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Microbial Association of Gastroduodenal Diseases with Special Reference to Epstein–Barr Virus and *Helicobacter pylori* and their Effect on Expression of miRNAs: A Review

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Abstract

Gastroduodenal diseases are very commonly encountered among patients who attend hospital with the complaints of dyspepsia. These diseases range from inflammatory ones like gastritis and peptic ulcer disease to neoplastic ones like gastric carcinoma and lymphoma. Gastroduodenal diseases may be caused by various factors of which microbial association with a bacterium called *Helicobacter pylori* and a virus called Epstein–Barr virus (EBV) is noteworthy. It has also been noted in various studies that the coinfection by these two organisms may also play a significant role in an exaggerated inflammatory response in cases of gastritis which may ultimately lead to carcinoma (Correa's cascade of carcinogenesis). As a result, it appears critical to identify both *H. pylori* and EBV in samples taken for biopsy in various gastroduodenal illnesses. Recent research has also suggested that these two separate etiologies may work together to cause gastric cancer, with miRNAs playing a key part in this process. This review presents the status of the current research on the association of gastroduodenal diseases with EBV and *H. pylori* along with the emerging context of the connection with miRNA expression. This will help to understand these complex etiologies having significant bearing on human health as well as highlight the need for intensive research in the subject.

Keywords: Gastroduodenal diseases, Microbiology, miRNA

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Introduction

Gastroduodenal diseases are frequent causes of clinical disease. The spectrum of gastroduodenal diseases is wide ranging from inflammatory lesions such as gastritis and peptic ulcer disease (PUD) to frankly malignant ones such as gastric carcinoma and lymphoma. However, until about the time of World War II, knowledge of gastrointestinal pathology was largely based on autopsy studies, which were often erroneous due to tissue autolysis. With the advent of fiber-optic endoscopy, mucosal biopsy samples can now be obtained from areas which were previously difficult to be sampled. Endoscopy also provides material for cytology (both lavage and brush). A pathologist is mainly concerned with the diagnostic aspects of endoscopy, although it can be therapeutic as well. Fiber-optic endoscopy has added to the abundance of tissue available to the pathologists for diagnosis and study of the pathogenesis of gastroduodenal disease. Surgical specimens and biopsy material from the gastrointestinal tract account for a considerable proportion of all the materials seen in any department of histopathology. Hence, today, gastrointestinal pathology is accepted as one of the largest sub-specialties within general histopathology.

Epidemiology of Gastroduodenal Diseases

Global Scenario

In Western countries, peptic ulcers develop in up to 10% of the general population at some point during their lifetime. In most European countries and the USA, duodenal ulcer is at least twice as common as gastric ulcer, and in some areas, this ratio reaches 5:1.[1] In contrast, gastric ulcer in Japan occurs 5–10 times more often than duodenal ulcer.[2] Studies regarding gastric cancer (GC) in the past few decades have shown marked geographical variations with high-risk areas in Japan, China, East Europe, and some countries in Latin America. Low-risk regions are North America, India, the Philippines, Africa, some parts of West Europe, and Australia.[2]

Indian Scenario

The epidemiological scenario of PUD is slightly different in India. Studies indicated the lifetime prevalence of PUD to be 11.22%; duodenal-to-gastric ulcer ratio being 17.1:1.[3] Both duodenal and gastric ulcers showed a male preponderance.[3] However, the overall incidence of GC in India is less compared to the global scenario and India falls under the low incidence region category for GC.[4,5] The 2010 reports from the National Cancer Registry Programme suggested that the mean age-adjusted rate (AAR) of GC varied from 3.0 to 13.2, with the highest rate being recorded in Chennai.[6-9] However, currently, the prevalence was found to be much higher in the north-eastern region of India with Mizoram occupying the first position among Indian states and fifth position globally with AAR of 46.3–70.2.[10,11]

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Microbial Etiology of Gastroduodenal Diseases

Of the diverse etiological associations of gastroduodenal disease, the most important is a bacterium named *Helicobacter pylori*.[12] Another significant microorganism which is implicated in gastric diseases, especially gastric carcinoma, is Epstein–Barr virus (EBV).[13] *H. pylori* causes chronic infection of the gastric mucosa (chronic gastritis) which happens to be the most common infection globally.[14] This chronic infection may cause genetic instability ultimately leading to GC. EBV, on the other hand, causes infectious mononucleosis, autoimmune diseases, Burkitt's lymphoma, nasopharyngeal carcinoma, and GC. The persistent infection may lead to the development of malignancies in the lymphoid and epithelial cells.[15]

