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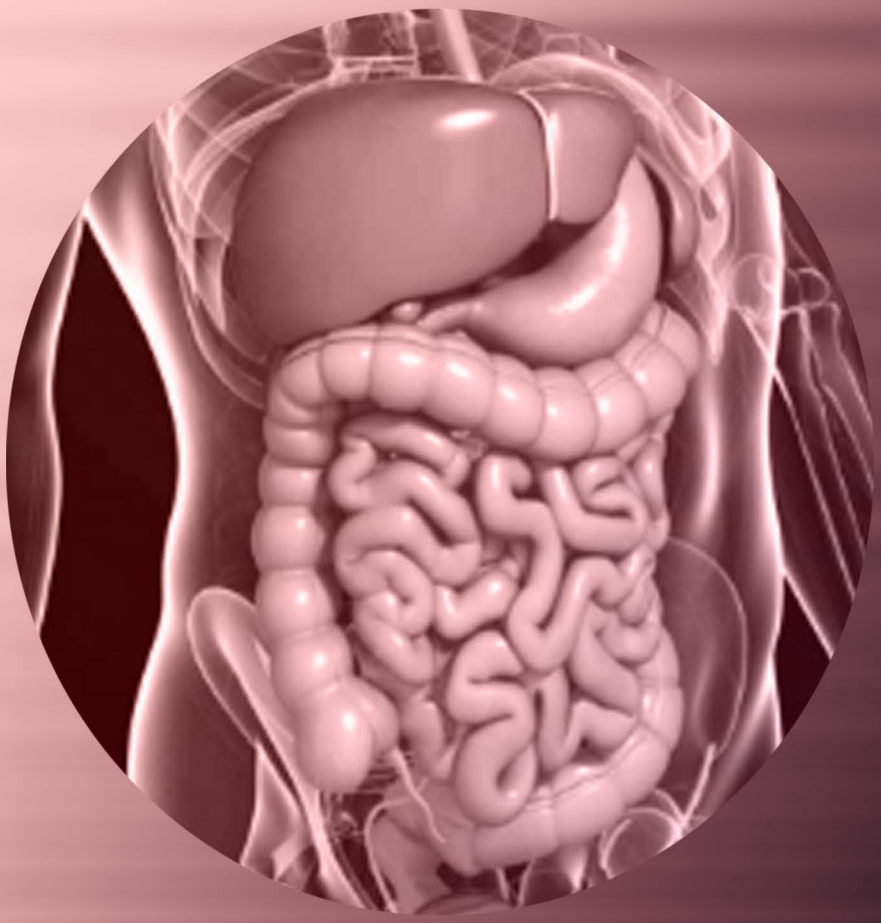

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Editorial

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Management of Boerhaave's Syndrome

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Boerhaave's syndrome or spontaneous oesophageal perforation is characterised by barogenic oesophageal injury. This leads to contamination of the pleural cavity with enteric contents and various degrees of injury.[1] The syndrome is named after Herman Boerhaave, a Dutch physician, who first described it in 1724. Incidence of spontaneous rupture amongst all oesophageal ruptures varies between 15-38%.[2,3] Mallory-Weiss tears are assumed to represent part of the spectrum of the spontaneous perforation, but it is likely that these mucosal injuries reflect shearing rather than barogenic trauma.[4] Spontaneous oesophageal perforations are associated with the highest mortality amongst all gastrointestinal perforations with an overall rate of nearly 30%. Early diagnosis and definitive surgical management as soon as possible after the presentation of symptoms, indisputably within the first 24 hours show the best outcomes. If treatment is started within 24 hours from the onset of symptoms, mortality rate is observed to be below 10%; after 24 hours, it is approximately 65% and after 48 hours, it reaches 75%-89%. If left untreated it rises up to 100%.[5,6]

Oral, water-soluble contrast medium is the most used and preferred method of investigation for visualisation of the oesophageal leak. Although this method helps in revealing most cases of oesophageal perforation, it may be associated with up to 66% false negative results and up to 90% sensitivity.[7] Water-soluble contrast should be used instead of barium contrast to prevent barium-related inflammation of the mediastinum if there is any perforation. If the initial contrast-swallowing study is negative, imaging can be repeated after 4-6 hours if the clinical suspicion still remains high. In approximately 90% of the cases, the area of perforation and contrast leak is at the left posterolateral aspect of the distal third of the oesophagus, usually within 2-3 cm above the oesophagogastric junction.[8] The complication risk of endoscopic assessment (OGD) is minimal and it excludes the diagnosis if normal. It also influences the management if underlying pathology, such as cancer is found and facilitates placement of nasojejunal feeding tube if required. Computed tomography (CT) with oral and intravenous contrast is frequently performed in critically ill patients. Apart from showing a leak, it reveals the degree of contamination and associated extra-oesophageal insult; in addition, it has a significant role in the decision-making process as well as post-operatively in patient assessment.[9]

Management can be conservative, surgical, endoscopic or a combination and hugely depends on the patient condition and timing of presentation. It is clear that surgery remains the main stay of treatment for Boerhaave's syndrome. Management of the condition should preferably be performed in a specialist oesophageal unit with high expertise. Admission to intensive care unit is usually necessary.

Non-operative management may be appropriate in a small number of patients who are diagnosed early, with minimal contamination and no mediastinitis. It can also be reserved for those with delayed diagnosis that have been stable. All patients should be nil by mouth and receive urgent respiratory and cardiovascular support plus opiate based analgesia. Intravenous fluids, urinary catheter and close fluid monitoring should be given. In addition, broad-spectrum antibiotics and antifungal plus intravenous proton pump inhibitors to lower the acid exposure are strongly recommended. Part of the management includes placement of intercostal chest drains

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and nasogastric tube, which should only be done under image guidance. Early enteral feeding is advised in all cases. Laparoscopic or open feeding jejunostomy and venting gastrostomy are advised for nutrition and drainage respectively. Although, usage of covered self-expandable stents as primary treatment has recently gained popularity, it still remains limited with sealing leakage failure rates of up to 50%.[10,11] Non-operative trans-oesophageal debridement with mediastinal irrigation has also been used with acceptable results. Nevertheless, there are limited cases described in the literature to reach any robust conclusions.[12]

Objective of surgery is to restore oesophageal integrity and prevent further soiling. Debridement, drainage and lavage are more important than the type of repair.[13] The majority of oesophageal perforations present with left chest contamination (90%) and a left posterolateral thoracotomy appropriate in most cases. Longitudinal oesophageal myotomy is advised as the mucosal injury is usually longer than the muscular one and mucosal debridement may additionally be necessary.[14] The controversy is with the late perforations by the time diagnosis is made the wound edges may have become oedematous, stiff or friable rendering primary repair risky due to the high rate of breakdown. Frequently, there is associated mediastinitis and empyema. In these cases, primary repair may not be feasible. Late perforations can therefore be managed with debridement of the pleural cavity and mediastinum, oesophagectomy and placement of feeding gastrostomy.[15] As a general rule, oesophageal resection is reserved for damage to diseased oesophagus, extensive trauma or delayed presentation; clinical judgment is of paramount importance and that is one of the main reasons why management of the condition in a specialist oesophageal unit is required. Immediate primary reconstruction with oesophago-gastric anastomosis in cases with minimal contamination or a delayed one (oesophageal diversion with cervical or tube oesophagostomy and reconstruction at a second stage) in profuse contamination and/or physiologic instability are the options.

