Official Publication of Philippine Society of Gastroenterology,
Philippine Society of Digestive Endoscopy and
Hepatology Society of the Philippines.

Philippine Journal of Gastroenterology

The Effect of Hyperbaric Oxygen Therapy (HBOT) on Liver Function and Fibrosis Using a Rat Model of Carbon Tetrachloride (CCl4)-Induced Liver Injury: An experimental study

Association of Spontaneous Bacterial Peritonitis and Use of Proton Pump Inhibitors among Patients with Liver Cirrhosis: A systematic review and meta-analysis

The Role of L-Carnitine in the Improvement of Liver Tests and Glycemic Control among Patients with Non-Alcoholic Fatty Liver Disease: A meta-analysis

The Yield of Combined Multichannel Intraluminal Impedance and pH monitoring (MII-pH monitoring) among Suspected Refractory Gastroesophageal Reflux Disease: A St. Luke's Medical Center experience

Analysis of Predictive Factors for R0 Resection, Bleeding and Recurrence of Colorectal Adenomas after Endoscopic Mucosal Resection

PJG first
published 2005

Vol 10 No. 1 2021 Issue
p-ISSN 1908 4900

The *Philippine Journal of Gastroenterology (PJG)* is the official scientific publication of the Philippine Society of Gastroenterology, Philippine Society of Digestive Endoscopy, and Hepatology Society of the Philippines. They are the owners and publishers of this journal.

The *PJG* is a peer-reviewed, open access, bi-annual publication, which aims to provide a platform for the exchange of ideas and information in the fields of gastroenterology, endoscopy and hepatology for the improvement of practice and patient care. It publishes original research and review articles, meta-analyses and systematic reviews, case reports and series, clinical guidelines, editorials, video articles, image articles as well as correspondence. No processing fees are charged.

The *PJG* adheres to the recommendations of the International Committee of Medical Journal Editors *(ICMJE) for the conduct, reporting, editing, and publication of scholarly work in medical journals (https://www.icmje.org).

For submission of articles and manuscripts please contact secretariat at (02)89287014 / (02)89283768, or at philgastro60@gmail.com. Instructions to authors and relevant forms are available.

The Philippine Journal of Gastroenterology

Vision Statement

The *Philippine Journal of Gastroenterology* will continue to contribute to the improvement of heath care and training in the fields of gastroenterology, digestive endoscopy, and hepatology, by serving as a platform for publication of quality local and foreign research. It will have online presence by 2021 and be globally indexed by 2024.

Mission Statement

We strive to always upgrade the quality of research in the fields of gastroenterology, digestive endoscopy, and hepatology, and pursue the sustainability of the journal's publications from bi-annual from 2020-2022, to quarterly by year 2023, until its global indexing status and beyond.

Core Values

Scientific integrity, Editorial independence, Ethical publication, Timeliness

Table of Contents

Guidelines for Liver Transplantation During Covid-19 Pandemic in the Philippines: Joint Statement of the Philippine Association of Hepato-Pancreato-Biliary Surgeons (PAHPBS) and the Hepatology Society of the Philippines

Maria Vanessa H. De Villa, MD, PhD [1]; Maria Teresa T. Panlilio, MD[2]; Ian Homer Y. Cua, MD[2]; Jade D. Jamias, MD[2]; Anthony Q. Yap, MD[1]; Amornetta P. Jordan-Casupang, MD[1]; Wendell Z. Espinosa, MD[2], Marco M. Sumo, MD[1]; Noruel Gerard A. Salvador, MD[1]; Jennielyn C. Agcaoili-Conde, MD[2]; Aldwin D. Ong, MD, MBA[2]; Catherine SC Teh, MD[1], Robert N. De Guzman, MD[2]

[1]Philippine Association of Hepato-Pancreato-Biliary Surgeons
[2]Hepatology Society of the Philippines

The world is experiencing the worst health crisis in the 21st century thus far. After the Spanish flu pandemic of 1918, which claimed an estimated 50 million lives, this SARS-CoV-2 pandemic ravaging the world now may prove to be equally detrimental, if not worse. There have been tremendous consequences not only in the health sector but in all other arenas of life as well, with perhaps greater impact in developing countries like the Philippines. National statistics report that there have been more than 405,000 infected individuals from February until early November 2020[1]. Fortunately, the local mortality rate is low at 2%[1], close to the current worldwide death toll of 2.4% [2].

The country was first hit in the first quarter of 2020 and this necessitated a total lockdown of the National Capital Region (NCR) and nearby provinces. It then suffered a second wave sometime midyear which required continued community quarantine measures. The pandemic has shifted the focus of health care delivery to COVID-19 cases and has affected organ donation and organ transplantation as well. Transplant-related activity stopped [3,4] because priority had to be given to COVID-19 patients and resources (ICU beds, PPEs, blood supply, pharmaceuticals, manpower) had to be channelled accordingly. Deceased donation was halted because of the challenges with SARS-CoV-2 PCR tests that could produce results in a few hours, as required in an emergency situation, and the uncertainties related to processing and procurement of organs from a deceased donor during a pandemic. The Philippine Network for Organ Sharing (PhilNOS) has remained silent throughout this health crisis. Nonetheless, patients will continue to get sick and suffer end-stage liver disease, whether it be acute or chronic illness, and therefore transplant activity must go on. Now, about nine months into the pandemic, health care providers have gained a better understanding of the pathogen and the disease it causes and therefore, have a better handle on the prevention of spread and management of the infected.[5,6] With lessons learned, it is then about time to resume transplant activity,[7,8,9] keeping in mind always the safety of the patients, their families and health care workers in the quest to restore health in all those afflicted.

The Philippine Association of Hepato-pancreato-biliary Surgeons (PAHPBS) and the Hepatology Society of the Philippines (HSP), the lead local professional societies dedicated to liver care, have then decided to partner in drawing guiding principles in the practice of liver transplantation during the COVID-19 pandemic, which is far from over. The following recommendations are hereby put forth to guide the resumption of adult and pediatric liver transplant activity in the country in these crucial times:

1. Liver transplantation (LT) is a life-saving procedure and must, therefore, be given priority. Liver transplant activity should continue unabated provided that the necessary resources to carry out

a transplant can be ensured, i.e., ICU beds, appropriate PPEs, blood product availability, and trained personnel.

2. Only accredited/experienced programs may continue LT activity during COVID-19 times.
3. The indications for LT shall remain the same.
4. LT shall be performed only in SARS-CoV-2 NEGATIVE donor-recipient pairs.
5. SARS-CoV-2 PCR tests with rapid turnaround time must be available for expeditious processing of potential multi-organ deceased donors and preparation of the intended recipient.
6. Preoperative candidate and living donor evaluation may continue.
7. As per standard protocol, vaccination against pneumococcus and influenza is encouraged prior to LT.
8. All patient candidates and potential live donors must be evaluated for COVID-19.
    a. Initial assessment shall be based on travel history, exposure and symptoms. If they are not COVID suspects based on these, the evaluation process may be carried out.
    b. SARS-CoV-2 PCR testing must be performed right before LT. The patient and potential donor shall be admitted 1-2 days prior to the procedure and the nasopharyngeal swab done. They shall remain in the hospital while waiting for the results. If both the recipient and live donor test negative for SARS-CoV-2, the LT may proceed. If either of the tests is positive, the LT shall be deferred, and the re-testing shall be undertaken in 14-28 days.
    c. If, for whatever reason, the contemplated LT does not proceed, a negative swab test result shall be considered valid for seven days, for as long as the patient remains confined in the hospital or in strict isolation. If the operation is rescheduled beyond the seven-day validity, the provision in letter (b) shall apply.
    d. Should either the candidate or potential donor present with symptoms any time during the evaluation process prior to the LT, SARS-CoV-2 testing should be performed as deemed appropriate and the patient managed accordingly.
9. Routine SARS-CoV-2 testing of members of the LT team prior to participation in an operation is not required. However, those who get exposed or develop symptoms should have themselves tested prior to the operation. The PCR test result is valid for seven days.
10. Since LT will be performed only in patients who test negative for COVID, Level 3 PPE during the surgery is deemed sufficient. Donning Level 4 PPE is optional and shall be left to the discretion of the health care worker and/or the respective infection control committees of the institution.
11. Proper preparation of the operating suites should follow institutional infection control standards.
12. Candidates who develop complications during the evaluation process or while waiting for suitable donors must be treated accordingly following set guidelines and precautions for the specific procedures, e.g., endoscopy for variceal bleeding, paracentesis for massive ascites, dialysis for renal failure, liver support measures for liver failure. They must be admitted accordingly and COVID-19 testing performed as per institutional guidelines.
13. Standard precautionary measures of wearing masks, face shields, frequent hand washing and physical distancing must be practiced at all times, especially during face-to-face interactions between patients/donors and the health care workers.
14. Limit outpatient consults to only patients who need to be seen in person.
    a. Consider alternative platforms such as telemedicine or phone consults, where appropriate.
    b. Candidates or post-transplant patients requiring urgent attention (e.g., variceal bleeding, hepatic encephalopathy, signs of infection or acute rejection), shall be advised to seek immediate consult at the emergency room, where standard of care will be provided.
15. Standard immunosuppression protocols shall be followed since only SARS-CoV-2-negative patients shall undergo LT. Immunosuppression shall be adjusted accordingly in post-transplant patients who contract COVID-19.

Joint Liver Transplant Committee

Philippine Association of Hepato-Pancreato-Biliary Surgeons

Maria Vanessa H. De Villa, *Chair*
Anthony Q. Yap
Amornetta P. Jordan-Casupang
Marco M. Sumo
Noruel Gerard A. Salvador
Aldwin D. Ong
Catherine SC Teh, *President 2020-2021*

Hepatology Society of the Philippines

Mara Teresa T. Panlilio, *Co-Chair*
Ian Homer Y. Cua
Jade D. Jamias
Wendell Z. Espinosa
Jennielyn C. Agcaoili-Conde
Roberto N. De Guzman, Jr., *President 2020-2021*

References

1. Department of Health COVID-19 Case Tracker. https://www.doh.gov.ph/2019-nCoV. Accessed 14 November 2020.
2. World Health Organization (WHO) COVID-19 Dashboard. https://covid19.who.int. Accessed 14 November 2020.
3. Philippine Society of Nephrology and Philippine Society of Transplant Surgeons. Unified Interim Guidelines of The Philippine Society of Nephrology and The Philippine Society for Transplant Surgeons for Kidney Transplantation during the Covid-19 Pandemic. Published 22 June 2020. https://psn.org.ph/wp-content/uploads/2020/07/UNIFIED-INTERIM-GUIDELINES-OF-THE-PHILIPPINE-SOCIETY-OF-NEPHROLOGY-AND-THE-PHILIPPINE-SOCIETY-FOR-TRANSPLANT-SURGEONS-FOR-KIDNEY-TRANSPLANTATION-DURING-THE-COVID-19-PANDEMIC.pdf. Accessed 1 December 2020.
4. Philippine Association of Hepatopancreaticobiliary Surgeons. A Guide to Clinicians: Liver Involvement in SARS-CoV2 Infection/COVID-19. https://pahpbs.org/wp-content/uploads/2020/11/CoVID-Impact-on-Liver-Page.pdf. Accessed 14 November 2020.
5. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed 25 November 2020.
6. Philippine Society for Microbiology and Infectious Diseases, Philippine College of Chest Physicians, Philippine College of Physicians, Philippine Rheumatology Association, and Philippine College of Hematology and Transfusion Medicine. Interim Guidance on the Clinical Management of Adult Patients with Suspected or Confirmed COVID-19 Infection. Version 3.1, Published 20 July 2020. https://www.psmid.org/wp-content/uploads/2020/07/Final-PCP-PSMID-PCCP-COVID-19-Guidelines-20July2020b.pdf. Accessed 14 November 2020.
7. American Association for the Study of the Liver. Clinical Best Practice Advice for Hepatology and Liver Transplant Providers during the COVID-19 Pandemic: AASLD Expert Panel Consensus Statement. Published 9 November 2020. https://www.aasld.org/sites/default/files/2020-11/AASLD-COVID19-ExpertPanelConsensusStatement-November092020.pdf. Accessed 14 November 2020.
8. Di Maira, Tommaso and Berenguer, Marina. COVID-19 and Liver Transplantation. Nature Reviews Gastroenterology and Hepatology. 2020 Sept; 526-528.
9. Webb G, Marjot T, Cook JA et al. Outcomes following SARS-CoV-2 infection in liver transplant recipients: an international registry study. Lancet Gastroenterol Hepatol. 2020 November; 5(11):1008-1016.

The Effect of Hyperbaric Oxygen Therapy (HBOT) on Liver Function and Fibrosis Using a Rat Model of Carbon Tetrachloride (CCl_4)-Induced Liver Injury: An Experimental Study

Navarro MJH
Bondoc EM
Cervantes JG
Cua IHY

Institute of Digestive and Liver Diseases
St. Luke's Medical Center-Quezon City, Philippines

Correspondence:
Dr. Marc Julius H. Navarro
marcnavarromd@gmail.com

Accepted for publication:
December 2020

Abstract

Significance: Hyperbaric Oxygen Therapy (HBOT) is an intervention in which an individual breathes in nearly 100% oxygen inside a hyperbaric chamber. Numerous studies support HBOT as an efficient therapeutic option to control progress of diseases due to its multi-modal properties. Currently, there is paucity of data with regard to the effect of HBOT on liver diseases. *Objective:* The objective of this study is to investigate the effect of HBOT on liver function and fibrosis using a rat model of carbon tetrachloride (CCl_4)-induced liver injury. *Methodology:* Fifty-one adult Sprague Dawley rats with CCl_4-induced liver injury were randomized into three groups: (1) Pilot (sacrificed immediately after liver injury induction), (2) Control (exposed to room air), and (3) Experimental (exposed to 12 consecutive 120-minute daily sessions of HBOT 2.8 ATA). Outcome measures are serologic parameters of liver function and histopathologic evaluation of liver fibrosis. *Results:* There is significant difference between control and hyperbaric oxygen-treated group in improving AST (*p*-value <0.001) and ALT (*p*-value <0.001) among rats with CCl_4-induced liver injury. On histopathologic evaluation, rats exposed to HBOT revealed very strong evidence of improving degree of hepatic fibrosis (*p*-value <0.001). Majority of rats (94%) exposed to HBOT revealed mild hepatic fibrosis. Rats in the control group showed 76% having moderate fibrosis and 24% having severe fibrosis. *Conclusion:* HBOT exhibited very strong evidence in improving ALT, AST and degree of hepatic fibrosis among adult Sprague Dawley rats with CCl_4-induced liver injury.

Keywords: experimental study, liver injury, liver fibrosis, HBOT

Introduction

Hyperbaric oxygen therapy (HBOT) is defined by the Undersea and Hyperbaric Medicine Society as an intervention in which an individual breathes in close to 100% oxygen intermittently while inside a hyperbaric chamber that is pressurized to greater than sea level pressure (one atmosphere absolute [ATA]).[1] Since its inception in 1662 and practical application in 1930, application of hyperbaric oxygen therapy has continued to elicit controversy.[2] During HBOT, the increased concentration and the partial pressure of oxygen provide increased oxygenation of the whole body.[3]

In recent decades, numerous studies supported HBOT as an efficient therapeutic option to control progress of various diseases due to its anti-inflammatory, anti-oxidant, anti-aging, and anti-bacterial properties, as well as its angiogenesis and regeneration effects. It is highly used in hypoxia-related injuries. HBOT has also been clinically established as a widely used therapy for patients with carbon monoxide poisoning, decompression sickness, arterial gas embolism and problematic wounds. HBOT is also an important adjunctive therapy to treat diseases accompanied by impaired oxygen delivery.

In the liver, HBOT has been studied in hepatic artery thrombosis, acute liver injury, non-alcoholic steatohepatitis, and liver-related cancer. The beneficial effects of HBOT in the liver are mainly attributed to its anti-oxidation, anti-inflammation, regeneration and heme oxygenase-1 (HO-1) properties, which seem to be closely involved in HBOT-mediated protection.[1]

Liver injury can be induced by diverse etiologies, which include hepatotropic viruses, chemicals, alcohol and drug abuse, autoimmune disorders, cholestasis, and metabolic diseases. Liver injury induced by carbon tetrachloride (CCl_4) causes oxidative stress via lipid peroxidation. It is metabolically activated by cytochrome p450 2E1, which produces trichloromethyl radicals. CCl_4 stimulates liver injury through hepatocellular DNA damage, inflammation, apoptosis and fibrosis. Further liver damage occurs from exposure to reactive oxygen radicals released from activated Kupffer cells.[1,3] Chronic insult to the liver through this mechanism can cause chronic liver disease.

Chronic liver disease (CLD) refers to a long-term pathological process of continuous destruction of liver parenchyma and its gradual substitution with fibrous tissue. It is a major cause of morbidity and mortality in many countries.[4,5] Liver fibrosis is a common result of the inflammation-damage-repair response following different types of chronic insult to the liver. In patients who develop liver fibrosis, the majority ultimately develop liver cirrhosis, decompensated liver disease, and hepatocellular carcinoma (HCC). HCC is a dominant complication of CLD and cirrhosis, with the third highest death rate among malignancies in the world.[5] Information on the stage of hepatic fibrosis is essential for making a prognosis and deciding on anti-fibrosis treatment.[4]

Currently, there is paucity of data with regard to the beneficial effects of HBOT on liver diseases. The main objective of this experimental study is to investigate the effect of HBOT on liver function and fibrosis, using a rat model of CCl_4-induced liver injury. Specifically, the study aims to (1) compare the serological parameters of liver function between experimental group rats (with CCl_4-induced liver injury exposed to HBOT) against controls (exposed to room air), particularly: alanine transaminase (ALT), alanine aspartate (AST), total bilirubin, conjugated bilirubin, unconjugated bilirubin, alkaline phosphatase, total protein, albumin, globulin, platelets, and prothrombin time; and (2) using the IASL (International Association for Study of the Liver) scoring system, compare the histologic stage for hepatic fibrosis of rat livers with CCl_4-induced injury exposed to HBOT (experimental group) as against those of rats with CCl_4-induced liver injury exposed to room air only (controls).

Sample Size Estimation

Sample size was computed using the formula $n=1+2C(s/d)^2$ based on the parameter assumptions of significance level at 0.05 with a power of 90%, and a constant C of 10.51 on a two-tailed alternative hypothesis. Sample size was calculated at 16.41 animals in each group. A total of 51 rats were used in the study, equally divided into three groups: 17 rats for the pilot study group (rats that were sacrificed immediately after carbon tetrachloride liver injury induction), 17 rats for the control group (rats with desired degree of hepatic injury and exposed to room air only), and 17 rats for the experimental group (rats with desired degree of hepatic injury and exposed to HBOT).

Methodology

Animal Maintenance and Regulatory Compliance

Rats were obtained from the Philippine Department of Science and Technology (DOST) in whose animal facility these animals were grown. The hospital Institutional Animal Care and Use Committee and the Bureau of Animal Industry approved the study in accordance with RA 8485 (Animal Welfare Act of the Philippines) and the institution's guide for use of laboratory animals.

During the course of the study the rats were maintained under St. Luke's Medical Center Research and Biotechnology (SLMC-RBD) animal testing quarantine protocol. They were housed in a separate area under standard conditions dictated by SLMC-RBD protocol, including cage cleaning method, humidity, ventilation, room temperature regulation at 22-24^{0}C and a 12-hour light/dark cycle.

All animals were fed with standard diet and water, but were fasted for eight hours prior to blood collection, hepatectomy and animal euthanasia.

Induction of Hepatic Injury

CCl_4-induced injury to the liver causes lipid peroxidation by trichloromethyl radicals leading to hepatocellular membrane damage. Liver injury was

produced according to the Ozdogan Protocol. CCl_4 was administered by the primary investigator together with the institution's resident veterinarian. Ten percent CCl_4 was dissolved in olive oil and given by intraperitoneal injection three times a week, according to the following schedule: 0.3 ml/kg in the first week, 0.7 ml/kg in the second week, and 1.0 ml/kg for the next two weeks.

Pilot Study Model to Establish Desired CCl_4-induced Liver Injury

To represent baseline liver function and establish histopathologic liver injury status prior to the start of experiment, rat model with CCl_4-induced liver injury was used. Seventeen adult rats weighing 250 to 300 grams comprised the pilot study group. After four weeks of hepatic injury induction, these rats underwent blood collection and hepatectomy followed by animal euthanasia. Blood and liver specimens were submitted for serologic and histologic evaluation.

Process flow for the study is shown in **Figure 1.**

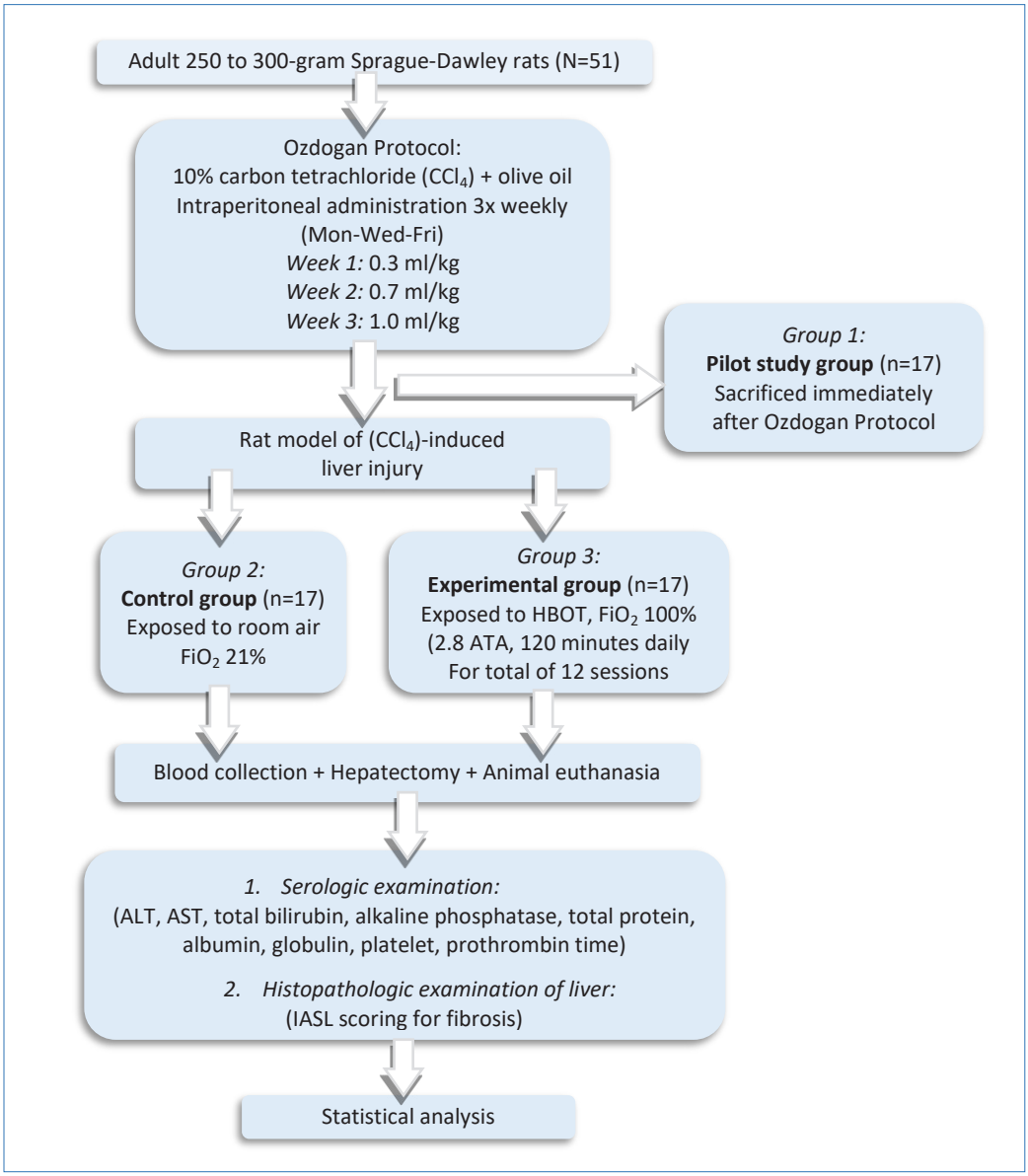


Figure 1. Process flow of study and distribution of subjects into (1) pilot study, (2) control group, and (3) experimental group

Description of Experiment Procedure

After four weeks of CCl_4 administration to induce the desired degree of hepatic injury, rats were assigned by simple randomization technique into two groups: 17 rats in the control group were exposed to room air; and 17 rats in the experimental group were exposed to HBOT.

Experimental and control rats were brought down from the Animal Facility to the St. Luke's Medical Center-Wound Care and Hyperbaric Oxygen Therapy Unit, where the HBOT monoplace chamber (*Perry Sigma 34*) (**Figure 2**) has been prepared and set up by the HBOT nurse. For the experimental group, treatment regimen with HBO was initiated (2.8 ATA for 120 minutes), done daily for a total of 12 sessions. Rats in the control group received no intervention.

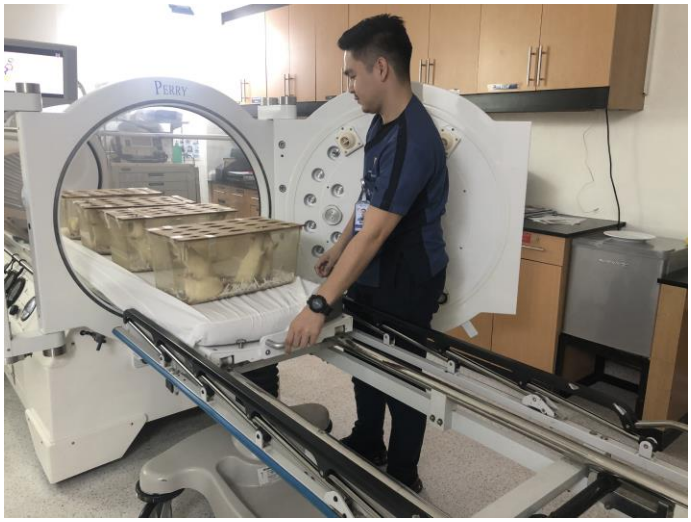


Figure 2. Experimental rats undergoing HBOT

After the 12th session, animals were ready to undergo blood extraction and hepatectomy followed by animal euthanasia. They were restrained in supine position and deeply anesthetized with tiletamine hydrochloride and zolazepam hydrochloride injection (Zoletil®) at a dose of 70 mg/kg, allowing them to breathe spontaneously during the procedure. Blood samples were obtained via intracardiac blood collection technique and sent to the laboratory for serologic evaluation of liver function. Hepatectomy was likewise performed by excision of the entire liver. Specimens were fixed in 10% formaldehyde and submitted for histopathological evaluation of liver fibrosis.

Hyperbaric Oxygen Therapy (HBOT)

The regimen dose used in this study (2.8 ATA, 120 minutes daily for total of 12 sessions), as patterned from previous HBOT studies in rats, is proven safe. This dose prevents animal mortality secondary to hyperbaric oxygen toxicity.[1,2,3] Previously reported hyperbaric oxygen toxicity in some earlier studies was due to limited clinical experience with hyperbaric oxygen application.[1] Toxicity was primarily due to the initiated free radical chain reaction by oxygen, which was then aggravated spontaneously with consequent lipid peroxidation, eventually leading to death. The condition leading to hyperbaric oxygen toxicity was observed with giving more than 3 ATA. In clinical application, however, HBOT is always controlled under 3 ATA.[1] Oxygen pressure is raised up to 10 to 15 times above its normal level when the patient breathes in 100% oxygen at 2.8 ATA.[3]

Animal Euthanasia

The pilot study group rats were immediately euthanized after the fourth week of induction of liver injury following Ozdogan protocol. The control group and experimental group rats were euthanized on the seventh week blood extraction and hepatectomy. The principal author together with the resident veterinarian

of St. Luke's Medical Center administered the carbon tetrachloride to induce liver injury, blood extraction, hepatectomy and animal euthanasia.

Blinding

A blinded medical technologist ran the blood samples for statistical analysis. A blinded veterinary pathologist performed the histopathologic evaluation of liver fibrosis using IASL Scoring. The biostatistician who analyzed the data was also blinded.

Description of Outcome Measures

Primary Outcome Measures:

1. Serological parameters of liver function

Blood samples were obtained using intracardiac blood collection technique before rats were euthanized. Serological parameters of liver function were the following: serum ALT (in *U/L*), AST (in *U/L*), total bilirubin (in *mg/dL*), conjugated bilirubin (in *mg/dL*), unconjugated bilirubin (in *mg/dL*), alkaline phosphatase (in *g/dL*), total protein (in *g/dL*), albumin (in *g/dL*), globulin (in *g/dL*), platelets (in 10^9/*L*), and prothrombin time (in *INR*). Test were done by a blinded medical technologist using the following commercially available machines of Diagnostic Veterinary Laboratories (DVL) as accredited by the Republic of the Philippines Department of Science and Technology:

- Hematology: *Mindray Vet Hematology Analyzer*, Mindray, Shenzhen, China;
- Clinical chemistry: *Fully Automated Biochemistry Analyzer*, E-lab Biological Science and Technology, Nanjing City, China;
- Coagulation factors: *Healvet Veterinary Coagulation Analyzer*, Guangzhou Wondfro Biotechnology, Guangzhou, China.