H. pylori

H. pylori is a curvilinear, non-sporing, Gram-negative bacillus with unipolar flagella which was discovered by Warren and Marshall in 1983. *H. pylori* infects close to 50% of the global population.[16] It can be spread through contaminated water or food or through sexual contact (mouth to mouth or orogenital).[17,18]

Pathogenetic traits of H. pylori

The specialized traits that allow it to flourish include: [12]

- Motility through flagella, which allows it to swim through the viscid mucus
- Elaboration of urease, which produces ammonia and carbon dioxide from endogenous urea, thereby buffering gastric acidity in the immediate vicinity if the bacillus
- Expression of bacterial adhesions (bab A which binds to fucosylated Lewis B blood group antigens) enhances binding to blood group O bearing cells
- Expression of toxins, Cag A, and Vac A.

H. pylori and its association with gastroduodenal diseases

H. pylori has been seen to play a critical role in major gastric and duodenal diseases such as: [12]

- Chronic gastritis
- PUD
- Gastric carcinoma
- Gastric lymphoma.

The study by Wyatt *et al*. (1995) shows that *H. pylori* is present in 90% of the patients with chronic gastritis affecting the antrum, 95% with duodenal ulcers, and 70% with gastric ulcers.[19] In 1994, the World Health Organization and International Agency for Research on Cancer have declared *H. pylori* as a Class I carcinogen.[20] It has been observed that it is associated with 50% of gastric adenocarcinoma and >80% of gastric lymphomas worldwide.[12] In India, recent reviews concluded that *H. pylori* is the primary cause of duodenal ulcer. With respect to gastric carcinoma, various epidemiological studies in India have shown a higher incidence in South India as compared with North India.[5] Although the prevalence of *H. pylori* infection in India is high (49.94–83.30%), the incidence of GC is comparatively low. This indicates mixed results for the association between *H. pylori* and GC in the Indian context.[21]

Correlation between clinical symptoms, endoscopic appearance, and histology is poor in many gastroduodenal diseases, for example, chronic gastritis. Therefore, gastric biopsy should be considered as an essential part of endoscopic examination even if no specific lesion is seen. *H. pylori* is present in 90% of the patients having chronic gastritis affecting the antrum.[22] The frequency of *H. pylori* infection in gastroduodenal diseases reported in literature[12,19,23-27] is compared in Table 1:

Various other studies by Lambert *et al*. (1986), O'Connor *et al*. (1987), and Rouvroy *et al*. (1987) also found *H. pylori* infection in over 90% of patients with duodenal ulcer and almost 70% of patients with gastric ulcer.[28-30] However, the study by Vu and Ng (2000) shows a lesser prevalence of *H. pylori* (85.1%) in case of duodenal ulcers. This is probably because the study evaluated only the point prevalence of *H. pylori* in PUD (over 6-month period) in a Singapore Hospital. Talley and Quan (2002) in their study reported that *H. pylori* infection in patients with non-ulcer dyspepsia ranges from 30 to 70%.[31] Roy *et al*. (2010) noted the prevalence of *H. pylori* in non-ulcer dyspepsia to be 41.66%, which falls in this range.[27]

Diagnosis of H. pylori

Chronic gastritis is defined as the presence of chronic mucosal inflammatory changes, leading to mucosal atrophy and intestinal metaplasia (IM), usually in the absence of erosions. The epithelial changes may become dysplastic and constitute a background for the development of carcinoma. This cascade of progression of disease from chronic gastritis to carcinoma is called Correa's cascade of multistep gastric carcinogenesis.[32]

Therefore, whenever possible, it is necessary to identify the cause of gastritis to check the progression to carcinoma. *H. pylori* being one such cause of chronic gastritis, the diagnosis with the help of the following tests is essential to formulate appropriate clinical strategies for better management of patients: [12]

a. Non-invasive tests:
   - Urea breath test
   - Stool for PCR to detect *H. pylori* antigen
   - Serology for the detection of *H. pylori* antibodies
b. Invasive tests:
   - Rapid urease biopsy test
   - Histopathological examination of punch biopsy samples supplemented with special stains and immunohistochemistry

Epstein–Barr virus (EBV)

It described almost half a century back, in patients with Burkitt's lymphoma, EBV happens to be the first virus linked to cancer in humans.[33] EBV spreads mainly through contact with saliva.[34] It has been noted that infection rates in underdeveloped countries

Table 1: Comparison of the frequency of association (%) of *Helicobacter pylori* with gastroduodenal diseases as reported in some representative studies

Studies	*Duodenal ulcer*	*Gastric ulcer*	*Malignancy*
Schrager *et al*. (1967)	>95%	65%	-
Blaser (1987)	70–90%	30–60%	-
Wyatt (1995)	95%	70%	51%
Vu and Ng (2000)	85.1%	67.9%	-
Kungge (2001)	90–95%	50–70%	-
Liu and Crawford (2004)	-	-	50%
Roy *et al*. (2016)	96%	61.9%	50%

are much higher than the developed countries and that this infection is usually acquired in early childhood.