Primary closure or closure over a *t*-tube is a suitable option, especially in cases with early presentation.[14] Primary repair is the most common procedure and should always be considered for those with early onset of symptoms. Single or 2 layered closure using absorbable sutures can be performed with an oesophageal bougie rarely utilised now-a-days.[16] Associated leak rate after primary repair performed within the first 24 hours of presentation is in the area of 20% and if treatment is delayed for more than 24 hours it can rise up to 50%.[17] Re-enforcing the repair with a patch of nearby tissue like pericardium, pleura or pericardial fat, may reduce the leak rate.[18] T-tube repair was developed in the 1970's mainly for cases with late presentation and it can work well for most repairs.[19] The concept is based on a controlled oesophago-cutaneous fistula, where a T-tube (6-10 mm in diameter) is placed through the tear with the limbs of the tube beyond the boundaries of the perforation. The oesophageal wall is approximated loosely over the tube with absorbable sutures. The tube is subsequently externalised and secured at the skin. Basal and occasionally apical chest drains are inserted. Oesophageal healing is monitored with contrast studies. T-tube is generally removed between 3-6 weeks, depending on the clinical and radiological progress. Sulpice et al[20] in his comparative study of T-tube repair and primary repair, found no difference between the two, even though the time from symptom onset to surgery was longer for the T-tube repair. In the era of endoscopic surgery, video-assisted thoracoscopic surgery (VATS) could not have been unutilised in the treatment of Boerhaave's syndrome. Haveman et al[21] showed that VATS can be used as the first choice and is a safe approach associated with a lower rate of complications and similar results in comparison with open surgery.[21] A Korean study, showed similar outcomes of thoracoscopic repair *versus* open repair, even at delayed presentation.[22]

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interst.

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Case Report

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Salvage Cryotherapy for Treatment of Persistent Barrett's Esophagus

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ABSTRACT

Background: A dysplastic Barrett's esophagus can lead to cancer if not treated by endoscopic eradication therapy. The current consensus is to eradicate the entire residual Barrett's esophagus that is visible during endoscopy by ablation therapy. However, a section of patients are resistant to this ablation therapy. They may need a second line of ablation therapy to eradicate the visible residual Barrett's esophagus.
Aims: To evaluate the efficacy of treatment with salvage cryotherapy of persistent Barrett's esophagus who failed to respond for ablation by radio frequency ablation.
Methods: Barrett's esophagus with high-grade dysplasia was initially treated with radiofrequency ablation (RFA) for at least 6 sessions to achieve complete eradication of Barrett's esophagus. Barrett's esophagus patients who failed to respond to the radiofrequency ablation was then treated with a different ablation therapy modality like a cryotherapy to achieve complete eradication of Barrett's esophagus.
Results: Two patients with Barrett's esophagus and high-grade dysplasia were included in the case series. The Barrett's esophagus in both the patients was treated with a mean of 6.5 sessions of radiofrequency ablation. Both the patients had persistent Barrett's esophagus despite the treatment with radiofrequency ablation as demonstrated by surveillance endoscopic biopsies. They were then treated with a mean of 4.5 sessions of cryotherapy as a salvage therapy. Surveillance endoscopy showed persistent Barrett's esophagus in both the patients despite being treated with cryotherapy. None of the patients had high-grade dysplasia in the surveillance endoscopy.
Conclusion: Salvage cryotherapy is futile in persistent Barrett's esophagus that had prior treatment failure with a different ablation therapy.

KEYWORDS: Cryotherapy; Radiofrequency ablation; Barrett's esophagus.

INTRODUCTION

Barrett's esophagus can predispose to esophageal adenocarcinoma after undergoing a histological transformation from non-dysplastic Barrett's esophagus to dysplastic Barrett's esophagus and subsequently into esophageal adenocarcinoma.[1] The annual incidence of esophageal adenocarcinoma arising from Barrett's esophagus is 0.12%-0.5%.[2]

The practice guidelines recommend eradication of the entire Barrett's esophagus with intra-mucosal adenocarcinoma, high-grade dysplasia and certain selective cases of low-grade dysplastic Barrett's esophagus. This is to prevent esophageal adenocarcinoma arising from Barrett's esophagus.[3] This is accomplished by endoscopic eradication therapy, which consists of initial endoscopic mucosal resection of all visible nodules in the Barrett's esophagus and subsequent eradication of residual Barrett's esophagus to achieve complete eradication of intestinal metaplasia (CE-IM). A CE-IM is defined as absence of endoscopic and histological evidence of intestinal metaplasia after treatment with ablation therapy. The risk of metachronous cancer is high if residual Barrett's esophagus is not completely eradicated.[4]

There are different therapies that are adopted for ablation of residual Barrett's esopha-

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gus like radio frequency ablation (RFA), cryotherapy, complete endoscopic mucosal resection, photodynamic therapy (PDT), argon plasma coagulation (APC) and multipolar electrocoagulation (MPEC). There are no randomized control trials between cryotherapy and RFA to treat Barrett's esophagus. The choice of ablative therapy to treat Barrett's esophagus depends on the preference of the endoscopist. However, RFA is being used predominantly due to its ease and low rate of post procedure complications.[5-7] A recent meta-analysis on 3802 patients showed efficacy of RFA in achieving CE-IM was 78% with a mean follow-up of 20.5 months and majority of these patients required 2-3 RFA sessions to achieve CE-IM.[8] Therefore, there is still 22% of the patients who will need a different ablation modality to achieve CE-IM in view of risk of metachronous cancer.

Cryotherapy is an option to use as salvage ablation therapy in patients who have failed to achieve CE-IM despite being treated with RFA. Two patients were treated with salvage cryotherapy and I would like to outline the experience of the outcomes of such a treatment.

Case 1

A 75-year-old male was initially diagnosed with a 10 cm Barrett's esophagus and a multifocal nodular high-grade dysplasia. His Prague score measured C10M10. He had no evidence of hiatus hernia. He underwent mucosal resection of the nodules in the Barrett's esophagus and his entire Barrett's esophagus was treated with RFA every 2 months for 1 year (Figure 1A). He was treated with 6 sessions of RFA in total over the course of 1 year. His surveillance endoscopy showed no evidence of nodules but there was persistent residual Barrett's esophagus of 8 cm. His Prague score was C7M8. He was considered to be having a persistent intestinal metaplasia due to failed RFA. His Barrett's esophagus was then treated with a liquid nitrogen cryotherapy every 2 months for 8 months (Figure 1B). He has been treated 4 times in total over the course of 8 months. His surveillance endoscopy showed a persistent Barrett's esophagus of 8 cm. His surveillance biopsies showed persistent intestinal metaplasia but there was no evidence of high-grade dysplasia or cancer.

Case 2

A 60-year-old male was evaluated for persistent symptoms of gastro-esophageal reflux. His endoscopy revealed a 12 cm nodular Barrett's esophagus. His Prague score was C11M12. He had a 5 cm hiatus hernia. His nodule was resected by EMR and the histology showed high-grade dysplasia. His residual Barrett's esophagus was treated with RFA every 2 months for 14 months. He had a total of 7 sessions of RFA. His surveillance endoscopy showed a persistent Barrett's esophagus of 9 cm. His Prague score in surveillance endoscopy was C8M9. There was no evidence of nodules. It was decided that his RFA treatment had failed and he had a persistent Barrett's esophagus. He was referred for surgery in view of his 5 cm hiatus hernia. However, he was deemed to be a poor surgical candidate in view of his other medical co-morbidities and was not considered for surgery. He was then treated with a liquid nitrogen cryotherapy every 2 months for 10 months for a total of 5 treatments. His surveillance endoscopy showed a persistent Barrett's esophagus of 9 cm and his Prague score was C8M9. His surveillance biopsies from the Barrett's esophagus did not show any evidence of high-grade dysplasia or cancer but showed persistent intestinal metaplasia.

DISCUSSION

Cryotherapy uses the principle of mucosal necrosis caused by ischemia and subsequent apoptosis leading to cell death.[9] It is a non-contact method of ablating Barrett's esophagus by spraying liquid nitrogen. Initially a orogastric tube is inserted into the stomach through the mouth and is connected to a continuous suction, to suction all the gas that is produced by conversion of liquid nitrogen. The gastroscope is then inserted alongside the

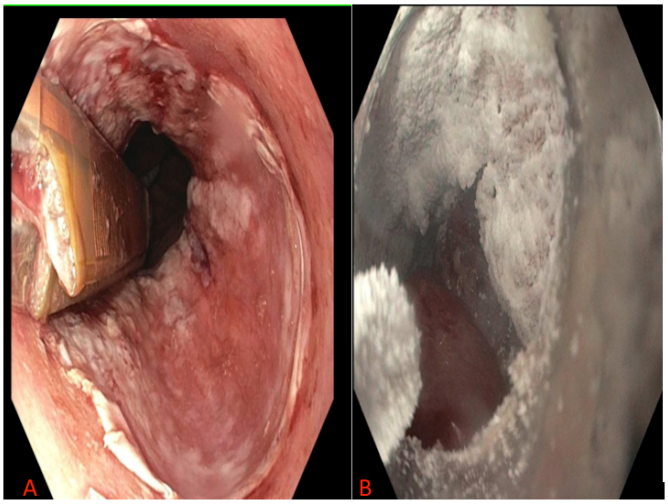

Figure 1: (A) Post-RFA treatment. (B) Post-cryotherapy treatment.

