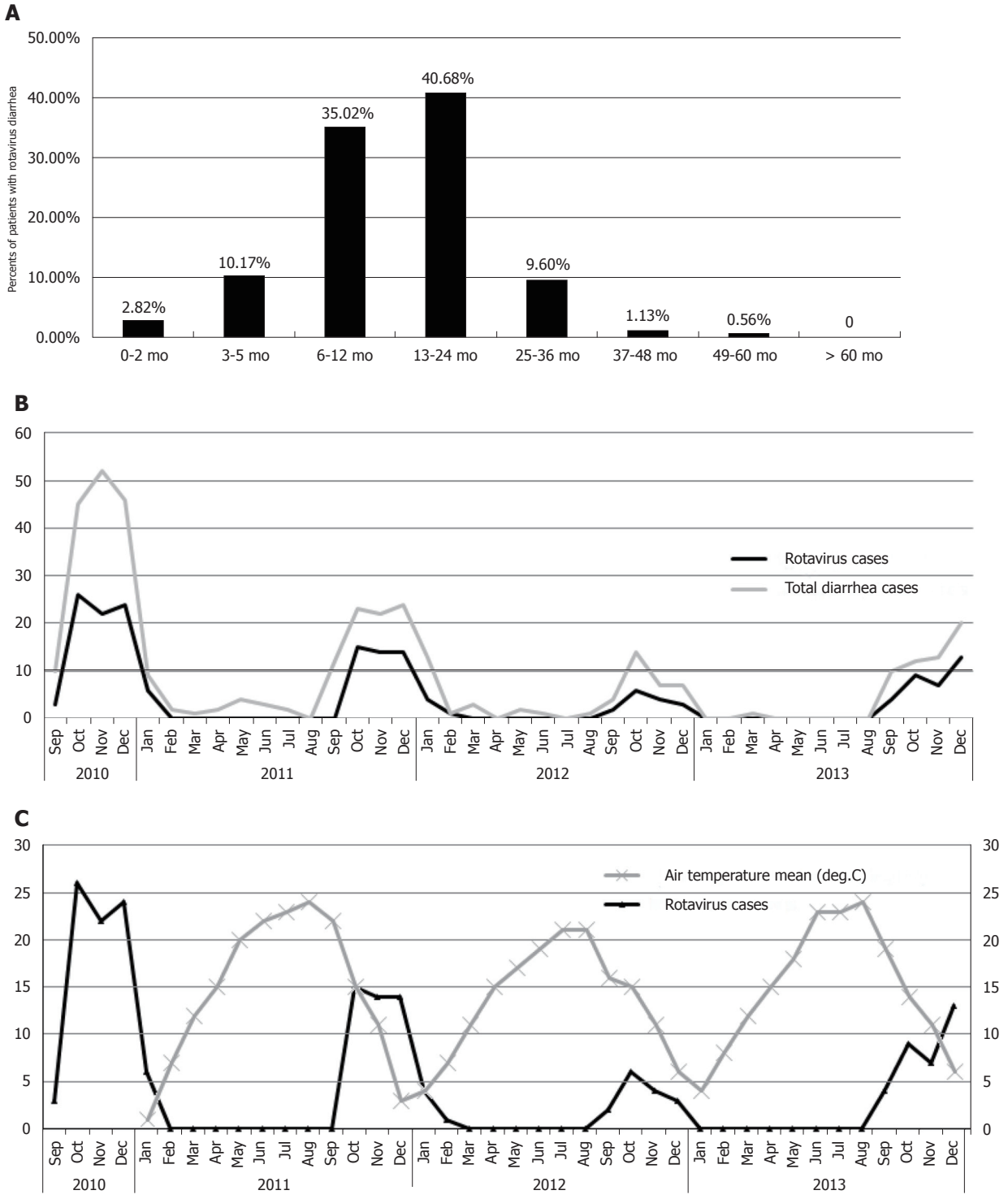


Figure 2 Age distribution and trends in number and proportion of gastroenteritis of rotavirus diarrhea cases from 2010 to 2013 in Yunnan, China. A: Age distribution of rotavirus diarrhea cases from 2010 to 2013; B: Trends in number and proportion of gastroenteritis by rotavirus antigen over the study period. The scale bar on the left represents the number of rotavirus cases and total diarrhea cases; C The mean daily temperature of Yunnan from January 2011 to December 2013 compared to the number of rotavirus cases.

segments of cell culture-adapted ZTR-68 strain were determined. The sequences deduced in this study are either identical or show a few changes from those that are already in GenBank for ZTR-68. Genotypes were assigned for each genome segment based on the nucleotide percent identity cut-off values defined by the RCWG and by submission to RotaC. Our analysis shows that ZTR-68 can be classified as a G1P[8] strain. Specifically, the gene segment 1 (*VP1*) of ZTR-68 showed 90.7%-99.2% nucleotide identity with the R1 genotype cluster. The sequence identity was found to be 98.6%-99.2%with strains Dhaka16-03, Dhaka6, and

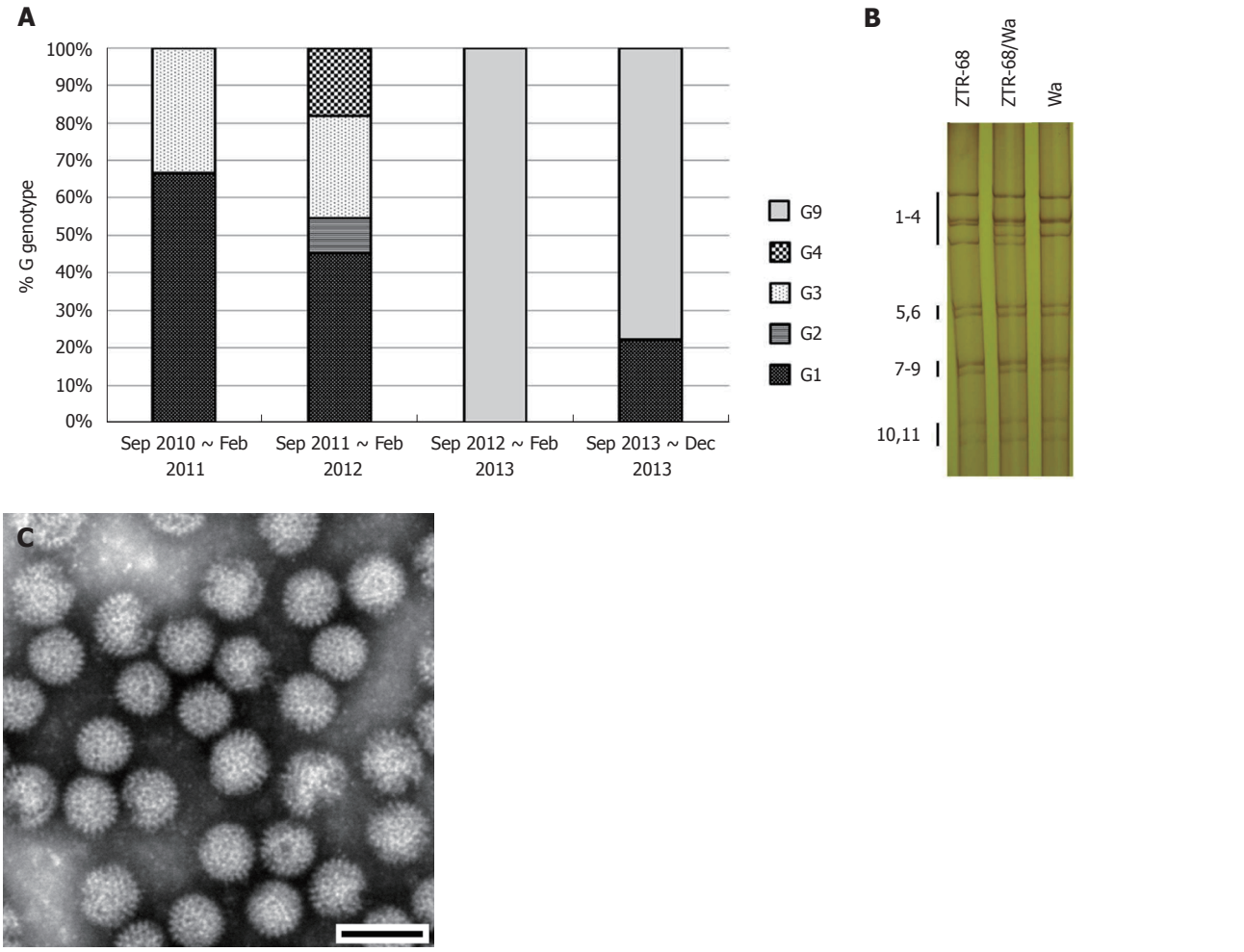
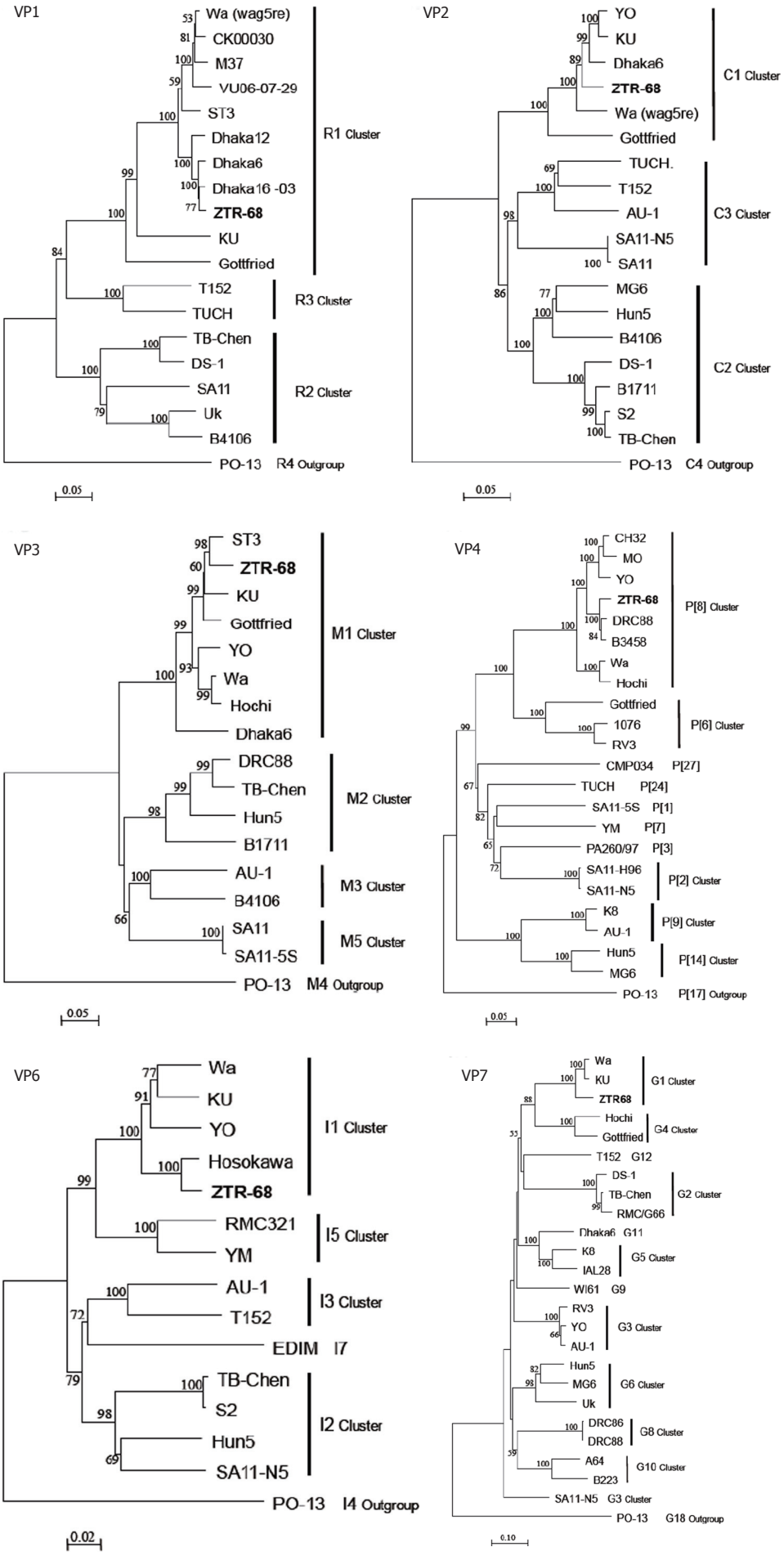


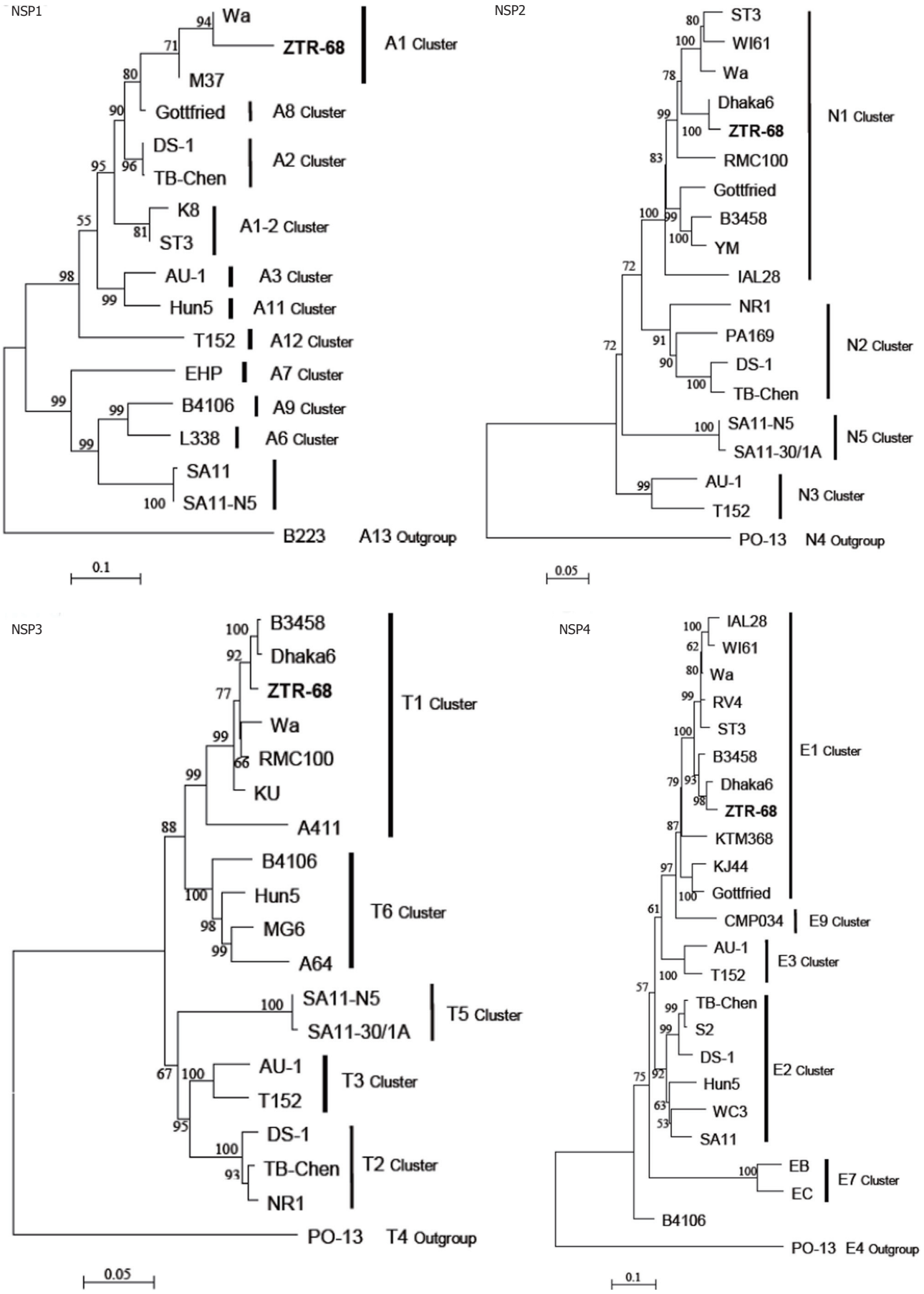
Figure 3 Rotavirus G genotype distribution and characterization of strain ZTR-68. A: Rotavirus G genotype distribution during rotavirus peak season (September to February) from 2010 to 2013, on the basis of rotavirus *VP7* genes sequences analysis; B: Electrophoretic pattern of ZTR-68/Wa. Electrophoretic migration pattern of RNA from rotavirus. Viral genomic dsRNAs extracted were separated in 10% polyacrylamide gels and visualized by silver staining. Numbers indicate the order of the ZTR-68 and Wa gene segments; C: Rotavirus strain ZTR-68, Bar = 100 nm.

Dhaka12 of genotype R1 lineage. Percent nucleotide sequence identities with other genotypes were in the range of 77.6%-85.8%. Sequence analysis of the *VP2* gene of rotavirus strain ZTR-68 showed genetic relatedness with the strains of the C1 genotype with 86.7%-95.2% nucleotide identity. The strain clustered with strains Wa, Dhaka6, YO, KU, and Gottfried. Percent nucleotide sequence identities with other genotypes (C2-C3) were in the range of 75.8%-77.5% (< the cut off value of 84%). Nucleotide sequence analysis of the *VP3* gene of rotavirus strain ZTR-68 showed genetic relatedness with the strains of M1 genotype with 86.0%-95.2% nucleotide identity. The sequence identity was found to be 95.2%, 91.9%, and 86% with strains ST3, Wa, and Dhaka6, respectively. Percent nucleotide sequence identities with other genotypes (M2-M5) were in the range of 69.6%-71.6% (< the cut off value 81%). Sequence analysis of the *VP4* gene of rotavirus strain ZTR-68 showed genetic relatedness with the strains of the P[8] genotype with 88.1%-97.3% nucleotide identity. The sequence identity was found to be 91.9%-97.1% with strains DRC88, B3458, MO, YO, and CH32 and 88.1%-89.1% with Wa and Hochi. Phylogenetically the strain ZTR-68 clustered with the strains of I1 genotype of the *VP6* gene indicating 91.8%-97.7% nucleotide sequence identity and identified to be closer to strain Hosokawa. The nucleotide identity with other genotypes (I2-I7) ranged from 80.2% to 85.2%. Sequence analysis of the *VP7* gene of rotavirus strain ZTR-68 showed genetic relatedness with the strains of G1 genotype with 91%-91.5% nucleotide identity. The sequence identity was found to be 91% and 91.5% with strains Wa and KU of genotype G1 lineage, respectively. Percent nucleotide sequence identities with other genotypes (G2-G10) were in the range of 53.7%-66.7% (< the cut off value 80%) (Figure 4).

