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MINIREVIEWS

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WJGP mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal pathophysiology and covering a wide range of topics including disorders of the esophagus, stomach and duodenum, small intestines, pancreas, biliary system, and liver.

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MINIREVIEWS

Application of electron microscopy in gastroenterology

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Abstract

Electron microscopy has long been used in research in the fields of life sciences and materials sciences. Transmission and scanning electron microscopy and energy-dispersive X-ray spectroscopy (EDX) analyses have also been performed in the field of gastroenterology. Electron microscopy and EDX enable (1) Observation of ultrastructural differences in esophageal epithelial cells in patients with gastroesophageal reflux and eosinophilic esophagitis; (2) Detection of lanthanum deposition in the stomach and duodenum; (3) Ultrastructural and elemental analyses of enteroliths and bezoars; (4) Detection and characterization of microorganisms in the gastrointestinal tract; (5) Diagnosis of gastrointestinal tumors with neuroendocrine differentiation; and (6) Analysis of gold nanoparticles potentially used in endoscopic photodynamic therapy. This review aims to foster a better understanding of electron microscopy applications by reviewing relevant clinical studies, basic research findings, and the state of current research carried out in gastroenterology science.

Key Words: Transmission electron microscopy; Scanning electron microscopy; Energy-dispersive X-ray spectrometry; Gastrointestinal disease, gastroesophageal reflux disease; Pathogens

Core Tip: This review provides an overview of transmission electron microscopy, scanning electron microscopy, and energy-dispersive X-ray spectrometry analyses used in the field of gastroenterology. Previously reported articles have been reviewed, with a focus on electron microscopy applications. The history and present trends in electron microscopy applications in patients and research associated with digestive system diseases are also summarized.

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INTRODUCTION

In light microscopy, visible light is used to obtain magnified views of the object. As the resolution is related to the wavelength of light used to image a specimen, the resolution of an optical microscope is theoretically limited to approximately 200 nm. Thus, nanostructures cannot be observed using light microscopy. In contrast, electron beams are used in electron microscopy. As the wavelength of an electron beam is shorter than that of visible light, electron microscopy has extremely high resolution and provides sharp, finely detailed images of the surface or interior of biological and nonbiological specimens. In addition, energy-dispersive X-ray spectroscopy (EDX), which is a chemical microanalysis technique used in conjunction with electron microscopy, enables the analysis of elements or chemical characterization of a sample. Since the development of the first prototype in 1931, electron microscopes have been widely used in various fields, such as physics, chemistry, engineering, biology, and medicine [1]. Based on its versatility, electron microscopy analysis has been used in several studies covering various aspects of clinical samples obtained from patients with gastrointestinal diseases. This paper briefly discusses the fundamentals of electron microscopy and reviews the literature concerning the application of electron microscopy in gastroenterology science.

ANALYTICAL METHODS IN ELECTRON MICROSCOPY

Analytical methods in electron microscopy can broadly be categorized into three types: Transmission electron microscopy, scanning electron microscopy (SEM), and EDX. The different types of electron microscopes used in these methods are related and often applied concurrently in the field of biology.

A transmission electron microscope irradiates a specimen with an electron beam. The object must be cut into very thin cross-sections because it is visualized through the spatial distribution of the transmitted electron beam. Although the use of transmission electron microscopy is limited to engineering science at the outset, it has been extensively used in the field of biology since the 1950s largely due to improvement of the microtome for ultrathin slice preparation using a diamond knife and the development of staining techniques based on heavy metals, such as osmium.

A scanning electron microscope produces an image using electrons reflected or generated from the surface of the specimen. The specimen is placed in a high vacuum state, and the surface is scanned with an electron beam focused by an electric or magnetic field. SEM produces a characteristic three-dimensional appearance that is useful for understanding the surface ultrastructure of a sample.

EDX is an X-ray system used to identify the elemental composition of a material. It has a semiconductor detector to detect the fluorescent X-rays generated when the primary X-ray beam illuminates the sample. The fluorescent X-rays emitted from the material have a spectrum of wavelengths characteristic of the types of atoms present in the specimen. EDX enables both qualitative and semiquantitative analyses of the elements based on the energy and number of generated electron-hole pairs. EDX is more suited for analyses of inorganic materials than organic materials.

In the field of gastroenterology, transmission and SEM and EDX analyses have been used to visualize cells (Figure 1) and pathogens, including parasites, bacteria, viruses, biofilms, and elements deposited in the gastrointestinal mucosa. Nonbiological materials, such as stents, powders, and bezoars, have also been analyzed at subnanometer resolution. In the following sections, we review examples of electron microscopy analyses in association with the pathophysiology of gastrointestinal disorders.