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orogastric tube and is placed just proximal to Barrett's esophagus. A spray cryotherapy catheter is passed through the gastroscope. The spray cryotherapy catheter is used to spray the liquid nitrogen onto the Barrett's esophagus. The lesion is frozen for 20 seconds at 196 °C and subsequently thawed for 60 seconds to ablate the Barrett's esophagus. This is done until the entire Barrett's esophagus is treated. This maneuver is then repeated 3 times for that session.

Cryotherapy has been predominantly used to treat residual Barrett's with no other prior ablative therapy.[10] A long-term follow-up of 2 years on 32 patients treated with cryotherapy reported a CE-IM in 84% and chronic eradication for dysplasia in 100% of the patients.[10] There has been one published literature of treating persistent Barrett's esophagus with a failed RFA. Sixteen patients with failed RFA were included in the study. Treatment with cryotherapy resulted in CE-IM in 31% and chronic eradication for dysplasia in 75% of the patients.[11] Three (19%) of these patients developed stricture, which responded to dilation.[11] Fortunately, there were no adverse events in our patient case series.

The above two cases highlights some of the challenges that are encountered during the ablation of Barrett's esophagus. Cryotherapy is a good ablation therapy based on available evidence.[8] However, it may not be a solution to salvage a prior failed ablation therapy of Barrett's esophagus. It is possible that the pathology of certain Barrett's esophagus is such that they do not respond to any ablation therapy, if they have already failed with one kind of ablation therapy. Failure of one ablation therapy predicted failure of second ablation therapy in the above set of patients. Reassuringly, none of our patients had recurrence of high-grade dysplasia or cancer. Therefore, continuous surveillance of persistent Barrett's esophagus is important to identify and treat metachronous cancers. This case series adds to the limited literature that is available. Further, larger multicenter studies with salvage cryotherapy is required to know if it is truly a futile option in the case of a prior failed ablation therapy. Also, longer follow-up is required to know the course of natural history of persistent Barrett's esophagus that has failed ablation therapy.

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Mini Review

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Overriding Elements in Colon Cancer Progression: Some Less Known Facts

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ABSTRACT

Colorectal cancer (CRC) is one of the most life threatening disease with escalating mortality and morbidity. Some known causative factors include lifestyle, alcohol etc. but there are other aspects like long standing colitis, inhibition of apoptotic proteins, chemokines and their receptors and inflammasomes which increase the chances of CRC progression. In a clinical setting of diseases like colitis and Crohn's disease, the risk of CRC varies on degree of inflammation, expression of chemokines and cytokines and other molecular alterations. Hence, the underlying molecular inductions determine the fate of CRC progression through extent of epithelial-mesenchymal transition, metastasis and invasive ability. The present view point will showcase some of the latent and notable considerations in CRC progression.

KEYWORDS: Colon cancer; Apoptosis; Chemokines; Inflammasomes; Inflammation.

INTRODUCTION

Colorectal cancer (CRC) is a heterogeneous disease causing more than 4 million deaths.[1] Apart from dietary factors, lifestyle and genetics, advancements from other ailments like inflammatory bowel disease are reasons for cause of CRC.[2] In several studies, it was affirmed that prevalence of CRC in patients with ulcerative colitis greatly varies from place and nature of race.[2] Amongst the molecular pathogenesis, inflammation has a direct link to CRC.[2] Not only by the assessment of mucosal biopsies revealed escalated levels of cell division and cell death, there were several conditions like epithelial mesenchymal transition (EMT) which had a higher role in cancer invasion rate or metastasis.[3]

Cytokines which are categorized under small proteins play an important role in cell signaling. In carcinogenic conditions like colitis associated cancers, cytokines were said to have a significant influence. Factors like nuclear factor-kappa B (NF-kB), tumor necrosis factor (TNF) etc. regulate cell cycle, apoptosis, reactive oxygen species and mutations.[4,5] Thus the present view point will list few perplexing conditions, inflammatory modulators and receptors which would aid in CRC progression.

HYPOXIA - THE ENVIRONMENT FOR PROGRESSION

Hypoxia plays a huge role in the augmentation of solid tumors. During hypoxia, there is restricted oxygen supply which is regulated by hypoxia-inducible factor (HIF). HIF is not only involved in transcriptional activity of several genes, but its high levels are associated with poor prognosis of CRC.[6] It was well evidenced that hypoxia promotes migration ability of cancer cells conditioning EMT.[7] Cytokines such as tumor necrosis factor-α (TNF-α) were said to have direct relation with HIF-α levels enhancement *via* NF-kB activation. Additionally, Forkhead Box M1 (FOXM1) also advances EMT as FOXM1 promotes urokinase-type plasminogen activator receptor (uPAR) and matrix metalloproteinase 2 & 9.[8] In the condition of CRC, endoplasmic reticulum (ER) stress is down-regulated by hypoxic condition thereby increasing the aggression of metastasis. It was clearly observed in several solid tumors that FOXM1 transcrip-

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tion factor was over expressed and ER stress was far reduced.[8] The condition of hypoxia can be attenuated either by sensitizing the cancerous cells to ER stress or blocking uPAR pathway. Figure 1 depicts the role of hypoxia in regulating mammalian target of rapamycin (mTOR) in articulating vascular proliferation. It is evident that dysfunction in the P13k/mTOR pathway leads to pathogenesis of CRC. The hypoxia inducing factors HIF-α and HIF-β trigger chemokines to initiate epithelial mesenchymal transition which is a lead step in metastasis.

CHEMOKINES AND THEIR RECEPTORS

Clinical evidences state that chemokine receptor 6 (CCR6) expressions was up-regulated in colorectal cancer.[9] Chemokines in general can regulate the movement of tumor cells. CCR7, CCR9, C-X-C chemokine receptor type 1 (CXCR1), and C-X-C chemokine receptor type 2 (CXCR2) are also detected in tumor cells and their ligands can induce the chemotaxis of the corresponding receptor-expressing cells. These chemokines act as inducers of invasion within the tumor and also in regards to movement to other organs. When chemokine (c-c motif) ligand 20 (CCL20) triggered p130 phosphorylation, there was an enhanced migration and proliferation of cancer cells. The expression of chemokine receptor C-X-C chemokine receptor type 4 (CXCR4) in CRC metastasis was well established. Factors like high invasion rate, development of liver metastasis and colon carcinoma micro metastasis of liver were reported by CXCR4.[9] Direct association of lymph node metastasis in CRC and CCR7 was observed in a study by Gunther et al.[10] Not only does chemokine-chemokine receptor interaction supports metastasis in colon cancer, CCR6-CCL20 interaction was found to be involved in various inflammatory and immune disorders.[11] CCR6 role in advanced colon cancer was clearly shown and also CCR6-CCL20 was reported to have involvement in EMT which resulted in poor outcome of colon cancer.[11] CCR6 was described as independent risk factor in liver metastasis. On the other hand, CCL20 levels were higher in colon cancer patients with liver metastasis compared with controls without metastasis.[12] In a study by Kawada and colleagues,[13] amongst the colon cancer samples with CXCR3 expression, there was significantly high lymph node metastasis recorded. CXCR3 expression levels were directly proportional to poor prognosis which clearly indicates that CXCR3 activation was concomitant with colon cancer metastasis preferentially to the draining lymph nodes with poorer prognosis.

mTOR's Role in Colon Cancer

Hyper activation of mechanistic target of rapamycin (mTOR) is due to signaling malfunctions upstream of mTOR in phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR pathway. Mutagenesis in PI3Kα occurs in late tumorigenesis which is generally evident in more than 30% of colon cancers.[14] mTORC1 inhibition had a role in improving regeneration capacity of intestinal stem cells but at the same time, abberant stimulation could trigger carcinogenesis. In humans, immunohistochemical studies on colorectal carcinoma samples revealed that mTORC1 signaling takes place in the early onset of tumorigenesis and plays a role in transforming normal cells to neoplastic. mTORC1 and mTORC2 were implicated in colorectal cancer biology to a greater extent.