IRV ZTR-68 induces increase of IgG and neutralizing antibodies levels

Immunogenicity of inactivated rotavirus vaccine prepared with ZTR-68 was examined by IgG ELISA and neutralization assay (Figure 5A and 5B). RV-specific


VP1
Wa (wag5re)
CK00030
M37
VU06-07-29
ST3
Dhaka12
Dhaka6
Dhaka16 -03
ZTR-68
KU
Gottfried
T152
TUCH
TB-Chen
DS-1
SA11
Uk
B4106
PO-13
R1 Cluster
R3 Cluster
R2 Cluster
R4 Outgroup
0.05
VP2
YO
KU
Dhaka6
ZTR-68
Wa (wag5re)
Gottfried
TUCH.
T152
AU-1
SA11-N5
SA11
MG6
Hun5
B4106
DS-1
B1711
S2
TB-Chen
PO-13
C1 Cluster
C3 Cluster
C2 Cluster
C4 Outgroup
0.05
VP3
ST3
ZTR-68
KU
Gottfried
YO
Wa
Hochi
Dhaka6
DRC88
TB-Chen
Hun5
B1711
AU-1
B4106
SA11
SA11-5S
PO-13
M1 Cluster
M2 Cluster
M3 Cluster
M5 Cluster
M4 Outgroup
0.05
VP4
CH32
MO
YO
ZTR-68
DRC88
B3458
Wa
Hochi
Gottfried
1076
RV3
CMP034 P[27]
TUCH P[24]
SA11-5S P[1]
YM P[7]
PA260/97 P[3]
SA11-H96
SA11-N5
K8
AU-1
Hun5
MG6
PO-13
P[8] Cluster
P[6] Cluster
P[2] Cluster
P[9] Cluster
P[14] Cluster
P[17] Outgroup
0.05
VP6
Wa
KU
YO
Hosokawa
ZTR-68
RMC321
YM
AU-1
T152
EDIM I7
TB-Chen
S2
Hun5
SA11-N5
PO-13
I1 Cluster
I5 Cluster
I3 Cluster
I2 Cluster
I4 Outgroup
0.02
VP7
Wa
KU
ZTR68
Hochi
Gottfried
T152 G12
DS-1
TB-Chen
RMC/G66
Dhaka6 G11
K8
IAL28
WI61 G9
RV3
YO
AU-1
Hun5
MG6
Uk
DRC86
DRC88
A64
B223
SA11-N5 G3 Cluster
PO-13
G1 Cluster
G4 Cluster
G2 Cluster
G5 Cluster
G3 Cluster
G6 Cluster
G8 Cluster
G10 Cluster
G18 Outgroup
0.10


NSP1
Wa
ZTR-68
M37
Gottfried
DS-1
TB-Chen
K8
ST3
AU-1
Hun5
T152
EHP
B4106
L338
SA11
SA11-N5
B223
A1 Cluster
A8 Cluster
A2 Cluster
A1-2 Cluster
A3 Cluster
A11 Cluster
A12 Cluster
A7 Cluster
A9 Cluster
A6 Cluster
A13 Outgroup
0.1
NSP2
ST3
WI61
Wa
Dhaka6
ZTR-68
RMC100
Gottfried
B3458
YM
IAL28
NR1
PA169
DS-1
TB-Chen
SA11-N5
SA11-30/1A
AU-1
T152
PO-13
N1 Cluster
N2 Cluster
N5 Cluster
N3 Cluster
N4 Outgroup
0.05
NSP3
B3458
Dhaka6
ZTR-68
Wa
RMC100
KU
A411
B4106
Hun5
MG6
A64
SA11-N5
SA11-30/1A
AU-1
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TB-Chen
NR1
PO-13
T1 Cluster
T6 Cluster
T5 Cluster
T3 Cluster
T2 Cluster
T4 Outgroup
0.05
NSP4
IAL28
WI61
Wa
RV4
ST3
B3458
Dhaka6
ZTR-68
KTM368
KJ44
Gottfried
CMP034
AU-1
T152
TB-Chen
S2
DS-1
Hun5
WC3
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E1 Cluster
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E7 Cluster
E4 Outgroup
0.1

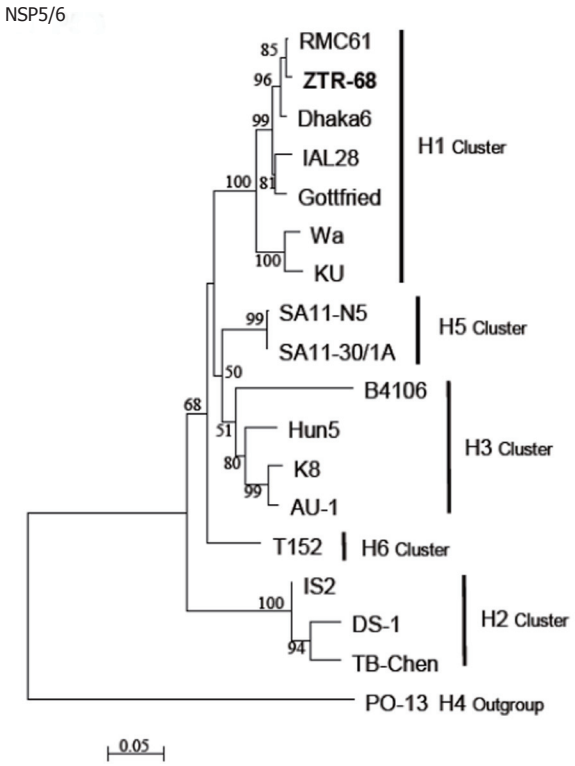

Figure 4 Phylogenetic trees of the all rotavirus 11 gene segments for ZTR-68. Phylogenetic trees of the genes coding for virus structural proteins and non-structural proteins. The scale bar represents 2%-10% nucleotide sequence difference. Bootstrap values of > 50% (for 1000 iterations) are shown.

IgG and neutralizing antibodies were detected after one dose vaccination, and its levels increased after two vaccinations. Ten micrograms of IRV induced lower levels of total IgG antibodies and neutralizing antibodies compared to 20 μg IRV. Both 10 μg and 20 μg of IRV induced increased IgG and neutralizing antibody levels. After the first vaccination (at 0 d), differences between 10 μg and 20 μg IRV were not statistically significant both in the IgG titer and neutralizing antibodies titer. In addition, a significant difference (P = 0.0043) was found in the IgG titer between 10 μg and 20 μg at 14 d and 28 d, but there was no significant difference (P = 0.0565) in the neutralizing antibodies titer. IRV ZTR-68 also could induce increased cross-typing anti-rotavirus antibodies anti-rotavirus strain Wa (G1P[8]), S2(G2P[4]), and SA11(G3P[2]) by the fluorescent antibody test (Figure 5C).

DISCUSSION

Rotavirus is recognized as a major public health problem for children in China[3,21-24]. Diarrhea remains the major common cause of Chinese children hospitalizations[11,25-28]. In Chinese rural areas, rotavirus caused about 33% of hospitalizations for severe pediatric gastroenteritis. The numbers of outpatient visits and all diarrhea episodes in the community are 28% and 7%, respectively[3,12,13,27,29,30]. The percentage of diarrhea patients hospitalized for rotavirus is consistent with findings in other Asian countries. In a 2004 study of rotavirus surveillance throughout Asia, 44%-53% of children hospitalized for gastroenteritis in Myanmar, Thailand, Indonesia, and Vietnam were detected as rotavirus-positive[31,32]. Our study demonstrated that the G1, G2, and G3 genotypes had a high prevalence from 2010 to 2011. However, the G9 genotype was the predominant cause of gastroenteritis in children in Yunnan, China, in 2012 and 2013. The most prevalent rotavirus G genotype shifted from G1–G4 and G9 to G1 + G9. Although the reason for this trend is unclear, the emergence of the G9 rotavirus has been documented in many studies over the last decade[33-35]. Previous studies have reported G3 as the most common strain in China[3,28], however, in this study, we found that G1 was predominant (43.3%), followed by G3 (21.7%), and G9 (17.4%). Notably, from 2010 to 2011, G1 + G3 were detected, while there was no G9 detected. However, from 2012 to 2013, G1 + G9 were predominant, and no G3 was detected. In this study, we observed an increase in the incidence of G9 rotavirus. In addition, G9P[4] rotavirus strains have been described as emerging in several Latin American countries[33,35]. The results of the phylogenetic sequence analyses demonstrating that some of the G9 rotavirus sequences detected in this study were closely related to sequences that originated in Ecuador may support this view. Some G9 rotavirus isolates were obtained during the surveillance, and full genomic sequencing and characterization will be performed in future studies.

Rotavirus diarrhea presents a serious health burden in China. A previous study suggested that every child in

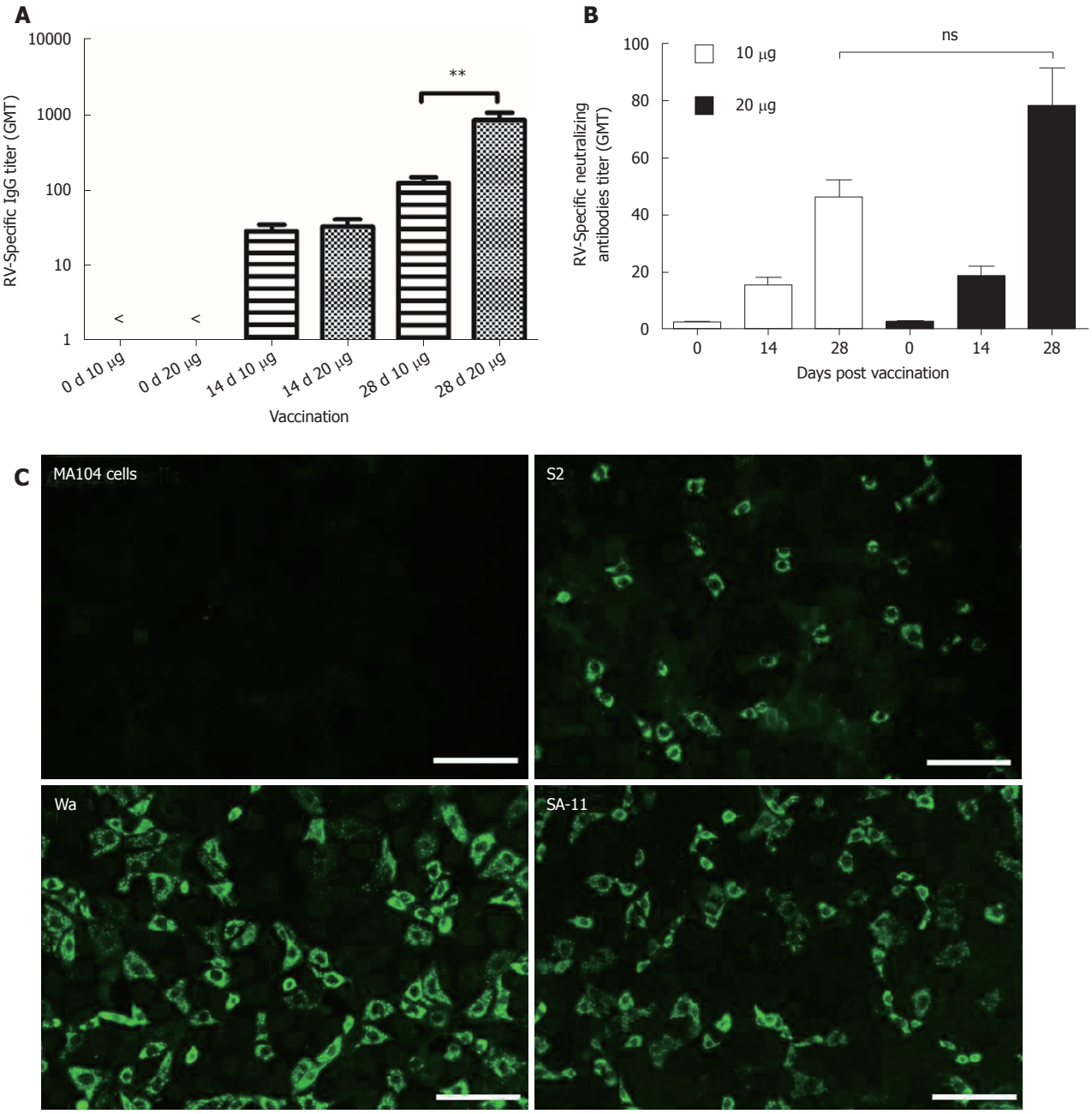

Figure 5 Increased IgG levels and neutralizing antibody levels in inactivated rotavirus ZTR-68. RV-specific IgG and neutralizing antibodies were detected in sera on days 0, 14, and 28. A: RV-specific IgG titer; B: Neutralizing antibodies titer. Both showed in GMT; C: Rotavirus vaccine (RV) specific antibodies detected by fluorescent cross-type rotavirus antibody test using rotavirus strain Wa(G1P[8]), S2(G2P[4]), and SA11(G3P[2]) for IRV ZTR-68. ns: not significant; $^{b}P \leqslant 0.01$.

China experiences at least one episode of diarrhea due to rotavirus[3]. The use of rotavirus vaccines should be part of a comprehensive strategy to control diarrheal disease[18]. WHO/UNICEF recommend that all children receive solutions of low-osmolality oral rehydration salts to prevent and treat dehydration due to diarrhea. Rotarix[36] and RotaTeq are two approved vaccines used worldwide[37]. The Lanzhou Lamb Rotavirus Vaccine has already been licensed and used in China for several years. However, the immunization program for rotavirus is unpopular in the area of Yunnan. Our study may be useful for decisions regarding the need for rotavirus vaccines for use in rural China, such as Zhaotong, Xiangyun, and Xishuangbanna. It is assumed that G1 type rotavirus may be the most common strain in these areas. However, some issues should be considered, such as the reason for the G3 and G9 shift over time and the impact of the changes in the genetic diversity of rotavirus on the use of a rotavirus vaccine in China.

Whole-genome sequencing has shown that human RVs with the genotype constellation of G1/G3/G4/G9/G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 or G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2 are globally dominant[7,38]. Based on all 11 rotavirus gene segments and the for-

mation of RCWG classification system, rotavirus ZTR-68 has been designated G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 and was known as Wa-like rotavirus group A (RVA). Human Wa-like RVA strains are believed to have a common ancestor with porcine RVA strains[39]. The ZTR-68 and its plaque clone 3C were fully adapted to grow in MA-104 and Vero cell cultures (data not shown). ZTR-68 was identified as a Group A rotavirus, which was a Wa-like strain. The characterization of ZTR-68 G and P types are similar to Wa, but different in RNA electropherotype. It was interesting that our data demonstrated several rotavirus isolates with high similarity to ZTR-68 during the period of this study. It is assumed that G1P[8] type rotavirus may be the most common strain in the Zhaotong area. But some issues should be considered, such as the reason that the G3 and G9 type shifted as time went on, and the impact of the variation of genetic diversity of rotavirus for the use of rotavirus vaccine in China. This newly isolated human Group A rotavirus, ZTR-68 is being used to establish a new candidate rotavirus vaccine for use in studying the immune effectiveness.

Unfortunately, we were not able to acquire purified G3 and G9 strains after several passages in cell culture. Determining the circulation of various rotavirus strains in this area is of interest. However, no P types were identified during the genotype detection, which is another limitation of our study. The usefulness of next-generation DNA sequencing has previously been evaluated for the direct detection of bovine rotavirus from fecal samples[40]. More meticulous rotavirus surveillance should be conducted in Yunnan, China. In this study, a high prevalence of the G1, G2, and G3 genotypes was detected from 2010 to 2012. However, the G9 genotype was the predominant cause of gastroenteritis in children in Yunnan in 2013. Our data will be useful for future efforts to formulate and develop a vaccine in China.

ARTICLE HIGHLIGHTS

Research background

Human rotavirus genotypes G1-G4 and G9 are major causes of acute gastroenteritis in children worldwide. Approximately 35000 children die per year in China due to rotavirus, which is the second highest rate of rotavirus deaths in the world.

Research motivation

In China, the elimination and control of the two kinds of diseases have experienced the process of alternating use of two kinds of vaccines, which proved to be feasible and effective. These successful experiences provide a theoretical basis and an approach to the development of an inactivated rotavirus vaccine. This newly isolated human Group A rotavirus, ZTR-68 is being used to establish a new candidate inactivated rotavirus vaccine for use in studying the immune effectiveness.

Research objectives

The aim of this study was to determine the distribution of rotavirus *VP7* gene in hospitalized children in Yunnan, China. A new candidate human inactivated rotavirus vaccine strain was isolated and characterized.

Research methods

A total of 366 stool specimens were collected from hospitalized children in hospitals in Yunnan Province from September 2010 to December 2013. The genomic RNA electropherotypes and the G genotypes of the rotaviruses were determined. A phylogenetic analysis of the *VP7* genes was performed. Rotavirus isolation was performed, and characterized by plaque, EM, and all gene sequence analysis. The sequences were deposited in GenBank under the following accession numbers: KM247264 - KM247286. ZTR-68 genomic sequences: JX509930 - JX509940. Quantification of antibodies for inactivated vaccine prepared with ZTR-68 were examined by ELISA and microneutralization assay.

Research results

Group A human rotavirus was detected in 177 of 366 stool samples using a colloidal gold device assay. The temporal distribution of rotavirus cases showed significant correlation with the mean air temperature. Rotaviruses were isolated from 13% of the rotavirus-positive samples. The predominant genotype was G1 (43.5%), G3 (21.7%), G9 (17.4%), G2 (4.3%), G4 (8.7%), and mixed (4.3%). A rotavirus strain was isolated from a rotavirus-positive stool sample of a 4-month-old child in The First People's Hospital of Zhaotong (2010) for use as a candidate human inactivated rotavirus vaccine strain and for further studies, which was designated ZTR-68. The genotype of 11 gene segments of strain ZTR-68 (RVA/Human-wt/CHN/ZTR-68/2010/G1P[8]) was characterized. The genotype constellation of strain ZTR-68 was identified as G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1. The *VP7* and *VP4* genotypes of strain ZTR-68 was similar to Wa-like strains.

Research conclusions

Group A human rotavirus was detected in 48.4% stool samples. A high prevalence of the G1, G2, and G3 genotypes was detected from 2010 to 2012. However, a dominant prevalence of the G9 genotype was identified as the cause of gastroenteritis in children in Yunnan, China, in 2013. A candidate human inactivated rotavirus vaccine strain, designated ZTR-68 was isolated, characterized, and showed immunogenicity.

Research perspectives

Our data will be useful for the future formulation and development of a vaccine in China.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Diagnostic value of elevated serum carbohydrate antigen 199 level in acute cholangitis secondary to choledocholithiasis

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Author contributions: Mei Y and Chen L contributed equally to this work; Mei Y, Chen L, Peng CJ and Leng K participated in study design, and drafted the manuscript; Wang J, Zeng PF, Wang GX, Li WP, Luo YQ, Du C, Liu K, Xiong K, Leng K and Feng CL participated in data collection and performed the statistical analysis; all authors have read and approved the final manuscript.

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STROBE statement: This report is presented as suggested by the STROBE statement, *i.e.*, according to the guidelines for reporting observational studies.

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Abstract

AIM

To investigate the diagnostic value of abnormal serum carbohydrate antigen 199 (CA199) level in acute cholan-

gitis secondary to choledocholithiasis.

METHODS

In this retrospective cohort study, the clinical data of 727 patients with choledocholithiasis admitted to the Third Affiliated Hospital of Zunyi Medical College from June 2011 to June 2017 were collected. Among these patients, 258 patients had secondary acute cholangitis and served as observation group, and the remaining 569 choledocholithiasis patients served as the control group. Serum liver function indexes and tumor markers were detected in both groups, and the receiver operating characteristic (ROC) curves were constructed for markers showing statistical significances. The cutoff value, sensitivity, and specificity of each marker were calculated according to the ROC curves.

RESULTS

The results of liver function tests showed no significant differences between the two groups ($P > 0.05$). Tumor markers including serum CA125, CA153, carcinoembryonic antigen, and alpha fetoprotein levels were also not significantly different ($P > 0.05$); however, the serum CA199 level was significantly higher in the observation group than in the control group ($P < 0.05$). The ROC curve analysis showed that the area under the curve was 0.885 (95%CI: 0.841-0.929) for CA199, and the cutoff value of 52.5 kU/L had the highest diagnostic accuracy, with a sensitivity of 86.8% and a specificity of 81.6%.

CONCLUSION

Abnormally elevated serum CA199 level has an important value in the diagnosis of acute cholangitis secondary to choledocholithiasis. It may be a specific inflammatory marker for acute cholangitis.

Key words: Carbohydrate antigen 199; Tumor marker; Choledocholithiasis; Inflammatory marker; Diagnosis; Acute cholangitis

Core tip: Acute cholangitis is an acute inflammatory response to increased bile duct pressure and bacterial infection following biliary obstruction, whereas biliary obstruction is mostly caused by choledocholithiasis. Failure of timely predicting the onset of acute cholangitis may lose the chance for a minimally invasive surgery and result in a high mortality of the patients. In this study, a total of 727 choledocholithiasis patients were included, and the results suggest that abnormally elevated serum carbohydrate antigen 199 level has an important value in the diagnosis of acute cholangitis secondary to choledocholithiasis. It may be a specific inflammatory marker for acute cholangitis. As a convenient and rapid test, it is worthy to be applied in clinical settings.

Mei Y, Chen L, Peng CJ, Wang J, Zeng PF, Wang GX, Li WP, Luo YQ, Du C, Liu K, Xiong K, Leng K, Feng CL, Jia JH. Diagnostic value of elevated serum carbohydrate antigen 199 level in acute cholangitis secondary to choledocholithiasis. *World J Clin Cases* 2018; 6(11): 441-446 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i11/441.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i11.441

INTRODUCTION

Acute cholangitis is an acute inflammatory response to increased bile duct pressure and bacterial infection following biliary obstruction, whereas biliary obstruction is mostly caused by choledocholithiasis[1,2]. Bacteria and endotoxin in the infected biliary system can enter the blood circulation quickly, causing secondary systemic inflammatory reaction, septic shock, sepsis, and other serious complications, which threaten the life of the patients. Therefore, failure of timely predicting the onset of acute cholangitis may result in a high incidence of acute cholangitis and high mortality of the patients[3]. Furthermore, the patients may lose the chance for endoscopic retrograde cholangiopancreatography (ERCP), which is a mainstay of treatment for choledocholithiasis, instead, they have to undergo an emergency surgery that is more difficult, more risky, and associated with poor prognosis. In our clinical practice, some of the patients with acute cholangitis often had abnormally elevated tumor markers. To explore the values of these markers in the early diagnosis of acute cholangitis, we retrospectively analyzed the clinical data of acute cholangitis patients who were treated in our hospital in recent years.

MATERIALS AND METHODS

General data

In this retrospective cohort study, the clinical data of 727 choledocholithiasis patients were collected. Among them, 258 patients had secondary acute cholangitis and served as observation group, and the remaining 569 patients with choledocholithiasis alone served as the control group. Choledocholithiasis was suggested by B-ultrasound, computed tomography (CT), or magnetic resonance cholangiopancreatography before surgery and confirmed during surgery. Acute cholangitis secondary to choledocholithiasis was diagnosed if Charcot's triad was found in these patients. The age, sex, diameter of common bile duct (CBD), number of CBD stones, complications and white blood cell (WBC) count were compared, with no significant differences between the two groups ($P < 0.05$) (Table 1).