2. Histopathological evaluation of liver fibrosis

Rat livers were extracted and sent for histopathology using basic hematoxylin and eosin (H&E) staining. A blinded veterinary pathologist compared the histopathological features of the samples using the International Association for Study of the Liver (IASL) scoring system for histopathological stage of fibrosis; *Grade 0:* No fibrosis; *Grade 1:* Mild fibrosis (periportal fibrotic expansion); *Grade 2:* Moderate fibrosis (periportal septae, more than one septum); *Grade 3:* Severe fibrosis (portal-central septae); and *Grade 4:* Cirrhosis. Standard pictographs of the micro-sections were obtained and analyzed using the same image editing software (Adobe Photoshop 7.0; Adobe Systems, Inc.).

Data Analysis

Frequency data were reported as counts and percentages while continuous data were reported using means and standard deviations. Statistical analysis of frequency data was conducted using Chi-square test. All continuous data were first tested for normality, then *t*-test for two independent groups assuming equal variance was used. All computations were done using Microsoft Excel data calculator.

Ethical Considerations

The hospital Institutional Animal Care and Use Committee (IACUC) and Bureau of Animal Industry approved the study in accordance with the RA 8485 (The Animal Welfare Act of the Philippines) and the institution's guide for use of laboratory animals. All anesthetic agents were administered according to the approved rodent anesthesia and analgesia formulary. Animal euthanasia was conducted humanely, using deep anesthesia.

Biosafety

The Biosafety Review Committee (BRC) of St. Luke's Medical Center approved the study in accordance with universal recommendations when handling hazardous substances. All personnel who handled CCl_4 during aliquot preparation, handling and administration to mice underwent biosafety/biosecurity training and certification (**Table 1**).

Precautions and Biosecurity

During handling and preparation of the CCl_4 aliquot, wearing of proper personal protective equipment was necessary, including the use of mask, gown, gloves, and goggles/eye shield. Material safety data sheets (MSDS) were available and accessible.

Facility Management

The study was conducted at St. Luke's Medical Center Molecular Diagnostics Laboratory and Animal Laboratory. Biosafety cabinet was not needed, as the preparation of aliquot was handled using a fume hood. In case of spillage of carbon tetrachloride, personnel

protection (protective clothing, safety goggles, rubber gloves and respiratory protective device) was safeguarded. Small quantities CCl_4 were disposed of by evaporation in a fume cupboard or in a safe, open area. Hand washing facilities and eye wash stations were available within the work area. Access to the laboratory was limited only to persons advised of the nature of the CCl_4 in this research.

Transport of Hazardous Material

Handling and preparation of the aliquot of carbon tetrachloride were done using a fume hood located at the St. Luke's Medical Center Molecular Diagnostics Laboratory. Once prepared, the aliquot of CCl_4 was placed in labelled, airtight container in a well-ventilated place at a temperature below 30°C and protected from light. This was transported to the St. Luke's Medical Center Animal Facility/Laboratory using triple packaging system, consisting of three layers: the primary receptacle, the secondary packaging and the outer packaging.

Disposal of Animal Carcasses

Carcasses of rats used in the study were autoclaved prior to disposal. They were then disposed of using sealable yellow plastic bags (infectious waste) even if these were not treated with any infectious agents.

During Hyperbaric Oxygen Therapy Session

At the HBOT Unit, the animals were placed in clean cages with plastic liners to catch fecal material and urine. In the event of a spill of infectious or potentially infectious material (rat urine/feces), the following spill clean-up procedure was used (WHO recommendation):

1. Use of gloves and protective clothing;
2. Spill covered with cloth or paper towels to contain it;
3. Disinfectant poured over paper towels and immediate surrounding area.
4. Disinfectant applied concentrically from the outer margin of the spill area working toward the center;
5. After appropriate amount of time (e.g., 30 min), materials cleared away. Broken glass or sharps collected using dustpan or stiff cardboard and deposited into a puncture-resistant container;
6. Cleaning and disinfection of the area of spillage (if necessary, steps 2 to 5 repeated);
7. Contaminated materials disposed of into a leak-proof, puncture-resistant waste disposal container;
8. After disinfection, competent authority informed.

Table 1. Project hazard carbon tetrachloride (CCl_4) management

Handling, Storage and Disposal	
Handling / Storage	Stored in labelled, air-tight containers in a well-ventilated place protected from light, at a room temperature below 30^0C, stored separately from chemically active materials.
Disposal	Small quantities of CCl_4 disposed of by evaporation in a fume cupboard or in a safe open area.
Preparation and Handling of CCl_4 Aliquot	
Location of CCl_4	St. Luke's Medical Center, Molecular Diagnostics Laboratory
Biosafety Level	Level 1
Containment Device	Fume hood
During Administration of CCl_4 Aliquot to Rats	
Location of CCl_4	St. Luke's Medical Center, Animal Laboratory
Biosafety Level	Level 1
Containment Device	Not needed

Results

This experimental study was conducted to investigate the effect of hyperbaric oxygen therapy (HBOT) on liver function and fibrosis using a rat model of carbon tetrachloride (CCl_4)-induced liver injury.

It is of noted that 17 rats under the pilot study group revealed significant hepatic damage, with mean ALT that was 11-12 times elevated than the upper limit of normal and mean AST that was 4-5 times elevated than the upper limit of normal. All other liver parameters were noted to be within acceptable limits (**Table 2**).

Table 2. Serologic parameters of liver function after CCl_4 administration

Liver Parameters	Normal Limit *(range)*	Pilot Study *(mean ± SD)*
ALT, *U/L*	17.5 – 30.2	388.67 ± 30.50
AST, *U/L*	45.7 -80.8	413.71 ± 35.99
Total bilirubin, *mg/dL*	0.20 – 0.55	0.32 ± 0.08
Alkaline phosphatase, *g/dL*	56.8 - 128	110.18 ± 16.34
Total protein, *g/dL*	5.1 – 6.5	6.07 ± 0.18
Albumin, *g/dL*	2.6 – 3.5	3.32 ± 0.08
Globulin, *g/dL*	2.5 – 3.0	2.75 ± 0.15
Platelet, 10^9*/dL*	923 -1580	1169.65 ± 168.37
Prothrombin time, *INR*	0.8 – 1.2	0.85 ± 0.05

Histopathologic evaluation to establish the degree of induced liver injury was also done, which revealed that the greater majority, around 88% of rats' liver, developed severe liver necrosis and only a little more than 10% had moderate liver necrosis (**Figure 3**).

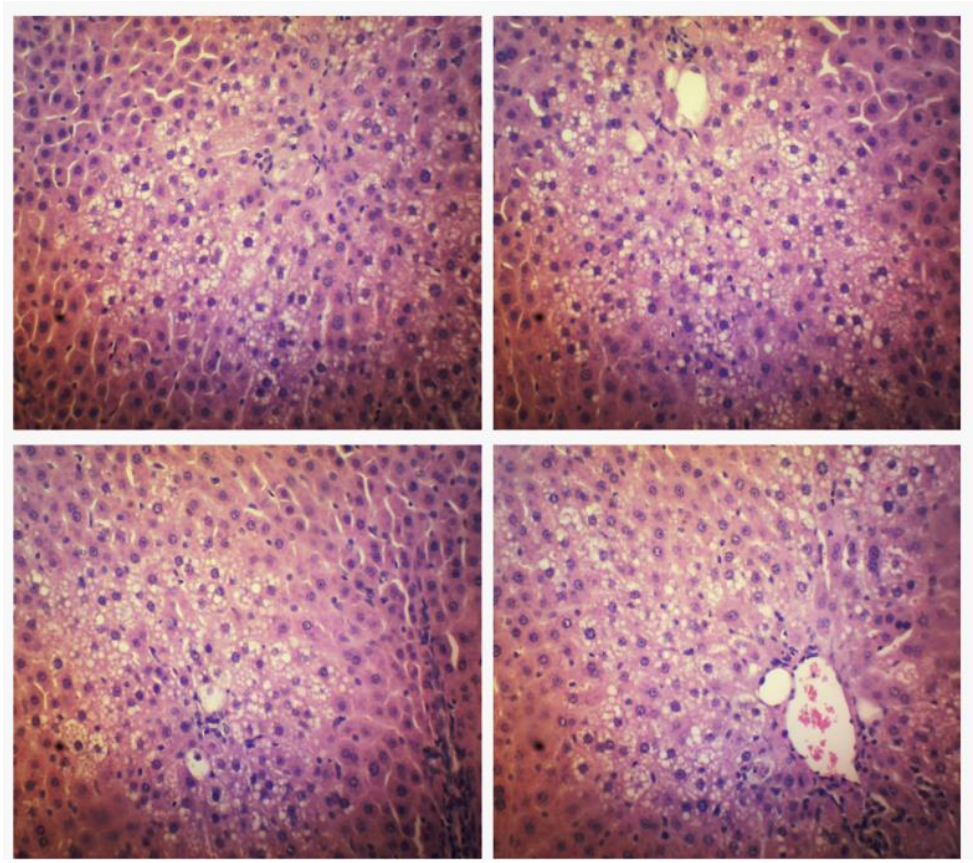


Figure 3. Histopathologic evaluation (H&E) of the liver after CCl_4 administration showing moderate (two out of 17 rats, 11.76%) and severe (15 out of 17 rats, 88.24%) liver necrosis.

This study showed that there is a significant difference between control and HBOT group in improving AST and ALT among rats with CCl_4-induced liver injury. Rats exposed to HBOT revealed very strong evidence of improved ALT and AST levels compared to controls. The mean ALT level of rats in the experimental group revealed 0-1 time more than the upper limit of normal, compared to controls which was 1-2 times

more than the upper limit of normal. On the other hand, the mean AST level of experimental group rats revealed 1-2 times elevated than the upper limit of normal compared to the controls which was 2-3 times elevated than the upper limit of normal. All other liver parameters revealed no significant difference between rats in the experimental and control groups (**Table 3**).

Table 3. Serologic parameters of liver function between experimental and control groups after CCl_4 administration

Liver Parameters	Normal Limit *range*	Experimental *mean ± SD*	Control *mean ± SD*	*p* value
ALT, *U/L*	17.5 – 30.2	55.28 ± 6.89	69.18 ± 7.90	**<.001**
AST, *U/L*	45.7 -80.8	195.53 ± 30.94	248.53 ± 28.31	**<.001**
Total bilirubin, *mg/dL*	0.20 – 0.55	0.33 ± 0.07	0.31 ± 0.12	0.293
Alkaline phosphatase, *g/dL*	56.8 - 128	81.29 ± 15.33	82.18 ± 10.57	0.435
Total protein, *g/dL*	5.1 – 6.5	6.10 ± 0.32	6.01 ± 0.27	0.176
Albumin, *g/dL*	2.6 – 3.5	3.21 ± 0.12	3.16 ± 0.11	0.162
Globulin, *g/dL*	2.5 – 3.0	2.89 ± 0.22	2.84 ± 0.22	0.235
Platelet, 10^9/*dL*	923 -1580	1313.00 ± 158.79	1288.06 ± 243.70	0.336
Prothrombin time, *INR*	0.8 – 1.2	0.81 ± 0.02	0.80 ± 0.00	0.166

Histopathologic evaluation to compare the degree of induced liver injury was also done. Rats exposed to HBOT revealed very strong evidence of improved liver fibrosis compared to controls. It can be noted that majority (94%) of rats exposed to HBOT revealed mild hepatic fibrosis. In contrst, 76% of rats in the control group developed moderate fibrosis, and 24% sustained severe fibrosis (**Table 4**).

Table 4. Histopathologic description of experimental and control groups

Fibrosis	Experimental *n (%)*	Control *n (%)*	*p* value
Mild	16 (94.12)	0 (0,00)	**<0.001**
Moderate	1 (5.88)	13 (76.47)	
Severe	0 (0.00)	4 (23.53)	

Discussion

Rats with hepatic injury induced by CCl_4 and exposed to HBOT revealed a very strong evidence of improved ALT, AST and degree of hepatic fibrosis compared to controls (*p*-value <0.001).

The above findings were compatible with the reported mechanism of CCl_4-induced liver injury, causing oxidative stress via lipid peroxidation by trichloromethyl radicals, which then leads to hepatocellular DNA damage, inflammation, apoptosis, and activation of hepatic stellate cells (HSCs) triggering fibrogenesis. This was comparable to the reported general mechanism scheme of oxidative stress induced by various factors on liver disease such as alcohol, drugs, viruses, environmental toxins, obesity and insulin resistance[1,3] (**Figure 4**).

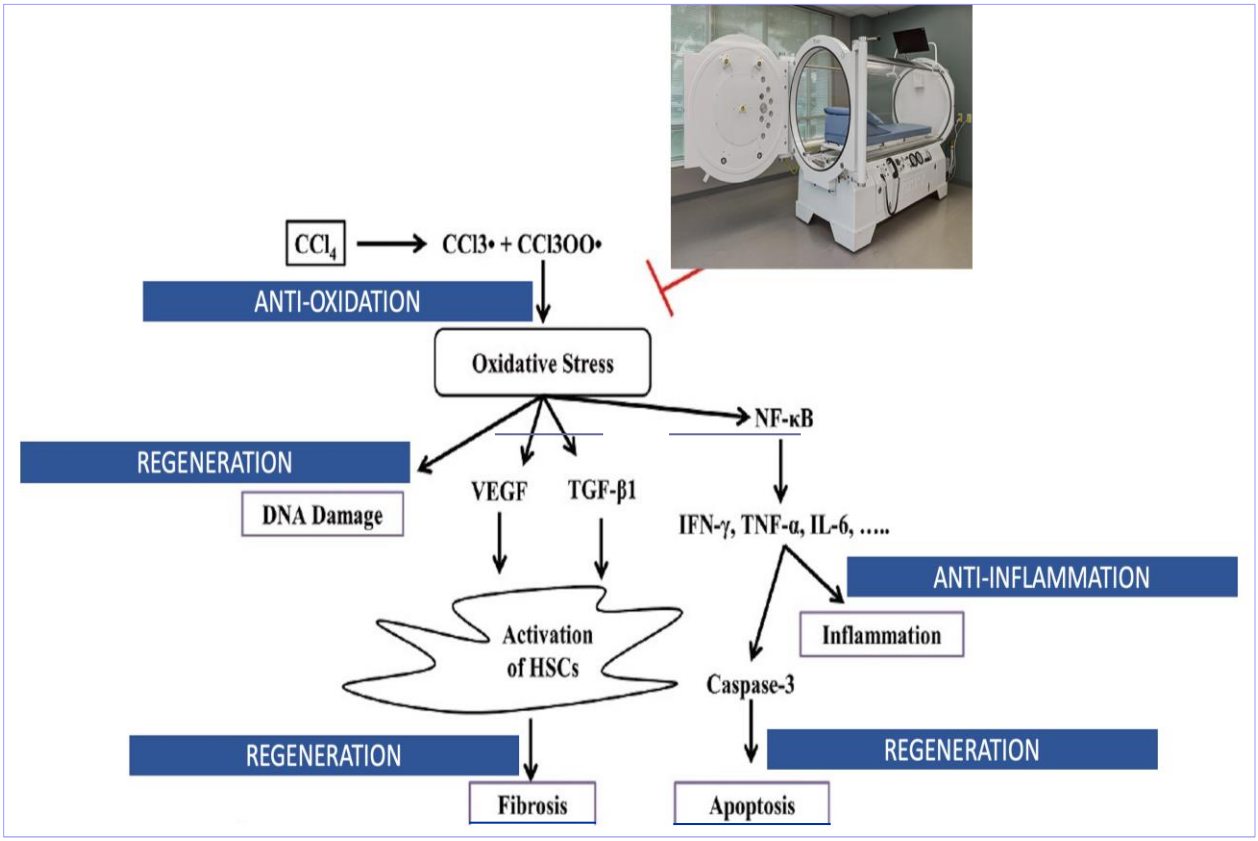


Figure 4. Mechanism of liver injury induced by CCl_4 and reported beneficial properties of HBOT (blue boxes)

Conclusion

HBOT exhibited very strong beneficial evidence in improving ALT, AST and degree of hepatic fibrosis among rats with CCl4-induced liver injury.

Authors' Contributions

MJH Navarro and EM Bondoc conceived and planned the experiment. MJH Navarro prepared the research proposal, methodology and performed needed calculations. JG Cervantes and IHY Cua verified analytical methods. MJH Navarro and EM Bondoc carried out the experiment. JG Cervantes and IHY Cua contributed to the interpretation of the results. MJH Navarro took the lead in writing the manuscript with support from EM Bondoc, JG Cervantes, and IHY Cua. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgements

This study was supported and funded by the Institute of Digestive and Liver Diseases (IDLD) and Research and Biotechnology (RBD), St. Luke's Medical Center-Quezon City, Philippines. The authors thank Dr. Hercules Baldos, Dr. Martin Samson, Dr. Mark Pierre Dimamay, Dr. Armin Masbang, Dr. Sherrie Isabel De Ocampo, and Dr. Sarah Bellido for their technical assistance.

Conflict of Interest

All JPG peer reviews are blinded. Dr. EM Bondoc, as co-author and at the same time JPG's editor-in-chief, inhibited himself from the review process and acceptance of this paper.

References

1. Sun Y, Wen Y, Shen C, Zhu Y, You W, Meng Y, et al. Hyperbaric oxygen therapy in liver diseases. International Journal of Medical Sciences. 2018; 782-287.
2. Muralidharan V and Christophi C. Hyperbaric oxygen therapy and liver transplantation. HBP. 2007; 174-182.
3. Ozdogan M, Ersoy E, Dundar K, Albayrak L, Devay S, Gundogdu H. Beneficial effect of hyperbaric oxygen on liver regeneration in cirrhosis. Journal of Surgical Research. 2005; 260-264.
4. Xie C, Ma B, Wang N, Wan L. Comparison of serological assessments in the diagnosis of liver fibrosis in bile duct ligation mice. Experimental Biology and Medicine. 2017; 1398-1404.
5. Kurokawa T and Ohkohchi N. Platelets in liver disease, cancer and regeneration. World Journal of Gastroenterology, Volume 23 Issue 18. 2017; 3228-3239.
6. Feldman M, Friedman L, Brandt L. Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 10th Edition. Elsevier Saunders Inc., Chapter 74, pages 1254-126.

Association of Spontaneous Bacterial Peritonitis and Use of Proton Pump Inhibitors among Patients with Liver Cirrhosis: A Systematic Review and Meta-Analysis

Sy-Janairo MLL[a]
Janairo JIB[b]
Mariano PMB[c]
Gopez-Cervantes JL[a]
Cua IHY[a]

[a]*Institute of Digestive and Liver Diseases St. Luke's Medical Center-Global City, Philippines*

[b]*Biology Department De La Salle University, Manila, Philippines*

[c]*Department of Medicine St. Luke's Medical Center-Quezon City, Philippines*

Correspondence:
Dr. Marianne Linley L. Sy-Janairo
mariannelinleyhsyjanairomd@gmail.com

Accepted for publication:
December 2020

Abstract

Background: Spontaneous bacterial peritonitis (SBP) is a frequent complication seen among cirrhotic patients resulting in increased hospitalization and has an estimated 30-day mortality of 33%. While the use of proton pump inhibitors (PPI) has been associated with higher incidence of SBP, previous studies provided conflicting conclusions. *Objective:* This study aims to re-assess the association between PPI use and SBP incidence with larger, updated data. *Methods:* Database of Medline, Cochrane, and Google scholar were used to search for relevant articles. Two reviewers independently assessed the quality of each paper. Disagreements were resolved by the third author. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was observed and complied with. Pooled odds ratios with 95% confidence intervals were calculated. Sub-group analysis was done to decrease heterogeneity. *Results:* Twenty-two studies (eight case-control, 13 cohort, and one randomized controlled trial) involving 10,828 patients were analyzed. Results showed a statistically significant association between SBP and PPI use (pooled odds ratio (OR): 2.03, 95% CI of 1.67-2.45), with substantial heterogeneity. Subgroup analysis involving cohort and randomized controlled trial revealed statistically significant association, although weaker (OR: 1.88 with 95% CI of 1.51-2.34, $p<0.00001$) and has substantial heterogeneity. For case-control studies, OR is 2.64 with 95% CI of 1.91-3.64. Pooled OR for high quality studies is 1.93 with 95% CI of 1.57-2.38, $p<0.00001$). The funnel plot was asymmetric suggesting publication bias. *Conclusion:* This meta-analysis showed there is statistically significant association, although weak, between higher incidence of SBP and PPI use. This updated meta-analysis suggests judicious use of PPI among cirrhotic patients with ascites.

Keywords: meta-analysis, peritonitis, proton pump inhibitor, liver cirrhosis

Introduction

Proton pump inhibitors (PPI) are one of the most commonly used medications. It is generally well-tolerated and safe, with few reported adverse effects. Studies have shown that there is an increasing overuse of PPIs in hospital and outpatient practices.[1-4] Because of this, there is now a growing concern regarding potential complications associated with long term use.[5-7]

Spontaneous bacterial peritonitis (SBP) is a common infection among patients with liver cirrhosis, and is associated with increased hospitalization and an estimated 30-day mortality of 33%.[8] Patients with liver cirrhosis are considered to be at higher risk for infection because of several factors, such as increased intestinal permeability resulting to bacterial translocation, and lower immune system due to complement deficiency, reticuloendothelial system depression, and leukocyte dysfunction.[9,10] The use of PPI among cirrhotic patients with ascites has been associated with higher incidence of SBP, possibly due to the suppression of the gastric acid secretion which may lead to increased bacterial colonization and altered gut flora. This may in turn

contribute to an even higher risk of bacterial overgrowth and translocation among this group of patients.[5,11,12] Furthermore, since PPIs are metabolized in the liver, changes in the pharmacokinetics among cirrhotic patients may occur, making them at higher risk for possible adverse effects.[13] Previous studies, however, including case control, cohort, and meta-analysis, provide conflicting results and conclusions. Several case control[14-16] and cohort studies[17,18] show association of SBP with PPI therapy among cirrhotic patients. In contrary, the study of Mandorfer et al.[19] and Terg et al.[20] conclude that there is no association between PPI use and higher risk of SBP. The latest meta-analysis by Yu et al.[21] also did not establish association between PPI use and higher incidence of SBP. After this meta-analysis, additional studies were published evaluating the association of PPI use and development of SBP. Considering the new data and information available, we aim to re-assess the association between PPI use and SBP incidence with larger and better quality data.

Methods

Literature Search

Two authors independently conducted a search for articles published until November 2019 in PUBMED (158 articles), the Cochrane Central Registry of Clinical Trials (four articles) and Google scholar (132 articles). The search entry terms used were: *proton pump or omeprazole or esomeprazole or lansoprazole or rabeprazole or pantoprazole AND spontaneous bacterial peritonitis OR peritonitis and cirrhosis*. The search was limited to human subjects. Different institutions from different countries were included to allow generalizability of the study. Bibliographies were also reviewed for articles which could qualify for the study.

Study Selection

All retrieved abstracts were independently reviewed by two authors. The full texts of potential articles were retrieved and reviewed by the same authors to determine eligibility. Disagreements were resolved by consensus with the third or, if necessary, by the fourth author. Inclusion criteria for the selection of relevant studies were: (i) those that reported the association between PPI therapy and SBP incidence (defined as ≥250 polymorphonuclear leukocytes/L in the ascitic fluid; (ii) randomized controlled trial (RCT), case–control or cohort articles; (iii) study population comprised adult patients (≥18 years); and (iv) articles reported relative risk (RR), odds ratio (OR), or hazard ratio (HR) at 95% confidence interval (CI), or the raw data to calculate them.

Exclusion criteria were as follows: (i) studies had no control group; (ii) studies included patients who experienced gastrointestinal bleeding, who were on antibiotic prophylaxis during the last two weeks prior to SBP, or liver transplant patients; (iii) papers were letters, commentaries, editorials, reviews and duplicate publications; and (iv) outcome is recurrent SBP. Outcome of interest is development of spontaneous bacterial peritonitis.

The study complied with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement[22].

Quality Assessment

Two reviewers independently graded the methodological quality of each included study using the Newcastle-Ottawa Scale (NOS)[23] for the non-randomized studies, and the Jadad scoring[24] system for the RCT article. Any disagreement about a particular study was resolved by consensus with a third and/or fourth investigator. NOS score ≥7 and Jadad score of 5 were considered high quality studies.

Statistical Analysis

Review Manager version 5.3 was used to conduct the meta-analysis. Results were presented as pooled ORs with 95% confidence interval. We assumed similarity between the OR and other relative measures, such as RR and HR, because SBP events and deaths were rare. When both the crude and the adjusted OR/RR values were offered, only the adjusted value was adopted for the meta-analysis. If only the raw data was reported, calculation for unadjusted OR was done. A random effects model was used with presumption of multiple potential sources of heterogeneity being present between the studies included. To address the heterogeneity from different study designs, subgroup analysis of studies was done. Statistical heterogeneity was evaluated using the Cochran Chi- square and the I^2 statistic. An I^2 value of >50% suggests significant heterogeneity.

Results

The search yielded 294 documents from database search, and four articles from bibliography search. After duplicates were removed, 194 articles were screened and full-text articles were reviewed. Out of the 55 screened-in articles, 31 were further excluded because they were either: a review/meta-analysis, no relevant data available, no standard definition of SBP, an abstract of an included study, and/or involved a different outcome. Two articles were published abstracts of conference proceedings. Twenty-two studies fulfilled eligibility criteria and were analyzed. **Figure 1** shows the flow of the selection process.

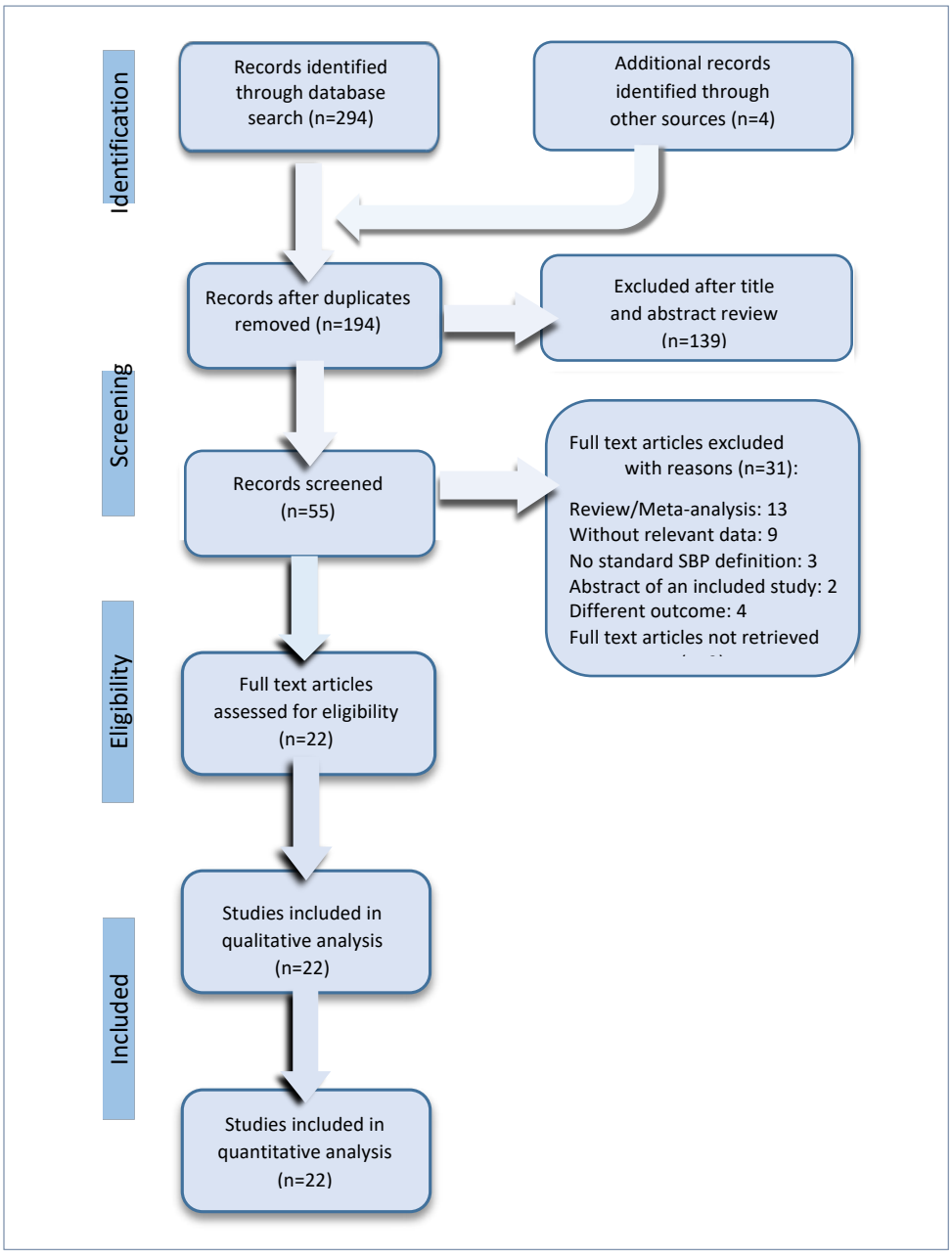


Figure 1. Flow diagram of the search strategy

Study Characteristics and Quality Assessment of Included Studies

The 22 studies included were published from 2008-2019, involving 10,828 patients. Most of the studies were conducted in the USA, Europe, and other studies were from Asia. The detailed characteristics of included studies are summarized in **Table 1**.