In the conditions of *in vivo* mTORC2 was down-regulated, there was a clear trend of reduction of proliferation and also there was reduction in the formation of tumor xenografts. EMT was also regulated by mTORC1 and mTORC2 and thus sequencing metastasis of CRC.[15-17]

Targeting kinases like polo-kinase 1 which is highly expressed in proliferating cells during G2 and M phase of cell cycle was regarded as a potential target. Besides, induction of apoptosis *via* mTOR suppression is a strategic therapeutic intervention. BI2536 was one amongst the potent polo-kinase 1 inhibitor used in combination with NVP-BEZ235 which is a dual PI3K/mTOR inhibitor.[18] These studies not only show the importance of epigenetic mechanisms in cancer signaling but also clearly denote the role of mTOR signaling in CRC progression.

Nod like receptors (Nlrp3) and their Role in CRC

Chronic inflammation is considered as a risk factor for the progression of CRC. Nod like receptor (Nlrp3) is a protein which assembles the inflammasome which is responsible for secretion of pro-inflammatory cytokines like IL-1β. This aids in cellular

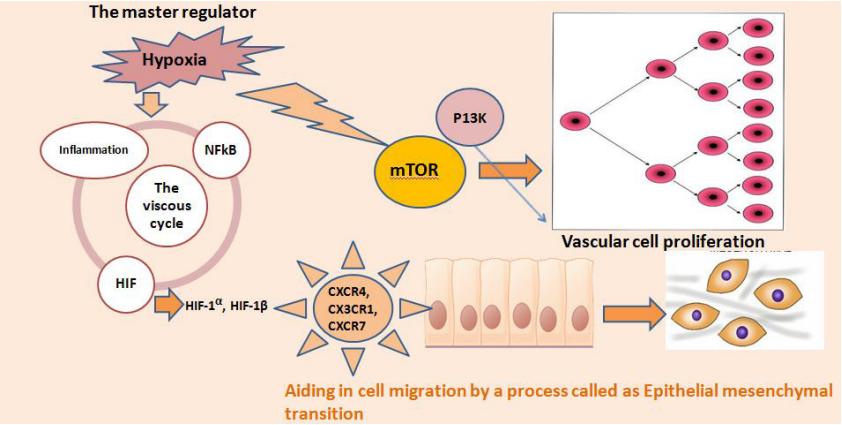

Figure 1: Correlation between hypoxia, chemokines and mTOR pathway and their role in carcinogenesis.

functions like repair *via* immune cell activation and triggering pro-inflammatory cytokines. Inoperative NLR activation results in cell proliferation and tumorigenesis.[18] Mice deficient of Nlrp3 were prone to dysplasia and tumor formation thus clearly stating the linkage between Nlrp3 deficiency and susceptibility to colitis associated carcinogenesis. Apoptosis associated speck like protein-1 (ASC) and caspase 1 are two major components of Nlrp3 which play a major role in inducing apoptosis of cancer cells. In experimental conditions of knocking out of these two major components of Nlrp3, the mice developed tumorigenesis. Interleukin 18 (IL-18) which is secreted by Nlrp3 exerts extensive anti-tumor activity and aids in epithelium repair. The fact that IL-18 offers its protection against colitis and carcinogenicity was described in mice models where caspase 1 and Nlrp3 were knocked out and there was development of CRC. It was well defined that IL-18 promotes enterocyte proliferation to repair chemically induced injury of colonic epithelium and also inhibits hyperplasia during chronic stages of colitis. Also in the acute conditions of disease, IL-18 offers restoration of barrier integrity by controlled proliferation of stem cells at the base of the intestinal crypt which indirectly helps in intestinal homeostasis. In experimental conditions of azoxymthane (AOM) or dextran sulfate sodium (DSS) induced colitis, phosphorylated levels of STAT1 were reduced in the colon where the restoration is possible by IL-18.[19-21] Nlrp3 inflammasome not only reduced the tumorigenesis but also suppressed liver colon cancer metastatic growth.[22]

SPINK1

Serine peptidase inhibitor kazal type-1 (SPINK1) has been investigated for its role in multiple human carcinomas especially CRC.[23] SPINK1 mutation was associated with pancreatitis. Epidermal growth factor receptor (EGFR) involves in intra-cellular message transduction. High expression of EGFR is also associated with poor prognosis. SPINK1 and epidermal growth factor (EGF) bind to EGFR and stimulate proliferation *via* mitogen-activated protein kinases (MAPK) which was recorded in pancreatic adenocarcinoma.[24] In a study by Chen et al,[24] the SPINK1 protein played a role in tumor proliferation and malignant transformation in CRC through the EGFR pathway. SPINK1 was also used as a marker for predicting tumors in response to anti-EGFR treatment in CRC patients.[25] Metalllothionein expression in colon cancer was analogous with poor survival and SPINK1 caused CRC progression by down regulating metallothioneins expression.[23]

COX2

The interest in cyclooxygenase (COX2) inhibitors as therapeutic interventions rose after its ability as an anti-carcinogenic agent with 40-50% reduction in CRC prevalence in non-steroidal anti-inflammatory drugs (NSAID) users.[26] COX2 over expression has been reported in numerous colorectal adenomas and adeno carcinomas. The direct relation of COX2 with intestinal cancers was well demonstrated in a study where the murine COX2 gene was deleted in APC Δ716 mice which resulted in reduction of intestinal polyps. Ideal tumorigenesis mechanisms of COX2 are inhibition of apoptosis, increased effectiveness of invasiveness etc. It is noteworthy that though COX2 is preponderant to the cytoplasm of cancerous epithelial cells, it is negatively expressed in normal epithelium. In regards to the mucosa, CRC samples were found to have more messenger RNA (mRNA) levels than normal mucosa.[27,28] In a clinical study involving 76 patients suffering with CRC, COX2 was conferred to advancement of the disease and decreased patient survival.[29] The underlying mechanism of COX2 associated carcinogenicity is by attenuation of the mitochondrial apoptotic pathway distinguished by reduced cytochrome C release and diminished caspase activity. There was an increased expression of anti-apoptotic genes Bcl-2 recorded in cell lines like HCT-15.[30] COX2 also was involved in death receptor (DR) pathway *via* DR expression in HCT-15 cells.[31] Targeting CRC with COX2 inhibitors may not be a complete solution though NSAID users had less probability of CRC.

CONCLUSION

Biological mechanism underlying CRC have been markedly improved in recent years with respect to therapeutic interventions selecting target receptors, inflammasomes, cytokines and other chemokines. Finding the right agent for CRC treatment was achieved by inducing apoptosis, reducing EMT and decelerating proliferation and invasiveness. Treatment for CRC is not only designed *via* symptomatic approach but also targeting signaling mechanisms which drive the disease. Thus, this article highlights some of the facets in CRC which when targeted or inhibited can turn out to be a potential treatment escalating the quality of life (QoL) and improved prognosis.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Research

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Analysis of the Intracellular Zinc in HCV Replicon

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ABSTRACT

Aim: In patients with chronic liver injury, metabolic disturbance of zinc was frequently observed and Hepatitis C virus (HCV) replication is crucially involved with zinc metabolism. *In vitro* HCV replication system, HCV replicon enabled us to analyze HCV replication *in vitro*. Here we aimed to quantitatively analyze the zinc in a HCV-infected hepatocyte, namely HCV replicon and control.
Methods: Genome-length HCV RNA-replicating cells, namely replicon cells (HCV-O cells) and control cells were treated with zinc salts and assayed their zinc content by in-air micro-PIXE analyzer. Metallothionein (MT) which is a major reserve of zinc was also analyzed by Cd-hem assay.
Results: 1) Micro-PIXE analysis revealed that zinc concentration was increased more in HCV-O cells than control. Additional zinc by zinc chloride administration enhanced the peak of zinc in both control and HCV replicon cells. Furthermore, the degree of zinc increment by additional zinc is more in HCV-O cells than control.
2) Cd-hem assay also demonstrated the increase of zinc in HCV-O cells and zinc stimulation further increased the metallothionein content in HCV-O cells.
Conclusion: In-air-micro-PIXE and Cd-hem assay revealed the increase of zinc and metallothionein in HCV-O. Metallothionein increased and additional zinc also increased more in HCV-O. Thus, HCV infection increased zinc at least partially through metallothionein.