Pathogenesis of EBV infection

EBV is a human herpesvirus which is known for establishing a latent infection in lymphoid cells and replicating in epithelial cells. EBV may cause various human cancers including gastric carcinoma. Several factors contribute to the development of cancer of which the EBV latent membrane protein 1 is a critical one in EBV pathogenesis as it induces cellular growth and affects cellular growth control mechanisms.[35]

EBV and gastroduodenal diseases

It is thought that after infection, EBV establishes a virus carrier state, and in about 90% of adults, this remains as a persistent infection.[36] Although EBV is best known to cause infectious mononucleosis in adolescents and young adults, the association with gastroduodenal diseases (especially gastric carcinoma) is also significant.

EBV-associated gastric carcinomas (EBVaGCs) are usually of the diffuse type.[37,38] Individuals with EBV-positive malignancies have a higher overall survival rate than those with EBV-negative tumors, according to an international pooled analysis of 4599 patients.[39] Shibata and Weiss first proposed the association of EBV with gastric carcinoma way back in 1992,[40] but it took more than 2 decades to get their work recognized (The Cancer Genome Atlas Research Network, 2014).[41] The Cancer Genome Atlas consortium, in its recently proposed molecular classification for GC, has recognized EBVaGC as one of the four proposed subtypes. This study proposed classifying GC on molecular basis: Tumors positive for EBV (8.8% samples), microsatellite unstable tumors (21.7% samples), genomically stable tumors (19.7% samples), and tumors with chromosomal instability (49.8% samples).[41]

Diagnosis of EBV infection

EBV can be identified in clinical samples by different methods, namely,

a. Serological Tests: Serological tests for EBV are mainly done to detect antibodies against specific antigens. Three specific antibodies tests are as follows: (a) Anti-viral capsid antigen antibodies immunoglobulin (Ig)M and IgG, (b) anti-early antigen (EA) antibody IgG, and (c) anti-EBV nuclear antigen (EBNA) 1 antibody, IgG[42,43]
b. PCR/Real-Time PCR-Based Detection: These methods are helpful in detecting the EBV DNA and the viral load. Because immunologic responses take time to manifest in the blood, these approaches are more sensitive and specific than serological procedures. According to several findings, EBV DNA is found in practically all cancer cells in EBV-positive cases.[44,45]

miRNAs and their Clinical Significance

Non-coding microRNAs (miRNAs) form a distinct class of RNA molecules that have significant characteristics for its use as a biomarker.[46] miRNAs are fairly stable and resist degradation, therefore, can be easily extracted from different samples such as biopsy tissues, blood, and feces.[47] miRNAs have shown differential expression in various gastroduodenal diseases including GC.[48]

miRNA Expression Pattern in *H. pylori* Infection

It has been noted that expression of miR-21 in tumor tissues and in blood of GC patients is increased significantly compared to controls.[49] Therefore, the role of miR-21 in tumorigenesis has been widely studied.[50-52] However, there has not been many studies which have addressed miRNA deregulation in the stomach related to *H. pylori* infection and different stages of Correa's cascade. Petrocca *et al*., in one of his studies, showed that chronic inflammation of the gastric mucosa was associated with alteration of seven miRNAs, specifically miR-155.[53] Other studies by Koch *et al*. and Oertli *et al*. have also revealed an essential role of miR-155 in modulation of *H. pylori*-triggered mucosal inflammation.[54,55]

Furthermore, Matsushima *et al*. profiled tissue samples from *H. pylori*-positive and -negative subjects and described a downregulation of 30 miRNAs and an upregulation of miR-223.[56] *H. pylori* eradication was associated with at least partial reversal of alterations of miRNA expression already 4 weeks after successful therapy.[56] Plasma analyses revealed also increased miR-223 levels in *H. pylori*-positive subjects.[57] Nevertheless, these miRNAs are not sufficiently studied in systematic manner in *H. pylori*-associated chronic gastritis or pre-neoplastic gastric conditions such as AG with and without IM. It was noted that *H. pylori*-positive gastric biopsies showed downregulation of let-7 miRNAs, particularly those infected with Cag A-positive strains.[58]