Table 1. Characteristics of included studies

Author	Year	Sample Size	# Patients on PPI Group	Single / Multi-Center	Follow Up (Months)	Adjusted/Matched Factors	Country
Case Control Studies (8)							
Bajaj[14]	2009	140	70	Single	-	CTP class, age, admission time period	USA
Campbell[25]	2008	116	43	Single	-	Age, bilirubin, INR, creatinine, MELD score, DM, gender, history of SBP, etiology of liver disease, race	USA
Choi[26]	2011	176	21	Single	-	CCTP class, age, MELD score, history of esophageal variceal bleeding	Korea
De Vos[27]	2013	102	38	Single	-	-	Belgium
Goel[16]	2011	130	91	Single	-	CTP class	USA
Kwon[15]	2014	1,140	129	Multi-	-	Age, MELD score	Korea
Miura[18]	2014	65	43	Single	-	Age, creatinine, platelets, albumin, total bilirubin	Japan
Ratelle[28]	2014	153	74	Single	-	Age, gender, year of admission, CTP class	Canada
Cohort Studies (13)							
Chang[29]	2015	947	-	Multi-	12	Age, sex, index date	Taiwan
Dam[30]	2016	865	340	Multi-	13	MELD, sodium, albumin, history of SBP	Denmark
Elzouki[31]	2018	333	171	Single	-	-	Qatar
Huang[32]	2016	3,060	1,870	Multi-	24	Age, sex, co-morbidities, ascites, hepatic encephalopathy, esophageal varices	Taiwan
Janka[33]	2019*	350	196	Single	60	Age, gender, co-morbidity, etiology of liver disease, MELD score, CTP class	Hungary
Mandorfer[19]	2014	607	520	Single	9.6	Age, HCC, history of variceal bleeding, varices, MELD score	Austria
Min[34]	2014	804	512	Single	25.1	Age, gender, etiology of liver disease, platelet count, AST, ALT,ALP, GGT, BUN, creatinine, serum sodium, serum albumin, total bilirubin, INR,CTP class	Korea
Miozzo[35]	2017	258	151	Single	60	CTP class	Brazil
O'leary[17]	2014	188	83	Multi-	6	-	N. America
Pacheco[36]	2017	113	44	Single	36	CTP class, ascites, chronic use of PPI, history of variceal bleeding	Mexico
Terg[37]	2015	384	165	Multi-	3	Age, gender, MELD score, CTP class, alcohol, HBV/HCV infection, encephalopathy, serum bilirubin, creatinine, peripheral leukocyte count, platelet count, protein in ascetic fluid	Argentina
Tergast[38]	2018	613	506	Single	0.9	-	Germany
Van Vlerken[10]	2012	84	52	Multi-	28	Age, CTP class	Netherlands
Randomized Control Trial (1)							
Hyat[39]	2018	200	100	Single	6	Age, gender	Pakistan

*published online

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CTP, Child-Turcotte-Pugh class; DM, diabetes mellitus; GGT, gamma-glutamyl transferase; HBV, hepatitis B virus; HCB, hepatitis C virus; HCC, hepatocellular carcinoma; INR, international normalized ratio (prothrombin time); MELD, model for end-stage liver disease

There was one randomized controlled trial, thirteen cohort studies, and eight case–control studies. Study duration ranged from 28 days to five years. Eighteen studies adjusted the impact of confounders when assessing the association between use of PPIs and development of SBP. Six studies were performed using a prospective cohort study design, five of which were conducted under multi-center settings. **Table 2** summarizes the risk of bias assessment for the included studies. Five out of the eight case-control studies were of high quality, and 12 out of 13 cohort studies were regarded as high quality. The only RCT trial was considered to have a moderate threat to validity, with a Jaded score of 2.

Table 2. Quality assessment of included studies

Author	Year	Quality Assessment Criteria	Score
Case Control Studies (8)			
Bajaj	2009	NOS*	7
Campbell	2008	NOS	6
Choi	2011	NOS	8
De Vos	2013	NOS	7
Goel	2011	NOS	7
Kwon	2014	NOS	8
Miura	2014	NOS	5
Ratella	2014	NOS	4
Cohort Studies (13)			
Chang	2015	NOS	8
Dam	2016	NOS	8
Elzouki	2018	NOS	6
Huang	2016	NOS	9
Janka	2019*	NOS	8
Mandorfer	2014	NOS	8
Min	2014	NOS	9
Miozzo	2017	NOS	8
O'leary	2014	NOS	8
Pacheco	2017	NOS	8
Terg	2015	NOS	9
Tergast	2018	NOS	7
Van Vlerken	2012	NOS	8
Randomized Control Trial (1)			
Hayat	2018	Jadad	2

*NOS: New Castle-Ottawa Scale

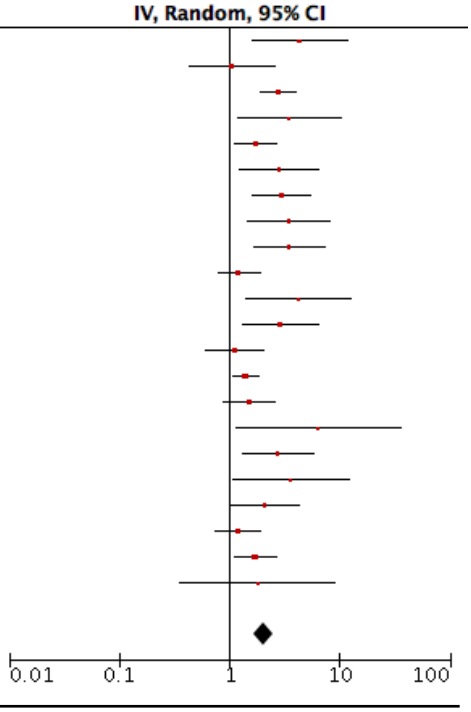
Meta-Analysis

The Forest plot (**Table 3**) showed pooled odds ratio of spontaneous bacterial peritonitis with proton pump inhibitor use among cirrhotic patients with ascites. The overall pooled odds ratio for the 22 studies is 2.03, with 95% CI of 1.67 to 2.45, and a statistically significant *p* value. Substantial heterogeneity was observed, as demonstrated by a significant Chi^2 *p*-value. Although the I^2 is less than 50%, moderate degree of heterogeneity was considered. Sensitivity analysis was done to decrease heterogeneity. One identified source of heterogeneity is the differences in the study design of the included articles, hence subgroup analysis for the different study design was done.

Table 3. Forest plot for association between PPI use and SBP incidence

Study or Subgroup	log[Odds Ratio]	SE	Weight	Odds Ratio IV, Random, 95% CI
Bajaj, 2009	1.4609	0.5088	2.8%	4.31 [1.59, 11.68]
Campbell 2008	0.0488	0.4555	3.3%	1.05 [0.43, 2.56]
Chang, 2015	1.0188	0.1924	7.8%	2.77 [1.90, 4.04]
Choi, 2011	1.2355	0.5529	2.4%	3.44 [1.16, 10.17]
Dam, 2016	0.5423	0.2281	7.0%	1.72 [1.10, 2.69]
de Vos, 2013	1.033	0.426	3.6%	2.81 [1.22, 6.47]
Elzouki, 2018	1.0852	0.3104	5.3%	2.96 [1.61, 5.44]
Goel, 2011	1.2384	0.4353	3.5%	3.45 [1.47, 8.10]
Hayat, 2018	1.2389	0.375	4.2%	3.45 [1.66, 7.20]
Huang, 2016	0.1906	0.2231	7.1%	1.21 [0.78, 1.87]
Janka, 2019	1.4469	0.5583	2.4%	4.25 [1.42, 12.69]
Kwon, 2014	1.0543	0.403	3.9%	2.87 [1.30, 6.32]
Mandorfer, 2014	0.1044	0.3139	5.2%	1.11 [0.60, 2.05]
Min, 2014	0.3365	0.1419	9.1%	1.40 [1.06, 1.85]
Miozzo, 2017	0.4055	0.2773	5.9%	1.50 [0.87, 2.58]
Miura, 2014	1.8579	0.8722	1.1%	6.41 [1.16, 35.42]
O'leary, 2014	1.0001	0.3775	4.2%	2.72 [1.30, 5.70]
Pacheco, 2017	1.2809	0.6189	2.0%	3.60 [1.07, 12.11]
Ratelle, 2014	0.7372	0.3561	4.5%	2.09 [1.04, 4.20]
Terg, 2015	0.1823	0.2398	6.7%	1.20 [0.75, 1.92]
Tergast, 2018	0.5306	0.2266	7.0%	1.70 [1.09, 2.65]
van Vlerken, 2012	0.5878	0.8269	1.2%	1.80 [0.36, 9.10]
Total (95% CI)			**100.0%**	**2.03 [1.67, 2.45]**

Heterogeneity: $Tau^2 = 0.08$; $Chi^2 = 39.99$, df = 21 (P = 0.007); $I^2 = 47\%$
Test for overall effect: Z = 7.24 (P < 0.00001)

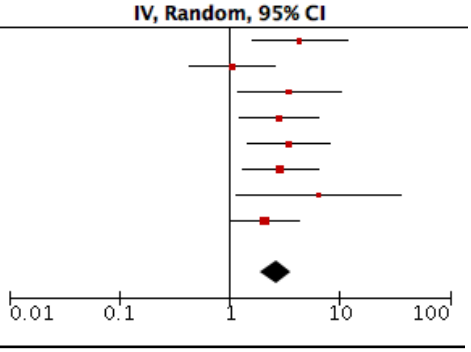


For case control studies (**Table 4**), the statistically significant pooled odds ratio of 2.64, with 95% CI of 1.91 to 3.64. Zero to minimal heterogeneity was observed in this subgroup analysis.

Table 4. Forest plot for association between PPI use an SBP incidence involving case controls

Study or Subgroup	log[Odds Ratio]	SE	Weight	Odds Ratio IV, Random, 95% CI
Bajaj, 2009	1.4609	0.5088	10.2%	4.31 [1.59, 11.68]
Campbell 2008	0.0488	0.4555	12.7%	1.05 [0.43, 2.56]
Choi, 2011	1.2355	0.5529	8.7%	3.44 [1.16, 10.17]
de Vos, 2013	1.033	0.426	14.5%	2.81 [1.22, 6.47]
Goel, 2011	1.2384	0.4353	13.9%	3.45 [1.47, 8.10]
Kwon, 2014	1.0543	0.403	16.1%	2.87 [1.30, 6.32]
Miura, 2014	1.8579	0.8722	3.5%	6.41 [1.16, 35.42]
Ratelle, 2014	0.7372	0.3561	20.5%	2.09 [1.04, 4.20]
Total (95% CI)			**100.0%**	**2.64 [1.91, 3.64]**

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 7.16$, df = 7 (P = 0.41); $I^2 = 2\%$
Test for overall effect: Z = 5.91 (P < 0.00001)



For the cohort studies and one randomized controlled trial (**Table 5**), the pooled odds ratio is 1.88, with 95% CI of 1.51 to 2.34, with a statistically significant *p* value. However, significant and substantial heterogeneity was observed. Another subgroup analysis was done in which a forest plot (**Table 6**) was created involving the 17 high quality studies. The pooled odds ratio is 1.93 with 95% CI of 1.57 to 2.38, and a statistically significant *p* value. Again, substantial heterogeneity was observed as demonstrated by a significant Chi^2 *p*-value. The pooled odds ratio is 1.93 with 95% CI of 1.57 to 2.38, and a statistically significant p value. Again, substantial heterogeneity was observed as demonstrated by a significant Chi^2 p-value.

Table 5. Forest plot for association between PPI use an SBP incidence involving cohort studies and randomized controlled trial

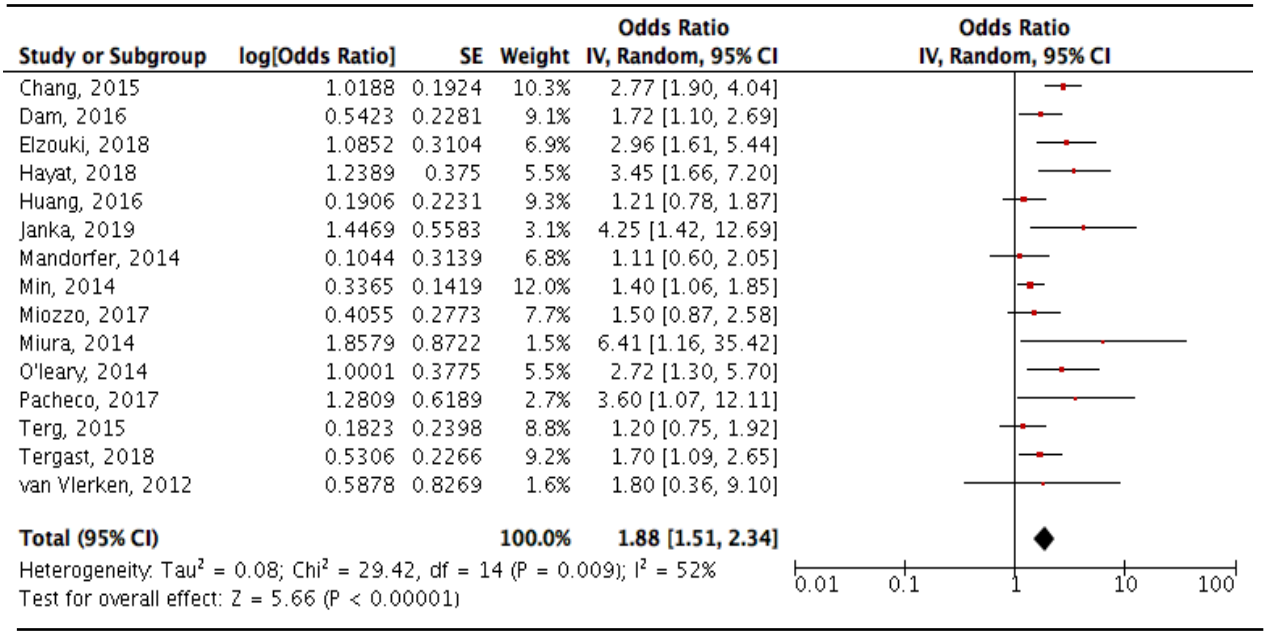
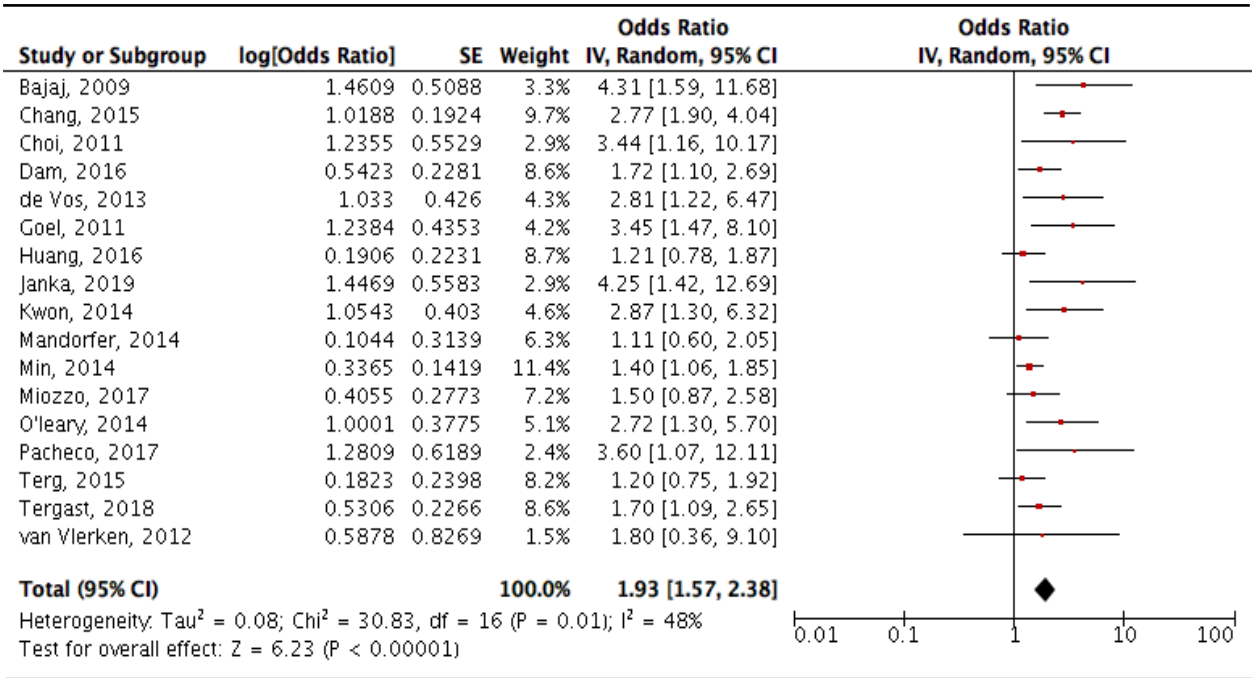


Table 6. Forest plot for association between PPI use an SBP incidence involving the 17 high quality studies



Publication bias was observed as seen in the asymmetrical funnel plot of included studies (**Figure 2**).

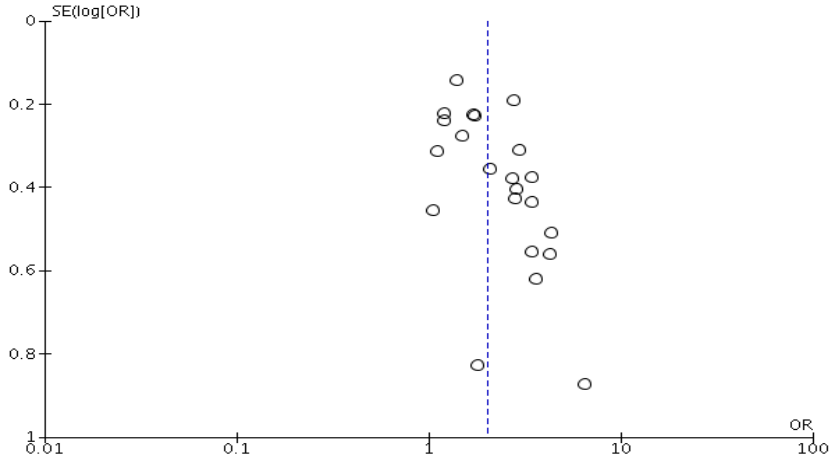


Figure 2. Funnel plot of included studies.

Discussion and Conclusion

One of the most commonly prescribed medications worldwide is proton pump inhibitors. Several studies have shown increasing inappropriate use of such drug. [1-1-4] Safety concerns regarding the use of PPIs have been raised especially among cirrhotic patients, one of which is the higher risk for bacterial peritonitis by increasing intestinal permeability predisposing to bacterial translocation, and lowering the immune system causing increased bacterial colonization in the gastrointestinal tract. Several studies were conducted in line with this but have shown inconclusive results. Newer studies were done to re-evaluate the causality of PPI use and development of SBP, hence an updated meta-analysis is important and warranted.

This study is the most updated and largest meta-analysis to date, including eight case-control studies, 13 cohort studies, and one randomized control trial. The meta-analysis of Yu et al.[21] included ten case control studies; however, the full text articles of the additional two studies were not retrieved; hence, was not included in this meta-analysis. The results of our study have demonstrated association of PPI with SBP occurrence with an overall pooled OR of 2.03, with 95% CI of 1.67 to 2.45, although a significant heterogeneity and publication bias was noted, as demonstrated by an asymmetrical funnel plot. To minimize the effect of heterogeneity and determine the robustness of the findings, sensitivity analysis was done. On the sensitivity analysis, the subgroup involving only the case-control studies has yielded the highest clinically significant pooled odds ratio with zero to minimal heterogeneity. Hence, the evidence for the observed association between PPI use and SBP occurrence remains to be weak, and could not establish causality. Since observations are made through time, results from cohort studies warrant more merit. Cohort studies may help determine potential causality on certain outcomes. For the cohort studies and one randomized controlled trial, a statistically significant pooled odds ratio of 1.88, with 95% CI of 1.51 to 2.34 was observed as compared to the statistically significant pooled odds ratio of 2.64, with 95% CI of 1.91 to 3.64 of the case control studies. Furthermore, the pooled OR for 17 high quality studies is 1.93 with 95% CI of 1.57 to 2.38, $p < 0.00001$. Hence, this systematic review and meta-analysis involving 10,828 patients from 21 observational studies and one randomized control trial has found statistically significant but quantitatively small associations between the use of PPI and SBP among cirrhotic patients with ascites. Our results reinforce the findings of previous meta analyses[21,40,41] that the association between incidence of SBP and PPI use is weak.

Several limitations were identified in our study. Most of the included studies were observational, and cannot establish causality with certainty. Hence,

more prospective studies are warranted to determine causal relationship of PPI use and SBP occurrence. Heterogeneity on the included studies and publication bias were observed. The differences in the population, such as age group of patients, Child-Pugh classification, and MELD score were possible confounders. Lastly, the indications for PPI use, and dosage and duration of PPI used were also not accounted for. It may be better if sensitivity analysis can be made according to duration and dosage of the PPI used. Further studies can be done to evaluate the association of PPI with SBP occurrence in comparison to the association of H2 receptor blocker with SBP occurrence, as to determine which gastric acid suppression therapy is safer to be given among cirrhotic patients.

In conclusion, this systematic review and meta-analysis showed that the evidence for PPI use leading to higher risk of SBP among cirrhotic patients with ascites is weak. Nevertheless, judicious use of PPI especially among this group of patients is highly recommended. Appropriate indication, duration, and dosage of PPI use is warranted.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Heidelbaugh JJ, Kim AH, Walker PC. Overutilization of proton-pump inhibitors: What the clinician needs to know. Therap Adv Gastroenterol. 2012; 5(4):219-32.
2. Chia CTW, Lim WP, Vu CKF. Inappropriate use of proton pump inhibitors in a local setting. Singapore Med J. 2014; 55(7):363-6.
3. Hálfdánarson Ó, Pottegård A, Björnsson ES, Lund SH, Ogmundsdottir MH, Steingrímsson E, et al. Proton-pump inhibitors among adults: A nationwide drug-utilization study. Therap Adv Gastroenterol. 2018 Jan 1; 11:1-11.
4. Haroon M, Yasin F, Gardezi SKM, Adeeb F, Walker F. Inappropriate use of proton pump inhibitors among medical inpatients: a questionnaire-based observational study. J R Soc Med Short Reports. 2013 Jul 31; 4(8).
5. Vaezi MF, Yang YX, Howden CW. Complications of Proton Pump Inhibitor Therapy. Gastroenterology [Internet]. 2017 Jul 1; 153(1):35-48. Available from: http://dx.doi.org/10.1053/j.gastro.2017.04.047.
6. Malfertheiner P, Kandulski A, Venerito M. Proton-pump inhibitors: Understanding the complications and risks. Vol. 14, Nature Reviews Gastroenterology and Hepatology. Nature Publishing Group. 2017; 697-710.
7. Kinoshita Y, Ishimura N, Ishihara S. Advantages and disadvantages of long-term proton pump inhibitor use. J Neurogastroenterol Motil. 2018; 24(2):182-96.
8. Oey RC, de Man RA, Erler NS, Verbon A, van Buuren HR. Microbiology and antibiotic susceptibility patterns in spontaneous bacterial peritonitis: A study of two Dutch cohorts at a 10-year interval. United Eur Gastroenterol J. 2018 May 1; 6(4):614-21.
9. Scarpellini E, Valenza V, Gabrielli M, Lauritano EC, Perotti G, Merra G, et al. Intestinal permeability in cirrhotic patients with and without spontaneous bacterial peritonitis: Is the ring closed. Am J Gastroenterol. 2010 Feb; 105(2):323-7.
10. van Vlerken LG, Huisman EJ, van Hoek B, Renooij W, de Rooij FWM, Siersema PD, et al. Bacterial infections in cirrhosis : role of proton pump inhibitors and intestinal permeability. Eur J Clin Invest. 2012 Jul; 42(7):760-7.
11. Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, et al. Proton pump inhibitors alter the composition of the gut microbiota. Gut. 2016 May 1; 65(5):749-56.
12. Jacobs C, Coss Adame E, Attaluri A, Valestin J, Rao SSC. Dysmotility and proton pump inhibitor use are independent risk factors for small intestinal bacterial and/or fungal overgrowth. Aliment Pharmacol Ther. 2013 Jun; 37(11):1103-11.
13. Weersink RA, Bouma M, Burger DM, Drenth JPH, Harkes-Idzinga SF, Hunfeld NGM, et al. Safe use of proton pump inhibitors in patients with cirrhosis. Br J Clin Pharmacol. 2018 Aug 1; 84(8):1806-20.
14. Bajaj JS, Zadvornova Y, Heuman DM, Hafeezullah M, Hoffmann RG, Sanyal AJ, et al. Association of proton pump inhibitor therapy with spontaneous bacterial peritonitis in cirrhotic patients with ascites. Am J Gastroenterol. 2009 May; 104(5):1130-1134.
15. Kwon JH, Koh SJ, Kim W, Jung YJ, Kim JW, Kim BG, et al. Mortality associated with proton pump inhibitors in cirrhotic patients with spontaneous bacterial peritonitis. J Gastroenterol Hepatol. 2014; 29(4):775-81.
16. Goel GA, Deshpande A, Lopez R, Hall GS, van Duin D, Carey WD. Increased Rate of Spontaneous Bacterial Peritonitis Among Cirrhotic Patients Receiving Pharmacologic Acid Suppression. Clin Gastroenterol Hepatol [Internet]. 2012 Apr; 10(4):422-7. Available from: http://dx.doi.org/10.1016/j.cgh.2011.11.019
17. O'Leary JG, Reddy KR, Wong F, Kamath PS, Patton HM, Biggins SW, et al. Long-term Use of Antibiotics and Proton Pump Inhibitors Predict Development of Infections in Patients With Cirrhosis. Clin

Gastroenterol Hepatol. 2014 Apr 1; 13(4):753-759.e2.
18. Miura K, Tanaka A, Yamamoto T, Adachi M, Takikawa H. Proton Pump Inhibitor Use is Associated with Spontaneous Bacterial Peritonitis in Patients with Liver Cirrhosis. Intern Med. 2014 May 15; 53(10):1037-42.
19. Mandorfer M, Bota S, Schwabl P, Bucsics T, Pfisterer N, Summereder C, et al. Proton pump inhibitor intake neither predisposes to spontaneous bacterial peritonitis or other infections nor increases mortality in patients with cirrhosis and ascites. PLoS One. 2014 Nov 1; 9(11).
20. Terg R, Casciato P, Garbe C, Cartier M, Stieben T, Mendizabal M, et al. Proton pump inhibitor therapy does not increase the incidence of spontaneous bacterial peritonitis in cirrhosis: A multicenter prospective study. J Hepatol [Internet]. 2015 May 1; 62(5):1056-60. Available from: http://dx.doi.org/10.1016/j.jhep.2014.11.036.
21. Yu T, Tang Y, Jiang L, Zheng Y, Xiong W, Lin L. bacterial peritonitis incidence and mortality : A meta-analysis. Dig Liver Dis [Internet]. 2015; 1-7. Available from: http://dx.doi.org/10.1016/j.dld.2015.12.009.
22. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009 Jul; 6(7).
23. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Non-Randomized Studies in Meta-Analysis. 2000.
24. Jadad AR, Andrew Moore R, Carroll D, Jenkinson C, John Reynolds DM, Gavaghan DJ, et al. Assessing the Quality of Reports of Randomized Clinical Trials: Is Blinding Necessary? Control Clin Trials. 1996; 17:1-12.
25. Campbell MS, Obstein K, Reddy KR, Yang YX. Association between proton pump inhibitor use and spontaneous bacterial peritonitis. Dig Dis Sci. 2008 Feb; 53(2):394-398.
26. Choi EJ, Lee HJ, Kim KO, Lee SH, Eun JR, Jang BI, et al. Association between acid suppressive therapy and spontaneous bacterial peritonitis in cirrhotic patients with ascites. Scand J Gastroenterol. 2011 May; 46(5):616-20.
27. de Vos M, De Vroey B, Garcia BG, Roy C, Kidd F, Henrion J, et al. Role of proton pump inhibitors in the occurrence and the prognosis of spontaneous bacterial peritonitis in cirrhotic patients with ascites. Liver Int. 2013 Oct; 33(9):1316-23.
28. Ratelle M, Perreault S, Villeneuve JJ, Tremblay L. Association between proton pump inhibitor use and spontaneous bacterial peritonitis in cirrhotic patients with ascites. Can J Gastroenterol Hepato. 2014; 28(6):330-5.
29. Chang SS, Lai CC, Lee MTG, Lee YC, Tsai YW, Hsu WT, et al. Risk of Spontaneous Bacterial Peritonitis Associated with Gastric Acid Suppression. Med (United States). 2015 Jun 7; 94(22):e944.
30. Dam G, Vilstrup H, Watson H, Jepsen P. Proton pump inhibitors as a risk factor for hepatic encephalopathy and spontaneous bacterial peritonitis in patients with cirrhosis with ascites. Hepatology. 2016 Oct 1; 64(4):1265-72.
31. Elzouki AN, Neffati N, Rasoul FA, Abdallah A, Othman M, Waness A. Increased Risk of Spontaneous Bacterial Peritonitis in Cirrhotic Patients Using Proton Pump Inhibitors. GE Port J Gastroenterol. 2019 Mar 1; 26(2).
32. Huang KW, Kuan YC, Luo JC, Lin CL, Liang JA, Kao CH. Impact of long-term gastric acid suppression on spontaneous bacterial peritonitis in patients with advanced decompensated liver cirrhosis. Eur J Intern Med [Internet]. 2016 Jul 1; 32:91-95. Available from: http://dx.doi.org/10.1016/j.ejim.2016.04.016.
33. Janka T, Tornai T, Borbély B, Tornai D, Altorjay I, Papp M, et al. Deleterious effect of proton pump inhibitors on the disease course of cirrhosis. Eur J Gastroenterol Hepatol. 2019 Aug; 1.
34. Min YW, Lim KS, Min BH, Gwak GY, Paik YH, Choi MS, et al. Proton pump inhibitor use significantly increases the risk of spontaneous bacterial peritonitis in 1965 patients with cirrhosis and ascites: A propensity score matched cohort study. Aliment Pharmacol Ther. 2014; 40(6):695-704.
35. Miozzo SAS, John JA, Appel-da-Silva MC, Dossin IA, Tovo C V., Mattos AA. Influence of proton pump inhibitors in the development of spontaneous bacterial peritonitis. World J Hepatol. 2017 Dec 18; 9(35):1278-85.
36. Lázaro-Pacheco IB, Servín-Caamaño AI, Pérez-Hernández JL, Rojas-Loureiro G, Servín-Abad L, Higuera-De La Tijera F. Proton pump inhibitors increases the overall risk of developing bacterial infections in patients with cirrhosis. Arq Gastroenterol. 2018 Mar; 55(1):28-32.
37. Terg R, Casciato P, Garbe C, Cartier M, Stieben T, Mendizabal M, et al. Proton pump inhibitor therapy does not increase the incidence of spontaneous bacterial peritonitis in cirrhosis: A multicenter prospective study. J Hepatol. 2015 May 1; 62(5):1056-60.
38. Tergast TL, Wranke A, Laser H, Gerbel S, Manns MP, Cornberg M, et al. Dose-dependent impact of proton pump inhibitors on the clinical course of spontaneous bacterial peritonitis. Liver Int. 2018 Sep 1; 38(9):1602-13.
39. Hayat MKF, Shah ZH, Hayat MKF, Imran MY, Qureshi IH. Comparative study of spontaneous bacterial

peritonitis in cirrhosis patients managed with and without proton pump inhibitors. Pakistan J Med Heal Sci. 2018 Apr 1; 12(2):598-601.