KEYWORDS: HCV; Zinc; PIXE.

ABBREVIATIONS: HCV: Hepatitis C virus; PIXE: Particle-Induced X-ray Emission; PBS: Phosphate-Buffered Saline; MT: Metallothinein; TIARA: Takasaki Ion Accelerator for Advanced Radiation Application.

INTRODUCTION

Hepatitis C virus (HCV) is one of the most prevalent cause of liver diseases with estimates placing nearly 3% of the world population, roughly 150 million people, as HCV-infected.[1] Persistent HCV infection eventually develops into liver cirrhosis or hepatocellular carcinoma and more than 700,000 people die every year from hepatitis C-related liver diseases.[2] On the other hand patients with chronic liver disease tend to be complicated with metabolic disturbance of trace element, namely the decrease of zinc and the increase of iron and copper.[3] We have reported zinc supplementation additively enhanced the effect of interferon (IFN) on the eradication of HCV.[4,5] Zinc is the component not only of non-structural protein NS3 of

HCV but also of an inducer of metallothionein, which is an anti-oxidant and a chelating agent of metals. As for iron, phlebotomy could improve the liver function due to iron depletion through the reduction of oxidative stress.[6] Copper increases and deposits the hepatocytes as liver disease progresses not only in Wilson's disease but chronic viral hepatitis.[3,7] *In vitro* assay of HCV has long been difficult to establish because no cell culture system could replicate HCV until Bartenschlager developed *in vitro* HCV-replication system called "replicon" which cannot produce the complete virion of HCV but efficiently expressed a part of structural and nonstructural proteins of HCV in Huh-7 hepatoma cell line.[8] This system is useful for pharmacological assessment of drug effect on HCV but in order to reproduce the cellular event by HCV replicon, we used the full genome HCV replicon founded by Kato N et al HCV-O.[9] A genome-length HCV RNA replication system may reflect the phenomenon that HCV-infected human liver undergoes. This replicon is more similar to HCV itself than the original one which does not contain core region but non-structured region.[9] In Gunma, Japan we have an innovative system to analyze the trace element, micro-particle induced X-ray emission, PIXE. Our aim is to clarify the relationship between trace element and HCV replication in replicon system using in air micro particle-induced X-ray emission (PIXE) system which is introduced precisely in the references.[10] The quantitative analysis is available only by using PIXE system in one cell.[10] Metallothionein which is the main zinc reserve in the cell[11,12] was also quantitatively analyzed by Cd-hem assay.[13]

MATERIALS AND METHODS

HCV-Replicon and Control Cell

HCV replicon was kindly provided from Professor Kato N, Okayama University as infected in Huh-7 cell lines, designated as O cells.[9] Briefly, O cells were introduced genome length HCV RNA into Huh-7 and real-time reverse transcriptase polymerase chain reaction detected HCV RNA in the cell and culture supernatant.[9] O cells were positively selected by neomycin and cultured in Dulbecco's modified Eagle's medium GIBCO Cat No.12320-032 (Gibco-BRL, Invitrogen Life Technology, Carlsbad, CA, USA) supplemented with 10% fetal calf serum, Bio-West (NW, USA) Cat No S1820, penicillin, and streptomycin, Gibco Cat No 15070-063 (complete DMEM).

Analysis of the Intracellular Zinc by in-Air micro-PIXE

Preparation of the samples for in-air micro-PIXE

Replicon cells and control cells were cultured with 1 ml of medium on Mylar foil (Graphix Plastics, OH, USA) overnight. Then the cells were pretreated with 100 microM of zinc chloride for 9 hours and were rinsed seven times with tris-hydroxymethylaminomethane (THAM, Sigma, Saint Louis, MO, USA) solution and were cryofixed with liquid nitrogen and dried in a vacuum for 24 hours. Finally, the samples were mounted onto the sample holder. Non treated cells were served as control.

Analysis of cellular trace elements using PIXE

A 3.0 meV proton beam, 1 micrometer spot size, accelerated by Takasaki Ion Accelerator for Advanced Radiation Application (TIARA) single-ended accelerator at JAEA-Takasaki, was used to analyze the subcellular elemental distribution in the cell samples. Micro-beam less than 1 micrometer makes an electron in the inner shell jump out from the orbit and another electron in outer shell moves to an inner orbit. Then characteristic X-ray having the particular energy is emitted and detected by the X-ray detector. An ion beam can be focused in a spot as small as 1 mm in a specimen with a set of quadruple magnets. Period and dimensions of the scan were set at 30 minutes and 70×70 micro square meter (μm^2) areas, respectively. Precise measurement conditions were reported previously.[11] Net count of element yield was calculated by the PC soft ware program. Because sulfur (S) count is regarded as the representative of the whole cell number.[10]

Cd-hem Assay

Metallothinein (MT) concentrations in HCV-O cells and control were assayed by a modified Cd-hem method described by Onosaka and Cherian.[12] In brief, $1x10^6$ cells/mL of both cells was cultured in plastic dish with a diameter of 10 cm. At 90% confluent, the cells were treated with Zn 100 micro M-alone, IFN50 U/mL+Zn100 micro M, IFN100 U/mL+Zn100 micro M or IFN100 U/mL-alone. Non-treated cells were used for control. In 24 h after the treatment, the cells were rinsed with phosphate-buffered saline (PBS), then were harvested and resolved with 1.0 ml of 0.25 M sucrose. The cells were ultra-sonicated, and then centrifuged 20,000 g for 30 min. Following the protein concentration assay, the recovered supernatant was used for MT assay. The 0.2-ml supernatant sample was mixed with 1.0 ml of 0.03 M Tris–HCl buffer (pH 7.8) containing 1 micro gram Cd and was incubated for 10 min. For removing non-MT-conjugated Cd, the sample was added 0.1 ml of 5% bovine–hemoglobin and was heated for 90 s at 95 centigrade. After cooling on ice, the sample was centrifuged at 10,000 g for 5 min. The addition of bovine-hemoglobin and the heart treatment were repeated three times. MT-conjugated Cd in the supernatant was determined by inductively coupled plasma/mass spectroscopy (ICP-MS) (ELAN6100, Perkinelmer, Japan). Mass-to charge ratio was 114. MT concentration was calculated by assuming that 1 mole of MT (6600) binds 7 mole Cd, and was described per milligram of protein. MT in control cells and O cells with and without additional zinc was compared as duplicate.

Statistical Analysis

Statistical analysis was performed by two-way analysis of variance. $p<0.05$ was accepted as statistically significant.

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RESULTS

Analysis of the Intracellular Zinc by In-Air Micro-PIXE

In air micro-PIXE analysis revealed the peak of phosphorus, potassium, iron, copper zinc treated differences of metal component between the two cells were observed for 30 minutes analysis. Cellular distribution of phosphorus shows the micro-PIXE exactly hits and detects the cells (Figure 1). Although iron and zinc located compatible with phosphorus contour, no significant differences were visually observed between HCV replicon-infected cells and control (Figure 2). Calibration of zinc concentration using control showed enhanced zinc incorporation was observed in HCV-O cells than control Huh-7. As for the quantitative analysis, the comparison of the densitometry counts of additional zinc and O cells to non treated Huh-7 was shown in Figure 3 and analyzed. Zinc tended to increase in non treated O cells compared to controls, it was not statistically significant. On the other hand, zinc was significantly increased in zinc treated O cells compared to zinc treated controls and non treated O cells. This change of zinc might demonstrate that HCV infection itself increases zinc and additional zinc further enhance zinc content in HCV infected cells concomitantly increased MT compared with control (Figure 4).

Cd-hem Assay for MT (Figure 4)

Intracellular MT was calculated by Cd-hem assay. Baseline expression of MT is higher in HCV-O cells than control cells. MT induction by zinc was 1.6 μg/mg proteins in Huh-7 and 2.4 μg/mg proteins in HCV-O. These increases are not statistically analyzed because the quantification was repeated twice.