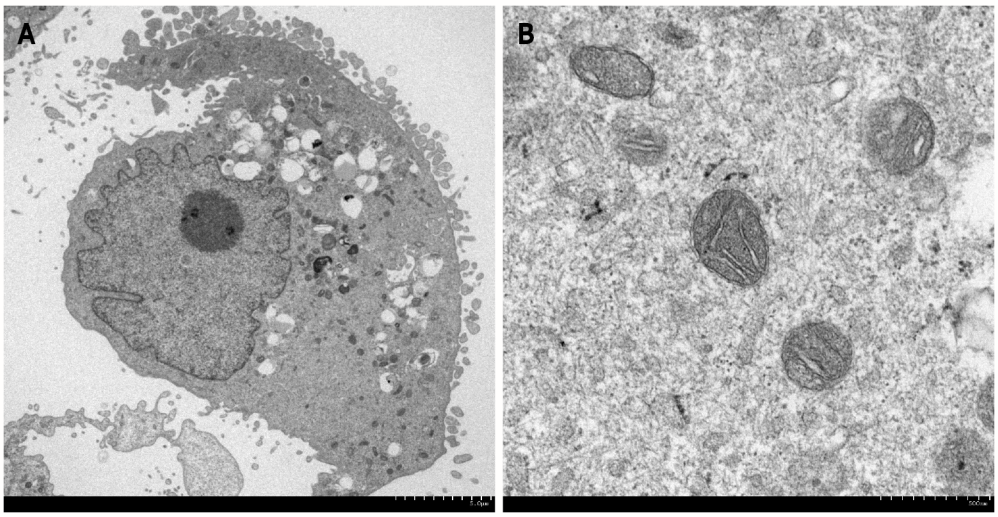

Figure 1 Transmission electron microscopy image. Transmission electron microscopy of the human cell line derived from gastric cancer (SH-10-TC) showing the morphology of cells and their organelles. A: Scale bars = 5 μm; B: Scale bars = 500 nm.

EXAMPLES OF ELECTRON MICROSCOPY ANALYSES

Intercellular spaces of the esophageal epithelium

The most typical example of electron microscopy analysis in gastroenterology is evaluation of the intracellular spaces of esophageal epithelial cells. Notably, some of the articles on this topic have been published in high-impact journals. Intercellular spaces in the esophageal epithelium are known to be dilated in patients with nonerosive reflux disease and in patients with esophagitis. Following several animal studies, endoscopic esophageal biopsy specimens taken from patients with (n = 11) and without (n = 13) recurrent heartburn were investigated in 1996 using transmission electron microscopy[2]. A dilated intercellular space diameter was observed in 8 of the 11 patients with heartburn, while none of the asymptomatic individuals exhibited this feature. Dilated intercellular space was also present in the normal-appearing, nonerosive mucosa of patients with symptomatic reflux disease. Other authors have provided further evidence that detached interepithelial cell junctions, which are observed as dilated intercellular spaces assessed by electron microscopy[3-5], correspond to early esophageal damage induced by acid reflux[6-8]. Dilatation of intercellular spaces in the esophageal epithelium is not observed in patients with functional heartburn, suggesting that this microscopic feature is specific to acid reflux[9]. Proton pump inhibitor therapy resulted in complete recovery of dilated intercellular spaces in > 90% of cases with nonerosive reflux disease and erosive esophagitis, indicating that the electron microscopy features are reversible[10,11].

Dilated intracellular spaces arise along the distal and proximal esophagus of patients with nonerosive reflux disease, suggesting that they may be an underlying mechanism accounting for the enhanced perception of proximal acid reflux[12]. Duodenal gastroesophageal reflux has also been reported to cause dilatation of intercellular spaces in the esophageal epithelium[3,13]. Similarly, in patients with laryngopharyngeal reflux and sore throat, this feature appears at the squamous basal and suprabasal levels in oropharyngeal biopsy specimens[14,15]. An investigation of patients with bronchial asthma[11, 16] and children with reflux-related cough[17] revealed that the intracellular spaces in the esophageal epithelium are significantly dilated compared with those in control patients, suggesting a pathophysiological correlation between gastroesophageal reflux and the development of these respiratory tract symptoms.

Although the width of the intracellular spaces can be measured using light microscopy[18], the sensitivity of light microscopy was 79.3%, and the specificity was 75.0%[19]. Owing to the inferior specificity of light microscopy analysis, electron microscopy seems to be more suitable for measuring intercellular spaces in the esophageal epithelium. Chu *et al*[20] reported the possible utility of in vivo confocal laser endomicroscopy to examine microalterations of the esophagus in patients with nonerosive reflux disease[20].

Eosinophilic esophagitis

Eosinophilic esophagitis is a chronic, allergic inflammatory condition of the esophagus. Dilated intracellular spaces are evident in the esophageal epithelium of patients with eosinophilic esophagitis, which are significantly reduced after treatment[21]. Transmission electron microscopy revealed a significant decrease in the number of desmosomes[22] and an increased autophagic vesicle content[23] in active eosinophilic esophagitis compared with observations in normal individuals and inactive