Criteria for case selection

Diagnostic criteria for acute cholangitis[4] was still dependent on clinical manifestations and auxiliary examinations. (A) Clinical manifestations included: (1) history of biliary disease; (2) fever and/or chills; (3) jaundice; and (4) abdominal pain (right upper quadrant or epigastric).

Table 1 Baseline data of patients

	Observation group (n = 158)	Control group (n = 569)	χ^2/t value	P value
Age (yr)	54.31 ± 12.54	51.65 ± 11.29	0.336	0.752
Gender				
Male	69	263	0.324	0.569
Female	89	306		
B-ultrasound				
Diameter of CBD (mm)	9.51 ± 2.14	9.85 ± 2.07	0.243	0.851
Number of CBD				
Single	107	372	0.302	0.583
Multiple	51	197		
Complications				
Diabetes mellitus	32	112	0.025	0.874
Hypertensive disease	36	101	2.049	0.152
Hyperlipidemia	13	41	0.188	0.665
WBC count	8.23 ± 1.82	8.68 ± 2.25	-1.702	0.092

CBD: Common bile duct; WBC: White blood cell.

(B) Laboratory data included: evidence of inflammatory response and abnormal liver function tests. (C) Imaging findings included: biliary dilatation, or evidence of an etiology (stricture, stone or stent). Diagnosis could be established if the followings were found: (1) Charcot's triad (2 + 3 + 4); (2) Two or more items in A + both items in B and item in C. The severity of acute cholangitis was divided into three grades: mild (grade Ⅰ), moderate (grade Ⅱ), and severe (grade Ⅲ).

Inclusion criteria: Control group: with confirmed choledocholithiasis; with typical manifestations of obstructive jaundice [total bilirubin (TBiL) > 17.1 μmol/L; increased direct bilirubin (DBil) level; DBiL/TBiL ratio > 50%)]; and without manifestation of acute biliary tract inflammation (including acute cholecystitis or cholangitis). Observation group: with confirmed choledocholithiasis; with the presence of acute cholangitis (grade Ⅰ or grade Ⅱ); and without severe biliary tract infection including acute suppurative cholecystitis or acute severe cholangitis (grade Ⅲ).

Exclusion criteria: (1) Accompanied with intrahepatic bile duct stones; (2) accompanied with malignant jaundice disease, including cholangiocarcinoma, gallbladder cancer, liver cancer, pancreatic head cancer, and/or ampulla carcinoma; (3) accompanied with acute pancreatitis, active hepatitis, and/or liver cirrhosis; and (4) accompanied with other systemic inflammatory diseases.

Methods of measurement

The baseline clinical data of both groups were analyzed. Serum liver function indicators aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase, alkaline phosphatase (ALP), total TBil, DBil, and tumor markers carbohydrate antigen 199 (CA199), CA125, CA153, carcinoembryonic antigen (CEA), and alpha fetoprotein (AFP) were detected in both groups. Receiver operating characteristic (ROC) curves were constructed for markers showing significant differences between the two groups. The cutoff value, sensitivity, and specificity of each marker were calculated according to the ROC curves.

Index analysis

Results of serum liver function indicators and tumor markers were compared between the two groups. The ROC curves were used to analyze the results.

RESULTS

Serum liver function indicators

The serum liver function indicators, including ALT, AST, TBil, DBil, glutamyl transpeptidase, and ALP, showed no significant differences between the observation group and the control group (P > 0.05) (Table 2).

Tumor markers

The serum CA199 level was significantly higher in the observation group than in the control group (P < 0.05). In contrast, serum levels of CA125, CA153, CEA, and AFP were all not significantly different (P > 0.05) (Table 3).

ROC curves

The area under the curve (AUC) was 0.885 (95%CI: 0.841-0.929) for CA199, and the cutoff value of 52.5 kU/L had the highest diagnostic accuracy, with a sensitivity of 86.8% and a specificity of 81.6% (Table 4 and Figure 1).

DISCUSSION

Choledocholithiasis is a common and frequently occurring disease in departments of surgery. Its incidence is particularly high in Southwest China and has been rising in recent years. The main treatments for choledocholithiasis include open or laparoscopic cholecystectomy and ERCP combined with endoscopic sphincterotomy[5-7]. Persistent presence of stones in the CBD may affect bile excretion, often leading to development of secondary acute cholangitis. Although there are various methods to diagnose CBD stones and examination techniques have been increasingly improved, there is no significant decline

Table 2 Comparisons of serum liver function indicators

	Observation group (n = 158)	Control group (n = 569)	t value	P value
ALT	127.51 ± 28.71	125.34 ± 27.25	0.235	0.867
AST	130.29 ± 27.87	132.37 ± 30.19	0.229	0.871
TBiL	101.06 ± 23.38	95.49 ± 22.42	0.824	0.206
DBiL	60.62 ± 11.73	55.31 ± 10.82	0.926	0.179
GGT	210.28 ± 43.75	186.35 ± 41.08	1.083	0.126
ALP	237.84 ± 52.97	218.47 ± 50.18	1.024	0.145

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBiL: Total bilirubin; DBiL: Direct bilirubin; GGT: Glutamyl transpeptidase; ALP: Alkaline phosphatase.

Table 3 Comparisons of tumor markers

	Observation group (n = 158)	Control group (n = 569)	t value	P value
CA199	90.25 ± 21.17	25.19 ± 7.06	25.597	0.000
CA125	26.73 ± 6.94	24.61 ± 6.45	0.527	0.487
CA153	10.07 ± 2.54	9.76 ± 2.24	0.353	0.728
CEA	2.67 ± 0.57	2.53 ± 0.15	0.301	0.779
AFP	3.18 ± 0.63	3.12 ± 0.61	0.208	0.894

CA: Carbohydrate antigen; CEA: Carcinoembryonic antigen; AFP: Alpha fetoprotein.

Table 4 Area under the receiver operating characteristic curve of carbohydrate antigen 199

Area	Std. error	Asymptotic sig.	Asymptotic 95%CI	Cutoff value	Sensitivity	Specificity
0.885	0.022	0.000	0.841-0.929	52.5	0.868	0.816

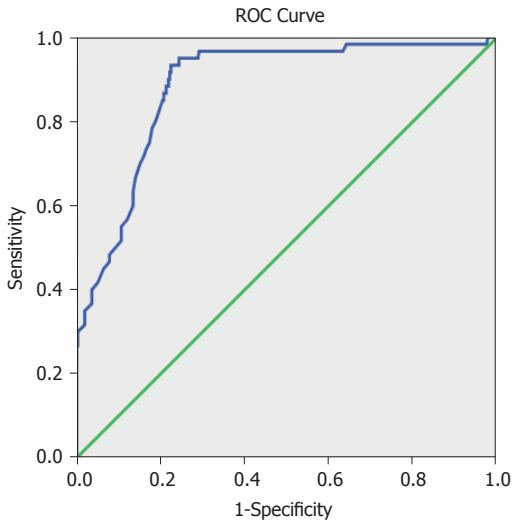

Figure 1 Receiver operating characteristic curve of carbohydrate antigen 199. ROC: Receiver operating characteristic.

in the incidence, severity, and mortality of acute cholangitis secondary to choledocholithiasis. The main reasons are the lack of objective evaluation markers and methods for the early diagnosis of acute cholangitis. Charcot's triad has long been used as a specific manifestation of acute cholangitis; however, its clinical practicality and reliability is questionable due to its low sensitivity (about 26%)[3], especially in elderly inpatients. Delayed or improper treatment can worsen the condition of the patients[8-9]. Therefore, it is of great clinical significance to identify the early predictors of acute cholangitis secondary to choledocholithiasis so as to develop rational surgical protocol avoid postoperative complications and reduce mortality.

According to the Tokyo criteria of acute cholangitis, it can be diagnosed if the clinical manifestations of Charcot's triad, *i.e.*, fever and/or chills, abdominal pain (right upper quadrant or epigastric), and jaundice are present[4]. Charcot's triad is the main clinical manifestation, whereas the auxiliary examinations mainly include the measurements of common inflammatory markers and the imaging of biliary obstruction. The commonly used inflammatory indicators include blood WBC count, neutrophil ratio, serum C-reactive protein, and procalcitonin. Imaging examinations for biliary obstruction included ultrasound, CT, MRI, endoscopic ultrasonography, and cholangiography. Unfortunately, up to date, well-recognized early predictors of acute cholangitis secondary to choledocholithiasis have been largely unavailable. CA199 is a mucin-like substance usually embedded on the surface of epithelial cells in the bile duct, pancreatic duct, stomach, and prostate. The serum CA199 level is low in normal subjects[10]. Numerous studies have demonstrated that the biological function of CA199 is mainly involved in cell proliferation, differentiation, signal transduction, apoptosis, and immune regulation. In particular, CA199 can promote leukocyte aggregation by regulating leukocyte migration and adhesion in inflammatory regions[11]. As one of the common tumor markers, CA199 is mainly

used in the auxiliary diagnosis and evaluation of disease progression and prognosis of pancreatic cancer, colorectal cancer, and other diseases[12-13]. In recent years, the abnormally elevated CA199 in some non-cancerous benign diseases has drawn increasing attention[14-16]. However, the role of elevated serum CA199 in the diagnosis of acute cholangitis secondary to choledocholithiasis deserves further studies.

In the present study, there was no statistically significant difference in the serum liver function indicators between the observation group and control group, suggesting that the changes of serum liver function indicators do not necessarily imply the occurrence of secondary acute cholangitis in patients with obstructive jaundice due to biliary duct stones. Comparisons of tumor markers showed that the levels of CA125, CA153, CEA, and AFP were not significantly different between the two groups, suggesting that the levels of these tumor markers were not directly related to the occurrence of acute cholangitis. However, our study found that the serum CA199 level in the observation group was significantly higher than in the control group, indicating that the abnormal elevation of serum CA199 level may have potential predictive value for the occurrence of acute cholangitis following choledocholithiasis, *i.e.*, CA199 may be a special inflammatory marker for the onset of acute cholangitis. The ROC curve analysis showed that the AUC was relatively large for CA199, and the cutoff value of 52.5 kU/L had the highest sensitivity and specificity. It is suggested that clinicians must deal with the disease early, avoid the occurrence of acute cholangitis, relieve the pain of patients, and improve the prognosis of the disease. The elevation of serum CA199 level in acute cholangitis patients may be related with the fact that inflammation stimulates the proliferation of bile duct epithelial cells, resulting in the increased secretion of CA199 and other inflammatory mediators. In addition, the accumulation of a large amount of CA199 in the bile duct, and the obstructed bile duct increase the bile duct pressure, leading to the backflow of CA199 into blood stream. Meanwhile, thickening of the bile duct wall during acute cholangitis lowers the ability of the bile duct in scavenging CA199 and other substances, leading to persistent high pressure in the bile duct, even destroying the peripheral vascular mucosal barrier, thus promoting backflow of CA199 into the bile duct.

In summary, abnormally elevated serum CA199 level may be a potentially useful marker for the early prediction of acute cholangitis secondary to choledocholithiasis. When the CA199 level reaches the cutoff value, physicians should be vigilant about the possibility of acute cholangitis, and timely and proper interventions should be carried out to avoid aggravation of the disease.

ARTICLE HIGHLIGHTS

Research background

Choledocholithiasis is a common and frequently occurring disease in departments of surgery. Persistent presence of stones in the common bile duct may affect bile excretion, often leading to development of secondary acute cholangitis. Failure of timely predicting the onset of acute cholangitis may lose the chance for a minimally invasive surgery and result in a high mortality of the patients.

Research motivation

It is of great clinical significance to explore the methods for the early diagnosis of acute cholangitis so as to develop rational surgical protocol, avoid postoperative complications and reduce mortality.

Research objectives

To investigate the diagnostic value of abnormal serum carbohydrate antigen 199 (CA199) level in acute cholangitis secondary to choledocholithiasis.

Research methods

In this retrospective cohort study, the clinical data of 727 patients with choledocholithiasis were collected. Serum liver function indexes and tumor markers were detected in both groups, and the ROC curves were constructed for markers showing statistical significances.

Research results

The serum CA199 level was significantly higher in the observation group than in the control group ($P < 0.05$). The receiver operating characteristic curve analysis showed that the area under the curve was 0.885 (95%CI: 0.841-0.929) for CA199, and the cutoff value of 52.5 kU/L had the highest diagnostic accuracy, with a sensitivity of 86.8% and a specificity of 81.6%.

Research conclusions

Abnormally elevated serum CA199 level has an important value in the diagnosis of acute cholangitis secondary to choledocholithiasis.

Research perspectives

Abnormally elevated serum CA199 level may be a potentially useful marker for the early prediction of acute cholangitis secondary to choledocholithiasis.

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CASE REPORT

Balo's concentric sclerosis in a patient with spontaneous remission based on magnetic resonance imaging: A case report and review of literature

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Author contributions: Ertuğrul Ö, Aluçlu MU and Çiçekçi E examined patient and collected clinical data; Ertuğrul Ö performed and analyzed radiologic imaging data; Tuncer MC and Ertuğrul Ö wrote the paper; Tuncer MC, Aluçlu MU and Ertuğrul Ö edited the manuscript and had final approval.

Informed consent statement: Informed written consent was obtained from the patient prior to all procedures described in the report as well as for the use of the patient's clinical information and images for published scientific works.

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Abstract

Balo's concentric sclerosis (BCS) is a rare monophasic demyelinating disease known as multiple sclerosis subtype and seen as a round lesion with variable hyper and hypodetoxification layers. Characteristic appearance can be seen as "bulb eye" or "onion bulb". The initial terminology for this neurological disorder was leukoencephalitis periaxialis concentrica; this is defined as a disease in which the white matter of the brain is destroyed in concentric layers in such a way as to leave the axial cylinders intact. This report presents a case of BCS with spontaneous healing of the patient and a mass lesion with concentric rings adjacent to the left lateral ventricle and the posterior portion of the corpus callosum with peripheral vasogenic edema. The neurological lesion of the patient was similar to the magnetic resonance imaging and clinical findings of the BCS.

Key words: Balo's concentric sclerosis; Multiple sclerosis; Demyelinating; Magnetic resonance imaging; Diffusion-weighted imaging

Core tip: This case report demonstrates that Balo's concentric sclerosis (BCS) a patient with a mass lesion containing concentric rings, BCS diagnosis was reported by magnetic resonance imaging. As supported in previously reported clinical trials, BCS is not always a fatal disease and supports the definition that it may be a self-limiting disease.

Ertuğrul Ö, Çiçekçi E, Tuncer MC, Aluçlu MU. Balo's concentric sclerosis in a patient with spontaneous remission based on magnetic resonance imaging: A case report and review of literature. *World J Clin Cases* 2018; 6(11): 447-454 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i11/447.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i11.447

INTRODUCTION

Balo's concentric sclerosis (BCS) is characterized radiologically and pathologically by demyelinating lesions with a concentric ring appearance formed by areas of demyelination alternating with relatively preserved myelin[1]. The lesions of BCS often occur in isolation or in association with clinically and radiologically more typical multiple sclerosis (MS). Historically, BCS was thought to be uniformly fatal and diagnosis was post-mortem, but in the magnetic resonance imaging (MRI) era, BCS can be detected intra vitam and, in many cases, has a favorable prognosis[2].

BCS was first described by Marburg in 1906, and in 1928, the Hungarian neuropathologist, Joseph Balo[3] published a report of a student with right hemiparesis followed by optic neuritis, who upon autopsy had demyelinated lesions described as encephalitis periaxialis concentrica. Traditionally, BCS has been grouped under one of the atypical forms of MS, with Marburg's disease, tumefactive demyelination, Schilder's disease, and acute haemorrhagic leukoencephalitis, although the contemporary status and usefulness of these categorizations are questionable apart from tumefactive demyelinations contentious. Tumefactive demyelinating lesions are more than 2 cm in size when viewed with MRI and may have an associated mass effect (45%) and/or edema (77%) with larger lesions generally having both more mass effect and edema[4]. Most tumefactive demyelinating lesions are focal and supratentorial, with a predilection for the frontal and parietal lobes, but they can present in other areas of the cerebral hemispheres as well as in the deep gray matter, brainstem, cerebellum, and spinal cord[4-6].

BCS is clinically indicated in clinical trials that may occur in a manner similar to MS. It is known that it can affect young people and children with mild dementia. However, it may be associated with altered behavior and focal central nervous system (CNS) deficits. Clinical trials have reported that BCS exhibits characteristic radiographic findings that aid in ante-mortem diagnosis[7]. BCS is clinically first reported to be a rapidly progressive and lethal condition[8], and subsequently reported clinical trials have demonstrated that anti-inflammatory corticosteroids are efficacious against BCS-associated neurological deficits. Because of this reason, it is known that MRI imaging allows early diagnosis and treatment by significantly affecting the course of the disease.

This acute idiopathic inflammatory demyelinating disease has a unique pathological and radiographic signature of concentric demyelination. The pattern can be quite striking upon MRI, with alternating concentric rings of T2 isointensity and hyperintensity related to advancing waves of demyelination. These may show gadolinium enhancement[9]. Lesions may be small or occupy large sections of a cerebral hemisphere and tend to spare the cortical U-fibers. Pathologically, there are rings of demyelination corresponding to areas of T2 hyperintensity with MRI alternating with rings of normal myelination or partial remyelination corresponding to areas of T2 isointensity. This renders the lesions with an onion bulb appearance[10]. Lesions can also be found in the basal ganglia, pons, cerebellum, and, very infrequently, the spinal cord and optic nerves[2]. Patients with this diagnosis were thought to have a fulminant course that was invariably fatal within a year. However, with the advent of MRI, certain cases detected via MRI have had favorable outcomes[2,11]. The concentric ring appearance is also not specific, with these types of lesions having also been described in the brainstem in a patient with neuromyelitis optica[12] and another with MS[13] as well as in patients with progressive multifocal leukoencephalopathy[14], cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy[15] and concomitant active hepatitis C and human herpes virus 6[16].

In this study, we reported a mass lesion with concentric rings adjacent to the left lateral ventricle and the posterior part of the corpus callosum with a peripheral vasogenic lesion in a patient with spontaneous remission with MRI imaging.

CASE REPORT

A 19-year-old woman complaining of night-raging nausea, blurred vision, and severe headache for seven days was seen in our clinic. Focal CNS deficiency was not detected in our patient. On cranial MRI, a mass with concentric circles and peripheral vesogenic edema located right lateral to the left lateral ventricle was seen in the posterior part of the corpus callosum (Figure 1). A significant increase was detected in the peripheries and central region of the lesion after contrast material injection (Figure 2). Diffusion-weighted imaging showed circular rings of

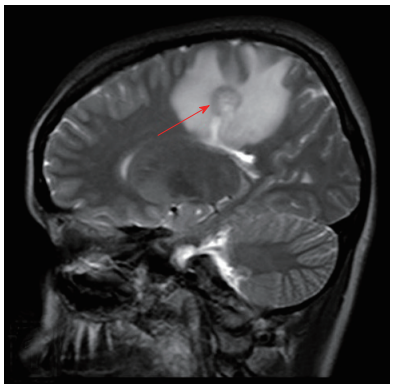

Figure 1 Sagittal T2-weighted magnetic resonance imaging showing a mass lesion with concentric rings located adjacent to the left lateral ventricle and posterior part of the corpus callosum with peripheral vasogenic edema.

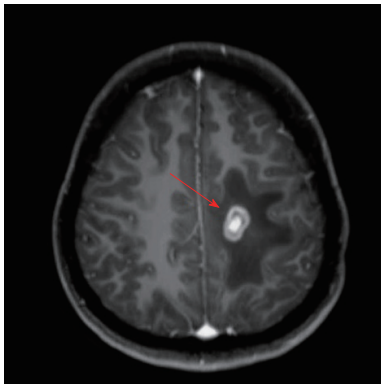

Figure 2 Axial T1-weighted image after administration of gadolinium-diethylenetriaminepentaacetic acid depicts prominent enhancement in the periphery and central area of the lesion.

hyperintensity, similar to the T2-weighted (T2W) images visible diffusion co-efficient maps showed a donut-shaped slow diffusion zone around a central nidus of facilitated diffusion (Figure 3). Single-voxel magnetic resonance spectroscopy was obtained from the left-enhancing centrum semiovale lesion. It indicated a decrease in the choline/N-acetyl aspartate ratio and mild lipid along with lactate peaks (Figure 4). No other lesions were seen. The patient underwent a fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT) and there were no pathological findings in favor of malignancy. Characteristic MRI findings suggested the diagnosis of BCS but the patient refused the treatment. After nine months, she was admitted to a neurology clinic for a severe headache. Interestingly, there was only a T2W linear signal intensity on the MRI (Figure 5). This case is very interesting for its spontaneous remission. Cases between 1985-2018 related to BCS can be seen in Table 1.