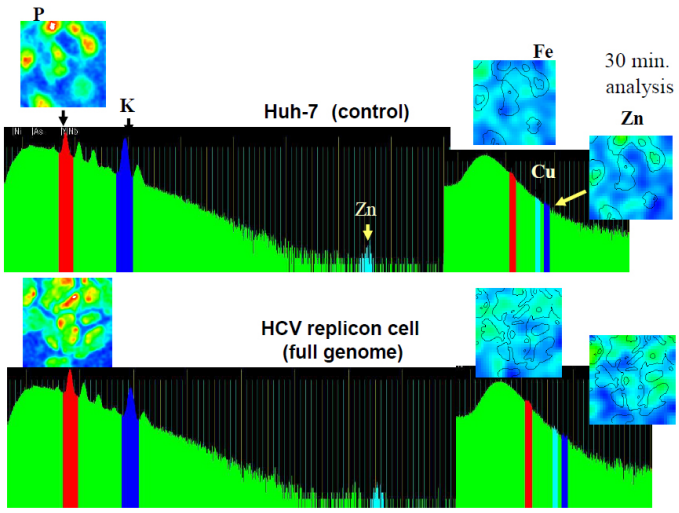

Figure 1: P, K, Fe and Zn content in HCV-O and Control (Huh7) analyzed by air micro-PIXE.

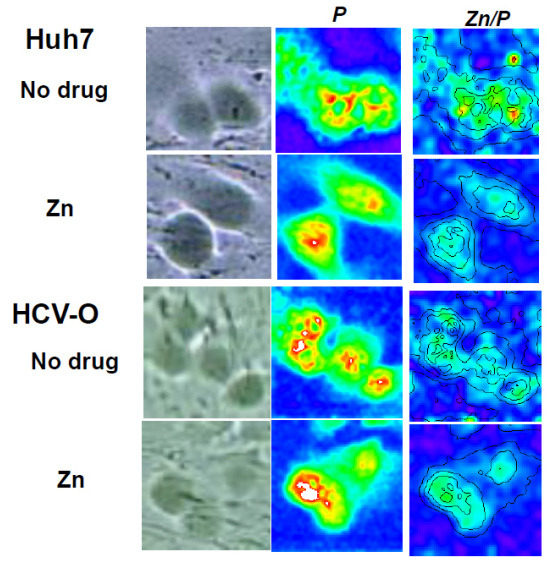

Figure 2: Zinc distribution detected by air micro-PIXE compared with P as cytoplasm, Br as nucleus in HCV-O and Control (Huh7).

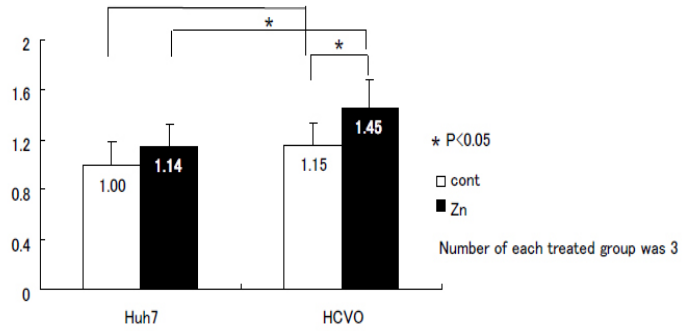

Figure 3: Calculation of zinc content by air-micro-PIXE callibrated by the control.

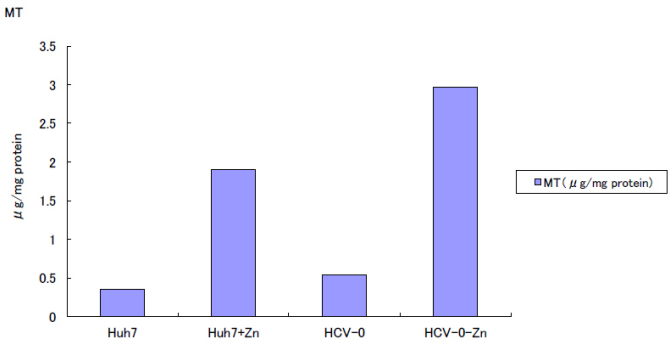

Figure 4: Quantification of metallothionein by Cd-hem assay.

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DISCUSSION

In patients with chronic liver disease, trace element in the liver and serum is changed in accordance with the severity of the disease.[1] In the trace elements, we focused on zinc because zinc is a key metal of HCV replication.[13] We have already revealed the zinc ion suppressed the HCV replication in HCV replicon[13] and zinc could be the possible treatment option for hepatitis C.[4,5] Until now, few precise report of metal distribution in one cell has been published because no dynamic observation of intracellular metal has been available. Micro-PIXE can overcome this technical difficulty. Nagamine et al[14] first reported zinc and MT distribution in hepatoma cell, line, HepG2 using micro PIXE. They concluded that zinc and interferon-beta collaboratively enhanced the MT expression and zinc in hepatoma cells. We have first demonstrated enhanced zinc in HCV replicon and using micro PIXE in this study. Micro PIXE analysis revealed the elevated zinc in the HCV replicon cells and the additional zinc more increased the zinc content in HCV replicon cells than control.

Non-structure coding region of HCV, namely, NS2/3 and NS3 protease contained zinc in their structure.[15] One of the reasons of zinc elevation in HCV infected cells may be the existence of zinc containing virus but those of more MT induction in HCV infected cells have not been clarified. *In vivo* studies have shown that MT expression was enhanced in chronic hepatitis C and interferon treatment decreased MT expression.[16] Our results are compatible with these reports that HCV itself can evoke MT expression in replicon cells.

The other possible mechanism of zinc increment in O cells might be the change of zinc transporter. Zinc transporter zip14 is reported to be up-regulated by interleukin-6.[17] The relationship of HCV and zinc transporter could be some keys of trace element disturbance.

Because serum zinc concentration decreased in the advanced stage of hepatitis C,[18] the increase of zinc in O cells might be the results of homeostatic HCV suppression in infected cells, such as hepatocytes. Anyhow further study is needed to elucidate the precise relationship of zinc and HCV.

Zinc distribution and relative concentration in one cell was visualized and quantified by air micro-PIXE analysis. According to the results, zinc is enhanced in HCV infected cells and further enhanced by additional zinc administration in cell culture. This enhancement is accompanied by metallothionein increase. These results might be preliminary but further study using this system will elucidate the relationship between HCV and trace element especially zinc.

CONCLUSION

This paper first demonstrated the zinc increase in HCV infected cells than control by micro-PIXE. One possible mechanism of this increment is MT enhancement.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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The Clinical or Radiographic Diagnosis of Gastroptosis: Still Relevant?

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ABSTRACT

Introduction: Gastroptosis was a frequent diagnosis in former days, however in current practice it is a rare diagnosis with uncertain etiology, with only a few number of studies to support it. Gastroptosis is diagnosed on a nupper gastrointestinal (GI) study by the downward displacement of the greater curve of the stomach below the level of the iliac crests in a standing position. Gastroptosis could possibly cause GI symptoms and delayed gastric emptying, but the relation is unknown, especially in children. This study addresses the association of GI symptoms and gastroptosis in children in the Netherlands.
Methods: We present a retrospective study of all children aged 6 to 18 years who underwent an upper GI study of the stomach in a 5-year period in our university children's hospital. Sixty of one hundred sixty-one children were included in the analysis, of whom seven appeared to have a radiological diagnosis of gastroptosis.
Results: GI symptoms were not significantly different between children with and without gastroptosis. Compared to the control group, there was a significant higher incidence of decreased gastric motility (p=0.004) in children with gastroptosis, as well as delayed gastric emptying (p=0.045).
Conclusion: Children with gastroptosis did not have specific GI symptoms. Gastroptosis was associated with decreased gastric motility and delayed gastric emptying, which could either be the cause of the gastroptosis or a consequence. The radiologic diagnosis gastroptosis had no consequence regarding treatment. Therefore, gastroptosis on an upper GI study seems clinically irrelevant.

KEY WORDS: Gastroptosis; Delayed gastric emptying; Impaired gastric motility.