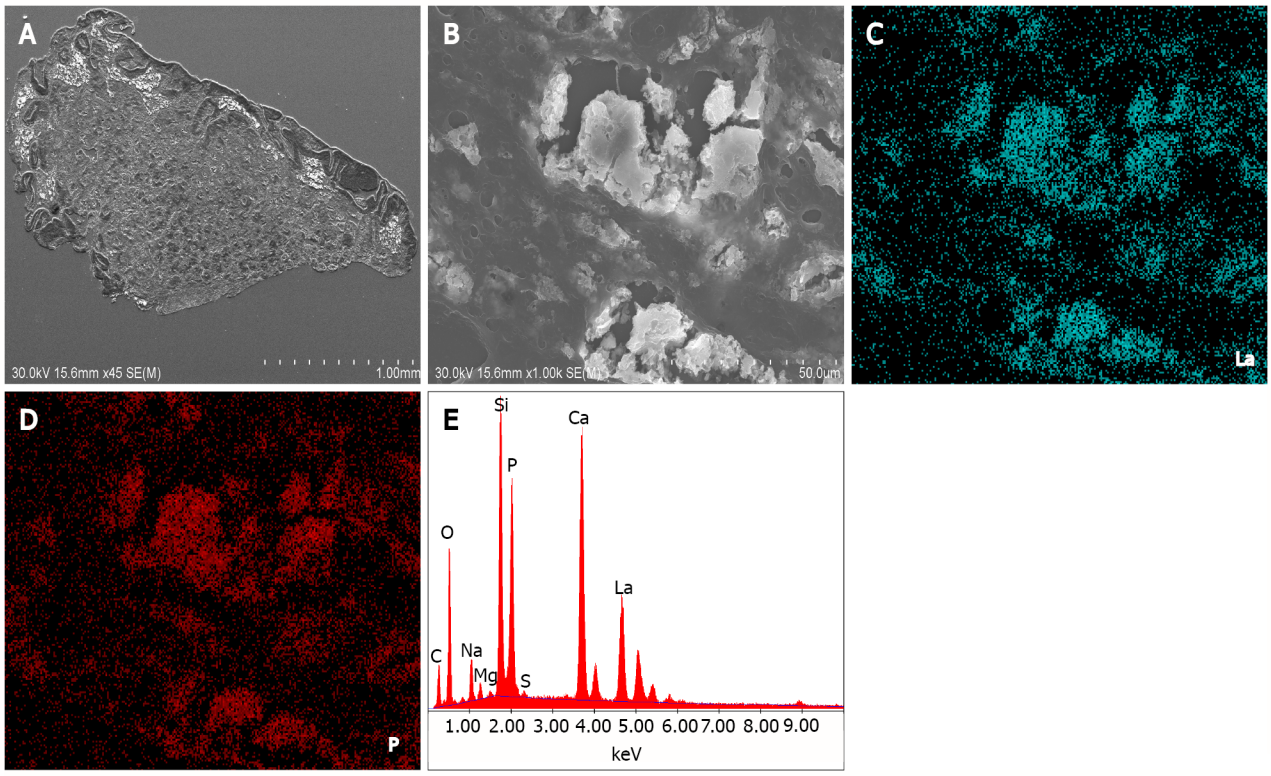

Figure 2 Transmission electron microscopy images and spectra obtained by energy-dispersive X-ray spectrometry. A: Lanthanum phosphate deposition in the gastric mucosa was diagnosed after analysis by scanning electron microscopy, which visualized deposited lanthanum as bright areas; B: Deposited lanthanum is composed of aggregates of particles; C and D: Elemental mapping showing the colocation of lanthanum (C) and phosphate (D); E: Energy-dispersive X-ray spectrometry.

eosinophilic esophagitis patients. Thus, electron microscopy may be useful for investigating the pathophysiology of eosinophilic esophagitis.

Lanthanum deposition

Lanthanum carbonate is a phosphate binder taken orally and is commonly used in patients with chronic kidney disease. Although its tolerability and safety profile have been reported in hemodialysis patients, lanthanum deposition in the gastric and duodenal mucosa of these patients, in the form of lanthanum phosphate, has been reported in the literature[24-28]. On light microscopy examination of hematoxylin and eosin-stained specimens, deposited lanthanum is visible as a fine, amorphous, eosinophilic material. SEM revealed bright areas in the deposited lanthanum (Figure 2A). Images at higher magnification showed deposition as the accumulation of minute particles (Figure 2B). EDX analysis provided evidence directly related to the presence of lanthanum and phosphate (Figure 2C). Elemental mapping by EDX revealed that lanthanum (Figure 2D) and phosphate (Figure 2E) showed an identical location to that of the bright areas on SEM. Although lanthanum deposition in the gastrointestinal tract can be clinically diagnosed with conventional light microscopy observation of the fine, amorphous, eosinophilic material and medication information from the patient's current or past use of lanthanum carbonate, SEM has advantages in the detection of deposited lanthanum, as it is easily identified as a bright area.

Enteroliths and bezoars

Enteroliths are calculi that occur in the intestines and include two types: "True" and "false" enteroliths. True enteroliths, for example, cholic acid and calcium stones, are generated from the sediments of substances found in enteric contents. False enteroliths, such as bezoars, gallstones, and foreign objects, are formed from indigestible substances stuck in the alimentary tract. Infrared spectroscopy is generally used to identify the chemical substances constituting enteroliths removed from patients. Electron microscopy and EDX have the advantages of imaging the microstructure and analyzing elements, allowing clarification of the nature of enteroliths.