40. Khan MA, Kamal S, Khan S, Lee WM, Howden CW, Ali M, et al. Systematic review and meta-analysis of the possible association between pharmacological gastric acid suppression and spontaneous bacterial peritonitis. Eur J Gastroenterol Hepatol. 2015 Oct 1; 27(11):1327-36.
41. Xu HB, Wang HD, Li CH, Ye S, Dong MS, Xia QJ, et al. Proton pump inhibitor use and risk of spontaneous bacterial peritonitis in cirrhotic patients: A systematic review and meta-analysis. Genet Mol Res. 2015 Jul 3; 14(3):7490-501.

The Role of L-Carnitine in the Improvement of Liver Tests and Glycemic Control among Patients with Non-Alcoholic Fatty Liver Disease: A Meta-Analysis

Li HWC
Olimba JLA
Tawasil AA
Salvaña AD

Division of Gastroenterology
Department of Medicine
University of the Philippines-Philippine General Hospital

Correspondence:
Dr. Henry Winston C. Li
winolps2488@gmail.com

Accepted for publication:
January 2021

Abstract

Background: Population-based cohort studies from Asia have reported rising prevalence of non-alcoholic fatty liver disease (NAFLD) from 10% to 24%. Philippine data report a rate of 12.2% with important co-morbidities such as obesity (56%) and diabetes mellitus (69%). Several interventions for NAFLD have emerged, among which L-carnitine has shown promise. *Objective:* This meta-analysis aimed to assess the role of carnitine in improving liver function and glycemic control among NAFLD patients. *Methodology:* Electronic search from databases (PubMed, Cochrane Library and Google Scholar) yielded five randomized controlled trials. Studies included adult patients with NAFLD diagnosed through clinical and/or histologic findings. Methodologic assessment of studies and statistical analyses were performed with Review Manager version 5.3. *Results:* Of 33 studies identified, five fulfilled the inclusion criteria with a total of 340 clinical subjects. Pooled analysis showed significant reduction in serum ALT and AST with mean differences of 34.64 $\pm$ 14.3 (p value = <0.0001) and 17.49 $\pm$ 9.88 (p value = 0.0005), respectively. No significant reduction on BMI and fasting blood sugar were demonstrated with mean differences of -0.10 $\pm$ 0.20 (p value = 0.31) and 2.31 $\pm$ 13.38 (p value = 0.73), respectively. Subgroup analysis based on treatment dose and duration showed unaltered results except for AST levels, which demonstrated greater reduction at carnitine dose of >500 mg/day. *Conclusion:* The use of L-carnitine resulted in lower ALT and AST levels, with dose-dependent reduction seen for AST. Intake of L-carnitine had no effect on glycemic control and BMI among NAFLD patients. Further studies involving more clinical subjects with histologic and radiologic assessments as outcomes are highly recommended.

Keywords: carnitine, carnitine-orotate, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, meta-analysis

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) is rising in concert with rising rates of obesity and diabetes mellitus, with an estimated 33.8% and 10.6% of the population meeting the criteria, respectively. Subsequent population-based cohort studies from China, Japan, and Korea have reported a prevalence of NAFLD ranging from 10% to 24% using ultrasonography.[1] Furthermore, this prevalence resembles our local statistics as evidenced by a cohort study published in 2008 stating a 12.2% rate of NAFLD based on clinical and ultrasonographic findings.[2] The study also emphasized an increased rate of obesity (56%) and diabetes (69%) across all of these populations. This emerging clinical condition holds a relevant impact since it is associated with various important co-morbidities that highly contribute to the burden of the disease, including diabetes mellitus, obesity and dyslipidemia.

Several mechanisms have been implicated in the development of NAFLD across its spectrum, ranging from steatosis to eventual liver cirrhosis. The popular "two-hit" hypothesis by which sequential progression

from isolated fatty liver (IFL) to non-alcoholic steatohepatitis (NASH) involves an initial lesion of hepatic steatosis followed by a second "hit" of oxidative stress resulting in liver injury.[3] Another etiology that greatly contributes to this process is insulin resistance, which has been associated with NAFLD. This metabolic state results in several changes in lipid metabolism including enhanced peripheral lipolysis, increased triglyceride synthesis and increased hepatic uptake of fatty acids. It is now recognized that patients who have steatohepatitis on liver biopsy specimens are at risk of progression to cirrhosis, and our understanding of the pathogenesis of NAFLD has evolved from the initial two-hit hypothesis concept to the introduction of emerging therapeutic interventions, including ursodeoxycholic acid, vitamin E and carnitine, to manage such disease condition.[4]

L-carnitine is a quaternary amine, which has been hypothesized to improve the outcome of NASH, because it reduces lipid levels, limits oxidative stress, and modulates inflammatory responses.[5] It performs a number of essential intracellular and metabolic functions, such as fatty acid transport between cytosol and mitochondria, detoxification of potentially toxic metabolites, regulation of the mitochondrial acyl-Co A/CoA ratio, and stabilization of cell membranes. Many studies have found that treatment with such drug has a substantial role in glucose tolerance, weight loss, fatty acid metabolism and insulin function.[6] Multiple randomized controlled trials have been published stating the beneficial effects of the use of L-carnitine in improving liver tests and glycemic control among patients with NAFLD.

The aim of this study is to synthesize data from pooled randomized controlled trials involving this emerging intervention, and to address the question of how effective the use of L-carnitine is in the improvement of liver tests and glycemic control among patients with NAFLD. The general objective of this research is to determine the efficacy of L-carnitine in the improvement of liver tests and glycemic control among patients with NAFLD. The following are the specific objectives: (1) To determine improvement in liver enzymes including AST, ALT among patients treated with L-carnitine versus the control group; (2) To determine improvement in glycemic control through serial fasting blood sugar (FBS) monitoring among NAFLD patients treated with L-carnitine versus the control group; (3) To determine the effects of L-carnitine on other anthropometric profiles including body mass index (BMI); and (4) To determine any possible adverse effects related to L-carnitine among patients with NALFD, if available.

Methodology

Database and Search Strategy

Electronic databases including PubMed, Cochrane Library and Google Scholar were used to retrieve articles from January 1986 (when the drug was approved by the US FDA for public use) up to November 2019. The following were the search terms/keywords used: L-carnitine, carnitine, carnitine-orotate, NASH, non-alcoholic steatohepatitis, NAFLD, and non-alcoholic fatty liver disease. No language and publication restrictions were used during the search of articles. We also obtained primary sources from hand searches with references encountered upon review of papers and original articles. Only original data were used in the meta-analysis.

Eligibility Criteria

The articles were considered eligible if the studies met the following criteria: randomized controlled trials (RCT) conducted among adult patients $\geq$18 years of age with NAFLD; use of either oral or intravenous L-carnitine or carnitine-orotate complex as the intervention compared to placebo or standard-of-care treatment (i.e., metformin for diabetes mellitus) with outcomes being change in the serum levels of liver enzymes, FBS, BMI, and other metabolic profiles if available. Studies which were non-RCTs (including retrospective studies and case reports or reviews) were excluded. Subsequently, patients known to have alcoholic fatty liver disease and significant alcohol consumption (20 gm/day for males and 10 gm/day for females), and hepatocellular carcinoma or cirrhosis related to other etiologies were excluded.

Selection of Studies

The study included trials discussing treatment effects of L-carnitine or L-carnitine orotate complex in the improvement of liver function and metabolic profile, including BMI and glycemic control, among patients with NAFLD. Three independent reviewers thoroughly assessed and identified available trials by

applying the inclusion and exclusion criteria mentioned above. Any disagreement in article inclusion and data extraction was solved by discussion and proper adjudication by the consultant co-author who stood as the fourth reviewer.

A comprehensive literature search was performed and we were able to identify 33 references from various electronic databases including the following: 18 from PubMed, nine from the Cochrane Library, and six from Google Scholar. Three additional articles were retrieved through hand search. Out of the 33 articles, six studies were fully reviewed and assessed for eligibility. One study was excluded due to its use of a different intervention in the placebo arm and the use of median as a measure among serial laboratory determinations. An effort to reach the investigators was made to obtain raw data for laboratory values but to no avail. Hence, this resulted in the analysis of five RCTs (see **Figure 1**).

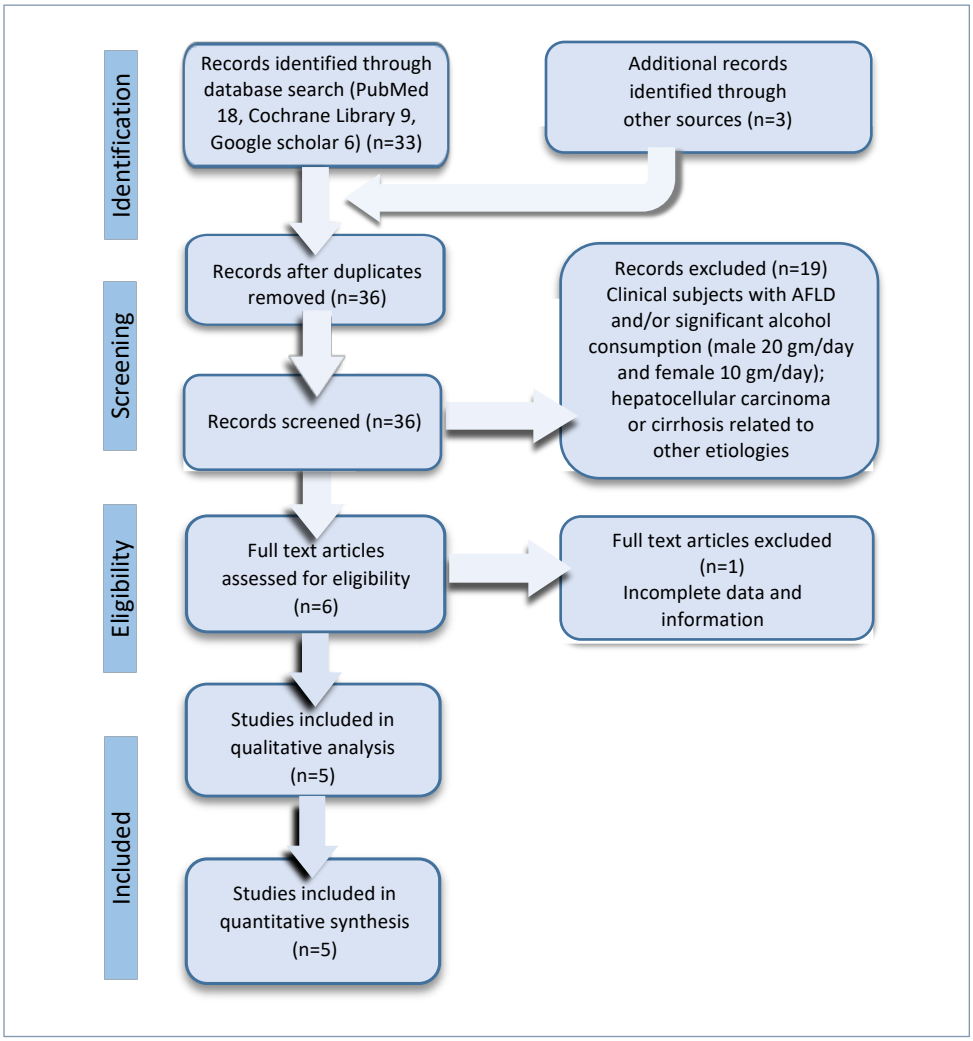


Figure 1. Flow diagram of the study selection

Quality Assessment of Selected Studies

The quality of included trials was duly assessed using the Cochrane Collaboration Risk of Bias Tool available in the Review Manager version 5.3 software. Domains including method of randomization, allocation concealment, blinding, follow-up rate and reporting bias were taken into account. They were evaluated by the investigators and classified as low, high or unclear

risk of bias. The qualities of the five randomized studies are summarized in **Figure 2**. Overall, there was essentially low risk of bias for most of the domains except for the method of allocation concealment which was not stated in the majority of the RCTs included.

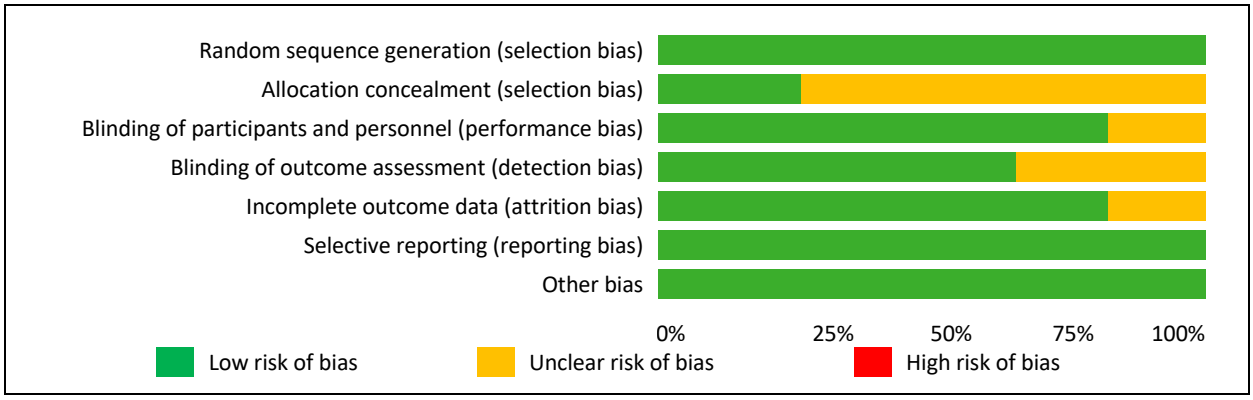


Figure 2. Risk of bias graph of included randomized controlled trials

Statistical Analysis

All statistical analyses were performed using Review Manager 5.3. Heterogeneity was assessed using the Chi-square statistic and a *p* value of less than 0.05 was considered to represent statistical significance. The degree of heterogeneity was determined by the I^2 value.

Additionally, sensitivity analyses were conducted to determine the stability of the overall effects by dividing the studies based on both treatment dose and treatment duration.

Results

Characteristics of Included Studies

The characteristics of the included studies that assessed the efficacy of L-carnitine or carnitine-orotate complex in the improvement of liver tests and glycemic control are shown in **Table 1**. Five studies presented results on improving liver tests as well as other metabolic parameters as outcomes, including glycemic control and BMI. Various doses and different dosing frequencies of L-carnitine or carnitine-orotate were used in the study ranging from 300 mg/day to 2,342 mg/day. Majority of the studies had treatment duration of 90 days, except for two studies extending up to 24 weeks or 180 days.

Table 1. Basic characteristics of included studies

	RCT1 Malaguarnera et al.[7]		RCT2 Hong et al.[8]		RCT3 Bae et al.[9]		RCT4 Alavinejad et al.[10]		RCT5 Somi et al.[11]	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
No. of participants	36	38	24	24	39	39	30	30	40	40
Country	Italy	Italy	Korea	Korea	Korea	Korea	Iran	Iran	Iran	Iran
Ave. Age (yrs)	47.9	47.8	51.5	52	51	52	60	59	40.3	41
Male (%)	53	56	69	69	64	74	78	65	83	83
Female (%)	47	34	31	31	36	24	22	35	17	17
BMI (kg/m^2)	26.5	26.5	27.2	27	28.2	26.7	28.6	29.5	29.4	28.6
FBS (mg/dL)	110.5	109.8	141	147.8	143.6	153.4	172	175	NS	NS
ALT (IU/L)	120.2	125.7	71.2	67.1	94.9	79.2	124	120	81.7	54.1
AST (U/L)	135.4	132.8	44.3	44.4	61.8	51.7	122.7	125.3	60.5	52.6

Treatment dosage	L-carnitine 2000 mg/day		Carnitine-orotate complex 300 mg/day (2 tabs TID)		Carnitine-orotate complex 2472 mg/day (824 mg TID)		L-carnitine 750 mg/day		L-carnitine 500 mg/day	
Outcomes measured	ALT, ɣ-GTP, albumin, lipid profile, insulin, C peptide, CRP, ALP		Primary: ALT; Secondary: FBS, Hba1c, AST, mtDNA, 8-hydroxydeoxyguanine		Primary: decline in ALT to normal range; Secondary: hepatic steatosis using HU via non-contrast CT, AST, FBS, HBa1c, HOMA-IR, HOMA-B, TG, LDL, HDL, weight, BMI		AST, ALT, TC, TG, FBS, Hba1c		Weight, BMI, AST, ALT	
Country	Italy		Korea		Korea		Iran		Iran	
No. of participants	36	38	24	24	39	39	30	30	40	40

Abbreviations: AST - aspartate aminotransferase; ALT – alanine aminotransferase; FBS – fasting blood sugar; BMI – body mass index; ɣ-GTP – gamma glutanyl transpeptidase; HOMA IR – homeostatic model assessment for insulin resistance; TC - total cholesterol; TG – total glyceride; LDL – low density lipoprotein mtDNA – mitochondrial DNA; CRP – C-reactive protein

The age of participants in each trial ranged from 40 to 60 years old. The total number of pooled subjects from the clinical trials summed up to 340 patients with 169 (49.7%) and 171 (50.3%) for the treatment and control groups, respectively. There were 234 (68.82%) male subjects and 106 (31.2%) female participants. Of note was the prevalence of an overweight BMI across all studies. The RCTs were generally conducted in Asian countries, including Iran and Korea, except for one study which was done in Italy.

Primary Outcomes

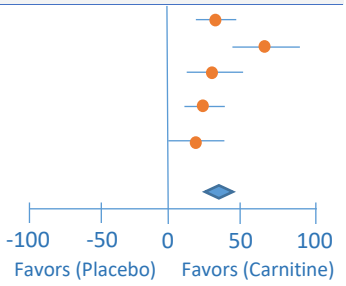
Change in Serum ALT Levels

Pooled analysis using random effects model of the five trials evaluating the role of L-carnitine or carnitine-orotate complex supplementation in the change of serum ALT levels showed a significant reduction with a mean difference of 34.64 (95% CI 20.34-48.94) and a *p* value of <0.00001. Significant heterogeneity was present with an I^2 of 87% (see **Table 2**).

Table 2. Forest plot on the change of serum ALT from baseline between treatment (carnitine) and control group

Study or Subgroup	Carnitine			Placebo			Weight (%)	Mean Difference IV, random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Alavinejad, 2016	41.9	12.43	30	5.0	12.45	30	23.3	36.90[30.60, 43.20]
Bae, 2015	73.7	38.7	39	5.38	37.1	39	18.2	68.32 [51.49, 85.15]
Hong, 2014	51.5	33.2	24	16.7	31.3	24	17.5	34.80 [16.55, 53.05]
Malaguarnera, 2009	58.4	22.6	36	37.4	12.1	38	22.5	21.00 [12.68, 29.32]
Somi, 2014	30.7	46.48	40	15.7	25.8	40	18.4	15.00 [-1.47, 31.47]
Total (95% CI)			**169**			**171**	**100**	**34.64 [20.34, 48.94]**

Heterogeneity: Tau^2 = 218.01; Chi^2 = 31.00, df = 4 (P <0.00001); I^2 = 87%
Test for overall effect: Z = 4.75 (P <0.00001)



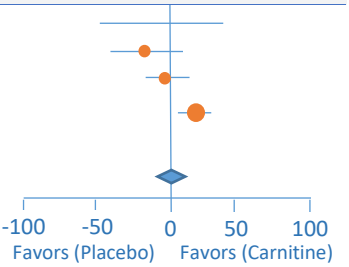
Change in Serum FBS Level

Four studies were included in the group analysis evaluating the effect of L-carnitine/carnitine-orotate in the glycemic control among patients with NAFLD. The test showed no significant difference between the experimental and the control group with a weighted mean difference of 2.31 (95% CI 11.6-15.69) and a *p* value of 0.73. The I^2 was 57% which represents moderate heterogeneity (see **Table 3**).

Table 3. Forest plot on the change of fasting blood sugar from baseline between treatment (carnitine) and control group

Study or Subgroup	Carnitine			Placebo			Weight	Mean Difference	Mean Difference
	Mean	SD	Total	Mean	SD	Total	(%)	IV, random, 95% CI	IV, random, 95% CI
Alavinejad, 2016	7	83.63	30	6	85	30	8.2	1.00[-41.67, 43.67]	
Bae, 2015	2.2	34.5	39	16.9	65.2	39	19.8	-14.70 [-37.85, 8.45]	
Hong, 2014	16.4	28	24	17.6	24.6	24	30.2	-1.20 [-16.11, 13.71]	
Malaguarnera, 2009	14.4	15.88	36	1.26	17.45	38	41.9	13.14 [5.54, 20.74]	
Total (95% CI)			**129**			**131**	**100**	**2.31 [-11.06, 15.69]**	

Heterogeneity: Tau^2 = 96.27; Chi^2 = 7.02, df = 3 (P = 0.07); I^2 = 57%
Test for overall effect: Z = 0.34 (P = 0.73)



Secondary Outcomes

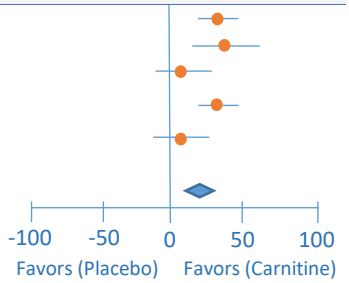
Change in Serum AST Levels

Grouped analysis of included trials evaluating change in serum AST levels among patients in the experimental group showed significant decrease from the baseline compared to placebo with a weighted mean difference of 17.49 (95% CI 7.61-27.36) and a *p* value of 0.003. There was substantial heterogeneity among the studies with an I^2 of 75% (see **Table 4**).

Table 4. Forest plot on the change of AST from baseline between treatment (carnitine) and control group

Study or Subgroup	Carnitine			Placebo			Weight	Mean Difference	Mean Difference
	Mean	SD	Total	Mean	SD	Total	(%)	IV, random, 95% CI	IV, random, 95% CI
Alavinejad, 2016	26.4	15. 3	30	0.8	11	30	25.60	25.60[18.86, 32.34]	
Bae, 2015	26.1	22.9	39	0.72	30.1	39	19.7	25.38 [13.51, 37.25]	
Hong, 2014	10.5	25.6	24	7.6	20.8	24	18.5	2.90 [-10.30, 16.10]	
Malaguarnera, 2009	71.7	22.9	36	46.1	24.94	38	20.6	25.60 [14.70, 36.50]	
Somi, 2014	15.6	36.34	40	13.1	32.26	40	16.8	2.50 [-12.56, 17.56]	
Total (95% CI)			**169**			**171**	**100**	**17.49 [7.61, 27.36]**	

Heterogeneity: Tau^2 = 92.26; Chi^2 = 16.00, df = 4 (P = 0.003); I^2 = 75%
Test for overall effect: Z = 3.47 (P = 0.0005)



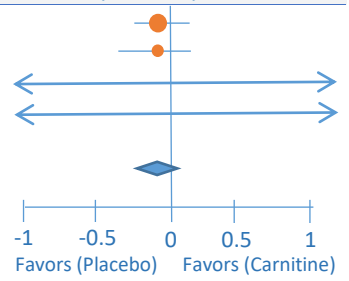
Change in Body Mass Index

Of the five studies, there were four RCTs that evaluated the use of L-carnitine in the improvement of anthropometric measurement including BMI, which is known to highly correlate with insulin resistance and NAFLD. In this analysis, no significant reduction was seen as noted by the mean difference of -0.10 (95% CI 0.30-0.10) and a *p* value of 0.31. The studies were deemed homogeneous with a Chi-square value of 0.40 and I^{2}of 0% (see **Table 5**).

Table 5. Forest plot on the change of BMI from baseline between treatment (carnitine) and control group

Study or Subgroup	Carnitine			Placebo			Weight	Mean Difference	Mean Difference
	Mean	SD	Total	Mean	SD	Total	(%)	IV, random, 95% CI	IV, random, 95% CI
Bae, 2015	0.14	0.58	39	0.25	0.25	39	69.9	-0.11[-0.35, 0.13]	
Hong, 2014	0.3	0.6	24	0.4	0.4	24	28.7	--0.10 [-0.47, 0.27]	
Malaguarnera, 2009	1.1	5.4	38	1.3	1.3	36	0.7	--0.20 [-2.59, 2.19]	
Somi, 2014	0.8	5.586	40	0.2	0.2	40	0.8	0.60 [-1.61, 2.81]	
Total (95% CI)			**141**			**139**	**100**	**-0.10 [-0.30, 0.10]**	

Heterogeneity: Tau^2 = 0.00; Chi^2 = 0.40, df = 3 (P = 0.94); I^2 = 0%
Test for overall effect: Z = 1.01 (P = 0.31)



Subgroup Analysis

A subgroup analysis was performed to assess whether results varied by the treatment dosage and duration of the intervention. We divided subgroups into treatment dosage comparing doses of the intervention between ≤500 mg/day and >500 mg/day. Treatment durations were divided into 90 and 180 days accordingly.

Change in Serum ALT Levels

The pooled analysis revealed that the significant reduction in ALT remained unaltered regardless of the treatment dose and duration. Between doses of >500 mg/day and ≤500 mg/day, ALT remained significantly reduced at a mean difference of 48.21 (*p* value 0.0001) and 24.49 (*p* value 0.01), respectively. In comparing subgroups based on treatment duration, improvement in serum ALT was still achieved with a weighted mean difference of 19.78 (*p* value of <0.00001) in 180 days. However, this was grossly less compared to the pooled analysis of three studies with treatment duration of 90 days with a weighted mean difference of 46.05 (*p* value of <0.00001) (see **Table 6**.)