DISCUSSION

It was stated that the case reports presented about the BCS were seen more in women[2,17-26]. However, it has been pointed out in scientific publications that BCS is more common in East Asian descent[17-20]. According to these studies, genetic and environmental factors should be considered with BCS. Many signs of Balo's disease are similar to MS symptoms. Headaches, seizures, muscle pain and spasms, muscle weakness, paralysis over time, difficulty speaking, different thinking or understanding, changes in behavior can be seen as clinical manifestations of BCS. And also, BCS symptoms show a similar clinical course, mostly with intracerebral mass lesions[11,17,18].

Preservation of cortical gray matter, cerebral white matter oligodendrocyte loss and demyelination are known pathological findings of BCS[3,20-24]. In the pathology of BCS tissue lesions, the number of oligodendrocytes in the demyelinated areas of the substantia alba layer was reduced, and the lesions were defined as a variation of the immunopathological pattern Ⅲ of MS[1,22].

The demyelinated ring appearance of BCS has been reported to include foamy macrophages, activated microglia, reactivated astrocytes and axonal loss areas, as is typically found in MS. It has been reported that hypoxia and demyelination of the edge of BCS lesions are related to the production of chemical mediators and cytokines by macrophages or microglia cells. This provides some protection against demyelination at the BCS lesion side, and as the lesion expands, the demyelination area appears to be a relatively preserved myelinated tissue[1].

Hypoxia-inducible factor 1α and heat-shock protein 70 are proteins that protect the myelin structure between the rings demyelinated in BCS lesions[25]. BCS lesions are larger than MS lesions in appearance. Different ring appearances are seen with a shape called onion bulb. The formation of this shape is related to relative myelin preservation and the loss of axon structure[1,26]. The myelin structure in BCS patients is rarely preserved. However, it is stated that this is actually a partial demyelination area[1,27]. When the pathological results of BCS lesions are examined, lymphocytic infiltrates around the vessel and demyelination area at different stages are reported[28]. Histological studies on MS lesions indicated that the areas of demyelination may closely resemble the appearance of BCS patients[22,29,30]. Because of this close anatomical resemblance, some BCS cases have been described as MS cases. Some of the BCS lesions have been found to have lost myelin-associated glycoprotein[1,22].

In MRI studies, BCS lesions may typically be multiple, isolated, and mixed, such as in MS lesions[31]. Concentric rings can be seen the most common alternative augment rings in the outer rings[32]. In T1-weighted MR scans of BCS lesions, the lesions are generally seen as light or dark (isointense or hypointense) concentric rings. However, in the T2W MRI sequences, it was stated that the density of the lamellae appearance around the lesion increased. Apart from these, it has been reported that the images of BCS lesions may have different geometric shapes[33,34]. The image intensity of MR sections on the outer margin of the BCS lesions was found to be higher[27,35]. It has been stated that in the MR sections of

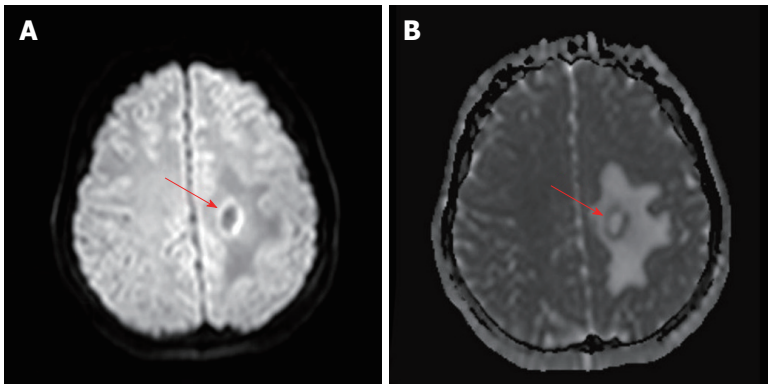

Figure 3 Apparent diffusion co-efficient maps portraying only a thin rim of restricted diffusion at the outer rim of the lesion, with facilitated diffusion centrally and at the outer edema. A: Diffusion weight images shows a thin rim of increased diffusion at the outer rim of the lesion; B: The outer rim is hypointense on the corresponding apparent diffusion coefficient map images, indicating true restriction.

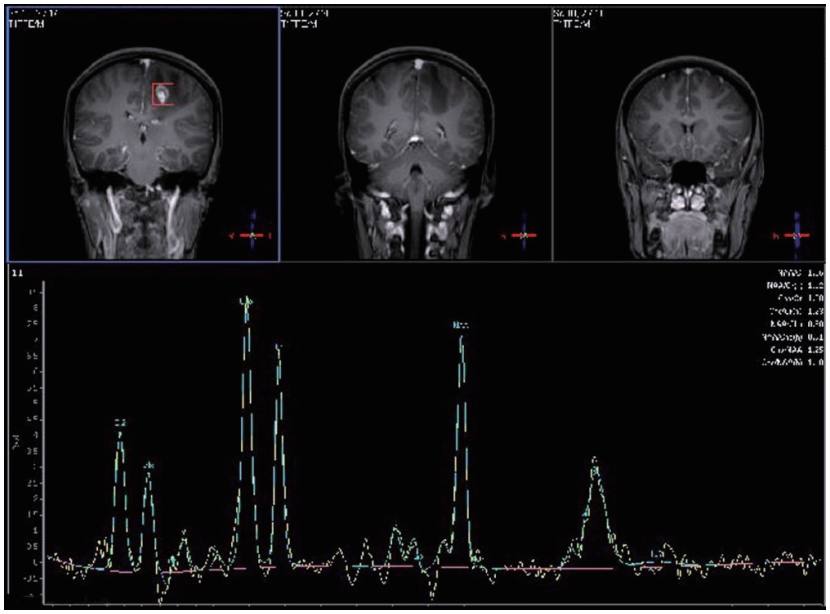

Figure 4 One hundred and forty-four millisecond single-voxel magnetic resonance spectroscopy was obtained from the left-enhancing centrum semiovale lesion. It showed a decrease in the choline/N-acetyl aspartate ratio along with mild lipid and lactate peaks.

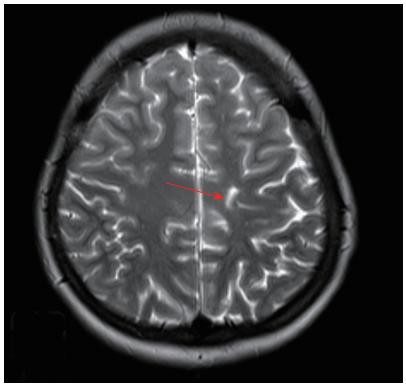

Figure 5 There was only a T2-weighted linear signal intensity with magnetic resonance imaging obtained after nine months.

the BCS lesions, the flow of the contras material may be in the peripheral direction. However, it was determined that the density of lesion layers increased in T2 weighted sections[7,21]. BCS lesions are frequently seen in the white matter layer (substantia alba) of cerebrum. And, subcortical U-fibres usually initially spared. However, BCS lesions have been reported in rhombencephalon and the basal ganglia[13,36-38]. In our case, T2 images revealed one adjacent hyperintense perioedematous concentric lesion at the left centrum semiovale and periventricular white matter spreading to the corpus callosum (Figure 1). Magnetic resonance spectroscopy of the patient indicated a decrease in the choline/N-acetyl aspartate ratio along with mild lipid and lactate peaks (Figure 4). Clinical studies on long-term follow-up of BCS lesions have shown that these lesions lost their ring appearance and turned into demyelinating areas. It has even been reported that the lesions may have a linear shape[7]. Other studies have shown that the classic concentric view of the BCS lesion can retain its structure for a long

Table 1 Cases related to Balo's concentric sclerosis between 1985-2018

Reference	Case number	Gender/age	Clinical presentation	Oligoclonal bands	Coexistence with injuries	Histopathological examination	Clinical progression
[7]	1	F/37	Monophase	NR	NR	Y	SH
	2	F/56	Monophase	NR	NR	Y	SH
	3	M/42	Monophase	NR	NR	N	SH
	4	F/33	Monophase	NR	NR	N	SH
[9]	1	F/56	Relapsing-Remitting	NR	N	Y	MH
[11]	1	M/51	Monophase	Negative	N	Y	SH
	2	F/20	Monophase	Negative	N	N	CH
	3	M/48	Monophase	Positive	Y	N	CH
	4	M/38	Monophase	Negative	N	N	CH
	5	F/15	Monophase	Negative	Y	N	SH
[12]	1	F/29	Relapsing-Remitting	NR	Y	N	MH
[13]	1	F/45	Relapsing-Remitting	Negative	N	N	SH
[15]	1	M/26	Progressive Primary	NR	N	N	SH
[17]	1	F/52	Monophase	NR	N	N	SH
	2	M/31	Monophase	NR	N	N	CH
	3	F/40	Relapsing-Remitting	NR	Y	N	SH
	4	M/31	Monophase	NR	N	N	CH
	5	F/23	Monophase	NR	Y	N	SH
	6	F/44	Relapsing-Remitting	NR	Y	N	SH
	7	F/43	Relapsing-Remitting	NR	Y	N	SH
[21]	1	M/43	Relapsing-Remitting	Negative	Y	N	MH
[23]	1	M/46	Progressive Primary	NR	NR	Y	D
	2	M/24	Progressive Primary	NR	NR	Y	D
	3	M/48	Progressive Primary	NR	NR	Y	D
	4	F/40	Progressive Primary	NR	NR	Y	D
	5	F/25	Progressive Primary	NR	NR	Y	D
	6	F/24	Progressive Primary	NR	NR	Y	D
[25]	1	F/NR	NR	NR	Y	Y	D
	2	F/NR	NR	NR	Y	Y	D
	3	F/NR	NR	NR	Y	Y	D
	4	F/NR	NR	NR	Y	Y	D
	5	F/NR	NR	NR	Y	Y	D
	6	F/NR	NR	NR	Y	Y	D
	7	F/NR	NR	NR	Y	Y	D
	8	F/NR	NR	NR	Y	Y	D
	9	F/NR	NR	NR	Y	Y	D
	10	M/NR	NR	NR	Y	Y	D
	11	M/NR	NR	NR	Y	Y	D
	12	M/NR	NR	NR	Y	Y	D
	13	M/NR	NR	NR	Y	Y	D
	14	M/NR	NR	NR	Y	Y	D
[34]	1	F/32	Monophase	NR	N	N	SH
[35]	1	F/45	Monophase	NR	N	N	SH
[37]	1	F/57	Progressive Secondary	Negative	Y	N	NH
[38]	1	F/54	Progressive Primary	Positive	Y	Y	D
[39]	1	F/31	Relapsing-Remitting	NR	Y	N	NR
[40]	1	M/28	Relapsing-Remitting	Negative	NR	Y	D
[41]	1	F/32	NR	NR	NR	NR	NR
[42]	1	F/28	NR	Negative	NR	N	NR
[43]	1	F/52	Monophase	Negative	N	Y	SH
[44]	1	M/4	Monophase	Negative	N	N	MH
[45]	1	F/45	Progressive Primary	Negative	N	N	LH
	2	M/36	Progressive Primary	Negative	N	N	LH
[46]	1	F/24	Relapsing-Remitting	NR	Y	Y	D
[47]	1	F/34	Monophase	NR	N	Y	NR
[48]	1	M/NR	Monophase	Negative	N	N	SH
	2	F/38	Progressive Primary	Positive	N	N	D
	3	M/40	Monophase	Negative	N	N	SH
[49]	1	F/23	Relapsing-Remitting	Negative	Y	N	MH
[50]	1	F/13	Relapsing-Remitting	Positive	N	N	SH
[51]	1	F/27	Relapsing-Remitting	Negative	N	Y	LH
[52]	1	F/37	Monophase	NR	NR	NR	MH
[53]	1	F/31	Monophase	NR	N	N	SH
	2	F/58	Monophase	NR	N	Y	LH
[54]	1	M/26	Progressive Primary	Positive	N	Y	LH
[55]	1	F/17	Relapsing-Remitting	Positive	N	N	MH

[56]	1	M/37	Monophase	Negative	Y	N	SH
[57]	1	M/49	Progressive Primary	NR	NR	Y	D
	2	M/23	Progressive Primary	NR	NR	Y	D
	3	F/28	Progressive Primary	NR	NR	Y	D
	4	F/40	Progressive Primary	NR	NR	Y	D
[58]	1	M/52	Relapsing-Remitting	NR	N	N	D
[59]	1	F/25	Progressive Primary	NR	NR	N	SH
[60]	1	F/21	Relapsing-Remitting	NR	NR	N	SH
	2	M/45	Relapsing-Remitting	NR	NR	N	CH
	3	M/35	Relapsing-Remitting	NR	NR	N	SH
	4	F/38	Progressive Primary	NR	NR	N	D
	5	M/43	Progressive Primary	NR	NR	N	MH
	6	F/33	Progressive Primary	NR	NR	N	MH
[61]	1	M/36	Monophase	Negative	NR	N	MH
	2	F/52	Progressive Primary	Negative	NR	N	LH
	3	M/56	Progressive Primary	Positive	NR	N	NH
[62]	1	M/24	Progressive Primary	Negative	NR	N	SH

M: Male; F: Female; Y: Yes; N: No; D: Dead; NR: Not reported; CH: Complete healing; SH: Significant healing; MH: Modarate healing; LH: Little healing; NH: No healing.

time[9], or that BCS lesions may lose their anatomical shape and appear as a classic demyelinating plaque.

In conclusion, in a patient with a mass lesion containing concentric rings, BCS diagnosis was reported by MRI imaging. As supported in previously reported clinical trials, BCS is not always a fatal disease and supports the definition that it may be a self-limiting disease. Although BCS is usually known to possess a fulminant demyelinating course, there are cases in the literature with favorable prognoses and occasionally cases with spontaneous remission[21]. The unexpected finding of spontaneous remission without any treatment was noted in this case. A mass lesion with concentric rings that we determined more than nine months later were seen with a linear signal intensity without any treatment during MRI (Figure 5).

ARTICLE HIGHLIGHTS

Case characteristics

In a 19-year-old woman complaining of night-raging nausea, blurred vision, and severe headache ongoing for a week was admitted in our clinic.

Clinical diagnosis

The patient underwent magnetic resonance imaging (MRI) examination at our hospital, which indicated a mass with concentric circles and peripheral vesogenic edema located right lateral to the left lateral ventricle was seen in the posterior part of the corpus callosum.

Differential diagnosis

The patient underwent a fluorodeoxyglucose positron emission tomography/computed tomography and there were no pathological findings in favor of malignancy.

Imaging diagnosis

MRI and single-voxel magnetic resonance spectroscopy were used in this case.

Treatment

The patient refused the treatment.

Related reports

Balo's concentric sclerosis (BCS) was first described by Marburg in 1906, and in 1928, the Hungarian neuropathologist, Joseph Balo, published a report of a student. Cases related to BCS between 1985-2018 were presented in this case report together with clinical findings and results.

Term explanation

BCS is a rare monophasic demyelinating disease known as multiple sclerosis subtype. BCS may rapidly progress to become severe and fatal.

Experiences and lessons

The unexpected finding of spontaneous remission without any treatment was reported by MRI in this case. Clinicians should consider BCS is not always a fatal disease.

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CASE REPORT

Neurofibroma discharged from the anus with stool: A case report and review of literature

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Author contributions: Cai SQ, Miao Y and Wang JJ designed the report; Chen ZM, Zhu JL, Wang MB collected the patient's clinical data; Wang MB and Cai SQ analyzed the data and wrote the paper.

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Abstract

Isolated neurofibromas that affect the gastrointestinal tract are rare and almost always manifest as neurofibromatosis type 1 or multiple endocrine neoplasia type 2b. In this paper, we present a case of a 24-year-old female with abdominal pain who discharged a neurofibroma in her stool without any blood on it. A colonoscopy showed multiple small polyps in the sigmoid colon and a nodule in the ileocecus. The pathology results and the immunohistochemical stains of the removed neoplasm from the ileocecus confirmed the diagnosis was a bowel neurofibroma. We report a rare case of ileocecal neurofibroma due to the patient's affected gastrointestinal tract, without any associated systemic syndrome other than a neurofibroma discharged in the stool.

Key words: Neurofibroma; Isolated; Gastrointestinal tract; Ileocecus; Clinical presentation

Core tip: Neurofibromas of the gastrointestinal tract are rare, and various types have been previously reported. However, to our knowledge, this is the first report of a neurofibroma discharged from the patient's intestine with stool without any other associated systemic syndromes.

Miao Y, Wang JJ, Chen ZM, Zhu JL, Wang MB, Cai SQ. Neurofibroma discharged from the anus with stool: A case report and review of literature. *World J Clin Cases* 2018; 6(11): 455-458 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i11/455.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i11.455

INTRODUCTION

Neurofibroma of the bowel can occur with multiple symptoms and has several different names, including intestinal neurofibromatosis, ganglioneuromatosis, diffuse plexiform neurofibromatosis, neuronal intestinal dysplasia, and diffuse colonic ganglioneuromatous polyposis. About a quarter of neurofibromatosis type-1 (NF1) and multiple endocrine-neoplasia type-2b (MEN2b) cases have been reported to be associated with gastrointestinal neurofibromatosis[1], while the occurrence of gastrointestinal neurofibromatosis alone was reported to be extremely rare[2,3]. Currently, whether neurofibromatosis of the bowel tract without any systemic syndrome is a distinct condition or simply a phenotypic manifestation of NF1 or MEN2b remains an open question.

CASE REPORT

A previously healthy 24-year-old female came to our hospital complaining of a month-long history of abdominal pain after meals. She stated that she had never developed dysphagia, diarrhea, nausea, vomiting, or fever during the month of abdominal pain. Forty days prior to her symptoms, she found an approximately 8 cm × 5 cm × 5 cm lump in her stool without any blood on it (Figure 1). The lump was sent to another hospital for biopsy, and the results showed a submucosal spindle-cell tumor with surface-tissue necrosis that was inclined toward leiomyoma (Figure 2). Slices of the lump were taken to our institution for immunohistochemical analysis, which indicated that it came from a submucosal neurofibroma. Three days later, the female was given a colonoscopy, which showed a neoplasm with a smooth surface at the ileocecus (Figure 3A) with polyps at the sigmoid colon (Figure 3B). After admitting the female as an inpatient, a computed tomography (CT) scan of the abdomen was performed, revealing a hypoattenuating tumor of the ascending colon (Figure 4).

The patient then underwent an exploratory laparotomy, with primary anastomosis, after optimization for removing the tumor. We found the 5 cm × 6 cm tumor on the ileocecus at surgery but did not find anything else on the affected bowel. Pathologic examination of the resected specimen revealed it was a submucosal spindle-cell tumor of the ileocecus (Figure 5A). Immunohistochemical stains of the resected specimen showed that it was CD117(-), CD34(-), Ki67(+) 1%, Actin(-), S100(+++), Desmin(-), CD10(-), and Dog(-) (Figure 5B). The pathology results confirmed the tumor to be a neurofibroma. The patient did well initially; however, on the 10th postoperative day, the female had an anastomotic fistula (Clavien-Dindo Class Ⅰ), and finally recovered well.

Figure 1 Photograph of the lump.

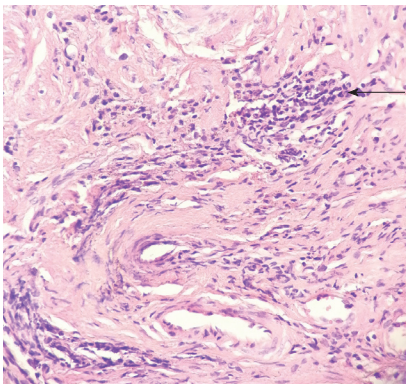

Figure 2 Proliferation of spindle cells in the lump (HE, × 200).

DISCUSSION

Isolated colonic neurofibromatosis is a benign neural tumor of the lower gastrointestinal tract. It can originate from the plexus of Meissner, the plexus of Auerbach, or even the serosa[4]. It can also be the onset manifestation of generalized systemic NF1 or MEN2b. Histologically, although isolated colonic neurofibromatosis manifests as a single or multiple high-degree of histologic differentiation of neoplasms or as a diffuse neuronal hyperplasia, it is commonly termed ganglioneuromatosis. In this case, the histology reports alone cannot specify whether it is NF1 or MEN2b because these conditions share some identical features[5].

The differing clinical symptoms found in neurofibromatosis of the hindgut tract depend on the lesion characteristics, such as the location, motility, and adjacent structures of the affected tract. Clinical presentation of the lesions can be abdominal pain[4], gut obstruction[6,7], palpable masses[8], constipation[9], or diarrhea[10].