Figure 3 shows examples of enteroliths and bezoars that we previously investigated. One patient had an enterolith in the stomach composed of bilirubin calcium, calcium carbonate, and fatty acid calcium [29] (Figure 3A and B). Another patient had a rare pharmacobezoar in the stomach, which was composed of magnesium oxide (Figure 3C–F)[30]. We also investigated the ultrastructure of the persimmon phytobezoar in the stomach (Figure 3G–I)[31]. Thus, electron microscopy and EDX analyses

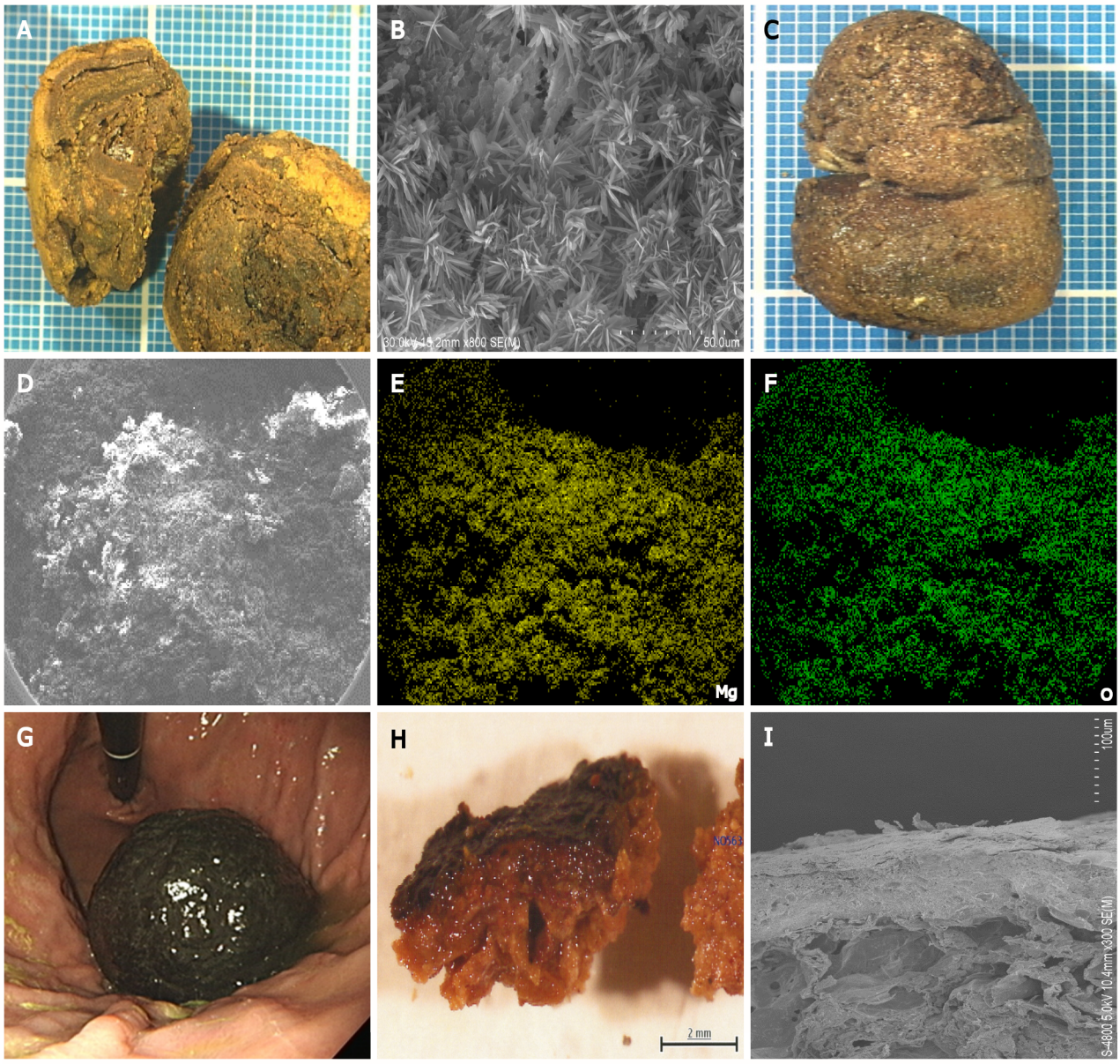

Figure 3 Images of enteroliths. A and B: An enterolith found in the stomach showing a laminar structure on the cut surface (A). Scanning electron microscopy demonstrating acicular crystals (B), suggesting epitaxial growth of the enterolith; C: Another patient had a pharmacobezoar composed of magnesium oxide in the stomach; D-F: Scanning electron microscopy showed a granular substance (D) and a diffuse distribution of magnesium (E) and oxide (F); G: A persimmon phytobezoar was observed in the stomach; H: The cut section shows that the color of the bezoar surface is black, and the interior is yellowish; I: On scanning electron microscopy, a high-density, continuous layer forming the exterior of the phytobezoar is evident on the cut surface, while sheet-like structures of curved or wiggly shapes constitute the inner part.

offer insights into the microstructure and elemental composition of enteroliths.