Table 6. Subgroup analysis comparing the effects of L-carnitine on serum ALT level based on treatment dose and duration

	No. of Studies	Weighted Mean Difference (95% CI)	*p* value within group	*p* value of heterogeneity	I^2
Treatment Dose					
>500 mg/day	3	48.21 (29.96, 66.46)	0.00001	0.003	0.83
≤500 mg/day	2	24.49 (5.11, 43.88)	0.01	0.11	0.60
Treatment Duration					
90 days	3	46.05 (26.73, 65.36)	<0.00001	0.002	0.84
180 days	2	19.78 (12.35, 27.21)	<0.00001	0.52	0.00

Change in Serum FBS Level

The subgroup analysis revealed no significant FBS reduction across all treatment doses and durations except for the treatment duration of 180 days. However, this comparison was only demonstrated by one trial[7] (see **Table 7**).

Table 7. Subgroup analysis comparing the effects of L-carnitine on serum FBS level based on treatment dose and duration

	No. of Studies	Weighted Mean Difference (95% CI)	*p* value within group	*p* value of heterogeneity	I^2
Treatment Dose					
>500 mg/day	3	2.21 (-18.05, 22.47)	0.83	0.07	0.62
≤500 mg/day	1	-1.2 (-16.11, 13.71)	0.87	N/A	N/A
Treatment Duration					
90 days	3	-4.67 (-16.7, 7.36)	0.45	0.61	0.0
180 days	1	13.14 (5.55, 20.74)	0.0007	N/A	N/A

Change in Serum AST Level

In this analysis, AST was proven to be more significantly reduced with the use of L-carnitine at a dosage of >500 mg/day, having a weighted mean difference of 25.56 (20.39, 30.72) and a *p* value of <0.00001, compared to the use of carnitine at ≤500 mg/day (WMD: 2.73 (-7.20, 12.65), *p* value of 0.59. Furthermore, the use of L-carnitine for 90 days showed greater reduction in AST than treatment for 180 days. (see **Table 8**).

Table 8. Subgroup analysis comparing the effects of L-carnitine on serum AST level based on treatment dose and duration

	No. of Studies	Weighted Mean Difference (95% CI)	*p* value within group	*p* value of heterogeneity	I^2
Treatment Dose					
>500 mg/day	3	25.56 (20.39, 30.72)	<0.00001	1.0	0.0
≤500 mg/day	2	2.73 (-7.20, 12.65)	0.59	0.97	0.0
Treatment Duration					
90 days	3	18.75 (5.64, 31.86)	0.005	0.009	0.79
180 days	2	14.6 (-7.95, 37.26)	0.20	0.01	0.83

Safety

Of the five studies included in the analysis, not one study measured or evaluated safety or adverse events as an outcome of interest.

Discussion

Apart from diet and lifestyle modification, various pharmacologic interventions have already emerged and studied to assess their use in the management of non-alcoholic fatty liver disease. In this meta-analysis, we were able to present the effects of L-carnitine in several surrogate markers of liver integrity, including AST, ALT and other metabolic profiles including glycemic control (fasting blood sugar) and anthropometric index (BMI) among patients with NAFLD. Previous systematic reviews done by Rad et al.[12] and Abolfathi et al.[13] included different subgroups of patients including subjects with pure cardiac, thyroid or other liver disorders. Among the studies mentioned, no analysis has been performed to evaluate the effects of L-carnitine on glycemic control, i.e., serum fasting blood sugar. To the best of our knowledge, this is the first meta-analysis that assessed the clinical characteristics mentioned.

The synthesis of data pooled from several RCTs confirmed the beneficial effects of L-carnitine in the improvement of liver function based on the serum markers measured (AST, ALT). Furthermore, the subgroup analysis was able to emphasize its consistent use across all treatment doses and duration except for the reduction of AST which is better achieved with a dose of >500 mg/day. The oral supplementation of L-carnitine (1-6 gm) has been reported to only have a biological availability of from 5% to 18%. This limited bioavailability is associated with the metabolization of L-carnitine by gut microbiota prior to absorption.[14] Hence, this unique pharmacokinetic property might explain its requirement for higher dosage in reducing ALT and AST levels.

The liver is a major organ responsible for metabolizing several substances which may produce reactive oxygen species (ROS) promoting oxidative stress. In patients with NAFLD, there is impairment of mitochondrial β-oxidation of fatty acids due to the functional and structural alteration produced by the clinical disease itself. This state causes further accumulation of ROS, thereby resulting in more hepatic

damage. In this light, the essential role of L-carnitine in the transfer of the long-chain fatty acids inside the mitochondria for β-oxidation might be a reason for reducing ALT and AST levels, which are markers of liver integrity. On the other hand, deficiency of L-carnitine results in the reduction of fatty acid transportation to mitochondria and facilitates accumulation in the cytosol relating to the pathogenesis of insulin resistance and poor glycemic control.[15] In this study, we also assessed its effect on such parameter through comparison of fasting blood sugars and the result failed to show significant reduction as analyzed.

Furthermore, obesity has been highly associated with NAFLD. Steatosis or increase in intrahepatic triglyceride content is the hallmark feature of the disease. It occurs when there is imbalance on the rate of hepatic fatty acid uptake from plasma and its *de novo* synthesis. In this study, the role of L-carnitine was determined in improvement of anthropometric index through change in BMI. This presumption reverts back to the essential function of L-carnitine in mobilization of fatty acids. Unfortunately, no effect was demonstrated in the grouped analysis. Safety was one of the variables we aimed to look into; however, not one study included it in their analysis of data.

Conclusion

The use of either L-carnitine or carnitine-orotate complex demonstrated reduction of liver enzymes (AST, ALT) among patients with non-alcoholic fatty liver disease, although heterogeneity among groups remained to exist even after subgroup analysis based on treatment doses and durations. Moreover, the study also showed more significant AST reduction with higher doses (>500 mg/day) of the drug. This study has also proven that L-carnitine has no significant reduction on other metabolic profiles including body mass index as well as glycemic control through FBS monitoring, as determined by the statistical analysis.

Implications in Research

The emergence of L-carnitine as a potential therapeutic intervention among NAFLD patients and its inclusion in several clinical trials signifies its relevance in clinical practice. Hence, we highly recommend to increase the number of clinical subjects on future RCTs to formulate a more robust and reproducible evidence. Furthermore, direct outcomes including both histologic (the gold standard for the diagnosis of NAFLD) and imaging measures must be evaluated on top of the clinical parameters so that its use may well be translated into practice. Allowing more observation time and longer duration of treatment are also encouraged to further assess the effectiveness of the intervention.

Conflict of Interest

All PJG peer reviews are blinded. Dr. AD Salvaña, as co-author and at the same time member of PJG's editorial staff, inhibited herself from the review process and acceptance of this paper.

References

1. Weldman M, Friedman L, Brandt L. Sleisenger and Fordtran's Gastrointestinal and Liver Disease 10th Edition, Volume 1, Pathophysiology and Diagnosis. Saunders, 2016.
2. De Lusong MAA, Labio E, Daez L, Gloria V. Non-alcoholic fatty liver disease in the Philippines: Comparable with other nations? World J of Gastroenterol. 2008 Feb 14; 14(6):913-917.
3. Day CP, James OFW. Steatohepatitis: a tale of two "hits"? Gastroenterology. 1998; 114:842-845.
4. Socha P, Horvath A, Vajro P, Dziechciarz P, Dhawan A, Szajewska H.; Pharmacological interventions for nonalcoholic fatty liver dsease in adults and in children: A systematic review. Journal of Pediatric Gastroenterology and Nutrition. May 2009; volume 48, issue 5:587-596.
5. Romano M, Vacante M, Cristaldi E, Colonna V, Gargante MP, Cammalleri L, Malaguarnera M. L-carnitine treatment reduces steatosis in patients with chronic hepatitis C treated with alpha-interferon and ribavirin. Dig Dis Sci. 2008; 53:1114-1121.
6. Molfino A. Caloric restriction and L-carnitine administration improves insulin sensitivity in patients with impaired glucose metabolism; Journal of Parenteral and Enteral Nutrition. 2010; 34(3):295-299.
7. Malaguarnera MM, Gargante MP, Russo C, Antic T, Vacante M, Malaguarnera M, Avitabile T, Li Volti G, Galvano F. L-carnitine supplementation to diet: A new tool in treatment of nonalcoholic steatohepatitis--a randomized and controlled clinical trial. Am J Gastroenterol. 2010 Jun; 105(6):1338-45. doi: 10.1038/ajg.2009.719.
8. Hong ES, Kim EK, Kang SM, Khang AR, Choi SH, Park KS, Jang HC, Lim S. Effect of carnitine-orotate complex on glucose metabolism and fatty liver: A double-blind, placebo controlled study. J Gastroenterol Hepatol. 2014; 29(7):1449-1457. doi: 10.1111/jgh.12536.

9. Bae JC, Lee WY, Yoon KH, Park JY, Son HS, Han KA, et al. Improvement of nonalcoholic fatty liver disease with carnitine-orotate complex in type 2 diabetes (CORONA): A randomized controlled trial. Diabetes Care. 2015; 38:1245-1252. doi:10.2337/dc14-2852, 2015.
10. Alavinejad P, Zakerkish M, Hajiani E, Hashemi SJ, Chobineh M, Moghaddam EK. Evaluation of L-carnitine efficay in the treatment of non-alcoholic fatty liver disease among diabetic patients: A randomized double blind pilot study. Journal of GHR. 2016 Oct; 2191-2195. doi: 10.17554/j.issn.2224-3992.2016.05.662, 2015.
11. Somi MH, Fatahi E, Panahi J, Havasian MR, Judaki A. The prescription of L-carnitine in the treatment of non-alcoholic fatty liver disease: A randomized and controlled clinical trial. Indian Journal of Research in Pharmacy and Biochemistry. July-August 2014; 1325-1331.
12. Rad EY, Eslampour E, Falahi E, Mardani M, Hekmatdoost A, Asbaghi O, Saboori S. Effects of carnitine supplementation on liver aminotransferase enzymes: A systematic review and meta-analysis of randomized controlled clinical trials. Indian Journal of Gastroenterology. 2019 Dec; 38(6):470-479. doi: 10.1007/s12664-019-00983-2. Epub 2019 Dec 11.
13. Abolfathi M, Mohd-Yusof BN, Hanipah ZN, Redzwan SM, Yusof LM, Khosroshahi MZ. The effects of carnitine supplementation on clinical characteristics of patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. Complementary Therapies in Medicine. 2020 January; volume 48. doi: https://doi.org/10.1016/j.ctim.2019.102273.
14. Evans AM, Fornasini G, Pharmacokinetics of L-carnitine. Clinical Pharmacology. 2003; 42(11):941-967. doi: 10.2165/00003088-200342110-00002.
15. Vidal-Casariego A, Burgos-Pelaez R, Martinez-Faedo C, Calvo-Gracia F, Valero-Zanuy MA, Luengo-Perez LM, Cuerda-Compes C. Metabolic effects of L-carnitine on type 2 diabetes mellitus: A systemic review and meta-analysis. Exp Clin Endocrinol Diabetes. 2013 Apr; 121(4):234-238. doi: 10.1055/s-0033-1333688. Epub 2013 Feb 21.

The Yield of Combined Multichannel Intraluminal Impedance and pH Monitoring (MII-pH Monitoring) among Suspected Refractory Gastroesophageal Reflux Disease: A St. Luke's Medical Center Experience

Navarro MJH
De Ocampo S

Institute of Digestive and Liver Diseases
St. Luke's Medical Center-Quezon City
Philippines

Correspondence:
Marc Julius Navarro
marcnavarromd@gmail.com

Accepted for publication:
January 2021

Abstract

Background: Multichannel intraluminal impedance and pH monitoring (MII-pH monitoring) allows accurate recording of gastroesophageal reflux at all pH levels. In the Philippines, there is no local data on the yield of these tests in the investigation of patients with refractory gastroesophageal reflux disease (GERD). *Objective:* The objective of this study is to determine the phenotypic presentation and diagnostic yield of MII-pH monitoring among Filipino patients with suspected refractory GERD. *Methodology:* This is a retrospective, cross-sectional study involving suspected refractory GERD patients who underwent MII-pH monitoring. *Results:* A total of 50 subjects were included. Most presented with typical symptoms of reflux, such as heartburn (44%) and regurgitation (40%). Most common study indication was refractoriness to proton pump inhibitors (PPI) (86%). Patients under phenotypic group of persistent acid reflux (abnormal acid exposure with positive symptom association) revealed prevalence of 20%. Majority were males, with esophagitis, and normal esophageal motor function. Patients under the phenotypic group of hypersensitive esophagus (normal acid exposure with positive symptom association), revealed a prevalence of 18%. Patients were mostly female who presented without esophagitis, 56% with normal manometric findings. Patients under the group of functional heartburn (normal acid exposure with negative symptom association) revealed prevalence of 62%. Majority were female, with normal manometric findings and without esophagitis. *Conclusion:* Among refractory GERD patients, functional heartburn was the most common diagnosis using MII-pH monitoring, showing higher prevalence compared to previous studies. MII-pH monitoring is helpful in the work-up of refractory GERD patients as it can redirect the course of management.

Keywords: retrospective, cross-sectional, MII-pH monitoring, refractory GERD

Introduction

Gastroesophageal reflux disease (GERD) is a common disorder with a high incidence rate of 10% to 20% of adults in the Western population, occurring at least once a week,[1,2] and is lower but increasing dramatically in Asian countries,[3,4] including Japan and the Philippines[5]. The Montreal worldwide consensus in 2005 defined GERD as ''a condition which develops when the reflux of stomach contents causes troublesome symptoms and/or complications,''[6] a definition that serves to differentiate normal individuals with occasional symptomatic reflux from patients with either life-altering symptoms (including extra-esophageal complaints) or asymptomatic reflux that produces mucosal injury and risk for neoplasia. Heartburn, estimated to occur daily in 7% of the U.S. population, is the most common symptom of GERD.[7] In addition to heartburn, regurgitation and difficulty swallowing are typical symptoms; while chronic cough, asthma, and laryngitis are atypical or extra-esophageal manifestations. While the clinical symptoms of heartburn and regurgitation are the most reliable for making a presumptive diagnosis based on history alone,

these are not as sensitive as most believe.

Symptom-based diagnosis of GERD is a problem, as demonstrated by Dent and colleagues, who found typical symptoms in only 49% of the patients with proven GERD.[8] Since the time that Alison described reflux esophagitis in 1946, the armamentarium of clinically available tools to diagnose GERD has become more sophisticated, as new technologies and advances have been introduced into the research and clinical arena.[6] This has been propelled by the necessity to probe into the nature of esophageal symptoms in the absence of endoscopic evidence of esophageal mucosal lesions and more recently to understand the causes of persistent esophageal symptoms among GERD patients already on potent acid suppression treatment.

In the evaluation of refractory reflux symptoms, the first step is assessing drug compliance and lifestyle modification. The second step is to increase the dose of the PPI. The third step involves investigation as to whether the GERD is due to a structural or functional cause. Structural evaluation includes endoscopy with biopsy and barium esophagogram, while functional assessment involves manometry, ambulatory pH-impedance monitoring and gastric scintigraphy.[9]

Esophageal manometry is a test wherein intraluminal sensors are positioned in the esophagus to quantify the contractile characteristics of the esophagus and segregate it into functional regions.[10] No manometric findings have adequate sensitivity and specificity to diagnose GERD. However, the test is useful in correctly positioning pH electrodes.

The current gold standard for gastroesophageal reflux testing is ambulatory pH monitoring, a method based on detection of changes in acid content in the esophageal lumen.[11] Johnson and DeMeester introduced ambulatory pH monitoring for the detection of reflux episodes in 1975. The DeMeester scoring system for 24-hour pH monitoring has 90.3% sensitivity and 90.0% specificity to diagnose GERD.[12] Since its introduction, pH-metry has become a commonly used technique for the evaluation of patients with symptoms suggestive of GERD and has been established as the gold standard for documenting gastroesophageal reflux. A fall below pH 4 in esophageal pH has been conventionally taken to indicate acid reflux. Although pH 4 tends to underestimate acid reflux, it is still considered the most appropriate threshold for clinical use.[13]

Intraluminal impedance monitoring is a method to assess intraluminal bolus transit without use of fluoroscopy.[14] It detects retrograde bolus movement and can determine the nature and proximal extent of reflux.[13]

MII-pH has been shown to allow accurate recording of gastroesophageal reflux at all pH levels and is emerging as a useful tool to study both acid and non-acid reflux.[15] The combined test increased the sensitivity of reflux monitoring to close to 90%.[3] The principles of pH impedance were first described in 1990 by Silny et al.[16] and depend on changes in resistance to alternating current (i.e., impedance) between two metal electrodes (impedance measuring segment) produced by the presence of bolus inside the esophageal lumen. MII-pH is able to detect bolus movement in the esophagus, both in the oral and aboral direction, and thus enables measurement of and distinction between swallows and reflux. Since MII registers retrograde flow of gastric contents into the esophagus in a pH-independent fashion, combining the technique with pH-metry allows the recognition of non-acid as well as acid reflux. Moreover, MII-pH monitoring provides detailed characterization of the reflux episode, including determination of the composition (gas, liquid, or mixed) and the height reached by the refluxate.[17]

In the Philippines, only our institution offers combined pH-impedance study and high-resolution esophageal manometry. To date, there is no local data on the yield of these tests in the investigation of the patients with suspected refractory GERD and its exact prevalence in the country, as well as on the phenotypic profiles of Filipino patients being referred for esophageal reflux monitoring.

The main objective of this study is to determine the phenotypic presentation and diagnostic yield of combined MII-pH monitoring among Filipino patients with suspected refractory GERD at St. Luke's Medical Center. Specifically, the study aims to (1) describe the demographic and clinical profiles (such as symptoms, EGD findings, response to PPI and manometric findings) of patients referred for MII-pH monitoring; (2) describe the result of combined MII-pH monitoring among patients with suspected refractory GERD; (3) describe patients' characteristics according to phenotypic group: persistent acid reflux, hypersensitive esophagus, and functional heartburn; and (4) determine the proportion of GERD, non-erosive reflux disease (NERD),

hypersensitive esophagus, and functional heartburn among patients with suspected refractory GERD.

Methodology

Study Design

This is a retrospective, cross-sectional chart review of adult patients with suspected refractory GERD who underwent combined MII-pH monitoring at St. Luke's Medical Center. Summary of study methodology is shown in **Figure 1**.

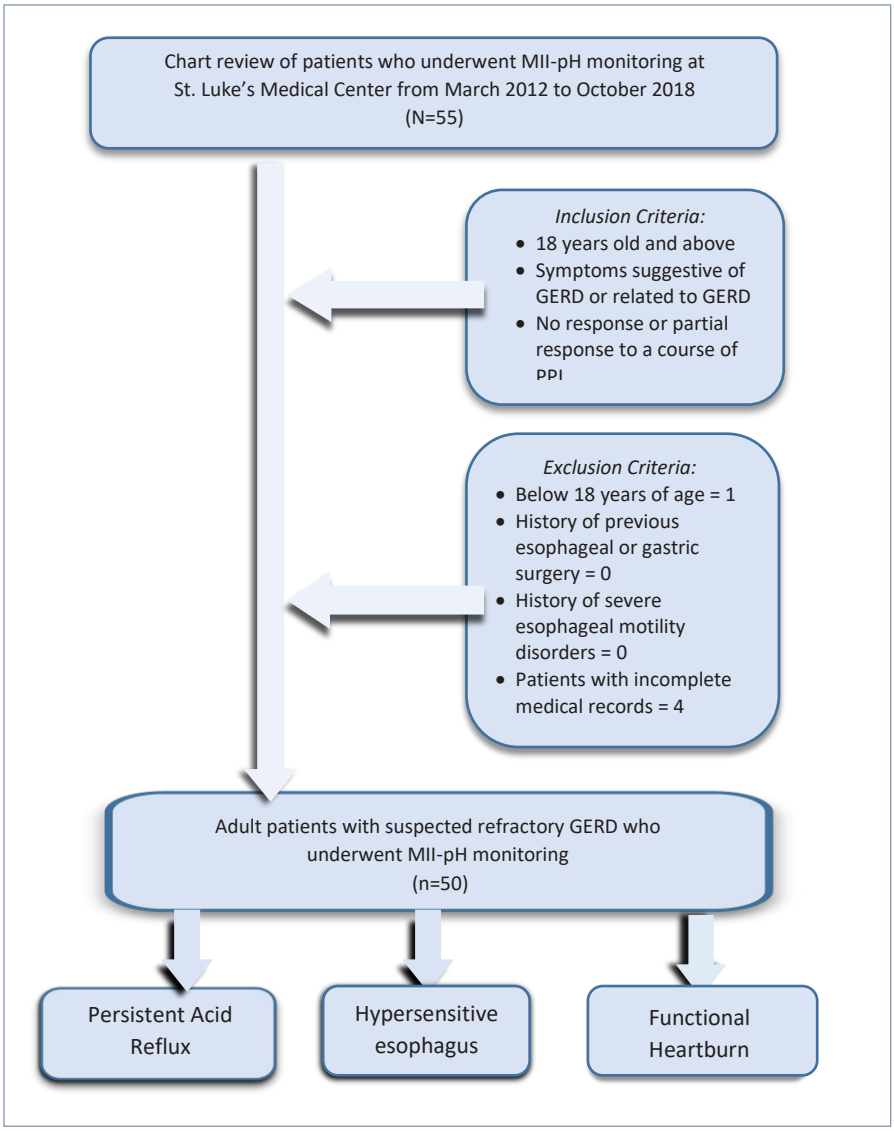


Figure 1. Flow diagram of study method

Patients aged ≥18 years old with symptoms suggestive or related to GERD with partial or no response to a course of PPI and referred for MII-pH monitoring from March 2012 to October 2018 were included in this study. The exclusion criteria were the following: history of previous esophageal or gastric surgery and severe esophageal motility disorders. Those with incomplete medical records were not included.

Study Setting and Time Coverage

This study was conducted at St. Luke's Medical Center (SLMC), a private hospital in Quezon City,

involving patients' records from March 2012 to October 2019.

Sampling and Sample Size

The researchers reviewed the chart of all patients with suspected refractory GERD referred for MII-pH monitoring at St. Luke's Medical Center for a period of six years between March 2012 and October 2018.

Outcomes Measured

The outcomes measured in this study were the proportions of patients with persistent acid reflux (i.e., patients with abnormal acid exposure with positive symptom association), those with hypersensitive esophagus (i.e., normal acid exposure with positive symptom association), and functional heartburn (i.e., normal acid exposure with negative symptom association).

Data Collection

Subject demographics (age, gender, height) and symptoms were gathered from data records accomplished prior to manometry and MII-pH. All subjects underwent esophagogastroduodenoscopy prior to the procedure. MII-pH study was performed either on or off acid suppression therapy depending on the indication of the study.

Ethical Considerations

The clinical protocol and all relevant documents were reviewed and approved by the SLMC Institutional Ethics Review Committee.

All study data were recorded and investigators were responsible for data protection and confidentiality. Anonymity of patient records was ensured. Each patient's document was coded and did not contain any identifying information. Investigators were also responsible for data integrity such as accuracy, completeness, legibility, and originality. All collected data were stored at the institution database and will be shredded one year after publication.

Data Management and Analysis

Impedance-pH Monitor

Esophageal impedance pH monitoring was performed using Digitrapper pH-Z system (Given Imaging, Sierra Scientific Instruments, Los Angeles, California), an ambulatory system, which includes a portable data-logger with impedance-pH catheters containing single-channel pH at 0 cm and 8 impedance electrodes (-3, -1, 1, 3, 5, 9, 11, 13). The impedance amplifier delivers AC voltage in a range of 1-2 kHz with resulting current flow variations in response to intraluminal impedance changes. All subjects underwent high-resolution esophageal manometry for LES localization and assessment of esophageal motor function. The impedance-pH catheter was inserted transnasally after topical anesthesia with 2% lidocaine and positioned in the esophageal body to record pH at 5 cm above the upper border of the manometrically determined LES. Subjects were advised to do the following prior to the procedure: fast for six hours, stop PPIs for a week, H2RAs for 48 hours, and antacids for 24 hours. Subjects were encouraged to maintain normal daily activities, eat usual meals and remain upright during the day. A diary was provided to record meal times, posture changes and symptoms. The study duration was 24 hours. Tracings were uploaded into a computer and displayed on a single screen for computer-assisted manual analysis (Accuview software).

Data Analysis

Demographic data were extracted from patient records. Analysis included identification, enumeration, and characterization of individual reflux events and esophageal exposure to volume and acid. The analysis of pH monitoring included the following parameters:

1. Total number of reflux episodes;
2. Total number of pathological episodes of reflux (pH <4 for more than five minutes);
3. Percentage of reflux time compared with total monitoring time (total reflux time);
4. Percentage of reflux time compared with the time while the patient was in the upright position (reflux time in erect position);
5. Percentage of reflux time compared with the time the patient was lying down (reflux time in supine position);
6. Johnson and DeMeester composite scoring system score[24] (based on the above-mentioned parameters).

Normal values of the above-mentioned parameters are the following:

1. Total reflux time up to 5%;

2. Reflux time in erect position up to 8%;
3. Reflux time in supine position up to 4%;
4. Johnson and deMeester score up to 14.72 (>6 or 7 up to 10).

The symptom index (SI) and the symptom-association probability (SAP) values were determined by the analysis software. SAP estimates the likelihood that symptoms are due to reflux by examining two-minute segments of the event and pH recorders. The numbers of two-minute segments with and without symptoms, and with and without reflux were tabulated. Fisher's exact test was performed by the software and SAP was calculated. A SAP greater than or equal to 95% is positive.

Statistical Analysis

Descriptive statistics for categorical variables were reported as frequency and percentage, whereas continuous variables were reported as mean and standard deviation, or median and range, as appropriate.

Results

This retrospective descriptive study was conducted to determine the phenotypic presentation and diagnostic yield of combined multichannel intraluminal impedance-pH monitoring among Filipino patients with suspected refractory GERD at St. Luke's Medical Center. The demographic and clinical profile is shown in **Table 1.**

Table 1. Demographic and clinical profile of patients (N=50)

Mean age in years	**mean (range)**
Male	44.66 (23-68)
Female	44.94 (24-65)
Height in cm, mean (range)	**156.54 (142.24-175.26)**
Typical symptoms	**n (%)**
Heartburn	22 (44)
Regurgitation	20 (40)
Atypical symptoms	**n (%)**
Non-cardiac chest pain	9 (18)
Chronic cough	2 (4)
Dysphagia	3 (6)
EGD Findings	**n (%)**
Negative esophagitis	38 (76)
Esophagitis	
A	4 (8)
B	0 (0)
C	0 (0)
D	0 (0)
Barrett's esophagus	0 (0)
Not known	8 (16)
Response to PPI	**n (%)**
Non-responsive	43 (86)
Partial response	7 (14)
Responsive	0 (0)
Therapy while on MII-pH monitoring	**n (%)**
On	8 (16)
Off	42 (84)
Manometric findings	**n (%)**
Normal	26 (52)
Hypotensive LES and peristalsis	9 (18)
Weak peristalsis with large break	4 (8)
Weak peristalsis with small break	6 (12)
Frequent failed peristalsis	4 (8)

Fifty patients were included in this study. Majority of the patients were in their 4th to 5th decade of life, with a mean age of 44.66 years. The mean height was 156.54 cm (ranging from 142.24 to 175.26 cm). Most of the patients presented with typical symptoms of reflux, specifically heartburn (44.00%) and regurgitation (40.00%), followed by atypical symptoms such as non-cardiac chest pain (18.00%), dysphagia (6.00%) and chronic cough (4.00%). In terms of EGD findings, 76% of patients had no signs of esophagitis upon examination and 8% presented with mild esophagitis (LA Grade A). The most common indication for referral was non-response to proton-pump inhibitors (86.00%). Most patients were off therapy (84.00%) during MII-pH monitoring. Manometric findings most commonly revealed normal esophageal motor function (52%) followed by hypotensive LES and peristalsis (18%), weak peristalsis with small break (12%), weak peristalsis with large break (8%), and frequent failed peristalsis (8%) (**Table 2**).

Table 2. Combined MII-pH summary of findings of included patients (N=50)

DeMeester score (n, %)	n (%)
<14.72	40 (80)
>14.72	10 (20)
Total number of reflux cases	mean (range)
Acid	10.58 (0-88)
Non-acid	11.52 (0-82)
% Total reflux time, minutes	n (%)
<5	47 (94)
>5	3 (6)
% reflux in supine	n (%)
<4	42 (84)
>4	8 (16)
% reflux in erect	n (%)
<8	49 (98)
>8	1 (2)

Of the 50 patients included in the study, 80% exhibited normal DeMeester score of <14.72. Mean total number of acid reflux episodes was 10.58 (ranging from zero to 88), and mean number of non-acid reflux episodes was 11.52 (ranging from zero to 82). For the percentage of reflux time, total acid exposure time was 94%, 98% during upright position and 84% during supine position. These were within physiologic limits in majority of patients. Excessive esophageal acid exposure was noted highest at supine position (eight of 50 patients, 16%) followed by total reflux time (6%) and during upright position (2%).

Table 3 shows the patients' characteristics according to phenotype. It is of note that patients with persistent acid reflux phenotype (i.e., abnormal acid exposure with positive symptom association) revealed overall prevalence of 20% (10 out of 50 patients). The mean age of patients in this group was 45, majority of whom were males (60%), around 40% presented with esophagitis on EGD, alongside with normal manometric findings (60%), and positive SI (60%) / positive SAP (100%).