In MEN2b, the development of medullary thyroid carcinoma, pheochromocytoma, and medullary carcinoma[11] is a clinical indicator besides what can be seen on the histological exam. In NF1, the development of

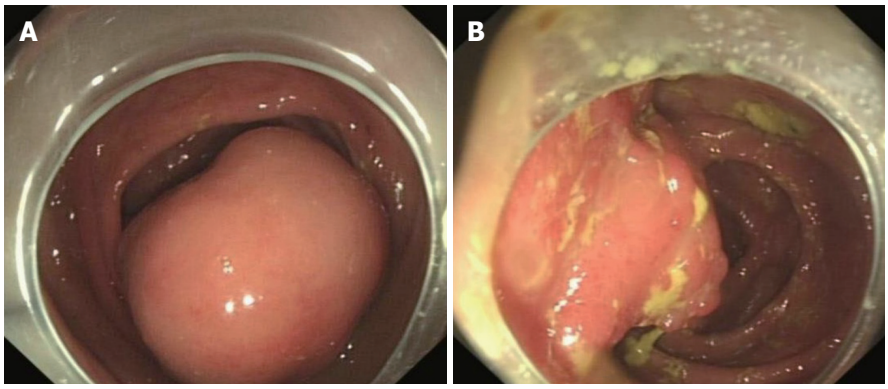

Figure 3 Endoscopic images of isolated nodularity of the ileocecus (A) and polyps of sigmoid colon (B).

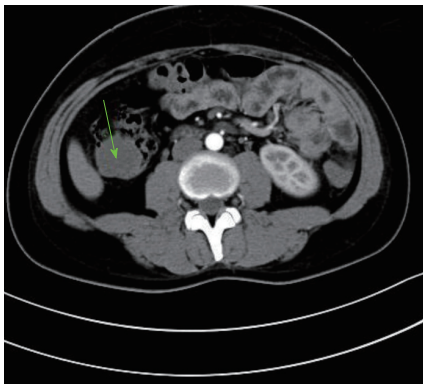

Figure 4 Photograph of computed tomography-scan abdomen shows hypoattenuating tumor of the ascending colon (green arrow).

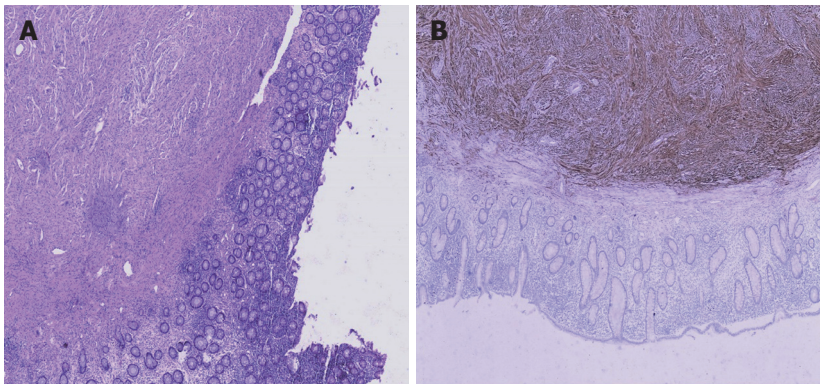

Figure 5 Photograph of resected colon (HE, × 200) (A) and immunohistochemical stain for S-100 protein (× 200) (B).

classic dermal neurofibromas, café-au-lait macules or Lisch nodules[12] is an additional clinical indicator. In the current case, our patient did not show any of the additional clinical features.

The diffuse form of colonic neurofibromatosis and Crohn's disease can mimic each other radiographically. Both colonic neurofibromatosis and Crohn's disease can appear as single or multiple thickened portions of the gastrointestinal tract on CT scans. In one report, a patient was clinically suspected of having Crohn's disease based on a CT scan, but the diagnosis was corrected to neurofibromatosis after histological exam of the resected bowel[9].

The etiology of isolated neurofibromatosis is still not clarified, but circulating nerve factors[13] and a neurofibromatosis gene mutation[14] were reported to be involved in hyperplasia of the nerve plexus. The primary treatment of isolated neurofibromatosis is surgical removal. Whether further therapy is required depends on the endoscopic findings and the histological exam.

In summary, we report a case of isolated neurofibromatosis with the onset of a lump in the patient's stool and without any other additional clinical features. Despite its rarity, the neurofibromatosis is the only clinical indicator in this case. We suspect that part or all of the neurofibroma underwent necrosis and fell into

the stool.

ARTICLE HIGHLIGHTS

Case characteristics

The unique character and only clinical symptom of this particular case is that the patient presented with a month-long history of abdominal pain after meals and a lump discharged from intestine with stool without any blood on it.

Clinical diagnosis

Ileocecal neoplasia.

Differential diagnosis

Appendicitis, cholecystitis, gastroenteritis, colon cancer, and bowel obstruction.

Imaging diagnosis

Ileocecal neoplasia.

Pathological diagnosis

Ileocecal neurofibroma.

Treatment

Ileocecectomy with primary anastomosis.

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CASE REPORT

Balloon dilator controls massive bleeding during endoscopic ultrasound-guided drainage for pancreatic pseudocyst: A case report and review of literature

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Author contributions: Wang BH and Jiang TA designed the report; Xie LT, Zhao QY, and Ying HJ collected the patient's clinical information; Wang BH and Xie LT analyzed the information and wrote the paper; all authors approved the final draft submitted.

Informed consent statement: Consent was obtained from the relatives of the patient for publication of this report and any accompanying images.

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Abstract

Pancreatic pseudocyst (PPC), a common sequela of acute or chronic pancreatitis, was defined by the revised Atlanta classification as "a collection." Endoscopic ultrasound (EUS)-guided drainage is often considered a standard first-line therapy for patients with symptomatic PPC. This effective approach exhibits 90%-100% technical success and 85%-98% clinical success. Bleeding is a deadly adverse event associated with EUS-guided drainage procedures, and the bleeding rate ranges from 3% to 14%. Hemostasis involves conservative treatment, endoscopy, interventional radiology-guided embolization and surgery. However, few studies have reported on EUS-guided drainage with massive, multiple hemorrhages related to severe pancreatogenic portal hypertension (PPH). Thus, the aim of this case report was to present a case using a balloon dilator to achieve successful hemostasis for PPH-related massive bleeding in EUS-guided drainage of PPC. To our knowledge, this method has not been previously reported.

Key words: Endoscopic ultrasound guided; Bleeding; Pancreatic pseudocyst; Balloon compression; Novel hemostasis

Core tip: There has been considerable research in recent years dedicated to the development of endoscopic ultrasound-guided drainage, which is viewed as the first-line therapy for the management of pancreatic pseudocyst due to the minimized invasiveness, lower mortality, better physical and mental condition of patient compared with surgical and percutaneous approaches. Although the procedure is safe and effective, bleeding is one of the deadly adverse events. This is the first report using a balloon dilator to control pancreatogenic portal hypertension-related bleeding in endoscopic ultrasound-guided drainage for pseudocyst.

Wang BH, Xie LT, Zhao QY, Ying HJ, Jiang TA. Balloon dilator controls massive bleeding during endoscopic ultrasound-guided drainage for pancreatic pseudocyst: A case report and review of literature. *World J Clin Cases* 2018; 6(11): 459-465 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i11/459.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i11.459

INTRODUCTION

Acute pancreatitis is the most common cause of hospitalization associated with gastrointestinal disease in the United States[1]. It is estimated that 5%-20% of pancreatitis episodes are complicated by the development of pancreatic pseudocyst (PPC)[2,3]. PPC, a common sequela of acute or chronic pancreatitis, was defined by the revised Atlanta classification as an encapsulated collection of fluid with a well-defined wall with minimal or no necrosis[4]. The majority of PPC cases was asymptomatic and may resolve spontaneously. However, PPCs can become symptomatic when they are infected or increase in size, which warrants intervention[5].

Previous retrospective and prospective studies have demonstrated that endoscopic ultrasound (EUS)-guided drainage for symptomatic PPC is increasingly used as a primary therapy with 90%-100% technical and 85%-98% clinical success rates[5,6]. However, hemorrhage is one of the deadly adverse events, and bleeding rates range from 3% to 14%[7,8]. Bleeding presents a challenging problem for physicians dealing with PPC. Management of this condition requires the cooperation of surgeons, endoscopists and radiologists. Historically, hemorrhages have been treated with conservative therapy, endoscopy, interventional radiology-guided embolization or surgery[9].

Herein, we present a meaningful case using a small balloon dilator to control PPH-related massive bleeding in EUS-guided drainage of PPC. To our knowledge, this method has not been previously reported.

CASE REPORT

A 55 years old woman presented with abdominal pain and distension resulting from PPC. She reported no nausea or vomiting. The patient had a complicated medical history (Supplementary file 1). She developed acute pancreatitis due to a gallstone and chronic cholecystitis 2.5 years ago. Then, the patient progressed from acute to chronic pancreatitis and PPC. Therefore, some treatment approaches, such as endoscopic nasopancreatic drainage (ENPD) and ultrasound-guided percutaneous drainage, were attempted. However, the PPC continually recurred. She visited our hospital again due to PPC growth and abdominal pain symptoms.

An abdominal contrast-enhanced computed tomography (CECT) scan (Figure 1) revealed a 7.4 cm × 6.2 cm pseudocyst in the tail of pancreas. Notably, most of the splenic vein compression, splenomegaly, perisplenic and gastric varices suggested severe varices due to pancreatogenic portal hypertension (PPH). In addition, pre-procedural magnetic resonance cholangiopancreatography (MRCP) revealed a homogeneous pseudocyst mass in the tail of the pancreas. Contact was observed between the pseudocyst and the pancreatic duct, and a high-intensity fluid tract was detected. Cross-sectional imaging and patient medical history helped to confirm the pseudocyst. Particularly, EUS (Figure 2) revealed that the cyst wall had a thickness of approximately 10 mm and good adhesion (within 10 mm) between the cyst wall and posterior gastric wall. After sufficient pre-procedural preparation, EUS-guided trans-gastric drainage was performed.

The patient underwent endoscopy performed by experienced interventional endoscopists using a linear array echoendoscope (Olympus Ltd, Tokyo, Japan) (Supplementary file 2). All procedures were performed while the patient was under general anesthesia. EUS imaging was used to determine the cyst puncture site and confirm the lack of intervening vessels at the puncture site. A 19-gauge needle was employed to perform the primary PPC puncture and access the cavity, which helped to create a fistula between the PPC and gastric lumen. Aspiration of PPC contents was then conducted to confirm the location, and the aspirate was microbiologically assessed. A 0.035 in guidewire was inserted through the needle and then coiled into the cyst cavity. The needle was withdrawn, while the guidewire remained in the cyst. Next, a 10F cystotome was utilized to dilate the fistula. Unfortunately, after we removed the cystotome, an acute, massive hemorrhage surrounding the fistula was noted under EUS, and the blood flow was similar to a stream (Figure 3A, video 1). Hemoglobin decreased by 2 g, and a blood transfusion was performed immediately. We transfused 1.5 units of fresh red cells, but the transfusion did not improve the situation.

Ultimately, we used a 10-mm balloon dilator (Boston Scientific, Natick, Mass) guided by digital subtraction angiography (DSA) to compress the bleeding area (Figure 4, schematic diagram). DSA was used to ensure that the balloon was placed in the correct location (Figure 5). We used a pressure pump to inject 1:1 contrast agent into the balloon to expand it to 1 cm. Suddenly,

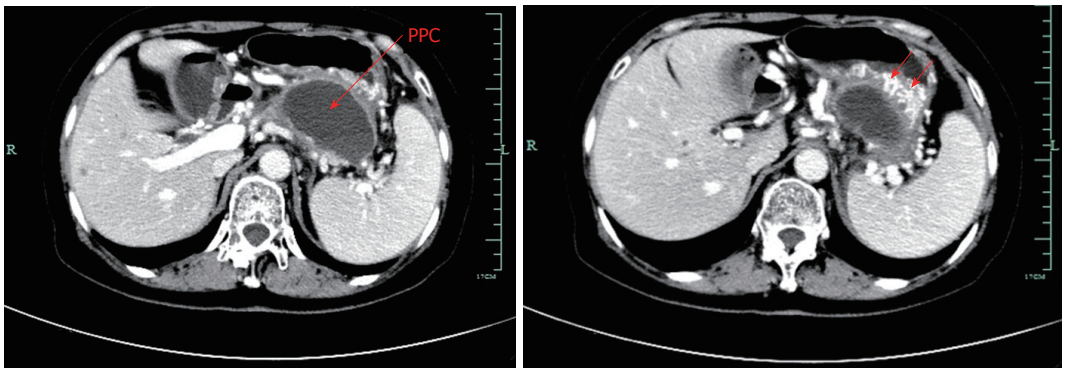

Figure 1 Pre-procedural contrast-enhanced computed tomography image. Computed tomography (CT) scan of the upper abdomen images revealed a 7.4 cm × 6.2 cm pancreatic pseudocyst in the tail of the pancreas, which was in close contact with the posterior wall of the stomach. Notably, splenic vein occlusion, splenomegaly and gastric varices were also observed.

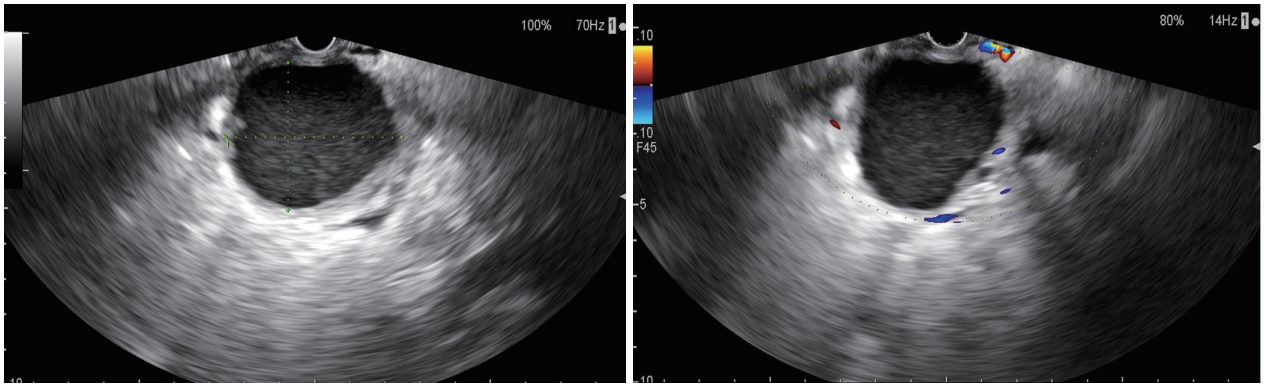

Figure 2 Endoscopic ultrasound image. These revealed a homogeneous pseudocyst mass, and the cyst wall had a thickness of approximately 10 mm. Good adhesion was noted between the cyst wall and the posterior gastric wall.

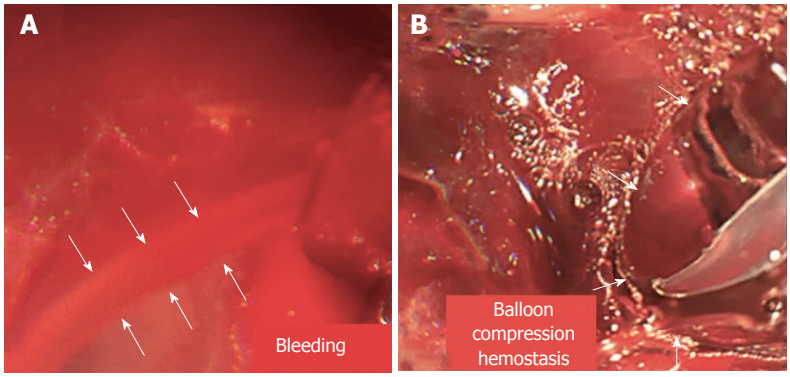

Figure 3 Endoscopic images. A: Endoscopic images during the procedure revealed acute and massive bleeding surrounding the fistula, and the blood flowed like a stream; B: Endoscopic images revealed that the massive bleeding was stopped *via* the compression of balloon dilator to achieve effective hemostasis, significantly impacting the patient's condition.

the "blood stream" was controlled (Figure 3B). After half an hour, we loosened the balloon, and bleeding was observed in EUS images. Thus, we continued compression for two hours. Surprisingly, the serious hemorrhage had completely stopped when we loosened the balloon again. Furthermore, the fistula was simultaneously dilated. One small balloon, as a dilator and an effective tool to achieve hemostasis, made a significant impact. Finally, a 1.0 cm double-pigtail plastic stent (DPPS) was successfully deployed. Considering the substantial bleeding, we decided to deploy only one stent to avoid excessive damage.

The patient underwent CECT after two months to evaluate resolution of the fluid collection. CECT revealed that the PPC completely resolved without any remaining fluid component, and the severe PPH was alleviated

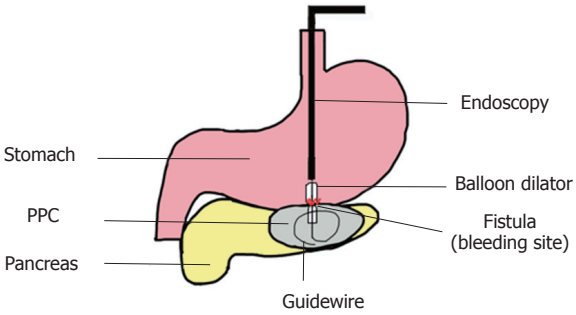

Figure 4 Schematic diagram of the procedure. A small balloon was expanded to 1 cm using a pressure pump, and contrast agent was administered. We used the balloon dilator to compress the bleeding area and achieve effective hemostasis.

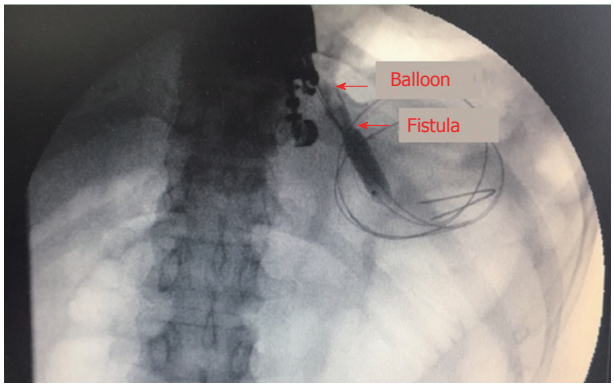

Figure 5 Image of digital subtraction angiography. It revealed a balloon dilator compressing the bleeding area. The digital subtraction angiography (DSA) guided and ensured that the balloon was in the correct location surrounding the fistula. DSA imaging also revealed balloon dilation of the tract.

(Figure 6). Ultimately, the stent was safely removed. Post-procedural CT indicated that the PPC lacked a stent and that PPH was significantly alleviated (Figure 7). The patient reported no bleeding and no recurrence at the six month follow-up.

DISCUSSION

PPC typically develops as a result of sequelae of acute or chronic pancreatitis, pancreatic trauma, malignancy, and surgery[10]. Ultrasound, CT, MRI (including MRCP) and EUS are routine diagnostic approaches for pancreatic fluid collections. A CT scan of PPC is the most commonly used modality for diagnosis, and EUS is used for observation and as a therapeutic procedure[11,12]. Rapid progression in the improvement of diagnostic methods enables examination with high sensitivity and specificity. The differentiation of PPC can be challenging and depends on high-quality imaging and thorough knowledge of medical history and disease pathophysiology (Supplementary file 3).

Currently, the management options for symptomatic PPC mainly include surgical, percutaneous and endoscopic drainage[13]. In a previous randomized trial comparing endoscopic and surgical drainage for a pseudocyst, Varadarajulu *et al*[14] revealed that these procedures had equal efficacy for PPC drainage. However, endoscopic treatment was associated with lower mortality, better physical and mental health of patients, shorter hospital stays, and lower cost. A recent systematic review and meta-analysis[15] concluded that endoscopic drainage rather than percutaneous drainage should be the preferred therapeutic method for PFC. In recent years, the use of EUS-guided drainage has gradually increased. Moreover, this procedure has been recognized as the first-line approach for managing symptomatic PPC[16].

In our case, the patient chose EUS-guided drainage for three reasons. First, the patient tried other treatments, including conservative approaches as well as endoscopic nasopancreatic and percutaneous methods. However, the PPC continuously recurred. Second, although another possible treatment for this patient was pseudocyst-jejunum anastomosis due to the connection between the pseudocyst and the pancreatic duct. Although surgery is the traditional therapy for PPC, it is associated with more complications, such as pancreatic fistula and intestinal fistula, longer length of recovery and hospital stays, and increased cost[17]. In contrast, EUS-guided drainage is a minimally invasive treatment for pseudocysts with fewer adverse events and shorter length of recovery[11]. Third, the patient experienced serious PPH. Surgery causes substantial trauma that could potentially injure the variceal vessels and increase the risk of bleeding. In consideration of all these factors, we selected EUS-guided drainage for this patient, and it was effective.