Pathogens including bacteria, parasites, and viruses in the gastrointestinal tract

Electron microscopy has been widely used in microbiology to elucidate the number, distribution, and adherence of microorganisms in clinical samples. One of the typical applications of electron microscopy for pathogens in gastroenterology is the detection of *Helicobacter* species, such as *Helicobacter pylori*[32-36] and *Helicobacter heilmannii*[37]. These bacteria have a spiral form, which is a distinct difference from other bacteria. Another example is *Tropheryma whipplei*[38-41], which causes the rare systemic infectious disorder Whipple's disease. Electron microscopy revealed that *Tropheryma whipplei* shows a characteristic trilamellar plasma membrane. Other rare pathogens identified by electron microscopy include anisakiasis[42], amoebiasis[43], intestinal spirochetosis[44], *Sutterella wadsworthensis*[45], *Giardia intestinalis*[46], and *Brachyspira aalborgi*[47].

A biofilm is a thick layer formed by microorganisms attached to the surface of a solid material or liquid. SEM has been used to visualize the shape and localization of biofilms and the steps of the biofilm formation process. For instance, several authors have investigated the efficiency of the cleaning, disinfection, and sterilization processes of biofilm-contaminated endoscopes[48,49].

Gastrointestinal tumors with neuroendocrine differentiation

Neuroendocrine and mixed neuroendocrine neoplasms can arise in most of the epithelial organs of the body and are not rare in the gastrointestinal tract. Transmission electron microscopy revealed that neuroendocrine tumor cells in the gastrointestinal tract contained numerous dense-core secretory granules of variable sizes and shapes in the cytoplasm. Because these neurosecretory granules are characteristic of neuroendocrine tumors, electron microscopy analysis has been used to support its diagnosis. For instance, neuroendocrine differentiation was assessed using electron microscopy images in cases of malignant peripheral nerve sheath tumors of the esophagus[50], gangliocytic paraganglioma in the duodenum[51], mixed acinar-endocrine carcinoma arising in the ampulla of Vater[52], combined adenocarcinoma and neuroendocrine tumors in the stomach[53], neuroendocrine carcinoma in the stomach[54], mixed acinar-endocrine neoplasm in the stomach[55], and large cell neuroendocrine carcinoma in the esophagogastric junction[56].

Gold nanoparticles potentially used in endoscopic photodynamic therapy

Based on the properties of absorption and scattering of electromagnetic radiation, gold nanoparticles are emerging as promising agents and are of particular interest for applications in photothermal therapy, in addition to efficient drug carriers and diagnostic agents. For instance, endoscopic fluorescence-guided near-infrared photothermal therapy using gold nanoparticles is in development for the treatment of gastrointestinal tumors[57]. The size, morphology, and composition of synthesized gold nanoparticles and their location within tissue can be assessed using transmission electron microscopy and EDX analysis[58].

CONCLUSION

Electron microscopy enables (1) Observation of ultrastructural differences in esophageal epithelial cells in patients with gastroesophageal reflux and eosinophilic esophagitis; (2) Detection of lanthanum deposition in the stomach and duodenum; (3) Ultrastructural and elemental analyses of enteroliths and bezoars; (4) Detection and characterization of microorganisms in the gastrointestinal tract; (5) Diagnosis of gastrointestinal tumors with neuroendocrine differentiation; and (6) Analysis of gold nanoparticles potentially used in endoscopic photodynamic therapy. Therefore, electron microscopy has had a profound impact on our knowledge and understanding of various digestive tract diseases. We hope that this article will help gastroenterologists widely utilize electron microscopy analysis for clinical diagnosis and basic research.

FOOTNOTES

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MINIREVIEWS

Risk assessment of hepatitis E transmission through tissue allografts

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Abstract

Hepatitis E virus (HEV) is a small non-enveloped single stranded RNA virus whose genotypes 3 and 4 have been associated with zoonotic transmission in industrialized countries. HEV infection is considered the main cause of acute hepatitis worldwide. In some cases, transfusion of blood components or organ transplantation have been reported as the source of infection. We have conducted a literature review on the risk of transmission through cell and tissue allografts. Although no case was found, measures to control this risk should be taken when donor profile (based upon geographical and behavioural data) recommended it. Issues to be considered in donor screening and tissue processing to assess and to reduce the risk of HEV transmission are approached.

Key Words: Hepatitis E; Tissue allograft; Risk assessment; Disease transmission; Donor screening; Bioburden reduction

Core Tip: This manuscript provide a novel perspective of the mode of transmission of hepatitis E virus (HEV). HEV is mainly transmitted *via* fecal-oral route, but in recent years other transmission routes have been reported, including blood-borne transmission. The processing of tissue allografts in duly accredited tissue banks provides safe and efficient products.

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INTRODUCTION

There are several types of human tissues which are commonly used as allografts: Bone, tendon, cartilage, skin, cornea, amniotic membrane, stem cells, heart valve, blood vessel, *etc.* Almost all surgical disciplines benefit of its availability. Thus, millions of human tissue transplants are performed worldwide every year[1].

One of the drawbacks of these procedures is the potential for donor to recipient disease transmission. Although the real incidence of tissue allograft transmitted infection is unknown, some articles have published cases of viral, bacterial and fungal infections transmitted by tissues[2-5]. Regarding the different infectious agents, hepatotropic viruses have represented traditionally the real *workhorse* in maintaining the safety of tissues used for transplantation.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) can cause acute and chronic hepatitis and potentially lead to the development of cirrhosis, liver cancer and death. In the European Union, estimated 4.7 million people have a chronic HBV infection, and 3.9 million people have chronic hepatitis C. Many of these infections may go undiagnosed as chronic infection is often asymptomatic and a hypothetical tissue donor could be a potential transmitter of the disease[6].