ABBREVIATIONS: BMI: Body Mass Index; NVNG: Nederlandse Vereniging voor Nucleaire Geneeskunde (Dutch Association of Nuclear Medicine); SDS: Standard Deviation Score; GI: Gastrointestinal.

INTRODUCTION

Gastroptosis was a frequent diagnosis in former days, however in current practice it is a rare diagnosis with uncertain etiology. Gastroptosis is diagnosed by the downward displacement of the stomach on an upper gastrointestinal (GI) study in a standing position, with the greater curve of the stomach partly projecting below the level of the iliac crests (Figure 1).[1,2] The physiological position of the stomach varies between individuals, and also in one subject depending on many factors like stomach tone, the degree of fullness of the stomach, the position of the subject (supine or upright) and the strain of the abdominal muscles.[3]

Gastroptosis may be associated with a variety of GI symptoms including epigastric pain or discomfort, early satiety and acid brash.[1-4] After a meal patients with gastroptosis may

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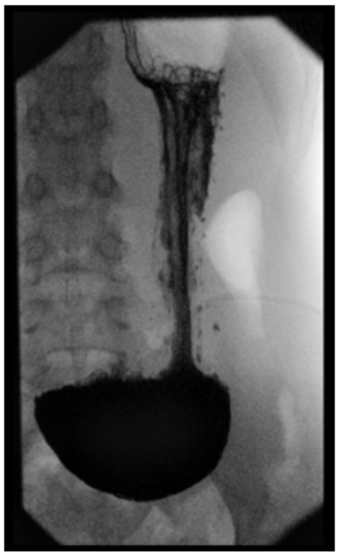
Figure 1: Gastroptosis on an Upper-Gastrointestinal Study.



suffer from nausea and discomfort.[1] Gastroptosis is also associated with delayed gastric emptying.[1,3] Delayed gastric emptying, altered antroduodenal motility, and impaired gastric accommodation have been proposed to explain symptoms of functional dyspepsia.[5-9] It is not known if gastroptosis itself leads to certain symptoms or if gastroptosis and related symptoms are the result of another disease. Symptoms related to gastroptosis are non-specific and could be caused by many other conditions.

In the past, surgical treatment was preferred.[10] Now-a-days only few cases have been reported in the literature.[1,3] In our university hospital, we recently were confronted with some children suffering from GI symptoms who had gastroptosis on the upper GI study, which brought this condition to our attention. Only small studies are available on gastroptosis, mostly on adults, and most of them are outdated.

It is not clear if gastroptosis is still a relevant diagnosis to explain certain GI symptoms.This study aims to study the possible association of gastroptosis and GI symptoms in children.

METHODS

Population

We retrospectively investigated the upper GI tract studies ofchildren aged 6 to 18 years in the Radboudumc Amalia Children's Hospital between March 2010 and March 2015. Children with a history of fundoplication were excluded, because of the change in stomach position and anatomy. Fundoplication can result in decreased gastric accommodation, which may be associated with postprandial fullness and dyspeptic symptoms,[9] which could bias the control group. Children with a history of or an indication for gastrostomy were also excluded.

The diagnosis of gastroptosis had to be established onan upper GI study in a standing position, therefore children who were studied only in supine position were excluded.

Data Collection

All data were extracted from electronic patient files (EPIC Hyperspace PRD 2014), including the age on the date of the upper GI study, gender, height standard deviation score (SDS), body mass index (BMI, kg/m²) and BMI SDS of within 2 months of the upper GI study. The BMI SDS represents the deviation in BMI from the mean BMI of the general population of children with the same age and gender. To be able to compare BMI between the children and the groups, BMI SDS was used to correct for gender and differences in age.

GI symptoms were assessed by investigation of the electronic patient files, particularly for a period of 6 months before the enema study was done. Possible symptoms of gastroptosis recorded in the database included epigastric pain or discomfort, acid brash or heartburn, nausea, constipation, recurrent diarrhea (>2 times a week), recurrent vomiting (>2 times a week), abdominal distension and early satiety.

Gastroptosis was diagnosed by anupper GI study either with barium or iodinated water soluble contrast, using pulsed fluoroscopy and/or radiographs, according to the insights of the performing pediatric radiologist. Images of the upper GI studywere studied for each child using the image viewer of Impax (Agfa). The diagnosis of gastroptosis was set by the investigator if the major curve of the stomach was partly located below the level of the iliac crests in a standing position, measured by the drawing of ahorizontal line between the iliac crests. In a supine position, the stomach could be positioned normally, while the stomach descends when the patient stands up.[11] To determine

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the position of the patient during the upper GI study, all images were investigated on the presence of air-fluid levels. Children with images only in supine position were excluded. The group of children with gastroptosis on the upper GI study was compared to the group of children without gastroptosis on the upper GI study (control group). All children meeting the inclusion criteria mentioned above without gastroptosis on the upper GI study in a standing position were considered as the control group.

In most children, gastroptosis was not diagnosed at the time of the upper GI study. In 5 patients the radiologist did mention the downward displacement of the stomach; however, it had no consequences for further investigation or treatment.

The presence of delayed gastric emptying was recorded in the database, as well as information about gastric motility. Gastric emptying was defined as delayed when it was diagnosed through a gastric emptying scintigraphy or when it was delayed during the upper GI study according to the opinion of the radiologist. For scintigraphy, children had to eat a pancake with 10 MBq technetium 99 m hepatate II colloid according to the recipe of the recommendations of the Dutch Association of Nuclear Medicine (NVNG).[12] Gastric emptying time was defined as delayed consistent with the same guidelines. Gastric motility was defined as impaired if it was reduced according to the opinion of the performing radiologist during the upper GI study.

Treatment and symptoms during treatment were recorded for all children. Conservative treatment was defined as expectant in combination with a diet. The period of follow-up was variable, depending on the time needed to set the treatment and the return of the children to the referring physician. The need for informed consent was waived by the institutional medical ethical review board.

Statistics

IBM SPSS Statistics version 21 (2012) was used for data analysis. Mann Whitney U-test was used to assess differences of age and BMI SDS between the group of children with gastroptosis and the control group. The significance of differences in incidences of GI symptoms, decreased gastric motility and delayed gastric emptying was assessed statistically by Fisher's exact test. For all analyses, $p<0.05$ was used for statistical significance.

RESULTS

One hundred sixty-one children between the age of 6 to 18 years underwent an upper GI study in our university hospital between March 2010 and March 2015. Children with fundoplication in history (N=18) and children having a gastrostomy or an indication for gastrostomy (N=41) were excluded. Two children were excluded because the results of the upper GI study were the only available data. Another 40 children were excluded because the results of the upper GI study showed the stomach only in a supine position. Of the 60 children with an upper GI study in standing position that were included, 7 children had gastroptosis (11.67%).

Table 1 provides an overview of all 60 included subjects. The age of the children with gastroptosis (14.14±2.48) was significantly higher compared to the age in the control group (11.43±3.39; $p=0.047$). We found no significant differences in gender between the two groups (boys 28.6% *vs*. girls 43.4%; $p=0.688$).

Overall, BMI SDS differed not significantly between the groups ($p=0.06$). However for girls, while BMI SDS was significantly lower in girls with gastroptosis compared to the control group ($p=0.027$), it was still within the normal range.

The indications for the performing of an upper GI study are shown in Table 2. For each child, more than one indication could be noted on the application form and these indications are demonstrated separately in the Table 2. There were no significant differences in indications.

GI symptoms were compared between children with gastroptosis and the control group. Results of the analysis of the incidence of symptoms between the groups are shown in Table 3. None of the symptoms had a significantly higher prevalence in children with or without gastroptosis.

Delayed gastric emptying was significantly more often present in children with gastroptosis than in the control group ($p=0.045$). Decreased gastric motility was identified significantly more often in the gastroptosis group ($p=0.004$). Of the 7 children with gastroptosis, 3 children had decreased gastric motility, of whom 2 also had delayed gastric emptying.

Table 1: Overview of Subjects Based on Upper GI Study.