However, some treatment methods related to EUS-guided drainage are associated with bleeding. A recent retrospective study assessed 103 pancreatic fluid collection patients treated by EUS-guided drainage. In total, five patients experienced bleeding (5%), and one patient died from splenic artery pseudoaneurysms[9]. In addition, stent erosion of the gastric wall was noted in one patient who was treated by cauterization for durable hemostasis under esophagogastroduodenoscopy (EGD). In another patient, collateral vessel bleeding was managed conservatively. Moreover, one patient experienced intracavity variceal bleeding and was treated by intracavity tamponade under endoscopy. In another multicenter study by Siddiqui[5], EUS-guided drainage of PPC was employed, and 4% of patients experienced bleeding. Two patients experienced severe bleeding due to inadvertent puncture of an artery, which was successfully treated with interventional radiology-guided coil embolization. Recently, Puri *et al*[18] performed EUS-guided cyst puncture and drainage on 40 patients with PPC. The success rate was 100%, and only one patient underwent surgery due to bleeding. The authors also believed that EUS-guided drainage of PPC was a safe, successful method.

In the present case, the patient experienced acute,

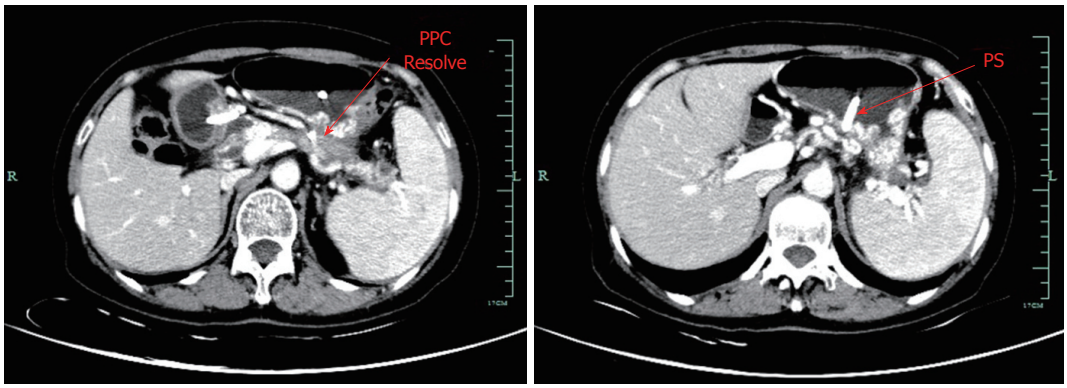

Figure 6 Follow-up contrast-enhanced computed tomography images. Computed tomography scan of the abdomen revealing a significant decrease in the size of the pseudocyst with a double pig plastic stent in position two months after endoscopy ultrasound-guided placement of a visible plastic stent between the stomach and residual pancreatic pseudocyst. The stent also effectively drained the pseudocyst and relieved the severely affected collateral vessels.

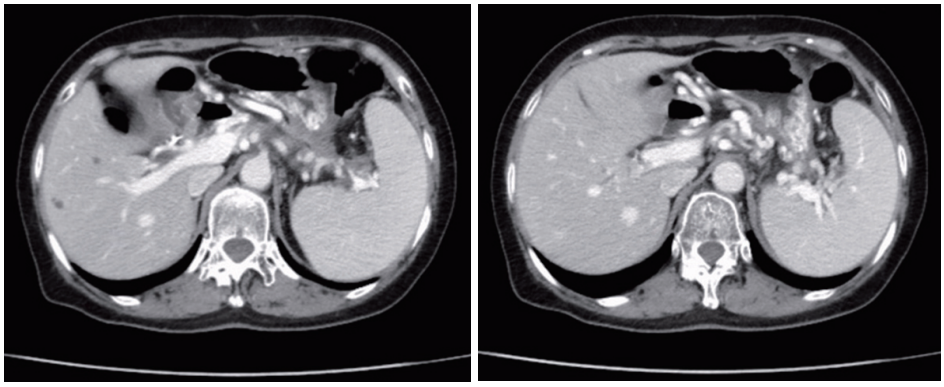

Figure 7 Follow-up contrast-enhanced computed tomography scan images. Contrast-enhanced computed tomography of the abdomen demonstrating that the pseudocyst was completely resolved three months after stent removal. No reoccurrence was noted, as indicated by the significant resolution of the serious perisplenic and gastric varices.

massive bleeding surrounding the puncture site, and surgery seemed to be an appropriate option. However, considering the serious situation with PPH, surgery could possibly damage the variceal vessels, while EUS with color Doppler ultrasound can identify the surrounding vessels and avoid intervening vessels at the puncture site[19]. Furthermore, because the bleeding was so severe, we needed a rapid, efficient, less damaging form of hemostasis to control it, and balloon compression quickly controlled the bleeding. In addition, the outcome of EUS appeared poor when the bleeding occurred like a "blood stream," and there were multiple, indefinite bleeding points. Thus, endoscopic cauterization or tamponade treatment and interventional embolization may have been impossible to perform. Under these conditions, balloon compression represented optimal hemostasis with the advantages of convenience, quickness, inexpensive cost, and minimal invasiveness. From this case, we learned the following. Although EUS-guided drainage for PPC is safe and effective, there are some adverse events, and interventional endoscopists need to prepare in advance to address different problems. In addition, management of bleeding requires integrated and multidisciplinary cooperation between surgeons, endoscopists, and radiologists.

In conclusion, balloon compression may represent a successful treatment for a fistula surrounding massive bleeding during EUS-guided drainage for PPC and provides a novel form of hemostasis. Although this method was effective in our patient, additional successful cases are needed to confirm the validity of this new hemostasis method in future studies.

ARTICLE HIGHLIGHTS

Case characteristics

A 55-year-old woman was referred to our hospital with abdominal pain and distension resulting from a history of pancreatic pseudocyst.

Clinical diagnosis

Pancreatic pseudocyst.

Differential diagnosis

Walled-off necrosis and pancreatic cystic tumors.

Laboratory diagnosis

No specific laboratory testing contributed to the diagnosis of the pancreatic

pseudocyst.

Imaging diagnosis
Abdominal contrast-enhanced computed tomography, magnetic resonance cholangiopancreatography and endoscopic ultrasound examinations showed a pseudocyst in the tail of pancreas.

Pathological diagnosis
Pancreatic pseudocyst.

Treatment
We performed endoscopic ultrasound-guided drainage with massive bleeding and used a balloon dilator to compress the bleeding sites.

Related reports
To our knowledge, using balloon compression to achieve effective hemostasis in EUS-guided drainage for pancreatogenic portal hypertension-related bleeding has not been previously reported.

Term explanation
Endoscopic ultrasound-guided drainage with stenting is recognized as the standard first-line approach for a symptomatic pancreatic pseudocyst.

Experiences and lessons
Balloon compression is a novel and effective form of hemostasis for endoscopic ultrasound-guided drainage with bleeding. Although this method was successful in this case, additional cases are needed to confirm our findings.

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CASE REPORT

Twin pregnancy with triple parathyroid adenoma: A case report and review of literature

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Abstract

Primary hyperparathyroidism (PHPT) is rare during pregnancy. A case of twin pregnancy with three simultaneous parathyroid adenomas at the same time has not been reported. Multiple parathyroid lesions are difficult to diagnose, as pregnant women who insist upon continuing

a pregnancy are not able to undergo 99mTc-sestamibi scintigraphy, so cases of PHPT are easily unobserved and often can have serious consequences for the patient and the fetus. Therefore, we reported a case of a 28-year-old woman mid-pregnancy with twins, who had hypercalcemia and was eventually diagnosed with twin pregnancy with PHPT due to a triple parathyroid adenoma, had good pregnancy outcomes after undergoing surgery in mid-pregnancy. Twin pregnancy with PHPT due to a triple parathyroid adenoma, as presented in this case, is very rare and surgery in mid-pregnancy is demonstrated here as safe. Intraoperative parathormone monitoring was and remains key to a successful operation.

Key words: Primary hyperparathyroidism; Pregnancy; Triple parathyroid adenoma; Surgery

Core tip: Primary hyperparathyroidism (PHPT) during pregnancy is rare and has been previously reported, but a case of PHPT in a twin pregnancy with three parathyroid adenomas at the same time has not been reported. Multiple parathyroid lesions are difficult to diagnose, and pregnant women who insist upon continuing a pregnancy are not suitable to undergo 99mTc-sestamibi scintigraphy. These results in a case of PHPT that is easily misdiagnosed and can have serious consequences for the patient and the fetus. Therefore, we reviewed the difficulties encountered during the diagnosis and treatment of this case to improve the understanding of this disease and reduce the incidence of incorrect and missed diagnoses.

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INTRODUCTION

Primary hyperparathyroidism (PHPT) is a rare disease characterized by parathyroid glands that secrete excessive parathyroid hormone (PTH), which causes disorders associated with calcium and phosphorus levels and bone metabolism. Parathyroid adenoma is the most common cause of PHPT. The prevalence of PHPT is 1/4000 and is found more commonly in women. The ratio of cases in males compared to female cases is about 1:3[1]. According to the literature, 85%-90% of PHPTs are solitary, and less than 2% of patients have 2 or more leisons[2]. PHPT with pregnancy is rarer[3], and can be masked by normal pregnancy conditions, resulting in it being easily misdiagnosis or incorrectly diagnosed. A consensus has not yet been reached regarding a treatment plan, as more conservative treatments or a surgical treatment are both associated with great risks. Treatment that is not timely, it can have serious consequences for both the mother and the fetus. Treatment of multiple parathyroid adenomas in pregnancy is totally a tricky proposition. There are no reports in the literature on a twin pregnancy complicated by multiple parathyroid adenomas. Therefore, this is the first report of a the diagnosis and treatment of a case of twin pregnancy where the mother experienced PHPT due to a triple parathyroid adenoma that was admitted to our hospital in November 2017 is reported for the first time.

CASE REPORT

The patient was a first-time mother who was 28-year-old. Because of suffering from "endometriosis", she has been trying to get pregnant for 2 years since she married, but she had not become pregnant due to ongoing endometriosis. She became pregnant through in-vitro fertilization and embryo transfer. At the 22nd week of pregnancy, she was admitted to the endocrinology department of our hospital on November 2, 2017, as she exhibited elevated serum levels of calcium for one month. The patient presented to the local hospital with severe vomiting, nausea, and general weakness at 18 wk of gestation. The obstetrical ultrasound showed a twin pregnancy, and a blood test showed that her blood potassium level was 1.5 mmol/L (normal range 3.5 to 5.30 mmol/L) and blood calcium level 2.79 mmol/L (normal range 2.00 to 2.60 mmol/L). Although symptoms were relieved after emergency rehydration and potassium supplementation, there is no immediate treatment for hypercalcemia. Therefore, it was found later that her blood potassium level had been corrected, but her blood calcium level gradually increased (3.47 mmol/L), so she was transferred to our hospital for further treatment. Physical examination: The patient's spirit is soft, the skin and sclera were not yellow, the superficial lymph nodes were not swollen, the limbs were not deformed, the thyroid gland was not swollen, the heart and lungs were not remarkably affected, the lower abdomen was bulging, no tenderness and rebound tenderness were observed, no lower extremity edema was observed, and no negative neuropathological signs were observed. Past disease history: The patient had no history of long-term use of vitamins A, D, and calcium, and had no family history of specific diseases. Supplementary examination: PTH 187 pg/mL (normal range 15-65 pg/mL), serum calcium level was 3.49 mmol/L, serum phosphorus level was 0.62 mmol/L (normal range 0.8-1.5 mmol/L), and alkaline phosphatase level was 76 mmol/L (35-100 u/L). Ultrasound on her neck revealed a moderately echogenic nodule (2.0 cm × 0.8 cm) on the dorsal side of the superior portion of the left thyroid gland, which first suggested a parathyroid adenoma. A nodule was observed in the inferior dorsal

lateral aspect of the right thyroid gland that was classified as a "3" on the TI-RADS evaluation scale. Admission diagnosis: twin pregnancy with PHPT that was possibly attributable to parathyroid adenomas. After admission, we performed fluid rehydration, diuresis, dilatation, and combined symptomatic supportive, such as calcitonin administration. Blood calcium and PTH levels decreased slightly but did not change much. After several discussions with the multiple-disciplinary team (MDT), and repeatedly communication with the patient, we told her about the advantages and disadvantages as they related to more conservative treatment options. Finally, the patient and her family chose surgical treatment.

After a fully preoperative preparation, resection of the parathyroid tumor was performed using a cervical plexus nerve block on November 16, 2017. During the operation, a tumor on the dorsal side of the superior lobe of the left thyroid was first observed (lesion 1), which was observed by ultrasound examination. No abnormal mass was found inferior lobe of the left thyroid. After resection of the above masses, blood PTH level was 131 pg/mL (preoperatively is 187 pg/mL) 10 min after resection, which failed to fall to an ideal value. Then, we began to explore the area behind the right thyroid lobe and found two more lesions: one in the superior dorsal lobe of the right thyroid (lesion 2) and one in the lateral aspect of the right lower lobe (lesion 3), with sizes of approximately 2.5 cm × 1.5 cm × 1.0 cm and 0.6 cm × 0.5 cm × 0.5 cm, respectively. Intraoperative puncture eluent results confirmed that the above lesions were parathyroid tissues, and the blood PTH level was 39 pg/mL 10 min after complete resection of the other two lesions (lesion 2 and 3), and freezing pathology also suggested that parathyroid adenoma should be first considered as the cause. To prevent postoperative hypocalcemia, relatively normal lesions (approximately 3 mm × 3 mm × 3 mm) were placed in the patient's forearm muscles. Postoperative PTH and serum calcium were within the reference values. Postoperative pathology suggested that the positioning of the lesions at positions of "upper left, lower right, upper right" is consistent with parathyroid adenomas. In follow-up, we found that the patient had undergone a cesarean delivery for 1 male and 1 female infant at 32 wk of gestation, and the newborns were basically normal for preterm infants except for the general performance in that they exhibited low weight. Blood calcium and PTH levels were normal during the follow-up of the patient and the infants.

DISCUSSION

Pregnancy with PHPT is rare[4]. There are only 150 cases of related diseases reported in the literature, and less than 10 cases reported in China. This is the first case of PHPT in a twin pregnancy with multiple parathyroid tumors. PHPT can lead to serious complications for both the mother and fetus[5]. Common complications for the mother include: hyperemesis, skeletal lesions, kidney stones, elevated blood pressure, and other similar complications. When serum calcium is extremely elevated, it can induce pancreatitis, and a high blood calcium crisis can lead to death. A total of 80% of patients who do not receive effective treatment will experience complications in the fetus that can include fetal growth restriction, low birth weight, premature birth, intrauterine fetal death, stillbirth and neonatal hand-foot convulsions. According to literature reports, the incidence of untreated maternal complications in PHPT is about 67% of all pregnancy-associated PHPT cases, and the incidence of fetal or neonatal complications is as high as 80%, and can cause fetal or neonatal death in as much as 30% of cases. Therefore, it is necessary to raise the awareness of the disease.

Most patients who experience PHPT while pregnant lack typical clinical manifestations. Clinical symptoms often only manifest as vomiting, polydipsia, increased nocturia, and other similar symptoms. These are easily to be confused with normal responses to pregnancy, thereby masking the condition and causing missed diagnoses. In this case, due to nausea, vomiting and general malaise were present, but subsequent examinations also revealed a progressive increase in serum levels of calcium, and parathyroid tumors were found after assessing PTH levels and were partly observed through ultrasound examination. Combined with other people's reports and our experience in this case[6,7], when a pregnant woman presents with the following symptoms: Hyperemesis, spasticity, pancreatitis, hypercalcemia, urinary system stones, this disease should be considered. If blood calcium and PTH levels are observed over time and can be combined with ultrasound, 99mTc-sestamibi scintigraphy, then the diagnosis of the disease is not difficult, and suggesting that raised awareness of the disease is most important for its correct diagnosis. However, it should be emphasized that 99mTc-sestamibi scintigraphy should be avoided for those who elect to continue with a pregnancy because of their associated risks of radiation exposure, as shown as example demonstrates. Additionally, the disease needs to be differentiated from other diseases, such as hypercalcemia that could be caused from a multiple myeloma or other similar causes, and secondary hyperparathyroidism, familial hyperparathyroidism syndrome, diseases that affect multiple endocrine glands, and other additional possible causes of these symptoms. These can be identified by combining laboratory and imaging tests with an oral history.

Current PHPT treatment guidelines do not give a clear treatment plan in PHPT associated with pregnancy as the incidence of PHPT in pregnancy is very low and most of the literature only reports individual cases. Norman *et al*[8] analyzed 32 women who had PHPT while pregnant and found that about 20% of patients who received surgical treatment during the middle of pregnancy had a good outcomes associated with the

pregnancy. When a PHPT patient remains untreated, loss of the pregnancy can occur in as many as 40% of untreated pregnancies. Therefore, it is considered that early diagnosis and active surgical treatment are necessary. However, some researchers[5] found through retrospective analysis that there was no statistically significant difference in the live birth rate compared to the rate of abortion in pregnancies associated with PHPT compared to normal pregnancies, and that only the cesarean section rate increased slightly. Therefore, parathyroid surgery should be considered, as PHPT does not reduce the risk of miscarriage in these patients, but surgery can still help reduce the complications in both the mother and the newborn. However, risks associated with surgery are also very high, including risks associated with anesthesia, postoperative bleeding, permanent hypoparathyroidism, and other similar complications. Any risk may have serious consequences for the mother, the child, or both. In addition, more conservative treatment may be considered for patients with PHPT who exhibit only mildly elevated serum calcium levels in PHPT, as studies suggesting that patients with asymptomatic PHPT with serum calcium levels below 11 mg/dL (2.75 mmol/L) should consider more conservative treatments, which include treatments like administration of calcitonin, and other similar options[9]. At present, the conservative treatment methods for PTPH mainly include rehydratation, Calcitonin, Bisphosphonates and Cinacalcet. The use of rehydratation and Calcitonin is relatively mature, and no adverse effects of the fetus have been reported in the literature. Bisphosphonates and Cinacalcet can cross the placenta and are embryotoxic at high doses[10]. Although these have been used in few cases with good results, but more evidence of safety is required[11]. However, conservative treatments and their effects are often limited, and drug resistance may occur. If pregnant women with PHPT do not receive effective treatment, the incidence of neonatal complications can reach up to 80% of all cases of PHPT in pregnancy. Even in conservatively treated patients, the incidence of neonatal complications can reach as high as 53%, of which 27% to 31% of these cases can result in neonatal deaths[12]. In summary, current evidence supports parathyroidectomy as the main treatment, performed preferably during the second trimester, when the serum calcium is above 11 mg/dL (2.75 mmol/L). In the patients with mild forms of PHPT, which are nowadays the most frequent, a conservative management is generally preferred[13]. We think the timing of surgical management of PHPT in pregnancy also should also think over acute maternal presentation, fetal status, maternal medical and surgical history, and the patient's response to medical management.

In this case, the patient's blood calcium and PTH levels were decreased somewhat but remained elevated overall after medical treatment. If we had continued with a more conservative treatment, there would have likely been an escape phenomenon, which can lead to severe consequences, such as: Refractory hypercalcemia, possibly a high calcium crisis, hematuria, coma, and death. Current research has confirmed that surgery performed during the second trimester of pregnancy is largely safe, but surgery performed in later stages of pregnancy can increase miscarriage rates. Our patient was in the second trimester, so it was the best time for surgery for this patient. Therefore, after a multidisciplinary discussion in the hospital, and repeatedly stressing advantages and disadvantages of each treatment option to the patient and her family, the patient chose surgery. Although most reports say that general anesthesia has little effect on the mother and the fetus, we still chose a nerve block in the cervical plexus to reduce possible harm to the mother and the fetus. Finally, in follow-up, we observed a good outcome relating to the pregnancy in this patient.

The surgical treatment of hyperparathyroidism is an organ-destructive surgery. Its success requires not only a return of normal PTH levels, but also an avoidance of overcorrection and prevention of permanent hypoparathyroidism. Multiple parathyroid lesions are more problematic in clinical treatment. The literature shows that 85%-90% of PHPT are solitary, and less than 2% of patients have 2 or more lesions[2]. Precise preoperative imaging to obtain the lesion's location is beneficial for a rapid search for parathyroid lesions, and it is also especially helpful for improving the success rate of the surgery, as it can reduce the time of the surgery and exposure to anesthesia, which is especially important for this patient. Ultrasound examination is a convenient, inexpensive and non-invasive examination method. The accuracy of preoperative localization of parathyroid lesions alone can be up to reach 77% (173/226)[14], which is comparable to the accuracy of a radionuclide scan plus MIBI imaging alone. The latter can be used when the ultrasound examination is negative or uncertain. The combination of these two examinations can increase the accuracy of positioning to 90%. Ultrasound, 99mTc-sestamibi scintigraphy, and MRI have their own advantages and disadvantages in PHPT diagnosis. Reasonable selection can complement each other. However, the literature reports and our experience in this case suggest that any imaging examination cannot ensure the visualization of all paraneoplastic lesions in these glands. More importantly, visualization of lesions depends on the surgeon's experience and careful exploration during surgery, especially in patients like the one in this case who was not able to undergo 99mTc-sestamibi scintigraphy. In addition, the immediate detection of changes in PTH levels during surgery is also an important measure for improving the success of the surgery[15]. PTH is metabolized by the liver and kidneys in vivo. Its half-life is only 3 to 5 min. High serum PTH reduction is the earliest change indicating success in surgical treatment of PHPT. The standard that is currently most widely accepted by the majority of scholars is the

Miami standard: The surgery is considered successful if the value of the PTH serum level drops to greater than or equal to 50% of the preoperative PTH value 10 min after the gland is removed. Therefore, being able to observe changes in PTH serum levels at 10 min after the surgical removal of the gland is critical for assessing the success of the operation. This case uses this criterion. After resection of the patient's supra-parathyroid tumor prompted by preoperative ultrasound, PTH levels in the blood (131 pg/mL) did not drop to the expected level, so we continued to explore the right neck and found two additional lesions. The intraoperative puncture of eluent results suggested that the two masses originated in the parathyroid gland. After the second complete resection of the two lesions, the levels of PTH in the blood dropped to 39 pg/mL, suggested the surgery had completely removed all parathyroid lesions.