Risk factors for HBV and HCV infection are now clearly established[7-11]. In recent decades, various factors have contributed towards changes in HBV and HCV epidemiology, including improvements in donor tissue safety. A rigorous evaluation of clinical, behavioral, and personal risks is now performed as it may completely exclude a donor[12,13]. In addition to this, all potential tissue donors must be tested for both serological anti-HBc, HBsAg anti-HCV and for HCV-HBV by nucleic acid testing. Based on both criteria, the risk of HCV and HCV transmission is currently very low established in 1 in 34000 for HBV and 1 in 42000 for HCV[14].

Hepatitis E virus (HEV) infection is one of the main causes of acute hepatitis in both developed and developing countries. This infectious disease has a high prevalence and incidence in Europe and has a greater clinical impact in vulnerable populations, such as immunosuppressed patients, pregnant women, and patients with underlying liver disease[10,15,16].

To date, there are no specific recommendations for the screening of this disease in blood, tissue, or organ donors, which may cause this route to be an important source of disease transmission.

INFORMATION RETRIEVAL SYSTEM

A search using the following search string: 'hepatitis E virus [Title/Abstract] OR HEV [Title/Abstract] NOT high endothelial venules [Title/Abstract]' was conducted. Applying these criteria on PubMed database (for articles published in last 20 years) 5485 records were recovered (Figure 1). This search was developed on 5th December 2020. Six hundred forty-three (11.7%) of them corresponded to reviews and 0.6% to systematic reviews (the first being published in 2009). When the search was restricted (using the Boolean operator *AND*) to the articles involving the word 'allograft', only 19 (0.3%) complied to the new condition. Seventy nine percent (15/19) of these last articles dealt only on organ transplantation, 2 on the transfusion of blood components (specially in relation to hematopoietic transplantation) and the other 2 were discarded because the reason for their recovery was the use of the acronym HEV (without description) to refer to high endothelial venules. Thus, to the best of our knowledge, the present paper is the first cross reference between HEV and tissue allografts.

HEV

HEV is a small non-enveloped positive-sense, single-stranded RNA virus, encased within an icosahedral capsid of between 27 and 34 nm in size belonging to the family Hepeviridae within the genus Orthohepevirus. Seven different genotypes have been described for the HEV. Five of them (1-4 and 7) can infect humans and the other two (5, 6) are found only in animals (boar). Genotypes 1 and 2 (HEV-1, HEV-2) have been found only in humans while genotypes 3 and 4 circulate in several animals (including pigs, rabbit, cattle, sheep, horse, boar, deer, and shellfish) and genotype 7 in camel. Genotypes 1 and 2 are directly transmitted fecal-orally, or indirectly, mainly *via* contaminated water. Genotypes 3 and 4 (HEV-3, HEV-4) are zoonotic infections with an animal reservoir, being indirectly transmitted through food (when consumed raw or undercooked) or by direct contact with infected animals. Thus, professionals who work in contact with animals or their wastes and carcasses (farmers, veterinarians, workers attending animals, slaughterers, traders, and suppliers) could be in higher risk of HEV infection[14,15-18]. In an effort to avoid inconsistencies when the HEV subtypes are named, Smith *et al*[19] have proposed standardization for the assignation of HEV sequences to each subtype. Likewise, the World Health Organization promoted the development of international standards for diagnostic assays[15,20].

Additionally to the host and mode of transmission, HEV genotypes also vary in geographical distribution. Genotype 1 is prevalent in Africa and Asia, whereas HEV-2 can be found in México and West Africa. Thus, HEV-1 and HEV-2 are responsible for HEV outbreaks in developing countries, with

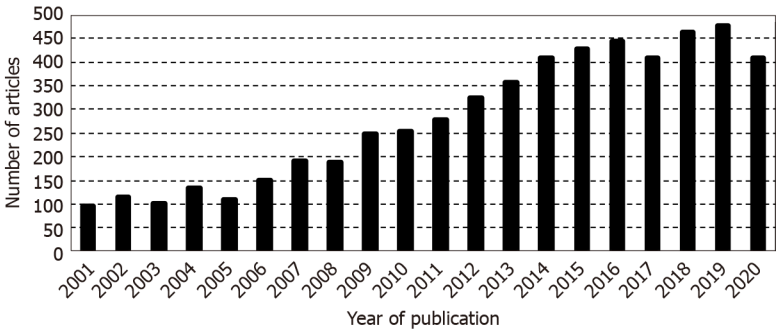

Figure 1 PubMed timeline results *per* year on hepatitis E virus.

limited sanitary conditions, due to contaminated drinking water. Genotypes 3 and 4 are associated with zoonotic transmission as autochthonous (locally acquired) infection in industrialized countries[21].