Patients of the hypersensitive esophagus phenotype of (i.e., normal acid exposure with positive symptom association) revealed overall prevalence of 18% (nine out of 50 patients). Patients involved were noted to be on their 4th to 5th decade of life, majority were females (77.78%), all of whom presented without esophagitis on EGD (100%), 55.56% with normal esophageal motor function on manometry, and all patients had positive SAP (100%)/negative SI (100%).

On the other hand, patients under the group of functional heartburn (i.e., normal acid exposure with negative symptom association) revealed overall prevalence of 62% (31 out of 50 patients). Most patients were in their 4th to 5th decade of life, majority were

females (52.94%), and presented with normal manometric findings (48.39%). All patients presented without esophagitis on EGD (100.00%) with negative SAP (100%)/negative SI (100%). In this study, overall prevalence of functional heartburn was higher compared to the reported 21% overall prevalence rate of Yamasaki, et.al.

Table 2. Patients' characteristics according to phenotype: persistent acid reflux, hypertensive esophagus, and functional heartburn

Characteristics	Persistent Acid Reflux (n=10)	Hypertensive Esophagus (n=9)	Functional heartburn (n=31)
Overall prevalence, n (%)	10 (20)	9 (18)	31 (62)
Age in years, mean age (range)	45 (30-68)	46 (31-64)	45 (23-65)
Male gender, n (%)	6 (60)	2 (22.22)	13 (41.94)
Esophagitis, n (%)	4 (40)	0 (0)	0 (0)
Total number of reflux, mean (range)	35 (3-88)	26 (3-50)	16 (0-82)
Symptom Index (SI), mean (range)	61 (0-100)	28 (0-100)	4 (0-49)
Positive SI, n (%)	6 (60)	O (0)	0 (0)
Symptom Association Probability (SAP), mean (range)	97 (91-100)	97 (91-99.7)	9 (0-88.1)
Positive SAP, n (%)	10 (100)	9 (100)	0 (0)
Manometric Findings, n (%)			
Normal	6 (60)	5 (55.56)	15 (48.39)
Hypotensive LES and peristalsis	4 (40)	0 (0)	5 (16.13)
Weak peristalsis with large break	0 (0)	0 (0)	4 (12.90)
Weak peristalsis with small break	0 (0)	2 (22.22)	4 (12.90)
Frequent failed peristalsis	0 (0)	2 (22.22)	3 (9.68)

Discussion

Acid suppression with PPI is the mainstay of treatment for GERD. However, despite treatment, there is still an estimated 10-40% of patients who fail to respond symptomatically. Although the definition for refractory GERD remains controversial, most authors have defined it as having poor response to PPI with <50% improvement in the chief complaint after at least 12 weeks of PPI therapy.[1]

In clinical practice, PPI failure has become the most common presentation of GERD-related symptoms. The underlying mechanisms for PPI failure include timing adherence and compliance, persistent esophageal acid exposure, and reflux hypersensitivity.

Intraluminal pH monitoring is now a widely accepted clinical tool for investigating refractory GERD. It is indicated in patients with typical GERD symptoms who fail four weeks of PPI therapy, those with atypical symptoms who fail six to eight weeks of PPI therapy, those being considered for endoscopic or surgical reflux therapy, and those who have undergone endoscopic or surgical reflux therapy and who continue to have GERD symptoms. In addition, the use of combined impedance-pH monitoring enables the detection of not only acid but also all types of reflux, and has been shown to substantially increase the diagnostic yield compared to pH alone.[2] In one of the largest studies which correlated impedance patterns and symptom occurrence in PPI non-responders, the impedance reflux profile in this subset of patients was heterogeneous and the majority of reflux events were not associated with symptoms. Thus, the treatment of patients with PPI failure should focus beyond reflux, such as visceral hypersensitivity and hypervigilance.[3]

Figure 2 shows the proportion of patients according to phenotypic group. Among suspected refractory GERD patients referred to our institution, diagnosis through MII-pH study revealed a majority of patients having functional heartburn (62%), which was higher compared

to the reported overall prevalence from other studies, followed by persistent acid reflux (20%) and hypersensitive esophagus (18%).

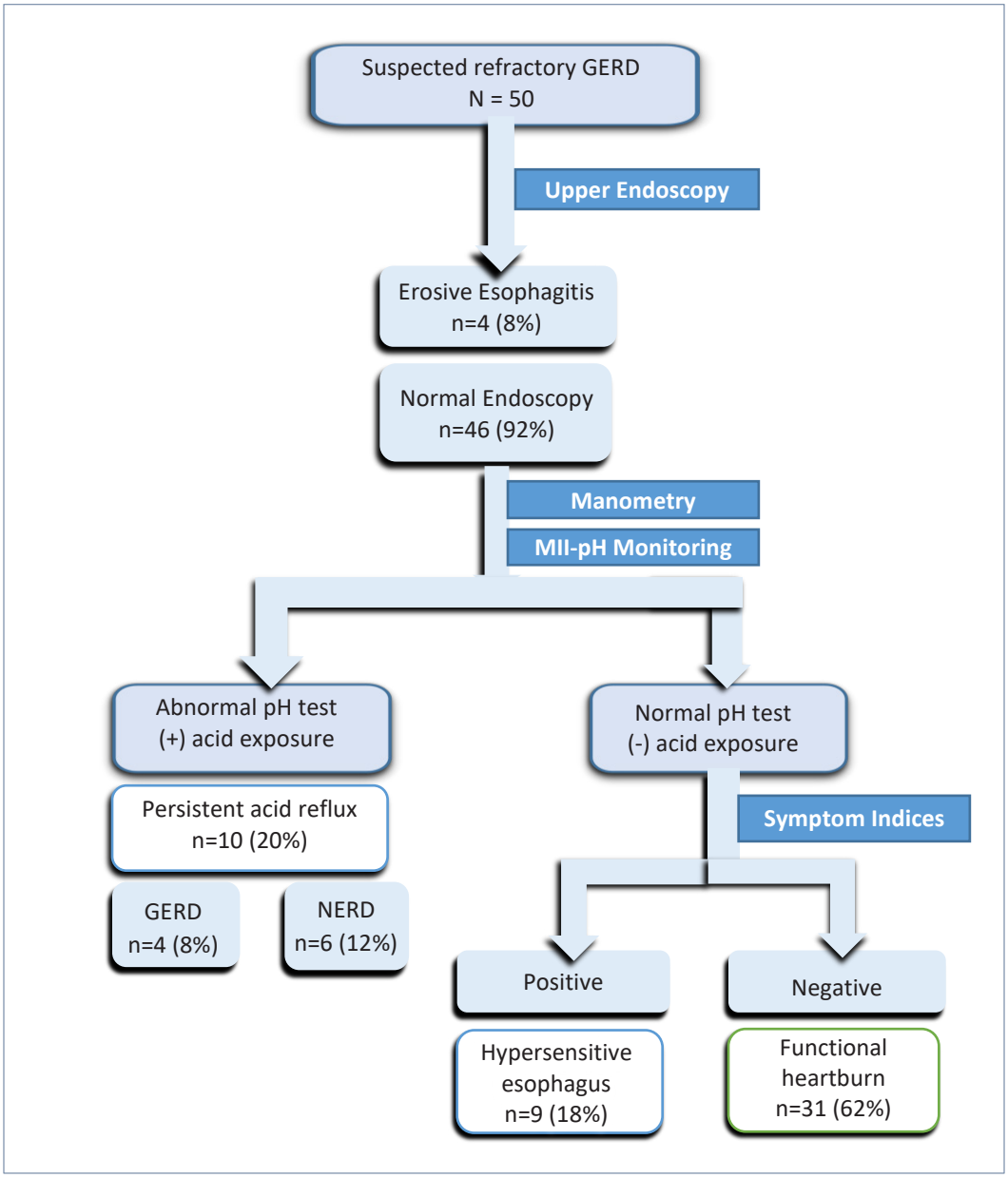


Figure 2. Proportion of patients according to phenotypic group

It can be noted that only 4 out of 50 patients (8.00%) revealed true refractory gastroesophageal reflux disease and 31 out of 50 patients (62.00%) with suspected refractory GERD belongs to the phenotypic group of functional heartburn.

In conclusion, MII-pH monitoring is helpful in the work-up of patients with suspected refractory GERD as it will redirect the course of management.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Kennedy T, Jones R. The prevalence of gastro-oesophageal reflux symptoms in a UK population and the consultation behaviour of patients with these symptoms. Aliment Pharmacol Ther. 2000; 14:1589-1594.
2. Locke GR 3rd, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ 3rd. Prevalence and clinical spectrum of gastroesophageal reflux: A population-based study in Olmsted County, Minnesota. Gastroenterology. 1997; 112:1448-1456.
3. Moayyedi P, Talley NJ. Gastroesophageal reflux disease. Lancet. 2006; 367:2086–100.
4. FujiwaraY, ArakawaT. Epidemiology and clinical characteristics of GERD in the Japanese population. J Gastroenterol. 2009; 44:518–34.
5. Fujiwara Y, Takahashi S, Arakawa T, et al. A 2008 questionnaire-based survey of gastroesophageal reflux disease and related diseases by physicians in East Asian countries. Digestion. 2009; 80:119-128.
6. Vakil N, van Zanten SV, Kahrilas P, et al. The Montreal definition and classification of gastroesophageal reflux disease. Am J Gastroenterol. 2006; 101:1900-1920.
7. Kahrilas PJ. Review article: gastro-oesophageal reflux disease as a functional gastrointestinal disorder. Aliment Pharmacol Ther. 2004; 20:50-55.
8. Johnson LF, Demeester TR. Twenty-four-hour pH monitoring of the distal esophagus. A quantitative measure of gastroesophageal reflux. Am J Gastroenterol. 1974; 62(4):325-32.
9. Subramanian C and Triadafilopoulos G. Refractory gastroesophageal reflux disease. Gastroenterology Report. 2014; 1-13.
10. Feldman M, Friedman L, Brandt L. Sleisenger and Fordham Gastrointestinal and Liver Disease 10th edition. 2014.
11. DeVault KR, Castell DO. Updated guidelines for the diagnosis and treatment of gastroesophageal reflux disease. Am J Gastroenterol. 1999; 94:1434-1442.
12. Johnson LF, DeMeester TR. Development of the 24-hour intraesophageal pH monitoring composite score system. J Clin Gastroenterol. 1986; 8:52-58.
13. Weusten BLAM, Roelofs JMM, Akkermans LMA, et al. Objective determination of pH thresholds in the analysis of 24h oesophageal pH monitoring. Eur J Clin Invest. 1996; 26:151-158.
14. Smout AJPM. Ambulatory monitoring of esophageal pH and pressure. In: Castell DO, Richter JE, editors. The Esophagus. 3rd edition. Philadelphia: Lippincott Williams & Wilkins. 1999; 119-133.
15. Vela MF, Camacho-Lobato L, Srinivasan R, Tutuian R, Katz P, Castell D. Intraesophageal impedance and pH measurement of acid and nonacid reflux: Effect of omeprazole. Gastroenterology. 2001; 120:1599-1606.
16. Silny J. Intraluminal multiple electric impedance procedure for measurement of gastrointestinal motility. J Gastrointest Motil. 1991; 3:151-162.
17. Shay S, Bomeli S, Richter J. Multichannel intraluminal impedance accurately detects fasting, recumbent reflux events and their clearing. Am J Physiol Gastrointest Liver Physiol. 2002; 283:G376-G383.

Analysis of Predictive Factors for R0 Resection, Bleeding and Recurrence of Colorectal Adenomas after Endoscopic Mucosal Resection

Aguila EJT
Co JT
Gopez-Cervantes JL
Cabral-Prodigalidad PAI
Caburnay AC
Lontok MADC

Institute of Digestive & Liver Diseases
St. Luke's Medical Center-Global City
Philippines

Correspondence:
Dr. Enrik John T. Aguila
enrikaguila@gmail.com

Accepted for publication:
January 2021

Abstract

Background: Larger colonic polyps require advanced resection techniques such as endoscopic mucosal resection (EMR) for safe and effective removal. There is a steady accumulation of scientific evidence with regard the technical aspects and long-term outcomes of colonic EMR compared with surgery. *Objective:* This study aimed to identify and analyze different factors predictive of clinical outcomes for patients undergoing EMR of colorectal lesions. *Methods:* This is a retrospective cohort study on all patients who underwent colorectal EMR from January 2015 to December 2018. The diagnostic yield of Japan NBI Expert Team (JNET) classification and clinical outcomes, namely, R0 resection, complications and recurrence of lesions, were studied. *Results:* Two hundred eighty-two patients were studied. The R0 resection rate was 96.3% (n=231) for lesions resected *en bloc*; 15.2% (n=43) presented with a complication, most commonly presenting as intra-procedural bleeding (n=36, 12.8%); and 10.7% (n=11) had recurrence post-EMR on surveillance colonoscopy. Main predictors of recurrence include a non-granular morphology of a resected polyp (cOR 2.621 [95% CI 1.0-6.84]) and piecemeal resection (cOR 2.306 [95% CI 1.06-5.04]). A larger lesion size of >20 mm was associated with both positive resection margin and post-EMR complications. The JNET classification exhibited good sensitivity for Type 1 (71.8%) and Type 2A (91.9%) and good specificity for Type 1 (96.9%) and Type 2B (95.5%). Accuracy was high for JNET Types 1 (91.02%), 2A (80.24%), and 2B (89.22%). *Conclusions:* EMR is an important advancement in the field of therapeutic endoscopy with good clinical outcomes. The JNET classification has a high diagnostic accuracy rate; hence is a good endoscopic tool for characterization of lesions.

Keywords: EMR, endoscopic mucosal resection, colorectal polyp, adenoma, JNET classification

Introduction

Colorectal cancer is the fourth most common cancer in the Philippines.[1] It is considered the third most deadly and fourth most commonly diagnosed cancer worldwide.[2] Nevertheless, deaths caused by colon cancer have been dropping steadily due to increased efforts in colon cancer screening that allows detection and removal of pre-cancerous and early malignant lesions, including endoscopic mucosal resection (EMR).[3]

EMR is a minimally invasive technique which has become the primary treatment of large (>10mm) laterally spreading lesions (LSLs) and polyps in the colon.[3] It is both diagnostic and therapeutic and enables complete removal and histologic assessment of the lesion. It is cost effective with high success rate, lower morbidity and mortality, and shorter length of hospital stay when compared to surgery. Due to these advantages, it is suggested that EMR should be considered as the first-line treatment for patients with colorectal lesions suspicious for neoplasia.

Different lesion and procedural factors have been reported to predict clinical outcomes and influence choice of resection strategy and endoscopic follow-up.[4] R0 resection, defined as removal of polyp with histologically assessed clear margins, is about 84% for

lesions <20 mm and 50% for lesions >20 mm, if removed via EMR.[5]

While EMR of colorectal neoplasms has been proven feasible and safe, it is associated with a small incidence of procedure-related complications such as bleeding (1-18%) and perforation (0.09-3.1%).[6-7] Literature has shown that certain patient and procedural factors could predict risk of bleeding and perforation.

Adenoma recurrence post-EMR is a major limitation in 10-55% of post-EMR patients.[8] Likewise, the necessity for strict endoscopic surveillance remains a significant challenge. Current guidelines recommend first follow-up colonoscopy at four to six months and a second colonoscopy at a subsequent interval after removal of adenomas through piecemeal EMR.[9] Retrospective studies showed several risk factors contributing to adenoma recurrence after EMR, which includes age >65 years, lesion size >30 mm, localization in the right-sided colon, non-pedunculated morphology, resection in piecemeal technique and tubular-villous histological features.[4] Recognition of these risk factors for tumor recurrence would aid us in predicting recurrence risk which may considerably reduce costs on colon cancer surveillance.

Magnification using narrow band imaging (NBI), which characterizes surface and vascular pattern, is a reliable method for differentiating neoplastic from non-neoplastic colorectal lesions.[10] The Japan NBI Expert Team (JNET) classification developed in 2014 is a proposed system considered to be useful for both expert and non-expert endoscopists in few validation studies.

This study determined the predictive factors for R0 resection, bleeding, and recurrence of colorectal adenomas after colonic EMR. In addition, this study determined the R0 resection rate, recurrence rate and complication rate (bleeding and perforation) of EMR in the institution where this research was done. The study determined the accuracy of the JNET classification in differentiating neoplastic from non-neoplastic lesions.

Methods

Subjects

A retrospective cohort study was conducted on adult patients aged 19 years and above who underwent colonic EMR with Olympus 190, 260 and 290 series high-definition colonoscopes at the Institute of Digestive and Liver Diseases of the St. Luke's Medical Center Global City, Philippines, within a four-year period from January 2015 to December 2018. Patients without existing medical records and colonoscopy or histopathology reports were excluded. Data on each patient for both their initial and surveillance colonoscopies were obtained and verified using hospital records and review of colonoscopy images and videos. Patient data included age of patient, gender, and admission. Data on lesion characterization included size, localization, morphology, JNET classification, submucosal fibrosis, histopathology, cauterized margins (R0 resection rate), and histopathology size of polyp. Procedural data included colonoscope used, technique of resection, adjunctive therapy used, duration of procedure, intra-procedural bleeding, delayed bleeding, perforation, bowel preparation quality (BPPS score), lifting agent, and antibiotic used. Post-procedural data were also collected from patients included in the study using their follow-up colonoscopy reports; and evidence of any delayed adverse events through their medical records. Patients with lesions suspicious for adenoma and biopsies which were positive for histologic recurrence on surveillance colonoscopies were considered recurrence.

The study was reviewed and approved by the Institutional Review Board (SL-19185) and complied with good clinical practice (ICH-GCP) regulations.

Outcome Measures

The primary outcome was the identification of factors predictive of R0 resection, bleeding, and recurrence of colorectal adenomas in patients who underwent colonic EMR. Secondary outcomes included the R0 resection rate, EMR-related complications, recurrence rate of colorectal adenomas, and the diagnostic accuracy of JNET classification in identifying adenomatous lesions.

Data Analysis

Descriptive statistics was used to summarize the general and clinical characteristics of the participants. Frequencies and proportions were used for nominal variables; mean and range for ordinal variables; and mean and standard deviation for interval/ratio variables. Independent samples T-test, Mann-Whitney U test and Fisher's Exact/Chi-square test were used to determine the difference of mean, median and

frequency between groups, respectively. Odds ratio and the corresponding 95% confidence interval from logistic regression were computed to determine the association between patient profile and rate of complete resection using *en bloc* technique, complication (bleeding or perforation), and resection status. Sensitivity, specificity, predictive values and overall diagnostic accuracy were measured to determine the reliability of the JNET classification in predicting histopathology.

Results

A total of 282 patients (males: n=161, 57%; mean age 60 ± 12 years) were included. Most underwent conventional EMR (n=266, 94%). Other techniques employed included cap-assisted (n=9, 3%), hybrid EMR (n=6, 2%), and underwater EMR (n=1, 0.4%). Grossly, neoplasms had a mean size of 12 mm (1-490 mm) and were mostly located in the descending part of the colon (n=134, 48%). Majority were sessile (n=211, 75%). Submucosal fibrosis was present in six patients (2%). Majority of lesions were seen on the left side of the colon at 47.5% (n=134) followed by the right side at 24.5% (n=69), rectum at 16.7% (n=47), and lastly in the transverse colon at 11.4% (n=32). Most identified lesions were sessile polyps (n=211, 74.8%). Histopathologic analysis revealed that most lesions were low-grade tubular adenoma (n=146, 51.8%), sessile serrated adenoma (n=40, 14.2%), and low-grade tubulovillous adenoma (n=25, 8.9%). The actual tumor sizes ranged from 3-40 mm, with a mean of 10 mm (**Table 1**). Of 174 lesions characterized using NBI, most (n=130, 74.7%) were JNET 2A. Most patients had adequate bowel preparation with a BPPS score of 9 (n=206, 73%). Seventy six patients (27%) had poor bowel preparation. Most procedures (n=220, 78%) were performed using Olympus 290. *En bloc* resection was achieved in 240 lesions (85%). Saline alone was the preferred lifting agent (n=156, 56%), followed by saline plus hyaluronic acid (n=66, 23%). Procedures lasted for a mean duration of 45 (14-263) minutes.

Table 1. Lesion characteristics (n=282)

Lesion Factors	Mean (Range) Frequency (%)
Size, mm	12 (1 to 40)
<10	79 (28.01)
10-20	169 (59.93)
>20	34 (12.06)
Localization	
Right side colon	69 (24.5)
Transverse colon	32 (11.4)
Left side colon	134 (47.5)
Rectum	47 (16.7)
Morphology	
Sessile	211 (74.8)
Semi-pedunculated	17 (6.0)
Pedunculated	23 (8.2)
Granular	5 (1.8)
Non-granular	26 (9.2)
JNET classification (n = 174)	
1	34 (19.5)
2A	130 (74.7)
2B	10 (5.8)
3	0
Submucosal fibrosis	6 (2.1)
Hyperplastic polyp	17 (6.0)
Adenoma	
Tubular	
Low-grade dysplasia	146 (51.8)
High-grade dysplasia	8 (2.8)
Tubulovillous adenoma	
Low-grade dysplasia	25 (8.9)
High-grade dysplasia	11 (3.9)
Serrated adenoma	
Sessile	40 (14.2)
Traditional	2 (0.7)
Adenocarcinoma	
Moderately differentiated	8 (2.8)
Well differentiated	3 (1.1)
Well-differentiated NET	9 (3.2)
Others	13 (4.6)
Positive cauterized margins	11 (3.9)
Histopathology size of polyp, mm	10 (3 to 40)

Positive resection margins were present in eleven (3.9%) lesions, of which nine were removed *en bloc* (**Table 2**). Complications were reported in 45 (16.0%) of cases. Intraprocedural bleeding was the most common (n=36, 12.8%). There was one case of perforation.

Table 2. Clinical Outcomes

	N / Total	Prevalence (95% CI)
Positive resection margin		
All patients	11 / 282	3.9 (2.17 to 6.93)
En bloc resection	9/240	3.8 (1.95 to 7.08)
Complications	45 / 282	16 (11.49 to 19.96)
Intra-procedural bleeding	36/282	12.8 (9.33 to 17.22)
Delayed bleeding	8/282	2.8 (1.42 to 5.59)
Perforation	1/282	0.4 (0.05 to 2.5)
Recurrence	11/103	10.7 (5.95 to 18.42)

Only 103 patients (37%) had follow-up colonoscopy in the institution of study (**Table 3**).

Table 3. Follow-up of endoscopy patients (n= 103)

Follow-up Data	Frequency (%)
Recurrence	11 (10.7)
Histopathology	
Tubular adenoma, LG[a]	8 (66.7)
Hyperplastic polyp	2 (16.7)
Tubulovillous adenoma, HG[b]	1 (8.3)
Others	1 (8.3)
Months since index EMR	
<4	7 (6.8)
4-6	16 (15.5)
6-12	27 (26.2)
>12	53 (51.5)
Endoscopist	
Same	92 (89.3)
Different	11 (10.7)

[a]LG: low-grade dysplasia; [b]HG: high-grade dysplasia

Most had their first surveillance colonoscopies beyond the recommended six-month period (n=80, 77%). Recurrence was noted in 11 patients (11%) with most recurrent lesions being low-grade tubular adenoma (n=8, 67%). Majority (n=92, 89%) of the surveillance colonoscopies were performed by the same endoscopist.

The diagnostic positive predictive value of JNET classification for 167 lesions after excluding non-adenomatous and benign lesions (e.g., leiomyoma, neuroendocrine tumor, and inflammatory polyp) where JNET was not applied, are enumerated in **Table 4**. Majority of JNET Type 1 lesions (n=28, 87.5%) were hyperplastic and sessile serrated polyp on histopathology. For JNET Type 2A lesions, 102 (81%) were low-grade intramucosal neoplasia. The diagnostic yield of JNET classification exhibited moderate to good sensitivity for Type 1 (71.8%) and Type 2A (91.9%), and good specificity for Type 1 (96.9%) and Type 2B (95.5%). Diagnostic accuracy was at 91.0% for Type 1, 80.2% for Type 2A and 89.2% for Type 2B.

Table 4. Diagnostic positive predictive value (PPV) of JNET classification

	Hyperplastic and Sessile Serrated Polyp (n = 39)	Low-Grade Intramucosal Neoplasia[a] (n = 111)	High-Grade Intramucosal Neoplasia[a] (n = 12)	Carcinoma (n = 5)
		Frequency (%)		
Type 1 (n = 32)	28 (87.5)	4 (12.5)	0 (0)	0 (0)
Type 2A (n = 126)	22 (8.7)	102 (81.0)	10 (7.9)	3 (2.4)
Type 2B (n = 9)	0 (0)	5 (55.6)	2 (22.2)	2 (22.2)

[a]Comprised of tubular and tubulovillous adenoma variant
Note: There were no Type 3 patients

Table 5. Diagnostic yield of JNET classification (n = 167)

	Sensitivity	Specificity	PPV	NPV	Accuracy
	% (95% CI); [Frequency/Total]				
Type 1 (non-neoplastic vs. neoplastic)	71.8 (55.1 to 85) [28/39]	96.9 (92.2 to 99.1) [124/128]	87.5 (71 to 96.5) [28/32]	91.9 (85.9 to 95.9) [124/135]	91.0 (85.62 to 94.89) [152/167]
Type 2A (LGN vs. others)	91.9 (85.2 to 96.2) [102/111]	57.1 (43.2 to 70.3) [32/56]	81 (73 to 87.4) [102/126]	78 (62.4 to 89.4) [32/41]	80.2 (73.4 to 86) [134/167]
Type 2B (HGN and shallow submucosal invasive cancer vs. others)	15.4 (1.9 to 45.4) [2/13]	95.5 (90.9 to 98.2) [147/154]	22.2 (2.8 to 60) [2/9]	93 (87.9 to 96.5) [147/158]	89.2 (83.5 to 93.5) [149/167]

Crudely, gross (endoscopic) lesion size >20 mm (cOR 16.375 [95% CI 1.965 to +Inf]), presence of submucosal fibrosis (cOR 15.617 [95% CI 1.22 to 132.98]), histopathologic size (cOR 1.1 [95/% CI 1.01 to 1.19]), and diagnosis of moderately differentiated adenocarcinoma (cOR 225.106 [95/% CI 17.11 to

14256.58]) were associated with greater odds of having a positive resection margin after *en bloc* resection (**Table 6**).

Table 6. Factors associated with a positive resection margin after *en bloc* resection (n=240)

	R > 0 (n = 9)	R = 0 (n = 231)	Crude Odds Ratio	*p*
	Mean ± SD; Frequency (%); Median (Range)		(95% CI)	
Lesion profile				
Gross size, mm	20 (10 to 40)	10 (1 to 35)	1.155 (1.06 to 1.26)	.001
<10	0	66 (28.57)	Reference	
21-40	4 (44.44)	20 (8.66)	16.375 (1.965 to +Inf.)	.008
Submucosal fibrosis	2 (22.22)	4 (1.73)	15.617 (1.22 to 132.98)	.035
Histopathology				
Moderately Differentiated adenocarcinoma	5 (55.56)	2 (0.87)	225.106 (17.11 to 14256.58)	<.001

Crude analysis showed the following factors to be associated with occurrence of bleeding or perforation: hybrid EMR technique (cOR 11.816 [95% CI 1.63-134.88]), gross tumor size >20mm (cOR 3.554 [95% CI 1.44-8.79), non-granular morphology (cOR 2.621 [95% CI 1.0-6.84]), histopathologic size (cOR 1.064 [95% CI 1.02-1.11]), piecemeal resection (cOR 2.306 [95% CI 1.06-5.04]), and use of saline and methylene blue as lifting agents (cOR 6.222 [95% CI 1.53-25.32]) (*Supplementary Table 1*[♣]).

In terms of recurrence, the only factors associated as per follow-up visit/s (n=103) were non-granular morphology (cOR 9.683 [95%CI 1.78 to 54.98]) and piecemeal resection (cOR 1.221 [95% CI 2.44 to 60.69]) (*Supplementary Table 2*[ε]).

Discussion

EMR is considered a safe and effective option for patients with complex colorectal lesions. It was developed for minimally invasive endoscopic removal of benign and early malignant lesions in the GI tract.[11] It is an advanced resection technique that is not routinely part of the general endoscopic training of gastroenterologists hence, requires dedicated training for a high-quality, safe and effective colorectal EMR.[3] According to the European Society of Gastrointestinal Endoscopy, the goals of EMR are to achieve a completely snare-resected lesion in the safest minimum number of pieces, with adequate margins and without need for adjunctive ablative techniques.[12]

Patient Characteristics

The study included 282 patients with a mean age of 60±12.36 years; majority of which were males (57%). This result is consistent and adheres to different screening colonoscopy guidelines, since neoplastic lesions are more commonly found in such age and in males.[9]

Lesion Characteristics

The study reported a mean endoscopic size of 12 mm (1-490 mm) which appears to be a slight overestimation of histopathologic size. Based on published data, endoscopists tend to overestimate lesions by 3 mm.[13] Such difference could have a significant impact on surveillance colonoscopy where lesions >10 mm in size are recommended to undergo follow-up after six months. A standardized polyp size measurement is recommended.