To avoid overcorrection and prevent the occurrence of permanent hypoparathyroidism, we chose a relatively normal lesion of about 3 mm × 3 mm × 3 mm in size for autologous transplantation in the forearm muscles in this patient, which was done according to the guidelines developed from the experiences from the treatment of patients with secondary hyperparathyroidism. After postoperative follow-up, blood PTH and serum calcium levels in this patient were within the normal range. However, in the case of similar patients, the need for transplantation of the parathyroid glands and the amount of transplanted tissue necessary must be further explored and studied. Additionally, the literature reports that about 5% of patients have ectopic parathyroid lesions. If this patient is involved in this situation, what should we do? PTH levels may remain substandard after all suspected lesions have been removed, but if the surgeon continues to probe blindly, it undoubtedly will increase the surgical complications and even possibly cause increased serious consequences. Recommendations for these cases require further research and lessons learned.

In summary, the possibility of PHPT should be considered during pregnancy when hypercalcemia, stones in the urinary tract, pancreatitis, prolonged irritability of pregnancy, or bone fractures occuring. Combined with experience of treatment in this patient, mid-pregnancy surgery has been previously observed as relatively safe. Early diagnosis and treatment of PHPT during pregnancy can result in better pregnancy outcomes. In addition, failure to perform surgery for PHPT surgery may result in a secondary surgery or permanent hypoparathyroidism, which may cause great pain to patients. In clinical practice, a rigorous diagnosis and treatment strategy must be established. Our experience suggests that imaging cannot be fully relied upon for diagnosis. The rapid detection of PTH levels using a process called intraoperative parathormone monitoring (IPM) after surgical resection is necessary and key for a successful operation, and the removal of diseased glands should return PTH levels to their ideal value. The treatment plan should allow for individual choices based on the patient's symptoms, serum calcium levels, the effectiveness of more conservative treatments, the size of the gestational age in the patient and the patient's willingness to receive the chosen treatment.

ARTICLE HIGHLIGHTS

Case characteristics

The patient presented with nausea, vomiting and general malaise and elevated serum calcium.

Clinical diagnosis

Physical examination showed the patient's spirit is soft, the lower abdomen is bulging.

Differential diagnosis

The differential diagnosis included multiple myeloma, secondary hyperparathyroidism, familial hyperparathyroidism syndrome and multiple endocrine gland diseases. The disease can be differentiated from other diseases by asking history and combining laboratory, imaging tests.

Laboratory diagnosis

Blood test results were as follows: Parathyroid hormone (PTH) 187 pg/mL, serum calcium 3.49 mmol/L, serum phosphorus 0.62 mmol/L, alkaline phosphatase 76 mmol/L.

Imaging diagnosis

Ultrasound on the neck showed a moderately echogenic nodule (2.0 cm × 0.8 cm) on the dorsal side of the upper left thyroid gland, which parathyroid adenoma was the first consideration. Thyroid right lower lobe dorsal lateral process nodule (TI-RADS 3).

Pathological diagnosis

Postoperative pathology suggested that "three lesions are all parathyroid adenomas".

Treatment

Treatment with parathyroid tumor resection the cervical plexus nerve block during mid-pregnancy.

Related reports

Primary hyperparathyroidism during pregnancy have been reported previously, they were all have a single parathyroid adenoma or choose to complete the radiological examination after termination of pregnancy. But we report a unique case of twin pregnancy with three parathyroid adenomas, without radiological examination, had good pregnancy outcomes after undergoing surgery during mid-pregnancy.

Term explanation

IPM: Intraoperative parathormone monitoring.

Experiences and lessons

Given the severity of the associated complications, every effort should be made to ensure prompt diagnosis and reasonable treatment. Do not rely on imaging diagnosis. Careful exploration, rapid detection of PTH after surgical resection and the removal of diseased glands to the ideal value, which called IPM is the key to successful operation.

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CASE REPORT

Unusual cause of lesions in the descending duodenum and liver: A case report and review of literature

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Abstract

The descending duodenum is rarely involved in *Schistosoma japonicum* (*S. japonicum*) infection. Here, we report a case of acute Schistosoma infection, which presented with abdominal pain, abdominal distension and irregular fever. Tumor-like lesions were observed in the descending duodenum. Simultaneously, heterogeneity in hepatic perfusion was demonstrated by dynamic computed tomography scanning. Biopsy of the descending duodenum showed the deposition of Schistosoma eggs. Following administration of the antihelminthic drug praziquantel, the patient showed rapid clinical improvement. In conclusion, we report a patient with acute *S. japonicum* infection presenting as tumor-like lesions in the descending duodenum and heterogeneity of blood perfusion in liver parenchyma.

Key words: *Schistosoma japonicum*; Heterogeneity; Duodenum; Tumor-like lesions; Liver

Core tip: *Schistosoma japonicum* (*S. japonicum*) is primarily found in the mesenteric veins and tends to involve the colon, rectum and liver. Here, we report a case of acute *S. japonicum* infection in a patient presenting with tumor-like lesions in the descending duodenum and heterogeneity of blood perfusion in liver parenchyma.

Xiao ZL, Xu KS, Song YH. Unusual cause of lesions in the descending duodenum and liver: A case report and review of literature. *World J Clin Cases* 2018; 6(11): 472-476 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i11/472.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i11.472

INTRODUCTION

Schistosomiasis is a disease caused by parasitic flatworms called schistosomes. The three major species of human schistosome are *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* (*S. japonicum*)[1]. *S. japonicum* is widespread in East Asia and the southwest Pacific region. It is primarily found in the mesenteric veins and tends to involve the colon, rectum and liver, and occasionally the duodenum. In this study, we report *S. japonicum* infection involving the descending duodenum presenting as tumor-like lesions. In addition, heterogeneity of blood perfusion in liver parenchyma was observed in the patient. This is the first report to describe patchy infiltration of *S. japonicum* in liver parenchyma.

CASE REPORT

A 47-year-old male farmer presented to our hospital with a 3 wk history of persistent abdominal pain, abdominal distension and irregular low fever. Abdominal examination revealed tenderness in the right upper quadrant with no rebound tenderness or guarding. The results of laboratory tests were as follows: leukocytes, 15450/mm^3; eosinophils, 28.7%; erythrocytes, 2.84 × 10^{12}/L; hemoglobin, 86 g/L. Liver function test findings were as follows: albumin, 24.6 g/L; alkaline phosphatase (ALP), 341 U/L (40-150 U/L); γ-glutamyl transferase (γ-GT), 94 U/L (17-53 U/L). The hepatitis B results were as follows: hepatitis B surface antigen, positive; hepatitis B virus (HBV) e antibody, positive; hepatitis B core antibody, positive. HBV DNA was undetectable, and hepatitis C virus antibody was negative. Erythrocyte sedimentation rate was 96 mm/h; autoantibodies were negative; immunoglobulins were within the normal range. Neither aerobic nor anaerobic bacteria were detected in blood culture. Tumor markers (carcinoembryonic antigen, alpha fetoprotein, carbohydrate antigen 199, carbohydrate antigen 72-4, prostate specific antigen, squamous cell carcinoma antigen) were within the normal range. The results of ascitic fluid examination were as follows: total protein, 26.0 g/L; albumin, 12.0 g/L; serum-ascites albumin gradient, 12.6 g/L; cell count, 450/mm^3; leukocytes, 254/mm^3; polymorphonuclear, 46/mm^3. A panel of ascitic tumor markers was within the normal range, and the remaining blood tests were normal. Upper gastrointestinal (GI) endoscopy indicated protrusive lesions in the esophagus (Figure 1A), swollen mucosa and protrusive lesions in the descending duodenum (Figure 1B). Endoscopic ultrasonography (EUS) revealed a hypoechoic mass in the muscularis mucosa of the esophagus (Figure 1C, arrow), thickening of the descending duodenal wall, destruction of the descending duodenal wall (Figure 1D, arrow), and ascites (not shown). Dynamic abdominal computed tomography (CT) scanning showed heterogeneous hypointensity in the liver (portal phase) (Figure 1E, arrow), thickening of the descending duodenal wall (Figure 1F, arrow), swollen mesentery around the arteries (regional increase in mesenteric fat density as a result of edema) (Figure 1G, arrowhead), and ascites (Figure 1G, arrow). Serological tests for anti-Schistosoma antibody (ELISA) were positive; a biopsy of the descending duodenum showed the deposition of Schistosoma eggs and infiltration of eosinophils (Figure 1H). The patient received the antihelminthic drug praziquantel. Two months later, the patient underwent a complete checkup, and his symptoms of abdominal pain, abdominal distension and fever had resolved. The results of laboratory tests were as follows: leukocytes, 9280/mm^3; eosinophils, 17.60%; erythrocytes, 4.6 × 10^{12}/L; hemoglobin, 142 g/L; albumin, 49.6 g/L; ALP, 169 U/L; γ-GT, 78 U/L. Upper GI endoscopy demonstrated protrusive lesions in the esophagus (Figure 2A) and normal mucosa (Figure 2B) in the descending duodenum. EUS showed a hypoechoic mass in the muscularis mucosa of the esophagus (no change, not shown), slight thickening and normal layer of the descending duodenal wall (Figure 2C, arrow). Dynamic abdominal CT scanning showed homogeneous hepatic perfusion on the portal phase (Figure 2D), slight thickening of the descending wall (Figure 2E, arrow), shrinkage of swollen mesentery to normal size (Figure 2F, arrowhead), and disappearance of ascites.

DISCUSSION

S. japonicum, which is widespread in China, is primarily found in the mesenteric veins and involves the colon, rectum and liver. Occasionally, the duodenum is involved in *S. japonicum* infection and is manifested as erosion, ulcer, bleeding and granular changes in the mucous membrane[2-5]. The patient in our study presented with tumor-like lesions in the descending duodenum, which has not been reported in the literature. EUS and CT revealed thickening and destruction of the descending duodenal wall, which resembled tumor-like lesions. Pathological examination demonstrated *S. japonicum* infection in the descending duodenum. The diagnosis of GI Schistosomiasis is established by histological evidences in clinical practice. Thus, endoscopic examination identifies the lesion of GI parasites, and pahological evidence from endoscopic biopsies define the diagnosis of parasites infection[6]. In addition, ELISA tests for anti-Schistosoma antibody demonstrated the infection of *S. japonicum* because *S. japonicum* is the only human blood fluke that occurs in China. Importantly, rapid

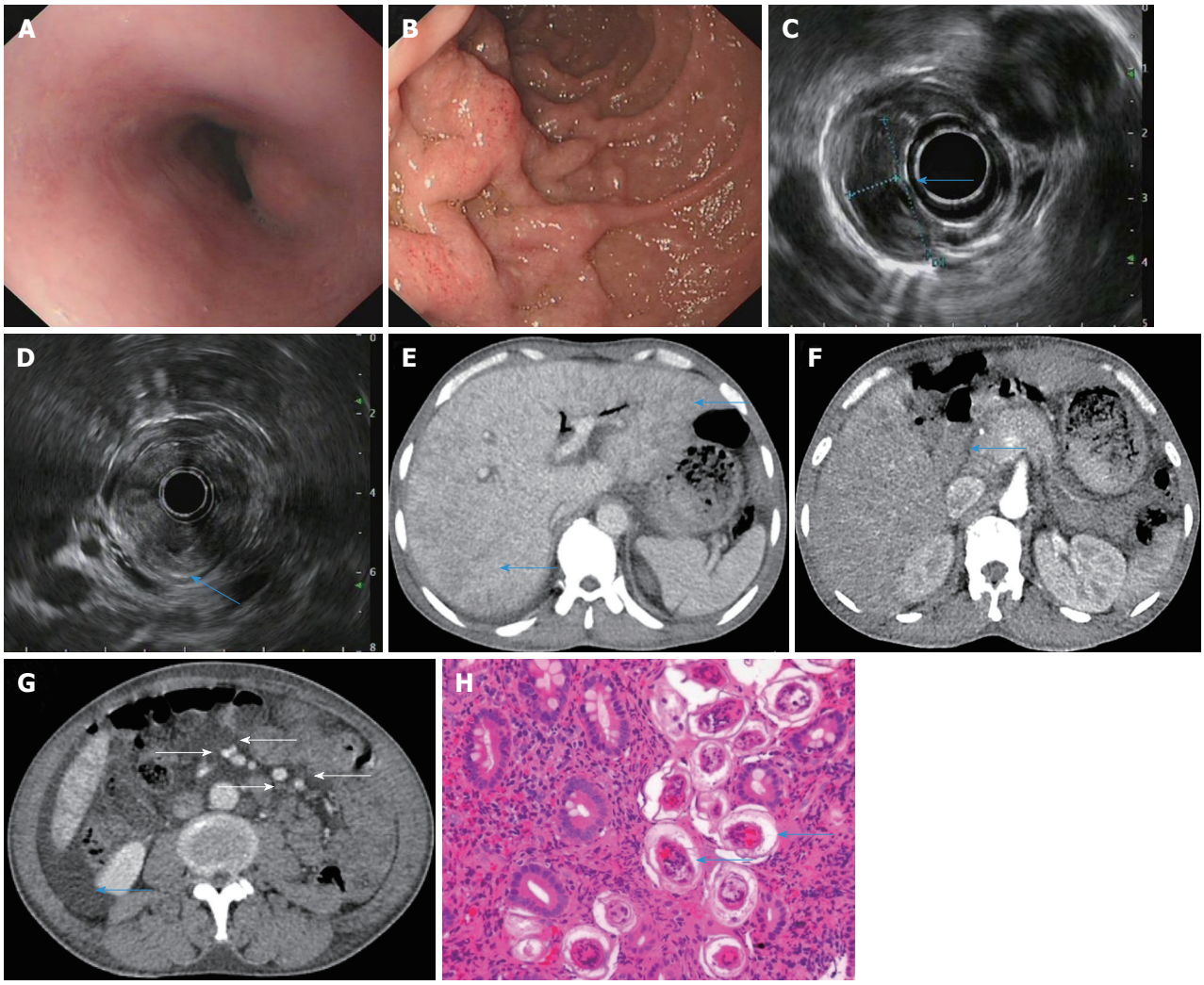

Figure 1 A 47-year-old male patient with acute *Schistosoma japonicum* infection underwent endoscopic examination, endoscopic ultrasonography and dynamic computed tomography scanning at the baseline visit. A, B: Upper gastrointestinal endoscopy showing protrusive lesions in the esophagus (A), swollen mucosa and protrusive lesions in the descending duodenum (B); C, D: Endoscopic ultrasonography revealing a hypoechoic mass in the muscularis mucosa of the esophagus (C, arrow), thickening of the descending duodenal wall, and destruction of the descending duodenal wall (D, arrow); E-G: Dynamic computed tomography showing heterogeneous hypointensity in the liver (E, arrow), thickening of the descending duodenal wall (F, arrow), swollen mesentery around the arteries (G, arrow), and ascites; H: Biopsy of the descending duodenum showing deposition of Schistosoma eggs.

improvement of the descending duodenal lesions was observed following administration of praziquantel. The clinical manifestations, pathological changes (uncalcified eggs) and the increase in eosinophils confirmed the diagnosis of acute *S. japonicum* infection in this patient.

In addition, heterogeneous hypointensity in liver parenchyma on the portal phase of dynamic CT was observed in the patient, which demonstrated a difference in blood perfusion of liver parenchyma. Patchy liver enhancement on the portal phase of dynamic CT was observed in the areas of liver parenchyma, which received better blood perfusion[7-9]. Patchy hepatic infiltration of *S. japonicum* gave rise to the heterogeneity of blood perfusion in liver parenchyma. Liver biopsy can provide direct evidence of *S. japonicum* infection in patients; however, liver biopsy was not performed in our patient due to extensive ascites. Following treatment with praziquantel, dynamic abdominal CT showed homogeneous hepatic perfusion on the portal phase. These findings showed that patchy infiltration of *S. japonicum* resulted in the heterogeneity of blood perfusion in the liver. Portal hypertension resulted from massive deposition of *S. japonicum* eggs in portal branches of the liver, which might give rise to ascites and heterogeneity in hepatic perfusion.

In conclusion, we report a patient with acute *S. japonicum* infection presenting as tumor-like lesions in the descending duodenum and heterogeneity of blood perfusion in liver parenchyma. Thus, in areas affected by *Schistosomiasis* epidemics, *S. japonicum* infection should be considered when patients have tumor-like lesions in the duodenum or hepatic heterogeneous hypointensity on dynamic CT. In addition, duodenal involvement in patients with *S. japonicum* infection should receive long-term follow-up to prevent the development of malignant lesions.

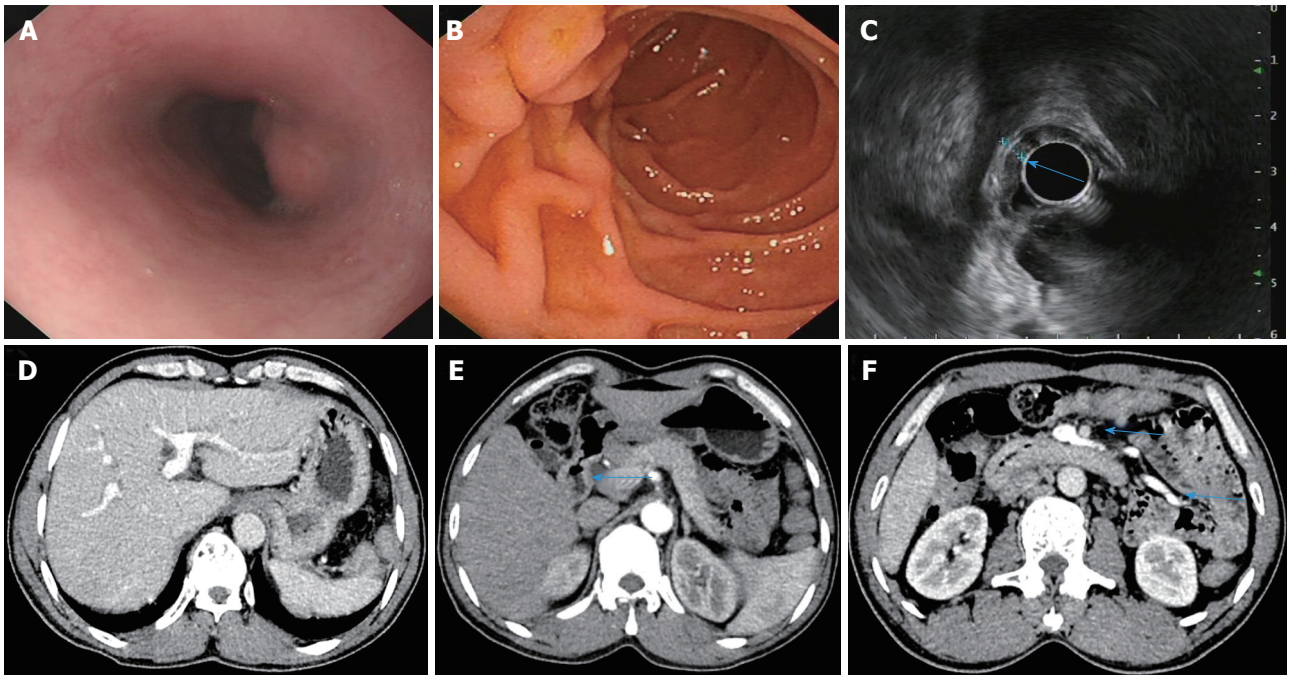

Figure 2 Following administration of praziquantel, the patient with acute *Schistosoma japonicum* infection received a complete check-up at the follow-up visit. A, B: Upper gastrointestinal endoscopy showing protrusive lesions of the esophagus (A) and normal mucosa in the descending duodenum (B); C: Endoscopic ultrasonography revealing slight thickening and normal layer of the descending duodenal wall; D-F: Dynamic abdominal computed tomography showing homogeneous hepatic perfusion on the portal phase (D), slight thickening of the descending wall (E, arrow), shrinkage of the swollen mesentery to normal size (F, arrow), and disappearance of ascites.