Clinical symptoms of HEV infection do not differ from other pathogens causing hepatitis. Therefore, diagnosis is performed by HEV RNA detection using real-time reverse transcription polymerase chain reaction with primers detecting all 4 genotypes affecting humans. Additionally, detection of HEV immunoglobulin (Ig) M and IgG antibodies is performed by enzyme linked immunosorbent assay. These data characterize HEV infection as acute (demonstration of specific IgM, rising levels of IgG, or detection of HEV RNA), passed, or chronic (positive results for HEV-RNA for more than 6 mo)[14,22]. Likewise, HEV antigen detection assay has been found to be used when HEV RNA testing is not available or time is limited[16]. Although HEV Ag shows low sensitivity with viral loads lower than 1000 copies/mL, it has shown good correlation with HEV-RNA, being useful in diagnosing infection in immunosuppressed patients[23,24]. Another issue to be considered, when Epstein-Barr virus and cytomegalovirus infection are present, is the risk of false positive results from anti-HEV IgM assays[25].

HEV transmission between persons by direct contact has proven very inefficient probably due to the high infective dose required[26]. Although it is associated with low mortality rates (< 1%) in the general population, this risk increases (approximately 20%) during pregnancy[14].

Although hepatitis viruses have been suggested to play a role of in the development of autoimmune hepatitis (AIH)[27], a large multicentre study did not find differences in the prevalence of anti-HEV IgG between AIH and healthy patients[28]. Additionally, they did not identify chronically HEV-infected patients within the AIH cohort.

HEV virions, as those of HAV (both hepatotropic virus but phylogenetically unrelated), are known to be non-enveloped in feces, but they circulate in the blood-stream coated in a lipid membrane. This kind of virus particles has been named quasi-enveloped virions[29].

The main risk factors on HEV infection to be considered for donor screening can be summarized in: Areas with limited access to essential services as water, sanitation, and health care facilities; Consumption of undercooked or raw foodstuffs from animals; Middle-aged and elderly men.

The severity of the consequences increases when these factors occur together with others related to the recipients, as pregnant women (because fulminant hepatitis occurs more frequently during pregnancy) or immunocompromised patients (as solid organ transplant recipients or patients receiving hematopoietic progenitor cell transplantation).

RISK ASSESSMENT OF HEPATITIS E TRANSMISSION THROUGH SUBSTANCES OF HUMAN ORIGIN

HEV is considered to be the most common cause of acute hepatitis worldwide[30]. Its infection typically follows a fairly routine clinical course with an incubation period of 2 wk to 6 wk, followed by a detectable viraemia in serum along to symptoms such as abdominal pain, vomiting, jaundice, *etc.* Usually, the disease course is self-limiting. As said before, some individual profiles can lead to a more severe hepatic complication.

Whereas HEV-3 infection in healthy humans is mostly asymptomatic, HEV 3 can induce chronic infection in immunocompromised individuals and acute on chronic liver failure in patients with underlying liver diseases. Recent data suggest that the number of reported cases of HEV infections in Europe increased significantly during recent years[31].

Although HEV is not routinely screened during blood donation in most countries, there have been prospective studies that have been conducted searching for markers of HEV infection in serum samples from potential blood donors to assess the local risk for transfusion related HEV[30,32]. The prevalence of detectable anti-HEV IgG positivity among blood donors varies among countries (Table 1). Nevertheless, data can also vary among geographical regions of the same country[40]. Moreover,

Table 1 Rates of anti-hepatitis E virus immunoglobulin G positivity in blood donors by country

Country	IgG positive rate (%)	Ref.
Argentina	11.3	Di Lello *et al*[33]
Austria	13.5	Fischer *et al*[34]
Bolivia	16.2	Konomi *et al*[35]
Brazil	7	Tengan *et al*[36]
China	30	Zhang *et al*[37]
Croatia	20.2	Miletić *et al*[38]
England	10	Beale *et al*[39]
France	22.4	Mansuy *et al*[40]
India	17.7	Tripathy *et al*[41]
Iran	8.1	Hesamizadeh *et al*[42]
Italy	8.7	Spada *et al*[43]
New Zealand	9.7	Hewitt *et al*[44]
Norway	14	Lange *et al*[45]
Poland	43.5	Grabarczyk *et al*[46]
Scotland	9.3	Thom *et al*[47]
Serbia	15	Petrović *et al*[48]
South Africa	42.8	Maponga *et al*[49]
Switzerland	20.4	Niederhauser *et al*[50]
Thailand	29.7	Jupattanasin *et al*[51]
The Netherlands	24	Alberts *et al*[52]
Uruguay	10	Bangueses *et al*[53]
United States	9.5	Stramer *et al*[54]

IgG: Immunoglobulin G.

differences can also be observed depending on the type of diagnostic assay used for the seroprevalence assessment[38,55].

A few cases of HEV infection have been reported to be transmitted by blood transfusion[56]. Since the first reported case of transmission human to human in Japan, some other cases have been reported in many countries[31]. In all of these, the HEV genomic sequence from blood donor and patient matched identically, confirming that the origin of the HEV infection was from the blood and had been transmitted to the patient by transfusion.

There are few data regarding the prevalence of HEV in organ transplant patients. HEV transmission through solid organ transplant have been reported after liver, heart, lung and kidney transplantation [57-60], although to date the risk of HEV infection transmitted by transplantation is unknown.

We did not find data regarding HEV transmission by tissue allografts.