Link A *et al*. found a strong association of both miR-155 and miR-223 expression with *H. pylori*-induced chronic gastritis, but not with *H. pylori*-negative gastritis. The same study did not find any difference in expression of miR-21 between *H. pylori*-positive and -negative subjects. However, it was also noted that subjects with *H. pylori*-negative gastritis showed only marginal changes in miR-155 and miR-223 expression compared to normal, non-inflamed mucosa.[58] A recent study by Hayashi *et al*. showed that *H. pylori* CagA can induce aberrant epigenetic silencing of let-7 expression contributing to Ras upregulation and carcinogenesis.[59] Yang *et al*. recently discovered that Cag A-positive *H. pylori* strains increase the production of NF-kB, which binds to the miR-223 promoter. ARID1A is a target of miR-223, which promotes cell proliferation and migration. In clinical samples, the researchers discovered that miR-223 is elevated in tumors, while ARID1A is dramatically downregulated in non-tumor neighboring mucosa from the same individuals.[60]

Taken together, host miRNAs play a key role in the inflammatory response to *H. pylori*, suggesting that they could serve as a link between *H. pylori,* chronic inflammation, and the advancement of pre-cancerous lesions. Therefore, the need for future studies has been felt to answer if and at what magnitude miR-155 and miR-223 may affect the gastric mucosa in response to *H. pylori* infection. These studies may also investigate the role of *H. pylori* strain-specific virulence factors in relation to miRNA expression in gastroduodenal diseases.

miRNA Expression in EBV Infection

EBV was the first described amongst all viruses to encode miRNAs.[61] EBV uses the host cell machinery to produce its miRNAs, analogous to the biogenesis of host cell miRNAs.[62] It has been observed that EBV miRNAs are largely encoded in two clusters. The first EBV miRNA

cluster is found within a transcript that codes for the BHRF1 protein and consists of three miRNA precursors (miR-BHRF1, 2, and 3), which yield four mature EBV miRNAs. The 5′ UTR (untranslated region) of the BHRF1 mRNA contains miR-BHRF1-1, while the 3′ UTR contains miR-BHRF12 and 3.[61,62] The second cluster is found in intronic portions of BART transcripts, and it consists of 22 miRNA precursors (miR-BART1-22) that produce 40 mature miRNAs.[63-65]

The expression of these miRNAs is influenced by the cell type and latency[66-69] state. During lytic infection and latency III, for example, miR-BHRF1s are expressed, but miR-BARTs are expressed in other EBV latency types.[63] In lymphoblastoid cell lines (latency III), miR-BHRF1s are expressed, but not in NPC.[66] In contrast to EBV-positive B lymphoma, miR-BARTs have much higher overall expression in EBV-infected lymphoblastoid cell lines, Burkitt's lymphoma, NPC, and EBVaGC. It is possible that the carcinogenic effect of EBV miRNAs is assigned to the BARTs because the expression of BHRF1 miRNAs is not consistently altered in tumors.[68] Marquitz *et al*. also demonstrated that in EBVaGC cell lines, viral BART miRNAs are significantly expressed.[70]

Kang *et al*. noted a high BART20-5p expression levels associated with worse recurrence-free survival of EBVaGC patients.[71] Therefore, it can be concluded that miR-BARTs play an important role in carcinogenesis. Other EBV BART-miRNAs which are expressed in EBVaGC cell lines include miR-BART1-3p, 5-5p, 7-3p, 15-3p, 19-3p, and 22-3p.[68,72] They have distinct pattern of expression in EBV-related epithelial malignancies compared to lymphoid malignancies.

However, in contrast to most published studies, some publications observed a controversial view on the strictly pro-tumorigenic role of miRBARTs. For example, it has also been proposed that miR-BART6-3p acts as a tumor suppressor miRNA. These findings which show that some miR-BARTs can possess anti-tumor activities have been poorly described till date and therefore need further studies in detail.

Few clinical case reports document an association between EBV and pediatric gastritis. For instance, EBV positivity has been reported in two young patients with dyspepsia[73] and also in patients with gastrointestinal symptoms.[74,75] These studies are particularly important because they have shown positivity to EBV by *in situ* hybridization and therefore demonstrated EBV infection in epithelial cells. There is also a case report in which gastritis to cancer progression was observed soon after a non-myeloablative hematopoietic stem cell transplantation in *H. pylori*-negative patient,[76] supporting a GC-triggering role for EBV starting from early inflammatory lesions. Therefore, studies addressing the EBV identification and expression of miRNAs may be very informative about the viral contribution to early gastric inflammatory lesions and their role in gastric carcinogenesis.