		Gastroptosis (N=7)	Non-gastroptosis (N=53)	*p*-value
Gender	**Boy Girl**	2 (28.6%) 5 (71.4%)	23 (43.4%) 30 (56.6%)	0.69
Age	**(mean±SD)**	14.14±2.48	11.43±3.39	0.047*
BMI SDS	**(mean±SD)**	-0.83±1.11	0.12±1.69	0.06

SD: standard deviation; BMI SDS: Body Mass Index Standard Deviation Score

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Table 2: Indications of the Barium Study in Children

		Gastroptosis (N=7)	Non-gastroptosis (N=53)
Indications barium study	Malrotation	6 (85.7%)	29 (54.7%)
	Hiatal hernia	0 (0%)	19 (35.8%)
	Gastroesophageal reflux	1 (14.3%)	13 (24.5%)
	Gastric motility	2 (28.6%)	10 (18.9%)
	Gastric emptying	1 (14.3%)	11 (20.8%)
	Esophagus abnormalities	0 (0%)	16 (30.2%)
	Passage/stenosis/stricture/obstruction	4 (57.1%)	11 (20.8%)
	Gastrointestinal inflammation	1 (14.3%)	0 (0%)

Table 3: Symptoms in Children with and without Gastroptosis.

		Gastroptosis, N=7 (%)	Non-gastroptosis, N=53 (%)	*p*-value
Symptoms				
Symptoms of acid brash	Present	4 (57.1%)	27 (50.9%)	1.000
Epigastric pain, discomfort	Present	7 (100%)	36 (67.9%)	0.18
Constipation	Present	1 (14.3%)	13 (24.5%)	1.00
Recurrent diarrhea	Present	0 (0%)	6 (11.3%)	1.00
Nausea	Present	5 (71.4%)	19 (35.8%)	0.10
Recurrent vomiting	Present	4 (57.1%)	16 (30.2%)	0.21
Early satiety	Present	2 (28.6%)	2 (3.8%)	0.06
Abdominal distension	Present	0 (0%)	1 (1.9%)	1.00
Upper GI tract study				
Decreased gastric motility	Present	3 (42.9%)	1 (1.9%)	0.004*
Delayed gastric emptying	Present	4 (57.1%)	10 (18.9%)	0.045*
***p*<0.05 Significant**				

One child with gastroptosis showed normal gastric emptying and normal gastric motility in supine position, but in a standing position there was no movement of food to the duodenum.

Treatment of the children with gastroptosis was symptomatically in all patients. Erythromycin was given in 4 patients, of whom one had to stop because of frequent vomiting as a side-effect, and one patient stopped because of the absence of improvement. Four children were treated with antacids, which reduced the symptoms in all patients, but not satisfactorily. In one patient with gastroptosis and delayed gastric emptying, symptoms recovered after treatment with Metronidazole for a Giardia lamblia infection. It is known that there is a relation between delayed gastric emptying and bacterial overgrowth, which could cause symptoms including diarrhea, bloating, malnutrition and weight loss.[13,14] In one patient, symptoms disappeared entirely after a diagnostic laparoscopy, with detaching of an adhesion of the duodenum. In none of the other 5 subjects symptoms disappeared entirely within a year, despite of the treatment.

In the control group, treatment was also symptomatically, mostly conservative. There was no indication for surgery. Two patients were treated with Metronidazole for a Dientamoeba Fragilis infection. Symptomatic treatments of both groups are compared in Table 4. Erythromycin was used significantly more often in children with gastroptosis, which is likely due to the higher incidence of delayed gastric emptying in children with gastroptosis.

Table 4: Management of Children with and without Gastroptosis.

Treatment	Gastroptosis, N=7 (%)	Non-gastroptosis, N=53 (%)	*p*-value
Strictly conservative	2 (28.6%)	28 (52.8%)	0.12
Macrogol	1 (14.3%)	6 (11.3%)	1.00
Antacids	4 (57.1%)	16 (30.2%)	0.35
Erythromycin	4 (57.1%)	1 (1.9%)	0.00*
**p*<0.05 Significant			

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DISCUSSION

This study demonstrates a higher incidence of decreased gastric motility and delayed gastric emptying in children with gastroptosis. GI symptoms, such as nausea, recurrent vomiting and acid brash, were not significantly more present in children with gastroptosis compared to the control group.

There are some mechanisms suggested to cause gastroptosis and GI symptoms. The downward displacement of the stomach in gastroptosis is suggested to be caused by relaxation or stretching of the muscles or by decrease of the muscle tone.[1] However, in this study GI symptoms were not significantly different in children with gastroptosis compared to the control group. This may be caused by the fact that the symptoms of gastroptosis are non-specific and applicable to several other conditions.

In the literature, gastroptosis seems to be associated with delayed gastric emptying.[1-3] In children, investigations to diagnose delayed gastric emptying are generally unreliable. Upper GI studies may demonstrate delayed gastric emptying, but sensitivity and specificity are low.[15] In our study, in most of the cases the result of the upper GI tract study was used to define delayed gastric emptying, when gastric emptying scintigraphy did not take place. This could have introduced a false interpretation, because results of gastric emptying and gastric motility, as seen on an upper GI study, are dependent on the interpretation of the radiologist, as there are no reference values for gastric emptying and gastric motility in upper GI studies. However, as this is a retrospective study, the performing radiologists were not aware of this study at the time of the upper GI studies.

Delayed gastric emptying seems to be associated with gastroptosis, but the mechanism by which they are associated is unknown. Natsis et al[3] suggested that gastroptosis could cause GI symptoms. They suggest that tubular structures are prone to kinking when internal organs are positioned low in the abdomen, which may cause a temporary obstruction of flow through these organs. This could lead to food remaining in the stomach, which can lead to GI symptoms.[3] Gastric emptying time seems to be prolonged when the stomach lays in a lower position.[3] Christianakis et al[1] suggested that delayed diagnosis or misdiagnosis of gastroparesis could possibly secondarily lead to gastroptosis, due to decrease of the muscle tone.

In this study, children with gastroptosis had a higher prevalence of decreased gastric motility. The presence of decreased gastric motility was determined subjectively by the radiologist who initially evaluated the upper GI study. Decreased gastric accommodation is associated with symptoms including early satiety, bloating, epigastric pain, weight loss and nausea.[5] Delayed gastric emptying, altered antroduodenal motility, and impaired gastric accommodation have been proposed to explain symptoms of functional dyspepsia.[5-9] However, despite of the higher incidence of delayed gastric emptying and decreased gastric motility in the group of children with gastroptosis, there was no difference in the presence of GI symptoms. This may be caused by the fact that the symptoms of gastroptosis are non-specific and applicable to several other conditions.

In the past, the preferred treatment of gastroptosis was surgical, because gastroptosis was thought to cause gastric dysfunction.[1,2] Barbat et al[10] suggested that the only indication for an operative procedure is when there is actual obstruction, which is rare. Now-a-days treatment is symptomatically, because it is uncertain that gastroptosis itself leads to GI symptoms.

There are some limitations to this study. The study is retrospective, so it depends on the availability and reliability of data, which is especially tricky when studying a dynamic upper GI study with only a couple of images saved. Results of gastric emptying and gastric motility depend on the radiologist, as there are no standard values available for gastric emptying and gastric motility in children in upper GI studies. Another issue is the limited number of patients of the study group. Of the 161 patients who met the inclusion criteria, 60 were included from which seven could be diagnosed with gastroptosis. Still, to our best knowledge this is the largest study on gastroptosis in children.

All children underwent an upper GI study for an indication related to GI issues, which causes the control group to be unequal to the general population. However the upper GI study indications were similar in cases with or without gastroptosis.

Regarding this study and the literature, the diagnosis of gastroptosis does not seem to be relevant anymore. There is no particular medical treatment for children with GI symptoms with gastroptosis. Also surgery of gastroptosis has become obsolete. Treatment is symptomatically as it is not clear if symptoms are caused by gastroptosis itself. Gastroptosis could possibly be part of a disease complex; however, it has no relevance on its own.

We conclude that gastroptosis can be part of the complex of findings in patients with GI symptoms; however, it is not diagnostic for a particular disease, nor does it influence the treatment. Therefore, gastroptosis on an upper GI study is an irrelevant finding.

COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of Interest: All authors declare no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Author's Contributions: All authors are responsible for study design and writing and revising the manuscript.

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