RISK ASSESSMENT OF HEPATITIS E TRANSMISSION THROUGH TISSUE ALLOGRAFTS

Damaged or absent tissues can be replaced by biological (autografts and allografts) or artificial substitutes. Nowadays, tissue banks offer great availability of different kind of human tissues to be used as allografts, with high standards of safety and efficiency. Therefore, studies analyzing the prevalence of HEV among tissue donors would be needed, in addition to other studies carried out in tissue recipients that could reveal its potential infectivity.

The drawbacks of these studies must be taken into account since many recipients of bone, valves or skin are also recipients of blood components. It is therefore important in a risk assessment procedure to know the degree of imputability that human tissues could have at the implants for HEV transmission. Additionally, these studies could also provide data to evaluate the probability of transmission. The Netherlands provided a definition for both transfusion-associated hepatitis E and transplant-associated

infection (Euro CDC). Based in that criteria, tissue transplant-associated HEV infection can be defined as "an acute hepatitis E within 6-8 wk after tissue transplantation (detected by HEV-RNA), where the donor was HEV-RNA positive and at least HEV ORF1/ORF2 hypervariable regions of donor and recipient strains are identical by sequencing".

It would be important to know the possible medium-long-term side effects for HEV regardless of the implant results. These studies could be obtained by the knowledge about their severity, in order to complete the risk assessment.

There are tissues which can be sterilized since cell viability is not relevant for their clinical efficiency or their biomechanical properties are not significantly altered by the procedure. Likewise, the avascular character of some tissues (as cornea) carries lower risk than vascularized ones (as heart valves).

As very simple forms of life (small size and absence of free water) viruses can be preserved by freezing, not requiring controlled cooling or use of cryoprotectants, as glycerol, dimethyl sulphoxide or polyethylene glycol (the only presence of albumin in the storage solution could be effective for virus cryoprotection). Although virus infectivity can be compromised with long term storage at -20 °C, temperatures ≤ -80 °C allow virus to survive. Additionally, virus can survive to several cycles of freezing/thawing[61]. Conversely, the process of drying and storing at room temperature (conditions associated to lyophilization), could lead to the collapse of the lipid membrane[62].

The storage in liquid nitrogen vs. vapour nitrogen has been related to higher risk of cross-contamination due to faulty seal, leak, or breakage of the containers (bags, cryovials, straws), by acting the liquid environment as vehicle for infectious agent diffusion[63,64].

It is mandatory for tissue banks that provide sterile tissue allograft to follow several steps as donor screening, microbiological testing, aseptic harvesting and processing, disinfection, and, finally, terminal sterilization. According with the standards of the International Atomic Energy Agency (IAEA)[65], sterilization is defined as a validated process to destroy, inactivate, or reduce microorganisms to a sterility assurance level (SAL) of 10^{-6}. Achieving this SAL by a validated process allows labelling of terminally sterilized allografts as sterile[66]. Validation refers to establishing documentary evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes, and shall include the following elements[65]: (1) Qualification of the tissue allografts and their packaging for sterilization; (2) Qualification of the irradiation facility; (3) Process qualification using a specified tissue allograft or simulated products in qualified equipment; (4) A certification procedure to review and approve documentation of (1)-(3); and (5) Activities performed to support maintenance of validation.

A validated procedure for the sterilization of tissue allografts must demonstrate efficacy against all classes of microorganisms, throughout the tissue volume and, additionally, must not adversely affect the biological and biomechanical properties which are critical for its clinical use. The inclusion of a terminal inactivation step provides safety against not usually tested viruses in donor screening, such as HEV.

Both enveloped and non-enveloped viruses containing either DNA or RNA have been inactivated by low dose gamma irradiation of musculoskeletal tissues[67]. Both directly (by ionizing radiation) and indirectly (due to aqueous free radicals as intermediaries in the transfer of radiation energy to biological molecules) effects are involved in the inactivation of allografts bioburden[68].

Ethylene oxide inactivates all classes of microorganisms by alkylation of nucleic acids and proteins. However, concerns regarding its potential toxicity have led to a decrease of its use[69].

HEV retained infectivity at temperatures up to 60 °C[70], and heating for 1 min at 70 °C yielded a log reduction of 0.48, which was increased up to 3.67 at 95 °C[71]. Thus, virus heat inactivation at 71 °C for, at least, 20 min has been suggested[72]. Using a Lobator sd-2 system (telos, Marburg, Germany) validated to achieve a temperature of 82.5 °C the centre of femoral heads with a diameter of ≤ 56 mm, Pruss *et al*[73] obtained a titre reduction (4 Log_{10} steps) of clinically relevant viruses.

Pruss *et al*[74] showed the treatment of spongiosa blocks with the peracetic acid-ethanol procedure as a methodology to sterilize bones (maximum thickness ≤ 15 mm). In this study, very slow inactivation kinetics for hepatitis A virus was observed. Thus, while a general reduction of virus titres by more than 4 log_{10} was determined, only HAV showed a reduction below that threshold (2.87), with residual infectivity.

CONCLUSION

Current evidence does not recommend to date the universal screening with HEV in tissue donors, although it could be advisable to include the revision of medical-social history about risk practices and in those cases be able to selectively screen for HEV.

FOOTNOTES

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