In terms of morphology, most tumors were sessile (n=211, 74.8%) located on the left side of the colon (n=134, 47.5%) and were low-grade tubular adenoma (n=146, 51.8%) on histopathologic examination. Knowing these characteristics are important since previous reports have shown their association with bleeding, perforation, and adenoma recurrence.[6-7] Lesions proximal to the hepatic flexure have 4.4 times

[♣]Supplementary tables on factors associated with bleeding or perforation (n=282) may be requested from the corresponding author.

[ε]Supplementary table on factors associated with recurrence (n=103) may be requested from the corresponding author.

higher risk of bleeding than the remainder of the colon.[14] Right-sided colon polyps are associated with increased risk of adenoma recurrence.[4] In addition, lesion characterization is important because it determines the appropriate resection technique, such as EMR for flat lesions like laterally spreading polyps.

JNET Classification and Histopathologic Results

In the study, the majority of patients (n=130, 74.7%) had a JNET classification of 2A. Diagnostic accuracy tests were used to determine the sensitivity, specificity, predictive value and accuracy of the JNET classification in predicting appropriate histopathology. It should be taken to consideration, however, that the 95% CI are wide for some of the values, or contain 50%, likely due to relatively small sample sizes and different scope models. The study results showed high sensitivity and specificity for Type 1 and Type 2A lesions but as the colorectal lesion becomes endoscopically complex on NBI, the sensitivity of the JNET classification decreases while the specificity remains high. This trend is similar with the published data, hence, supports current knowledge on the variability of diagnosis of JNET Type 2B lesions among endoscopists.[10]

In general, this study shows that the JNET classification is useful in a clinical setting. It can be deduced that endoscopists start to have varied endoscopic diagnosis for lesions that are JNET Type 2A and 2B at least. While adequate examination should be emphasized for lesions regardless of JNET type, this study supports current findings that a more meticulous evaluation is necessary for lesions classified as JNET Type 2B. These lesions are more likely to be high-grade adenoma or intramucosal cancer.[15] Type of resection and management would differ for each lesion and the JNET system aids in its classification.

EMR Techniques

There were different EMR techniques employed by the endoscopists in the study. In terms of technique, the conventional EMR technique was the most utilized (n=266, 94.6%). Other resection techniques such as cap-assisted (3.2%), hybrid EMR (2.1%), underwater EMR (0.4%) were performed in a minority of patients. Cap-assisted EMR was usually performed in rectal submucosal lesions resembling neuroendocrine tumors. Hybrid EMR, on the other hand, was performed on larger-sized lesions intended for endoscopic submucosal dissection but eventually aborted.

Various lifting agents are utilized in EMR in order to adequately resect the lesion and minimize complications. In our institution, most endoscopists use normal saline (n=158; 56%) because of its availability. However, normal saline is quickly absorbed and could only lift the lesion for a short time. Other agents that may be used are hypertonic saline, hyaluronic acid, and 4% succinylated gelatin. These agents offer an advantage over normal saline as these generally lift the lesions for a prolonged duration. These lifting agents may be mixed with epinephrine at a dilution of 1:10,000 as prophylaxis for post-EMR bleeding. In our center, 26 (9%) of resections utilized addition of epinephrine.

Clinical Outcomes

1. R0 Resection

In this study, eleven (3.9%) lesions, nine of which were removed *en bloc*, had a positive resection margin (**Table 6**). The R0 resection rate or resected lesions with histologically assessed clear margins is 96.3% for lesions resected *en bloc*. This is similar to several reports that showed endoscopic resection is successful in 70-100%.[6] Piecemeal resection is generally associated with a positive resection margin. Hence, *en bloc* snare excision is the principal approach for larger lesions up to 20-25 mm and it is associated with lower rates of recurrence compared with piecemeal resection.[3] However, this study has shown that even *en bloc* resections could have positive resection margins. Risk factors associated with greater odds of having a positive resection margin include a large lesion (size >20 mm), presence of submucosal fibrosis, and diagnosis of moderately differentiated adenocarcinoma. It is also notable that six patients (2.1%) had submucosal fibrosis characterized as 'positive non-lifting sign' or the inability to adequately lift the lesion after submucosal injection of a lifting agent. Submucosal fibrosis is predictive of incomplete polyp resection which can potentially be a mucosal tumor and thus the unsuitability of performing EMR.[16]

2. Bleeding and Perforation

Complications during or after EMR are inevitable but they can be managed readily and safely. In the study, 45 patients (16.0%) experienced complications, most commonly intraprocedural bleeding. Bleeding and perforation are the major complications associated with

EMR.[6] The most common complication of EMR is bleeding with a wide-ranging incidence of 1-18%.[7] Generally, factors affecting the risk of post-procedural bleeding include lesion size, flat morphology, location, co-morbidities, coagulation status and lesion histology. However, the reported risk factors are inconsistent across different reports. In this study, the overall bleeding rate was 15.6% with 12.8% (n=36) intra-procedural and 2.8% (n=8) delayed. Risk factors for bleeding or perforation include using a hybrid EMR technique, gross tumor size of >20 mm, non-granular morphology of a laterally spreading tumor, actual size of histopathology of >20mm, and the use of saline and methylene blue as lifting agents. Based on multivariate analysis, the odds of intra-procedural bleeding were increased with a lesion size of more than 20 mm and piecemeal resection. The larger lesion size and piecemeal resection as risk factors for bleeding and perforation are consistent with published studies.[6-7] The use of saline and methylene blue most likely served as a risk factor in this study because of the lack of adequate lift of the lesions compared to other lifting agents (e.g., hyaluronic acid) which are capable of lifting the lesions longer.

Delayed bleeding occurring after the procedure requiring hospital re-admission or intervention is considered a significant post-endoscopic resection bleeding.[12] Studies showed that it can occur in up to 7% of patients after EMR and that is mainly observed between 2-7 days after the EMR.[14] Endoscopic intervention is required for ongoing or recurrent bleeding. In this study, 2.8% had delayed bleeding, most of which occurred within the first 48 hours after resection. Hemoclipping was done on the post-EMR defects to control the bleeding.

Perforation is the most serious complication of EMR. Peritonitis can occur if perforation is not managed accordingly. According to a meta-analysis by De Ceglie, EMR-related perforation rate was reported at 0.09% to 3.1%.[7] It can be readily managed by endoscopic clip closure when recognized intraprocedurally. In this study, only one patient (0.35%) had a perforation after resection of a descending colon polyp measuring 2.5 cm through a hybrid EMR technique. The perforation was managed with 12 hemoclips and one resolution clip deployed on the post-polypectomy site and administration of intravenous piperacillin-tazobactam.

3. Recurrence

Recurrence after colorectal EMR is considered the greatest drawback of EMR and it occurs in 10-30% of patients.[17] In this study, 10.7% (n=11) had lesions suspicious of recurrence post-EMR on their surveillance colonoscopy. It should however be noted that only 36.5% (n=103) had follow-up colonoscopies in the study; hence, the recurrence rate may be under-reported. Risk factors associated with recurrence on follow-up visits include non-granular morphology (cOR 9.683 [95%CI 1.78 to 54.98]) and piecemeal resection (cOR 1.221 [95% CI 2.44 to 60.69]). A study by Uraoka, et al. (2006) showed that laterally spreading tumors of non-granular morphology had higher potential for malignancy with greater submucosal depth compared with the granular type.[17] These LSTs should hence be removed *en bloc*. Piecemeal resections as earlier discussed have higher rates of recurrence compared to *en bloc* snare excision especially for larger lesions. In general, given the possibility of recurrence of lesions post-EMR, it is recommended to have structured surveillance protocol with at least one done 6-12 months post-EMR.[3] Time interval could change depending on the characteristic of the resected lesion.

Follow-Up

The aim of follow-up after colorectal EMR is the early detection of local recurrence and metachronous lesions.[18] In the study, there is a poor follow-up rate for all patients who underwent EMR. Only 36.5% or 103 patients returned for follow-up, most of which were >12 months post-procedure, or 53% of the study population, followed by 27% at 6-12 months after the index EMR. The most plausible reason for a low follow-up rate is that bulk of the patients was only referrals to the institute for EMR; and their surveillance colonoscopies performed in referring hospitals. Furthermore, a fraction of the patients are of terminal age; hence, follow-up was no longer recommended, unless the lesion on the index EMR warrants surveillance. Some of the patients also expired for unrelated reasons along the window period before their next surveillance.

Limitations and Recommendations

This study was limited by its single-center, retrospective study design. A multicenter collaboration of that do large-volume EMRs would validate and yield

more conclusive results. A prospective study design could control confounding variables and would have fewer potential sources of bias. Given the study's retrospective design, there are also several important factors not analyzed such as co-morbidities or use of anticoagulants in relation to post-polypectomy bleeding. Inclusion of these in further studies is recommended. The study is also limited in its sampling as it used a total enumeration scheme. This may affect the results yielding outcomes of statistical insignificance.

Conclusion

In conclusion, EMR remains a technically challenging procedure that requires considerable skill and experience. It is an important advancement in the field of therapeutic endoscopy with good clinical outcomes sparing patients from surgery with an R0 resection rate at 96.3%, low complication rate at 15.2%, and low recurrence rate at 10.7%. A larger lesion size of >20 mm is associated with both positive resection margin and post-EMR complications. Recurrence rates were noted to be higher in lesions with a non-granular morphology of a resected LST and piecemeal necrosis. In these cases, we recommend a greater resection margin or a more specialized resection technique such as endoscopic submucosal dissection to possibly reduce the risk of recurrence.

Acknowledgement

We would like to thank the Kobe University (Osaka, Japan), Prof. Tsukasa Ishida, and Prof. Hideyuki Shiomi for their continuous support in the institute and for training our gastroenterologists in doing advanced endoscopic procedures.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Laudico AV, Mirasol-Lumague MR, Medina V, et al. 2015. Philippine cancer facts and estimates. Philippine Cancer Society, 2015. Retrieved from http://www.philcancer.org.ph/ wp-content/uploads/2017/07/2015-PCS-Ca-Facts-Estimates_CAN090516.pdf
2. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Przeglad Gastroenterologiczny. 2019; 14(2): 89-103.
3. Klein A, Bourke MJ. How to perform high-quality endoscopic mucosal resection during colonoscopy. Gastroenterology. 2017; 152: 466-471.
4. Briedigkeit A, Sultanie O, Sido B, Dumoulin FL. Endoscopic mucosal resection of colorectal adenomas > 20mm: Risk factors for recurrence. World J Gastrointest Endosc. 2016 Mar 10; 8(5): 276-281.
5. Tajika N, Niwa Y, Bhatia V, et al. Comparison of endoscopic submucosal dissection and endoscopic mucosal resection for large colorectal tumors. Eur J Gastroenterol Hepatol. 2011; 23: 1042-29.
6. Hong JY, Kweon SS, Lee J, et al. Risk factors for procedure-related complications after endoscopic resection of colorectal laterally spreading tumors. Medicine. 2018 Oct; 97 (41).
7. De Ceglie A, Hassan C, Mangiavillano B, et al. Endoscopic mucosal resection and endoscopic submucosal dissection for colorectal lesions: A systematic review. Crit Rev Oncol Hematol. 2016; 104: 138-55.
8. Khashab M, Eid E, Rusche M, Rex DK. Incidence and predictors of "late" recurrences after endoscopic piecemeal resection of large sessile adenomas. Gastrointest Endosc. 2009 Aug; 70(2): 344-9.
9. Hassan C, Quintero E, Dumonceau J-M, et al. Post-polypectomy colonoscopy surveillance: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. Georg Thieme Verlag KG. 2013 Sep 12; 45(10): 842-64.
10. Kobayashi S, Yamada M, Takamaru H, et al. Diagnostic yield of the Japan NBI Expert Team (JNET) classification for endoscopic diagnosis of superficial colorectal neoplasms in a large-scale clinical practice database. United European Gastroenterol J. 2019 Aug; 7(7): 914-923.
11. Hwang JH, Konda V, Abu Dayyeh BK, et al. Endoscopic mucosal resection. Gastrointest Endosc. 2015; 82(2): 215-226.
12. Ferlitsch M, Moss A, Hassan C, et al. Colorectal polypectomy and endoscopic mucosal resection (EMR): European society of gastrointestinal endoscopy (ESGE) clinical guideline. Endoscopy. 2017; 49.
13. Izzy M, Asif Virk M, Saund A, et al. Accuracy of endoscopists' estimate of polyp size: A continuous dilemma. World J Gastrointest Endosc. 2015 July 10; 7(8): 824-829.
14. Metz AJ, Bourke MJ, Moss A, et al. Factors that predict bleeding following endoscopic mucosal resection of large colonic lesions. Endoscopy. 2011; 43: 506-511.

15. Kato M, Abe K, Kubosawa Y, et al. Validation of treatment algorithm based on the Japan narrow-band imaging expert team classification for sub-centimeter colorectal polyps. Endosc Int Open. 2018; 6(8): E934-E940.
16. Kim EK, Han DS, Ro Y, et al. The submucosal fibrosis: What does it mean for colorectal endoscopic submucosal dissection? Intest Res. 2016 Oct; 14(4): 358-364.
17. Uraoka T, Saito Y, Matsuda T, et al. Endoscopic indications for endoscopic mucosal resection of laterally spreading tumors in the colorectum. Gut. 2006; 55: 1592-1597.
18. Tanaka S, Kashida H, Saito Y, et al. JGES guidelines for colorectal endoscopic submucosal dissection/endoscopic mucosal resection. Dig Endosc. 2015; 27: 417-434.

A Case of Primary Omental Infarction in an Adult Female Presenting as Right Upper Quadrant Pain

Mansukhani SMH
Bañez VP

Section of Gastroenterology and Digestive Endoscopy
Manila Doctors Hospital
Manila, Philippines

Correspondence:
Dr. Sujata May Mansukhani
suji_mansukhani@yahoo.com

Accepted for publication:
January 2021

Abstract

Background: Primary omental infarction is a rare cause of abdominal pain that may mimic other disease entities hindering timely management. Only over 400 cases have been documented and as of this writing, there are no published local data. *Clinical Presentation:* A 55-year-old hypertensive Filipino female presented with a five-day history of epigastric pain radiating to the right upper quadrant. Examination revealed direct right upper quadrant tenderness with Murphy's sign. Work-up revealed leukocytosis and elevated total and indirect bilirubin. Initial impression was acute cholecystitis. Abdominal ultrasound showed normal gallbladder and biliary tree. Inconclusive findings prompted a contrast-enhanced MRI of the upper abdomen with MRCP which showed a well-defined lobulated mass-like lesion with thin rim enhancement in the right upper anterior peritoneal cavity with ascites, inflammatory changes and edema of the overlying subcutaneous fatty layer, indicative of omental infarction or panniculitis. *Management and Outcome:* Exploratory laparotomy with omentectomy was done revealing a concavity between the subcostal area and segments IV/V of the liver where the omentum was trapped. The omentum was hyperemic, thickened with central fat necrosis, and adherent to the anterior abdominal wall, greater omentum and proximal transverse colon. Histopathology revealed fibro-collagenous tissue and adipose tissue with chronic inflammation, hemorrhage and congestion. She was discharged stable after three days. *Recommendations:* Awareness of this disease, its mimics and diagnostic strategies are keys to early diagnosis, treatment, and prevention of complications.

Keywords: case report, omental infarction, primary omental infarction, omental torsion

Introduction

Omental infarction is a rare cause of abdominal pain in adults with an incidence of less than four cases per 1000 cases of appendicitis.[1] Only over 400 cases have been documented and, as of this writing, there are no published local data. We report a case of primary omental infarction in a 55-year old Filipino female mimicking acute cholecystitis.

Case Presentation

A 55-year old Filipino female presented with five-day history of epigastric pain radiating to the right upper quadrant. She self-medicated with antispasmodics and antacids with no relief of pain. On physical examination, she had stable vital signs, afebrile, with a body mass index (BMI) of 33 kg/m^2. Abdominal examination revealed direct right upper quadrant tenderness with Murphy's sign. Complete blood count showed a slightly elevated WBCs (11.88 X 10^9/L) with predominance of neutrophils (0.69). Blood chemistries showed direct hyperbilirubinemia (20.68 umol/L vs. total bilirubin 23 umol/L). Amylase, lipase, prothrombin time, creatinine,

sodium, potassium, SGPT and SGOT were normal. Initial impression was acute cholecystitis. She was placed on nothing-per-orem diet and initiated on intravenous hydration. Ciprofloxacin 400 mg IV every 12 hours, tramadol 50 mg IV, and hyoscine N-butylbromide 10 mg IV every eight hours were given. There was no relief of pain despite pain medications. Ultrasound of the whole abdomen revealed fatty liver, normal gallbladder, biliary tree, pancreas, spleen, kidneys, uterus and urinary bladder. Work-up could not explain the persistent pain. Since the primary consideration was a biliary disease, contrast-enhanced upper MRI of the upper abdomen with MRCP was requested. It showed omental infarction or panniculitis with minimal ascites, inflammatory changes and mild gallbladder sludge (**Figure 1**).

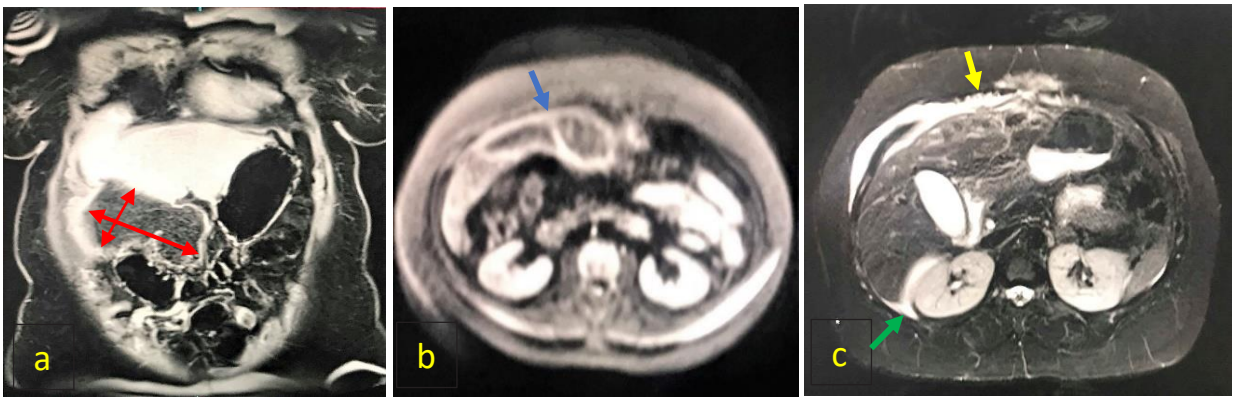


Figure 1. MRI with MRCP of the upper abdomen with contrast. T1 image: Well-defined lobulated mass-like lesion with fatty and edematous signals within the right upper anterior peritoneal cavity measuring 3.4 cm x 11.2 cm x 8.8 cm (red arrows). (a) *DWI image.* The well-defined lobulated mass lesion exhibits a thin enhancing rim (blue arrow). It is located anterior to the left hepatic lobe and superior to the transverse colon. (c) *T2 TIRM image*. Minimal perihepatic and perisplenic fluid (green arrow), inflammatory changes involving the right anterolateral abdominal wall with moderate edema of the overlying subcutaneous layer (yellow arrow).

The patient underwent diagnostic laparoscopy showing a concavity between the subcostal area and liver where the omentum was trapped, resulting to a hyperemic and thickened omentum with areas of necrosis. This prompted conversion to exploratory laparotomy with omentectomy (**Figure 2**).

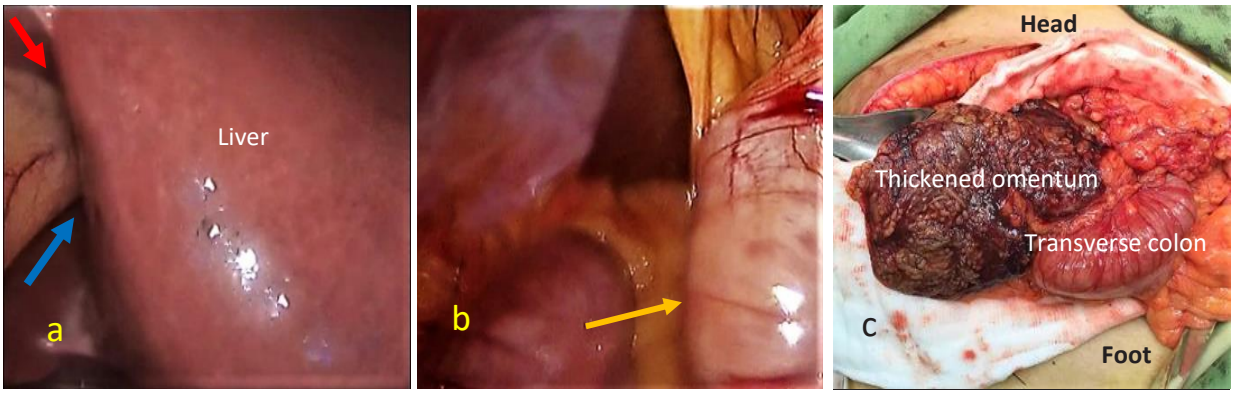


Figure 2. Intraoperative findings. (a) 3 cm x 4 cm concavity between the subcostal area and segments IV/V of the liver (blue arrow) with trapped omentum within (red arrow); (b) Omentum adherent to the proximal transverse colon (yellow arrow), anterior abdominal wall and greater omentum causing it to be displaced and pulled towards the infarcted area; (c) hyperemic and thickened omentum with areas of necrosis.

The patient tolerated the procedure well and was discharged stable after three days. Histopathologic findings revealed fibrocollagenous tissue and adipose tissue with chronic inflammation, hemorrhage and congestion consistent with omental infarction (**Figure 3**).

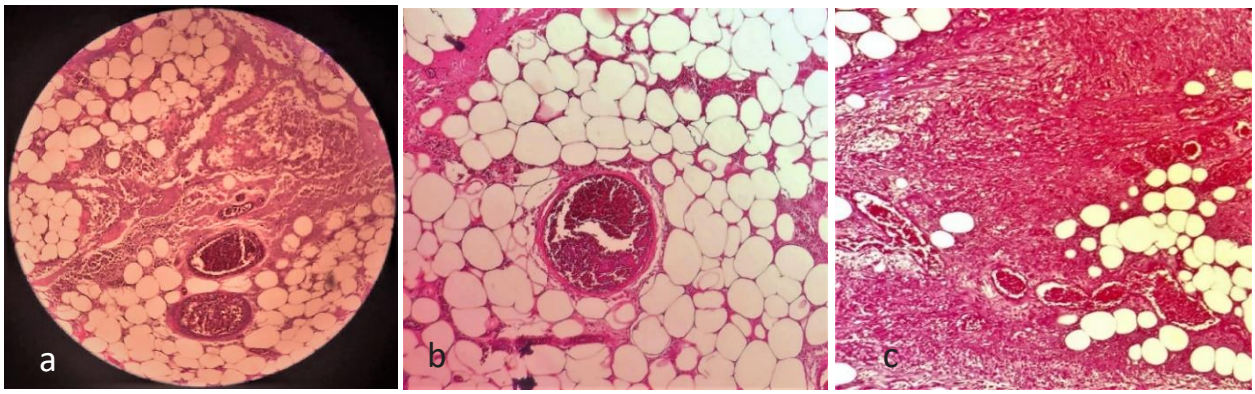


Figure 3. Histopathology, H & E stain. (a) & (b) Low power magnification showing numerous mononuclear inflammatory infiltrates admixed with red blood cells; (c) high power magnification showing vessels and blood-filled surrounding stroma indicating congestion and hemorrhagic change.

Discussion

Omental infarction results from venous stasis leading to edema and congestion of the omental vessels causing hemorrhagic necrosis and extravasation into the interstitium with thrombosis in the omental veins, thus, resulting to peritoneal irritation and pain.[3]

Omental infarction may occur with or without torsion of the greater omentum. Infarctions with torsion are classified either as: (a) idiopathic, or (b) secondary; which is associated with intra-abdominal adhesions, hernia and tumor. Omental infarctions without torsion are usually caused by hypercoagulability and vascular abnormalities.[3] Primary omental infarction (also known as idiopathic segmental infarction of the greater omentum or ISIGO) occurs when a mobile segment of the omentum rotates around a proximal fixed point in the absence of any intra-abdominal pathology. Torsion may be triggered by compression of the greater omentum between the liver and abdominal wall after local trauma, excessive exercise, occupational vibration, increased intra-abdominal pressure, sudden body movement, laxative use and acute changes in body position.[1,2,4] Anatomic malformations, such as bifid or accessory omentum and redundant omental veins, may also predispose to primary omental infarction.[2]

Obesity is a well-documented risk factor for primary omental infarction. In one study, almost 70% of patients with omental infarction were obese.[5] It is hypothesized that fatty accumulation in the omentum impedes the distal right epiploic artery and the additional structural mass potentially precipitates torsion.[1] Our patient had a BMI of 33 kg/m^2, classifying her as Obese Class I according to the WHO criteria, predisposing the development of primary omental infarction.

The major symptom of omental infarction is sudden onset pain over the right flank and lower abdomen which does not radiate to the abdominal wall.[4,5] Physical examination may demonstrate signs of local peritonitis and laboratory work-ups may show non-specific inflammatory response.[2] Computed tomography is the imaging modality of choice with high sensitivity (90%) and specificity.[6,7] The most common diagnostic finding is an ill-defined heterogeneous fat density with surrounding inflammatory changes.[1,6] Ultrasound is utilized to rule out other more common conditions such as acute cholecystitis.[7] Findings are usually normal. However, in <50% of cases, omental infarction is suggested by a hyperechoic, incompressible, ovoid mass.[8] In this case, since the initial impression was a biliary disease, MRI with MRCP of the abdomen was performed instead of a CT scan. Abdominal MRI is rarely used. On review of literature,

only a single report employed the use of this imaging modality. The MRI findings in this study showed a focal region of heterogeneous fat with surrounding inflammation and fluid,[9] similar to our patient.

Omental infarction is managed conservatively or via laparoscopic excision. Conservative treatment with bed rest, analgesics, anti-inflammatory medications, and fluid resuscitation are for hemodynamically stable patients.[1,2,7] Antibiotic prophylaxis is often used in the setting of conservative management when there is a risk of secondary infection of the infarcted fat.[3] Early surgical intervention via omental necrosectomy, on the other hand, reduces the duration of abdominal pain and incidence of complications, namely: necrosis, abscess formation and adhesions.[1]

Summary

Omental infarction is a rare cause of abdominal pain and is often diagnosed intraoperatively. Reports of this uncommon disease entity could raise awareness for clinicians to investigate other causes of acute abdominal pain, to employ appropriate imaging techniques in cases of uncertain diagnosis, and institute appropriate management to avoid complications.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Barai KP and Knight BC. Diagnosis and management of idiopathic omental infarction: A case report. International Journal of Surgery Case Reports. 2011; 2(6):138-140. doi: 10.1016/j.ijscr.2011.02.014.
2. Buell KG, Burke-Smith A, Patel V, Watfah J. Omental infarction: The great impersonator. The Cureus Journal of Medical Science. 2017; 9(12):e1940. doi: 10.7759/cureus.1940.
3. Ong WM, Matheson J, Chandra R, Stella DL. Omental infarction: A case of whole omental infarct. ANZ Journal of Surgery. 2015; 88(1-2):107-108. doi: 10.1111/ans.13215.
4. Katagiri H, Honjo K, Nasu M, Fujisawa M, Kojima K. Omental infarction due to omental torsion. Case Reports in Surgery. 2013. doi: 10.1155/2013/373810.
5. Occhionorelli S, Zese M, Cappellari L, Stano R, Vasquez G. Acute abdomen due to primary omental torsion and infarction. Case Reports in Surgery. 2014. doi: 10.1155/2014/208382.
6. Lindley S, Peyser P. Idiopathic omental infarction: One for conservative or surgical management? Journal of Surgical Case Reports. 2018. doi: 10.1093/jscr/rjx095.
7. Abdulaziz A, Zalbany TE, Al Sayed AR, Ansari AA H. Idiopathic omental infarction, diagnosed and managed laparoscopically: A case report. Case Reports in Surgery. 2013. doi: 10.1155/2013/193546.
8. Sun XW, Luo B, Lin HW. A rare case of acute primary omental infarction. Journal of Emergencies, Trauma, Shock. 2017; 10(1):31-33. doi: 10.4103/0974-2700.199522.
9. Sandusky MF, Herliczek TW. Pediatric omental infarction. Medicine and Health Rhode Island Medical Imaging Part I. 2012; 95(2):40-41.