H. pylori-EBV Coinfection

H. pylori-EBV Coinfection and their role in Gastroduodenal Pathologies

It has been observed by different researchers that about 45% of the world population have a probability of having coinfection with *H. pylori* and EBV and a significant proportion of them has shown to develop gastric carcinoma along with many other gastroduodenal diseases. There have been lot of studies done individually on EBV and *H. pylori* but very limited literature is available on coinfection of both the pathogens. Various studies done recently have also pointed out that EBV and *H. pylori* coinfection increases the occurrence of gastric carcinoma compared to infection with individual organisms.

With this background, it seems important to demonstrate the presence of both *H. pylori* and EBV in samples taken for biopsy in various gastroduodenal pathologies.[77] Recent research has also revealed that these two separate etiologies may work together to induce GC and that a large number of miRNAs may play a key part in this process. Therefore, studies which focus on the association of gastroduodenal diseases with these two organisms along with their effect on miRNA expression on the gastric mucosa would really be beneficial.

H. pylori-EBV Coinfection and miRNA Expression

The mechanism of interaction between *H. pylori* and EBV and their synergistic role in the pathogenesis of gastric diseases is not clearly known. As both pathogens induce severe inflammatory response, chronic inflammation has been thought to be the common theme. Cárdenas-Mondragón *et al*. have shown that the inflammatory response was more intense in coinfection than in patients infected with *H. pylori* or EBV alone.[78] It was also noted that when gastric carcinomas developed in coinfected patients, these were usually of intestinal type and associated with premalignant lesions[78] in contrast to EBVaGC which is predominantly of diffuse type. Therefore, it may be inferred that EBV probably acts in conjunction with *H. pylori* by inducing additive inflammatory response resulting in exaggerated inflammation.

It has been observed by various studies that 95% of population have EBV in latent stage[79] and the risk of gastric carcinoma increases significantly with *H. pylori* and EBV coinfection.[78] *H. pylori* and EBV account for approximately 80% and 10%, respectively, of all gastric carcinomas worldwide. EBV-associated GC is predominantly located in the cardia (58%) followed by non-cardia (42%),[80] while GC associated with only *H. pylori* is mostly non-cardia type of adenocarcinoma.[81]

Several researches looked into the link between EBV and *H. pylori* infection of the stomach and discovered that infection with both is linked to stomach cancer development in GC cell lines, which is likely due to the activation of the NF-kb and MAP kinases oncogenic pathways.[82-85] Although the spectrum of miRNAs generated in both infections appears to differ significantly *in vivo* and, particularly, *in vitro*, it seems reasonable from a clinical standpoint that both infections could work together to promote cancer formation and, therefore, might have a common link with respect to their pattern of miRNA expression.

The carcinogenic potential of *H. pylori* infection is associated with an induction of miR-155 which is also related to the presence of the cag A pathogenicity island.[54] These findings could explain why GC formation is only linked to Cag A-positive strains. miR-155-5p overexpression is also implicated in *H. pylori*-induced inflammation and carcinogenesis, according to the current research.[86] Overexpression of miR-155 has also been seen in *H. pylori*-associated gastroduodenal illnesses and GC, according to studies.[87-89] In B cells, miR-155 regulation is dependent on the activator protein 1 pathway, which appears to allow EBV to remain in immune cells. Bacterial LPS exposure also promotes miR-155 production in immune cells.[88,89] B cells express the CD21 receptor, which is a known EBV receptor. These infected B cells

now infiltrate the chronically inflamed tissue of the stomach, and cell-to-cell contact between B cells and gastric epithelial cells may result in viral entrance into gastric epithelial cells, triggering the carcinogenic potential of the virus. The mechanism underlying this occurrence is still unknown, but miRNA activation may play a role in other key pathways throughout similar activities.[48,90]

Thus, this interesting miRNA molecule may be of particular importance for evaluating the risk of gastric carcinoma in patients of non-neoplastic inflammatory gastric diseases, and in the early stages of cancer, it could also be used as a diagnostic biomarker.

Conclusion

This review evaluated several articles related to the topic and tried to establish a common link between coinfection of the gastric mucosa with *H. pylori* and EBV and their effect on the miRNA expression. The possibility of establishing miRNAs as biomarkers of coinfection with *H. pylori* and EBV may open up horizons for more personalized therapy in this era of evidence-based medicine. It might then be possible to decide whether treating EBV-associated gastroduodenal diseases with anti-*H. pylori* regime would offer any further therapeutic benefit to the patient.[91] The understanding of the pathogenesis of EBV-positive GC, its association with *H. pylori* infection, and miRNA expression is in its primitive stage. Therefore, further research and detailed study to evaluate the complete range of miRNAs (and specifically the overall quantity of miR-155 expression) at the cellular level are needed to understand the carcinogenic potential of *H. pylori*- and EBV-associated cancer disorders.

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