ARTICLE HIGHLIGHTS

Case characteristics

A 47-year-old male farmer presented with persistent abdominal pain, abdominal distension and irregular low fever.

Clinical diagnosis

The diagnosis of parasitic disease was made by serological test or histological examinations.

Differential diagnosis

Differential diagnosis with malignant lesions due to the thickening and the destruction of the descending duodenal wall.

Laboratory diagnosis

Serological tests for anti-Schistosoma antibody (ELISA) were positive.

Imaging diagnosis

Dynamic abdominal computed tomography (CT) scanning showed heterogeneous hypointensity in the liver, thickening of the descending duodenal wall, swollen mesentery around the arteries, and ascites.

Pathological diagnosis

Pathologic examination of the descending duodenum showed the deposition of Schistosoma eggs and infiltration of eosinophils.

Treatment

The patient received the antihelminthic drug praziquantel.

Related reports

The infection of *Schistosoma japonicum* (*S. japonicum*) is primarily found in the mesenteric veins and tends to involve the colon, rectum and liver, and occasionally the duodenum.

Experiences and lessons

S. japonicum infection should be considered when patients have tumor-like lesions in the duodenum or hepatic heterogeneous hypointensity on dynamic CT.

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CASE REPORT

Isolated myeloid sarcoma in the pancreas and orbit: A case report and review of literature

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Author contributions: Zhu T participated in the design of the report, analysed the data, and wrote the paper; Xi XY collected the medical imaging materials; Dong HJ designed the report and performed the preliminary revision of the article.

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Abstract

Myeloid sarcoma (MS) is a type of extramedullary solid haematological tumour. Myeloid sarcoma is classified into two types based on whether onset of the disease is complicated by haematologic diseases: extramedullary infiltration of leukaemia (leukaemic MS) and isolated myeloid sarcoma. The incidence of isolated myeloid sarcoma is low. In particular, isolated myeloid sarcoma involving the pancreas is extremely rare and prone to misdiagnosis. This case report describes the long and eventful diagnostic process of a case of myeloid sarcoma involving the pancreas and orbit. Due to a lack of typical clinical manifestations and imaging characteristics, the patient underwent several rounds of treatment without a confirmed diagnosis. Eventually, the final diagnosis was pathologically confirmed using several types of biopsies and immunohistochemical detection. To date, this type of disease has not been reported in the literature. This case report describes the detailed diagnostic process and discusses the strategies used for diagnosis, which will facilitate the diagnosis of such diseases in the future.

Key words: Immunohistochemistry; Isolated myeloid sarcoma; Granulocytic sarcoma; Pancreas; Orbit

Core tip: Although isolated myeloid sarcoma is difficult to diagnose, multi-site lesions provide indications for diagnosis. This case report describes isolated myeloid sarcoma occurring in both the pancreas and the orbit.

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INTRODUCTION

Myeloid sarcoma (MS) is also known as extramedullary myeloid tumour, granulocytic sarcoma, or chloroma. The 2001 World Health Organization classification of tumours of lymphatic and haematopoietic tissues unified these terms under MS[1]. MS is a type of malignant solid tumour that forms from the infiltration of the organs and tissues outside the bone marrow by immature myeloid cells[2]. MS mostly occurs in the lymph nodes, bones, periosteum, soft tissues, and skin[3] but rarely occurs in the gastrointestinal tract, orbits, and pancreas[4-6]. This case report describes the diagnosis of a case of isolated MS occurring simultaneously in the pancreas and orbit and aims to provide strategies for clinical diagnosis in such cases.

CASE REPORT

The patient was a 36-year-old male, who was admitted to the hospital due to persistent abdominal pain. This patient had no remarkable medical history. Starting in July 2015, the patient experienced repeated episodes of unexplained upper abdominal pain and experienced no other accompanying symptoms. After review of the patient's past medical records, multiple laboratory tests at other hospitals indicated elevated levels of pancreatic enzymes ranging from 213-341 IU/L (less than 3-fold of the upper limit of the normal range [35-140 IU/L]) combined with slight exudation around the pancreas, and gallstones. His condition improved when he was treated for pancreatitis. As pancreatitis caused by gallstones could not be excluded, cholecystectomy was performed. After surgery, his symptoms repeatedly relapsed. In October 2015, right eyelid oedema occurred intermittently, which was not treated. In April 2016, the right eye became increasingly swollen until protrusion of the eyeball occurred. Orbital computed tomography (CT) and magnetic resonance imaging (MRI) were performed at other hospitals and indicated space-occupying lesions, but no further treatment was given. In May 2016, the patient again experienced abdominal pain and was diagnosed at the Department of Gastroenterology at a hospital specializing in the standardization of residency training. Laboratory tests showed slightly elevated liver and pancreatic enzymes and normal routine blood and autoantibody test results. Levels of carbohydrate antigen 19-9, carcinoembryonic antigen (CEA), and other tumour markers were also normal. A contrast-enhanced abdominal CT scan indicated a blur gap between fat around the pancreatic tail and between the spleen and stomach. A circle-like cystic low-density shadow was observed in the gap between the stomach and pancreas without clear enhancement. The pancreatic duct and the bile ducts inside and outside the liver were also slightly dilated (Figure 1A). The possibility of pancreatitis was considered. However, the patient's abdominal pain relapsed repeatedly, and the evidence for "pancreatitis" was not sufficient. His medical history and related tests excluded the possibility of pancreatitis caused by drugs, metabolism, and autoimmunity. Therefore, endoscopic ultrasound was performed, the results of which indicated an uneven echo of the pancreatic tail and body. Echo-free fluid exudation was observed around the pancreatic tail, and a hypoechoic, solid space-occupying lesion was visible at the uncinate process with uneven echo. Scattered hyperechoic areas were also observed. A pancreatic head space-occupying lesion and degeneration due to pancreatitis were considered. Needle aspiration biopsy of the space-occupying lesion of the pancreatic uncinate process was performed using an endoscopic ultrasound. The pathologic analysis revealed local tissue necrosis, and the patient exhibited obstructive jaundice after the operation. Endoscopic retrograde cholangiopancreatography was performed to place a metallic stent, and dark-red tissue discharge was observed during the procedure. The tissue was sent for pathological examination, but no tumour cells were detected. The patient's symptoms were treated, and he was discharged after they improved.

In July 2016, the patient's abdominal pain recurred. Positron emission tomography CT (PET-CT) indicated low-density lesions resembling lumps in the pancreatic tail. The shadow had a ring shape and glucose metabolism was abnormally high. Therefore, the lesion was considered an inflammatory pseudotumour. The pancreatic head region exhibited shadowy patches, glucose metabolism was slightly changed, and another CT performed using the same machine showed a space-occupying lesion in the corresponding region as well as an abnormal density change. Thus, the lesion was considered a benign lesion (possibly an inflammatory lesion). Glucose metabolism was abnormally high in the capsule area that formed a cord outside the right orbit, and a benign lesion was diagnosed (possibly an inflammatory pseudotumour). The aetiology of the lesion in this patient remained unclear, and the possibility of a haematologic malignancy was considered. A bone marrow

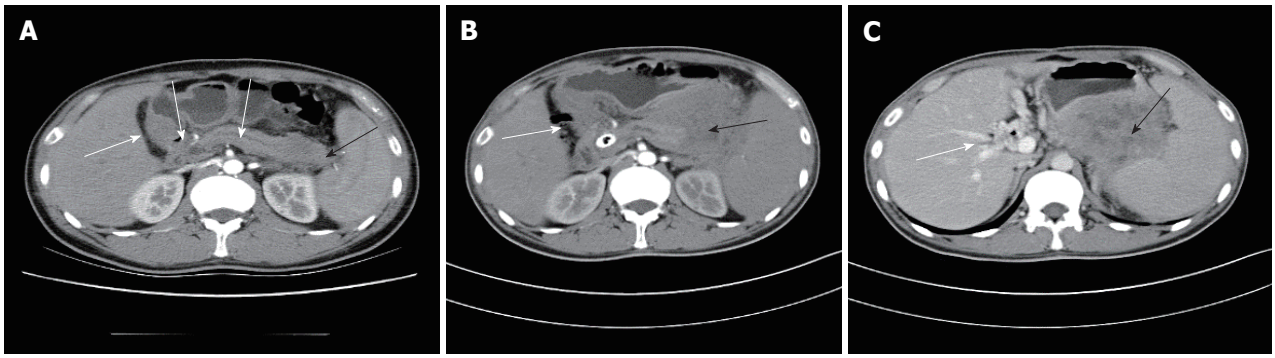

Figure 1 Abdominal computed tomography scan. A. Axial projection shows the pancreatic duct, intrahepatic, and extrahepatic bile duct dilation (white arrow heads) and low-density pancreatic mass (black arrow head); B. Axial arterial phase projection showing intrahepatic bile duct dilatation and pneumobilia (white arrow head) and pancreatic mass enlargement and enhancement (black arrow head); C. Axial portal phase projection showing portal cavernous transformation (white arrow head) and partial enhancement of the pancreatic mass and areas of low density in the center.

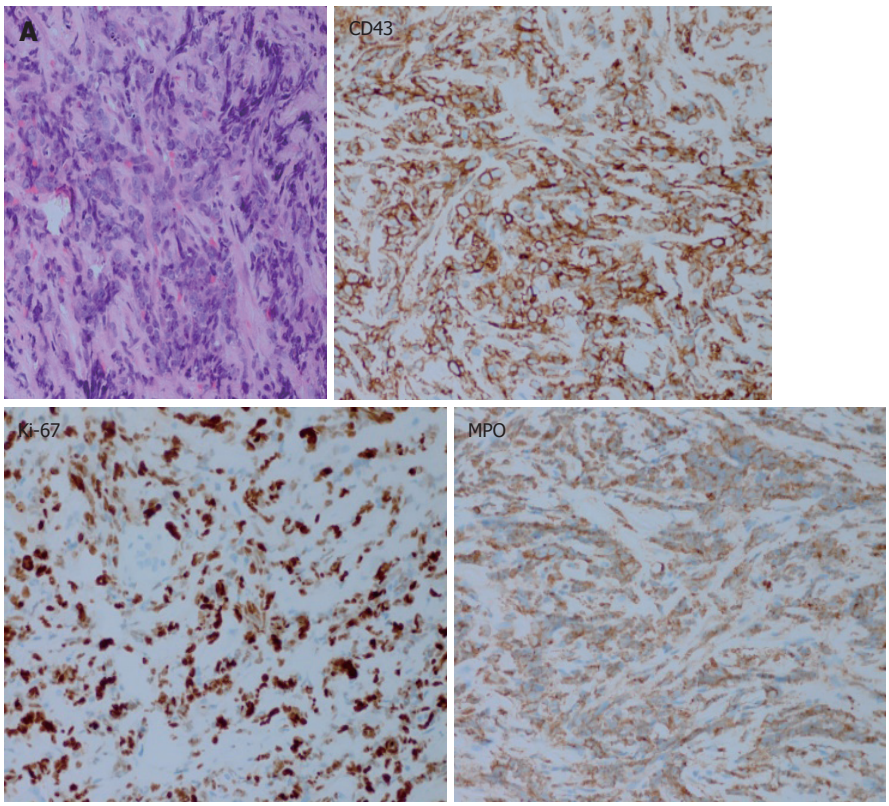

Figure 2 Haematoxylin and eosin stainin (magnification × 400) shows heterotypic cells arranged in a line that appear flaky and demonstrate infiltrative growth (A), the inserts show CD43, Ki-67, and MPO expression.

biopsy was performed, but obvious abnormalities were not detected. Therefore, percutaneous needle aspiration biopsy of the hypoechoic area of the pancreatic tail was conducted under ultrasonography guidance. The result showed heterotypic cells with a flaky appearance arranged in cord-like shape indicating infiltrate growth (Figure 2A), and the pathologic analysis indicated a malignant tumour. In addition, the morphology suggested a poorly differentiated adenocarcinoma. However, considering the long medical history of the patient and because tumour markers and imaging findings did not support the diagnosis of pancreatic cancer, immunohistochemistry was performed after a consultation with pathologists. The results showed that the lesion was negative for CEA(M), CEA(P), CK(AE1/AE3), CD56, CgA, Syn, Bcl-2, Bcl-6, CD10, CD20, CD3, and MUM1, and the lesion was positive for Ki-67 (+ 80%), P53 (approximately 50%), CD43, and MPO (Figure 2). These results supported the diagnosis of myeloid leukaemia with pancreatic involvement. Bone marrow puncture and biopsy were performed again, and the results were almost normal. No further treatment was administered.

In October 2016, the patient's right eye protruded significantly and was accompanied by severe pain; the

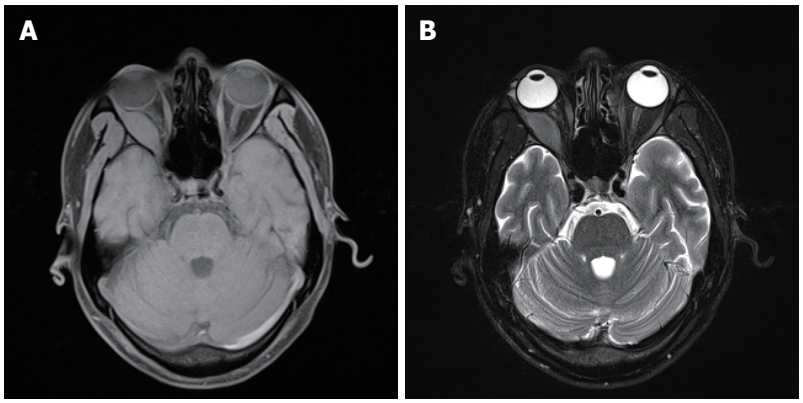

Figure 3 The results of T1-weighted (A) and T2-weighted (B) magnetic resonance imaging scans of the orbit show a uniform signal-intensity mass in the right lateral rectus area and right optic nerve compression with displacement.

eyelids were also unable to close. The patient underwent surgical treatment at another hospital, and the pathologic analysis suggested a malignant tumour. Although the combined immunodetection did not support an epithelial-derived tumour, myeloid leukaemia and osteosarcoma were still considered. After a consultation with pathologists at our hospital and the addition of a myeloperoxidase (MPO) (+) test, which was combined with other immunohistochemical results, myeloid sarcoma was considered. By then, the diagnosis of myeloid sarcoma was clear, and the patient was transferred to the Department of Haematology for treatment. On November 23, 2016, a CT examination revealed a mass shadow with a diameter of approximately 8.6 cm in the pancreatic tail. The density was not uniform, and a contrast-enhanced CT scanning showed significant enhancement. The demarcation between the lesion and the posterior wall of the stomach and the splenic hilum was not clear. The intrahepatic general bile duct exhibited dilation and pneumatosis, and spongy-like changes were observed in the portal vein (Figure 1B, C). An IDA chemotherapy regimen was initiated in December. After three cycles of chemotherapy, the disease progressed. Radiochemotherapy (the lesion in the pancreatic tail, 24Gy/12F) was then given. However, in April 2017, the patient experienced pain due to the distention of the right orbit. An orbital MRI scan (T1-weighted imaging (Figure 3A) and T2-weighted imaging (Figure 3B) showed a uniform-signal intensity mass in the right lateral rectus area and right optic nerve compression with displacement, suggesting recurrence. No abnormalities were observed on bone puncture. The patient was then treated with radiotherapy again, during which multiple masses developed throughout the body. Ultrasound results suggested the possibility of the infiltration. Bone puncture was performed again, and the result suggested that primary granulocytes accounted for 38.4% of the lesion. Consequently, the patient was switched to DAC (decitabine) + CAG chemotherapy regimen. By February 2018, bone puncture results indicated remission, but the extramedullary disease continued to progress.

DISCUSSION

MS often occurs simultaneously with acute myeloid leukaemia (AML) as the extramedullary manifestation of AML or during extramedullary relapse after remission of AML following treatment,that is extramedullary infiltration of leukaemia (leukaemic MS). However the isolated MS(non-leukaemic MS) is clinically rare and prone to misdiagnosis. Isolated MS refers to the occurrence of MS in the absence of any medical history of blood pathology at the onset of the disease. The lesion simply manifests as an extramedullary mass, which does not develop into AML within 30 d. This type of MS is very rare[7] and accounts for only 2%-14% of all AML cases[8]. Since isolated MS does not have a specific clinical manifestation, approximately 75%-86% of patients with this disease are misdiagnosed during the initial diagnosis[9]. The pancreas is rarely involved in cases of isolated MS, and the rate of misdiagnosis is therefore very high. The patient in this report visited several hospitals, and his diagnosis was confirmed after 15 mo.

The patient in this report visited various hospitals and was subjected to many laboratory tests due to persistent upper abdominal pain. He was treated for pancreatitis based on increased levels of pancreatic enzymes (less than 3-times the upper limit of the normal value), and his condition improved. Initially, abdominal CT also indicated the possibility of pancreatitis, but his medical history and related examinations had already excluded pancreatitis induced by drugs, alcohol, metabolism, and infection as well as biliary pancreatitis. Additionally, a pancreatic space-occupying lesion developed with progression of the disease. Therefore, further identification and diagnosis appeared to be particularly important. Was the pancreatic space-occupying lesion indicative of pancreatitis, pancreatic cancer, or some other disease? The patient also had an orbital space-occupying lesion; should the disease be explained with

"monism" or "dualism"? The patient's medical history, contrast-enhanced CT, PET-CT, and the tumour marker expression levels did not support the diagnosis of pancreatic cancer, and mass-forming pancreatitis was not excluded. If "monism" had been used to explain the disease, the diseases that simultaneously involve multiple systems, include autoimmune diseases, vasculitis-related diseases, multiple endocrine-related diseases, and rare tumour and blood system diseases, would not have be excluded. This case involved both the pancreas and the orbit. First, IgG4-related autoimmune diseases were considered. However, no IgG4 was found in the serum, puncture specimen IgG4 staining was negative, and other autoimmune diseases had already been ruled out. Pathology is the gold standard for diagnosis. Accordingly, in this case, endoscopic ultrasound-guided pancreatic biopsy and ultrasound-guided pancreatic biopsy were performed. The initial pathology result showed local necrotic tissue. Additional pathological findings suggested the possibility of poorly differentiated adenocarcinoma. Finally, after a consultation with pathologists, we further investigated endocrine-related and blood system disease-related immunohistochemistry and the disease was eventually diagnosed.

Early diagnosis and treatment can delay the progression of isolated MS to AML, which may result in a relatively good prognosis. However, as the clinical manifestations are primarily extramedullary mass-related symptoms that lack specific imaging and pathological features, diagnosing isolated MS is difficult. In these cases, immunohistochemistry is the key to the diagnosis of isolated MS. MPO, lysozyme, CD68, and other myeloid cell-related markers are the most sensitive and effective markers for MS[10]. Studies have found that MPO, CD68, CD20, and CD43 detection can confirm the diagnosis up to 96% of MS cases[11]. The key to the diagnosis of this patient was also immunohistochemistry.

Patients with isolated MS of the pancreas often visit the Department of Gastroenterology for abdominal symptoms, and they are often misdiagnosed with chronic pancreatitis or pancreatic tumours. As the first attending physician in this case, we must have a sense of differentiation for atypical cases and select a reasonable diagnostic method in a timely manner. In this case, the patient was diagnosed multiple times before the disease was confirmed, and his diagnosis was the result of the joint efforts of many disciplines, including gastroenterology, radiology, and pathology. In addition, although simultaneous occurrence of lesions in the pancreas and orbit is rare, detection of lesions at multiple locations has also provided guidance for the clinical consideration of related rare diseases. The aim of this paper is to provide ideas for clinical diagnosis. However, as this type of case is rare, studies of additional cases are needed to develop a deeper understanding of this disease.

ARTICLE HIGHLIGHTS

Case characteristics

A 36-year-old male with symptoms of recurrent abdominal pain and intermittent right eyelid oedema exhibiting space-occupying lesions in the pancreas and orbit.

Clinical diagnosis

Isolated myeloid sarcoma.

Differential diagnosis

Pancreatitis, pancreatic cancer, and lymphoma should be excluded.

Laboratory diagnosis

Normal tumour marker levels, slightly elevated liver and pancreatic enzymes, and normal routine blood and autoantibody test results.

Pathological diagnosis

A malignant tumour positive for Ki-67 (+ 80%), P53 (approximately 50%), CD43, and MPO was indicated.

Treatment

Chemotherapy combined with radiochemotherapy.

Related reports

A case of isolated myeloid sarcoma occurring simultaneously in the pancreas and orbit has never been reported.

Term explanation

Isolated myeloid sarcoma.

Experiences and lessons

This case contributes to deepening our understanding of the diagnosis of isolated myeloid sarcoma. We should combine various laboratory test results, especially pathologic and immunohistochemical results, to assist in diagnosis. Surgery is not recommended for these cases, and minimally invasive methods are preferred for pathological examinations because surgery may delay treatment and affect the prognosis of patients. In addition, we should have a sense of differentiation for atypical cases, and the monism explanation should be considered first for multi-site lesions.

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