CT-Guided Percutaneous Endoscopic Gastrostomy (PEG) Tube Replacement in a Post-Partial Gastrectomy Patient: A Pioneering Experience

Francisco CPD
Gopez-Cervantes JL
Co JT

Institute of Digestive and Liver Diseases
St. Luke's Medical Center-Global City
Philippines

Correspondence:
Dr. Carlos Paolo D. Francisco
carlosfranciscomd@yahoo.com

Accepted for publication:
January 2021

Abstract

Background: Percutaneous endoscopic gastrostomy (PEG) provides effective long-term access for enteral feeding. One indication for PEG is inadequate enteral intake due to dysphagia secondary to a neoplasia or neurologic disorder. However, history of previous partial gastrectomy is considered a relative contraindication for PEG due to limited gastric remnant. Computed tomography (CT)-guided PEG is an alternative technique in cases where endoscopic placement is not ideal. *Objective:* To demonstrate the feasibility of PEG tube placement in post-gastrectomy patients. *Case Presentation:* An 84-year-old female who previously had partial gastrectomy with Billroth 2 anastomosis and who has had multiple hospitalizations due to recurrent pneumonia is presented. Initial endoscopic evaluation showed unremarkable esophageal and remaining proximal gastric mucosa with intact gastrojejunostomy anastomosis. Prior to puncture, identification by CT scan of the left pleura, diaphragm in the superior aspect of the stomach, and the anastomotic site between the stomach and jejunum was done. Guided by CT scan, PEG tube was inserted by pull-through technique under intravenous sedation and local anesthesia at the puncture site. Initiation of enteral feeding was tolerated without untoward event within 24 hours after the procedure. Full intermittent feeding was achieved on the fourth postoperative day. *Conclusion:* With this first-hand experience, we have shown the greater advantage of CT-guided PEG over endoscopy alone in a previous gastric surgery patient. Radiologic guidance provides better anatomic orientation, preventing accidental puncture of adjacent organs and reduces the risk of tube misplacement. CT-guided PEG is a safe alternative procedure prior to surgical tube placement.

Keywords: case report, percutaneous endoscopic gastrostomy, PEG tube, CT-guided, partial gastrectomy

Introduction

Percutaneous endoscopic gastrostomy (PEG) is the preferred feeding route in patients with functioning gastrointestinal (GI) tract requiring prolonged enteral nutrition but with contraindications to feeding per orem. It provides superior access to the GI tract and is favorable over surgical methods because of its lower cost and lesser risk of morbidity and mortality.[1] Main indications of PEG tube placement are nutritional support for inadequate enteral intake due to dysphagia, and for gastric decompression.[1,2] Some of the common conditions for which patients are referred for PEG include cerebrovascular disease, Parkinson's disease, dementia, head trauma, head and neck malignancies, esophageal cancer and critically ill patients in the intensive care.[1]

Although PEG tube insertion is simpler and less invasive compared to surgical gastrostomy, several complications may still occur. In a prospective study by Blomberg and colleagues, the common complications encountered within two weeks after PEG insertion were abdominal pain (13%), peristomal infection (11%), diarrhea (11%) and leakage (10%).[3] However, a single

intravenous dose of a broad-spectrum antibiotic given 30 minutes before PEG has been proven effective in reducing the incidence of peristomal infections.[4] In a meta-analysis by Jafri et al., penicillin-based prophylaxis should be the antibiotic of choice in preventing peristomal infections, with a relative risk reduction of 62% and absolute risk reduction of 13%.[5] Other reported complications include pneumoperitoneum, inadvertent tube removal, peritonitis, tube blockage, aspiration pneumonia, metastatic seeding and perforation. Mortality after PEG has also been documented but is usually due to the patient's underlying co-morbidities.[1]

PEG was first introduced in 1980 as an alternative to feeding tube placement by surgical methods. The pull-string method by Gauderer and Ponsky is the most widely used technique in PEG tube insertion. This method uses a string that is inserted through a needle with a plastic sheath in the abdominal wall into the stomach, grasped with an endoscopic snare and then pulled through the esophagus and mouth. Afterwards, the string is fixed to the external end of the feeding tube and the tube is pulled from the mouth to the esophagus, stomach and then out though the abdominal wall.[1,6,7]

In addition to endoscopic and surgical gastrostomy, feeding tubes can also be placed percutaneously, guided by fluoroscopy or computed tomography (CT) scan. These radiologically-guided gastrostomy can be done using either the push-type or pull-type method. Both procedures are completed after contrast injection in order to confirm correct intraluminal tube position and to exclude extravasation.[8,9]

The decision for PEG tube placement should be individualized, not only to improve the patient's survival and nutritional status, but also to improve quality of life. Patients should be carefully screened prior to PEG insertion. Failure of transillumination and inadequate indentation of the proposed site with a finger should constitute a contraindication to tube placement at that site.[10]

Absolute contraindications of PEG tube insertion include serious coagulation disorders, hemodynamic instability, sepsis, severe ascites, peritonitis, marked peritoneal carcinomatosis, history of total gastrectomy, gastric outlet obstruction, and lack of informed consent. On the other hand, conditions such as the presence of gastric varices, hepatomegaly, splenomegaly, non-obstructing oropharyngeal or esophageal malignancy, large hiatal hernia, and history of partial gastrectomy are considered relative contraindications.[1,11]

In cases where endoscopic placement alone is not successful, a combined endoscopic and radiologic approach may improve anatomic orientation and accuracy.[2]

To our knowledge, there is no local data available on CT-guided PEG tube placement in the Philippines. This case report discusses the successful utilization of CT scan in tandem with upper endoscopy for PEG tube insertion in a geriatric patient who previously had partial gastrectomy.

Case Report

An 84-year-old Filipino female was referred due to aspiration pneumonia. She has hypertension, chronic kidney disease, rheumatoid arthritis and a history of partial gastrectomy (Billroth 2) a decade ago for an unrecalled indication. For the past year, she had several consults due to recurrent episodes of pneumonia, sometimes requiring hospital admissions. Her baseline functional capacity was assisted with most activities of daily living. Feeding tube insertion was recommended; hence, referred to our subspecialty for PEG.

Pre-endoscopic whole abdominal CT scan with intravenous contrast showed wall thickening of the gastric remnant with its anterior wall measured to be 2.3 cm away from the anterior abdominal wall (**Figure 1A**), but without any intervening bowel loops in between. Esophagogastroduodenoscopy showed an unremarkable esophagus and the remaining 25 cm of the proximal stomach with intact gastro-jejunostomy anastomosis. The PEG site was identified by transillumination, finger indentation and CT scan guidance. A gauge 22 spinal needle was punctured from the skin to the stomach under CT scan as well as gastroscope guidance. Lidocaine was infiltrated at the target site followed by a one-centimeter percutaneous incision. A French 24 PEG tube was placed by pull-through technique. Second look endoscopy showed the mushroom tip of the PEG tube in place, anchored at three-centimeter level. Repeat whole abdominal CT scan confirmed the placement of the PEG tube and showed the tube traversing the left upper anterior abdominal wall with the tip within the stomach (**Figure 1B**). There was no evidence of pneumoperitoneum or

abnormal fluid collection post-procedure. Trial tube feeding was tolerated after 24 hours with full, intermittent feeding achieved after four days.

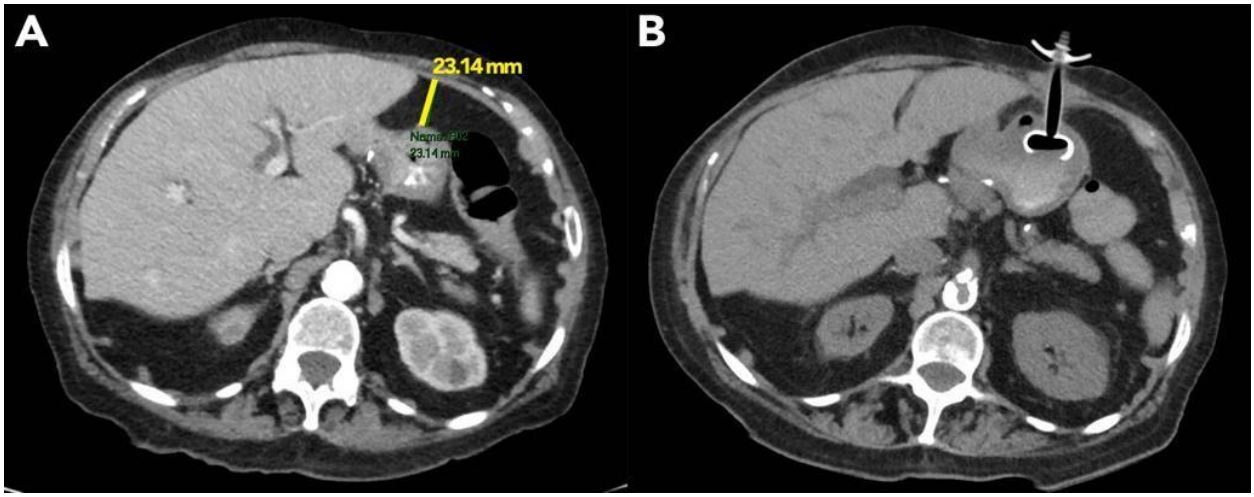


Figure 1. CT scan images. A: pre-PEG insertion. B: post-PEG insertion.

Discussion

PEG is a safe and well-tolerated procedure that provides effective long-term enteral feeding access for patients with inadequate nutrition who cannot tolerate oral intake because of malignant or neurologic conditions. Currently, it has more than 95% success rate when a safe site has been identified. Transillumination and adequate finger indentation of the proposed site are mandatory. Failure to fulfill these prerequisites should constitute a contraindication to PEG tube placement due to risk of organ injury.[12] Other contraindications of PEG include previous esophageal or gastric surgery, obesity, hepatosplenomegaly, peritoneal carcinomatosis, portal hypertension, peritonitis and gastric varices.[1,2]

Our patient was referred to the gastroenterology service for PEG tube insertion due to recurrent episodes of pneumonia from aspiration. In a study by Marumo et al., esophageal reflux of gastric contents and swallowing dysfunction were found to be the most important risk factors for aspiration pneumonia following gastrectomy.[13] Compared with nasogastric tube (NGT), PEG is associated with significantly lower incidence of aspiration pneumonia.[14] History of previous gastrectomy, however, has been described as a relative contraindication for PEG. This is due to the limited gastric remnant and the high posterior subcostal positioning of the stomach which prevents adequate approximation to the anterior abdominal wall.[15,16]

Performing PEG in a patient with partial gastrectomy requires skill and experience. In the study by Singh et al., a significantly longer procedure time was observed when PEG was done in a subtotal gastrectomy patient by a gastroenterologist with less experience compared with a gastroenterologist with more than ten years of experience (80 minutes vs. 20 minutes).[15]

CT-guided PEG is an alternative technique in cases where a purely endoscopic method of tube placement is not possible. It is a safe procedure done initially with acquisition of CT image slices to guide the choice of access prior to the endoscopic gastrostomy. The simultaneous endoscopy and CT guidance allows for an excellent anatomic orientation resulting in a reduced risk of tube mis-placement.[2] It has low risk of complications, which include pneumoperitoneum, aspiration, hemorrhage, perforation, wound infection and peritonitis. Post-insertion care includes adequate pain relief, daily wound care and regular flushing.[1]

This case is notable as CT-guided PEG represents an alternative technique for gastroenterologists in providing long-term enteral nutrition in patients for whom endoscopic method alone is difficult. Locally, there has been no published report yet documenting the use of this procedure. These findings therefore serve as a vital addition to the development of current

management of post-partial gastrectomy patients requiring long-term enteral feeding.

Acknowledgement

We thank the Philippine Society of Gastroenterology for accepting this paper for presentation at the Philippine Society of Gastroenterology Joint Research and Research Education Digital Research Forum held last July 31, 2020.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Rahnemai-Azar AA, Rahnemaiazar AA, Naghshizadian, Kurtz A, Farkas DT. Percutaneous endoscopic gastrostomy: Indications, technique, complications and management. World J Gastroenterol. 2014; 20(24):7739-7751.
2. Albrecht H, Hagel AF, Schlechtweg P, Foertsch T, Neurath MF, Mudter J. Computed tomography-guided percutaneous gastrostomy/jejunostomy for feeding and decompression. Nutr Clin Pract. 2017; 32(2):212-218. doi:10.1177/0884533616653806.
3. Blomberg J, Lagergren J, Martin L, Mattsson F, Lagergren P. Complications after percutaneous endoscopic gastrostomy in a prospective study. Scandinavian Journal of Gastroenterology. 2012; 47:737-742.
4. Sharma VC, Howden CW. Meta-analysis of randomized controlled trials of antibiotic prophylaxis before percutaneous endoscopic gastrostomy. Am J Gastroenterol. 2000; 95(11):3133-3136.
5. Jafri NS, Mahid SS, Minor KS, Idstein SR, Hornung CA, Glandiuk S. Meta-analysis: Antibiotic prophylaxis to prevent peristomal infection following percutaneous endoscopic gastrostomy. Aliment pharmacol Ther. 2007; 25:647-656.
6. Gauderer M, Ponsky J, Izant R. Gastrostomy without laparotomy: A percutaneous endoscopic technique. Journal of Pediatric Surgery. 1980; (6):872-875.
7. Hogan R, DeMarco D, Hamilton J, Walker C, Polter D. Percutaneous endoscopic gastrostomy--to push or pull: A prospective randomized trial. Gastrointest Endosc. 1986; 32(4):253-258.
8. Karthikumar B, Keshava S, Moses V, Chiramel G, Ahmed M, Mammen S. Percutaneous gastrostomy placement by intervention radiology: Techniques and outcome. Indian J Radiol Imaging. 2018; 28(2):225-231.
9. Tyng CJ, Santos EF, Guerra LF, Bitencourt AG, Barbosa PN, Chojniak R. Computed tomography-guided percutaneous gastrostomy: Initial experience at a cancer center. Radiol Bras. 2017; 50(2):109-114.
10. Spelsberg FW, Hoffmann R, Lang RA,Winter H, Weidenhagen R, Reiser M, et al. CT fluoroscopy guided percutaneous gastrostomy or jejunostomy without or with simultaneous endoscopy in otherwise untreatable patients. Surg Endosc. 2013; 27:1186-1195.
11. Hussain A, Woolfrey S, Massey J, Geddes A, Cox J. Percutaneous endoscopic gastrostomy. Postgrad Med J. 1996; 72:581-585.
12. Foutch PG, Talbert GA, Waring JP, Sanowski RA. Percutaneous endoscopic gastrostomy in patients with prior abdominal surgery: virtues of the safe tract. Am J Gastroenterol. 1988; 83(2):147-150.
13. Marumo K, Homma S, Fukuchi Y. Postgastrectomy aspiration pneumonia. Chest. 1995; 107:453-456.
14. Chang W, Huang H, Lin H, Tsai C. Percutaneous endoscopic gastrostomy versus nasogastric tube feeding: Oropharyngeal dysphagia increases risk for pneumonia requiring hospital admission. Nutrients. 2019; 11(12): 2969.
15. Singh P, Kahn D, Greenberg R, Indaram A, Pooran N, Bank S. Feasibility and safety of percutaneous endoscopuc gastrostomy in patients with subtotal gastrectomy. Endoscopy. 2003; 35:311-314.
16. Kurchin A, Halloran W, Kornfield R. Assessing the feasibility of percutaneous endoscopic gastrostomy following gastrectomy. Gastrointest Endosc. 1988; 34:366-367.

Septic Pylephlebitis of the Inferior Mesenteric Vein Secondary to Diverticulitis: A Case Report

Cabilao-Maala S
Garcia ACR
Valdez CM
Macatula T

The Medical City
Ortigas Avenue, Pasig City
Philippines

Correspondence:
Dr. Stephanie Cabilao-Maala
cabz_stephanie@yahoo.com

Accepted for publication:
January 2021

Abstract

Background: Pylephebitis is a rare complication of intraabdominal infections, with diverticulitis being the most common inciting condition. Due to its high mortality rate, a high index of suspicion is needed, and proper diagnostic work-up including radiologic imaging is important. *Case Presentation:* We report a 56-year-old Filipino male, who presented with a one-week history of fever, chills and abdominal bloatedness. Physical examination showed icteric sclerae and epigastric tenderness. Laboratory tests showed leukocytosis and liver function typical of a cholestatic jaundice. Ultrasound of the abdomen showed acalculous cholecystitis and abdominal CT scan revealed sigmoid diverticulitis with pylephlebitis of the inferior mesenteric vein. He was started on intravenous piperacillin-tazobactam and heparin drip. Antibiotics were later shifted to intravenous meropenem and oral warfarin with eventual resolution of both the thrombosis and the diverticulitis. *Recommendation:* Septic pylephlebitis is associated with high morbidity and mortality. Early diagnosis with appropriate imaging is important in order to initiate life-saving therapy with antibiotics and anticoagulation.

Keywords: anticoagulation, septic pylephlebitis, sigmoid diverticulitis, thrombophlebitis

Introduction

Colonic diverticulitis is defined as inflammation and/or infection of the diverticulum. Symptoms depend on the severity of inflammation as well as the location of the diverticulum. Approximately 25% of patients would have associated complications such as abscess, obstruction, fistula or perforation.[1] Pylephlebitis or thrombophlebitis of the portomesenteric venous system is a rare complication of diverticulitis, and can potentially cause death if not recognized early on.

Case Report

A 56-year old hypertensive Filipino male presented to the emergency room due to a one-week history of dyspnea, fever, chills, easy fatigability and abdominal bloatedness. Vital signs revealed blood pressure of 140/90, heart rate of 100 beats per minute, respiratory rate of 18 per minute, and a temperature of 37.4^{0}C. SpO_2 was 95% at room air. Further examination revealed icteric sclerae, regular heart rhythm, bibasal crackles without wheezing, and epigastric and left lower quadrant tenderness. Laboratories showed leukocytosis (12.6 cells/L) with 6% band forms and 77% neutrophil predominance. Creatinine was slightly elevated at 1.38 mg/dl. Electrolytes were within normal limits. Liver function tests showed elevated total bilirubin at 6.48 mg/dl, direct bilirubin at 4.83 mg/dl, indirect bilirubin at 2.96 mg/dl, alkaline phosphatase at 299 mg/dl, normal ALT and AST at 52 IU/L and 95 IU/L, respectively.

Cholestatic jaundice with a probable intraabdominal infection was entertained. He was started on intravenous (IV) ceftriaxone and metronidazole. Ultrasound of the upper abdomen revealed a thickened gallbladder wall without any intraluminal stones or

densities, indicating acalculous cholecystitis. A CT scan of whole abdomen with IV contrast showed the presence of multiple saccular outpouchings at the ascending, transverse, descending and sigmoid colon with surrounding fat stranding densities at the distal descending and proximal sigmoid colonic segments, indicating acute diverticulitis (**Figure 1**). Furthermore, contrast study showed poor opacification of the inferior mesenteric vein (IMV) with surrounding fat stranding densities along the course of the IMV and its tributaries including the left colic vein and sigmoidan veins. This was indicative of thrombophlebitis of the IMV, left colic and sigmoidan veins.

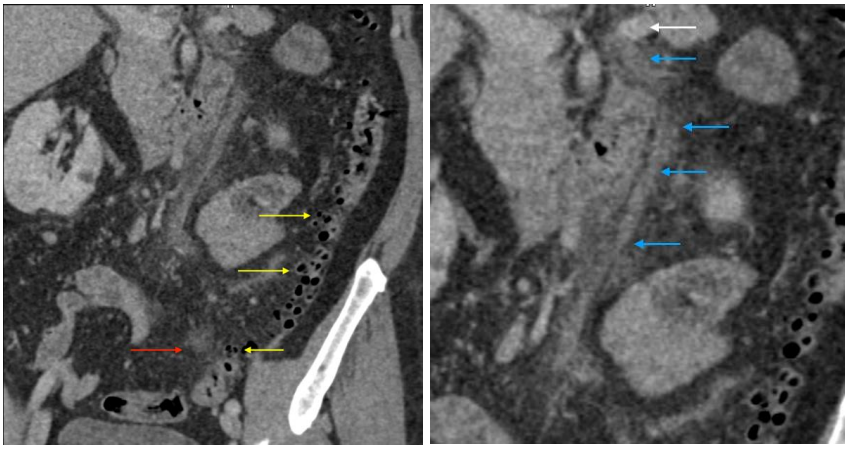


Figure 1. Contrast CT scan findings. (A) Multiple saccular outpouchings (yellow arrow) at the ascending, descending and sigmoid colon with fat stranding (red arrow) noted at the distal descending and proximal sigmoid colon. (B) Contrast study showing poor opacification of the inferior mesenteric vein (blue arrow) with surrounding fat stranding densities (white arrow) along the course of the IMV and its tributaries.

Anticoagulation with heparin drip was started for the thrombophlebitis. Antibiotics were shifted to piperacillin/tazobactam for empiric coverage and later to meropenem. Blood cultures were taken, and no organisms were isolated. Work-up for a hypercoagulable state included protein C, protein S, anti-thrombin III, antiphospholipid antibodies, lupus anticoagulant and factor V, the results of which were all inconclusive. There was noticeable improvement of the patient's symptoms, with lysis of fever and resolution of abdominal pain, jaundice and bloatedness. After completing 14 days of IV antibiotics, he was discharged stable and advised to continue anticoagulation with plans to continue oral warfarin for six months. A follow-up CT scan (**Figure 2**) was done four weeks later and showed resolution of both diverticulitis and the IMV thrombosis.

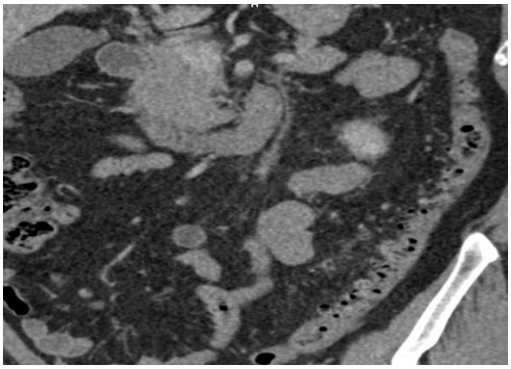


Figure 2. Follow-up CT scan (four weeks from initial CT): resolution of acute diverticulitis and thrombosis in IMV.

Discussion

Pylephlebitis is an infective suppurative thrombosis of the portal vein that develops secondary to an intraabdominal or pelvic infection draining into the portal venous system.[2] The right portal vein is identified as the most common site of thrombosis (33%), whereas the inferior mesenteric vein is the least common site (8%). Appendicitis was considered the most common inciting infection;[3] however, more recently, diverticulitis and other intra-abdominal infections have taken over. It is usually polymicrobial, and *Bacteroides fragilis* is the most commonly isolated organism in blood.[4] The symptoms are nonspecific; however, abdominal pain and fever are the most common findings at presentation.[5] Furthermore, mortality rate is high especially if complicated by hepatic abscess or bowel ischemia. Other nonspecific clinical features include fatigue, malaise, chills, nausea, vomiting diarrhea, anorexia, and weight loss. More advanced signs include hepatomegaly and jaundice. Laboratory testing is very nonspecific and no specific laboratory test points to the presence of pylephlebitis. Leukocytosis may be a common early finding, but both normal and decreased white blood cell counts have been noted in the literature. Liver function test abnormalities may or may not be present. In one study, blood cultures were found to be positive in 44% of patients[6] whereas other studies have shown the rate to be between 50 and 88%.[7] Pylephlebitis is diagnosed primarily by radiographic means. Doppler ultrasound and contrast-enhanced CT facilitate early diagnosis. Ultrasound may show portal vein thrombosis, and contrast-enhanced CT scan can display intraabdominal processes like appendicitis and diverticulitis as well as mesenteric and portal vein thrombosis, liver abscesses, and bowel ischemia.[8]

Antibiotics should be initiated once pylephlebitis is suspected and should cover for gram-negative bacilli, anaerobes, and aerobes. Antibiotics such as metronidazole, gentamicin, piperacillin, ceftizoxime, imipenem, and ampicillin have been associated with success; however, no empiric antibiotic regimen has been established.[9] Duration of antibiotic therapy hasn't been established either. In a report by Lim et al., there was clinical improvement in a patient with septic pylephlebitis after monotherapy with imipenem for two weeks.[10] On the other hand, results of a prior case series suggests a minimum of four weeks of antibiotic therapy.[2] Hepatic abscesses were found to be a frequent complication of pyelephlebitis and administering antibiotics for at least four weeks seemed prudent as developing abscesses may not be visualized on CT scan.

Our present case was treated initially with ceftriaxone and metronidazole, later on shifted to piperacillin/tazobactam, then finally to meropenem, since despite negative blood cultures, the patient had persistent fever episodes and abdominal pain. He was given a total of 14 days of IV antibiotics as in-patient and was sent home off antibiotics. Administration of antibiotics for less than the usual four weeks in the literature may still be as successful for as long as proper coverage is achieved. In this particular case, two weeks of antibiotic treatment (IV meropenem) was sufficient to give complete symptomatic relief together with anticoagulation.

Several case series and case reports describe the use of anticoagulation as part of their management; however, there is no clear consensus on its role in the treatment of pylephlebitis. Early anticoagulation in mesenteric and portal vein thrombosis is considered to minimize complications such as bowel ischemia and infarction. According to Baril et al., anticoagulation was recommended to be given only in patients with documented coagulation disorder or disease-associated hypercoagulable state.[11] Plemmons et al. noted a 100% survival rate among patients who received heparin, compared to 60% survival among those who did not.[2] Kanellopoulou et al. noted that the early use of anticoagulation in portal vein thrombosis may minimize serious sequelae and speed up recanalization.[4] Our patient underwent tests for hypercoagulable state. The results, however, were inconclusive; and no hypercoagulable state or thrombophilia was present. Anticoagulation was started, initially with heparin drip, eventually bridged to oral warfarin, which the patient continued as outpatient for six months.

No guidelines exist for when a repeat imaging should be done. Some suggest repeat imaging after completion of antibiotics. On follow-up, our patient had a repeat contrast abdominal CT scan which showed resolution of both diverticulitis and inferior mesenteric vein thrombosis. Although universally fatal in the pre-antibiotic era, the outcome of this infection has improved somewhat with modern diagnostic and therapeutic modalities.

Surgery is not usually required for the management of pylephlebitis. However, surgical drainage of the precipitating focus may be necessary in some cases.[12]

Conclusion

A case of septic pylephlebitis of the inferior mesenteric vein secondary to diverticulitis has been presented. Pylephlebitis is a rare complication of intraabdominal infections. Due to its high mortality rate, a high index of suspicion is needed, and proper diagnostic work-up, including radiologic imaging, is important. To the best of our knowledge, there has been no published study on its incidence in the Philippine setting. Ultimately, it should be treated aggressively and comprehensively with the objective of avoiding visceral ischemia, liver abscess, and chronic portal hypertension. Antibiotics are essential because uncontrolled infection, not visceral ischemia, is the primary threat to the patient's life.

In the context of data from case reports and series, further prospective randomized studies are recommended to establish duration of antibiotic use and to assess the need for anticoagulation.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Parks TG. Natural history of diverticular disease of the colon. A review of 521 cases. Br Med J. 1969; 4:639.
2. Plemmons RM, Dooley DP, Longfield RN. Septic thrombophlebitis of the portal vein (pylephlebitis): diagnosis and management in the modern era. Clin Infect Dis. 1995; 21:1114.
3. Falkowski AL, Cathomas G, Zerz A, Rasch H, and Tarr PE. Pylephlebitis of a variant mesenteric vein complicating sigmoid diverticulitis. Journal of Radiology Case Reports. 2014; vol. 8, no. 2:37-45.
4. Kanellopoulou T, Alexopoulou A, Theodossiades G, et al. Pylephlebitis: an overview of non-cirrhotic cases and factors related to outcome. Scandinavian Journal of Infectious Disease. 2010; 42:804.
5. Kashiura M, Sugiyama K, Akashi A, and Hamabe Y, Diverticulitis-induced pylephlebitis possibly misdiagnosed as biliary duct obstruction. Acute Medicine & Surgery. 2016; vol. 3, no. 4: 404-406.
6. Choudhry AJ, Baghdadi YMK, Amr MA, Alzghari MJ, Jenkins DH, and Zielinski MD. Pylephlebitis: A review of 95 cases. Journal of Gastrointestinal Surgery. 2015; vol. 20, no. 3: 656-661.
7. Wong K, Weisman DS, Patrice KA. Pylephlebitis: A rare complication of an intra-abdominal infection. Journal of community hospital internal medicine perspectives. 2013; 3(2).
8. Castro R, Fernandes T, Oliveira MI, and Castro M. Acute appendicitis complicated by pylephlebitis: A case report. Case Reports in Radiology. 2013; Article ID 627521, 3 pages.
9. Rea JD, Jundt JP, and Jamison RL. Pylephlebitis: Keep it in your differential diagnosis. American Journal of Surgery. 2010; vol. 200: e69-e71.
10. Lim, HE et al. Pylephlebitis associated with appendicitis. The Korean journal of internal medicine. 1999; vol. 14, 1:73-76.
11. Baril N, Wren S, Radin R, Ralls P, & Stain S. The role of anticoagulation in pylephlebitis. The American Journal of Surgery. 1996; 172(5):449-453. doi:10.1016/s0002-9610(96)00220-6.
12. Saxena R, Adolph M, Ziegler JR, et al. Pylephlebitis: A case report and review of outcome in the antibiotic era. Am J Gastroenterol. 1996; 91:1251.