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Review Article

The 'African Enigma' Revisited

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Abstract

IBS, Helicobacter Pylori (HP) is a Gram-negative bacteria that selectively colonizes the gastric epithelium. The bacteria have been associated with peptic ulcer disease (PUD), gastric adenocarcinoma, and mucosa associated lymphoid tissue lymphoma. The association between HP and gastric cancer (GC) remains a matter of intense debate owing to the fact that the consequences of the infection depends on multiple genetic and environmental interactions. The 'African Enigma' which was reported more than two decades ago, describes the high prevalence of HP with an apparently low incidence of PUD and GC in Africa. Despite the dramatic advances in our understanding of the gut microbiome since the Enigma was first described, the real causes remain unknown. This review will discuss the many attempts to explain the African Enigma, such as role of HP in the gastric microbiome, the interactions between HP and parasitic infections and the unique African diet which is rich in antioxidants, and the reasons why some have denied its existence.

Keywords: Helicobacter Pylori; Gastric Cancer; African Enigma

Introduction

Up until relatively recently, the stomach was considered a sterile environment due to the sterilizing effect of gastric acid. Peptic ulcers were considered to be caused by acid hypersecretion and treatment was acid suppression or surgical removal of the acid producing parietal cells. The landmark studies of Marshall and Clark [1] revolutionized our knowledge of gastric physiology and ulcerogenesis when they demonstrated that bacteria existed in the stomach and were associated with ulceration. The predominant bacteria identified, was Helicobacter pylori (HP). Furthermore, the removal of HP by triple antibiotics established the first 'curative' medical therapy for peptic ulceration.

HP is a gram-negative bacterial pathogen that selectively colonizes the gastric epithelium and is best associated with chronic active gastritis, but also with peptic ulcer disease (PUD), gastric adenocarcinoma, and mucosa associated lymphoid tissue (MALT) lymphoma. Since 1994, HP has been recognized as a type I carcinogen for gastric cancer (GC) development by the International Agency for Research on Cancer (IARC) [2]. Interestingly, the association between HP and gastric complications is not strong, and it is important to note that the majority of people in the world harbor HP in their stomachs with no proven consequences. Perhaps the highest incidence is in Africa, where the 'normal' state is to carry HP. Despite this, peptic ulceration and gastric cancer are uncommon, leading to the term the "African Enigma". After the original observations by Holcombe [3], researchers from other geographical locations such as Thailand, India, Bangladesh, Pakistan, Iran, Saudi Arabian countries, Israel

and Malaysia [4-7] have also confirmed the high prevalence of the HP infection along with low incidence of GC and even named it the 'Asian Enigma' [5].

Our recent results [8], have confirmed that HP is almost ubiquitous in rural Africans compared to African American controls, confirming our previous work that showed that trend [9]. The chronic HP infection in rural African might explain the high Ki67 mucosal proliferation that we observed in gastric mucosa of the studied group (Figure 1). But, despite the high HP colonization in rural Africans, the GLOBOCAN 2012 data shows that GC in South Africa is not amongst the five most common cancers in the country [10].

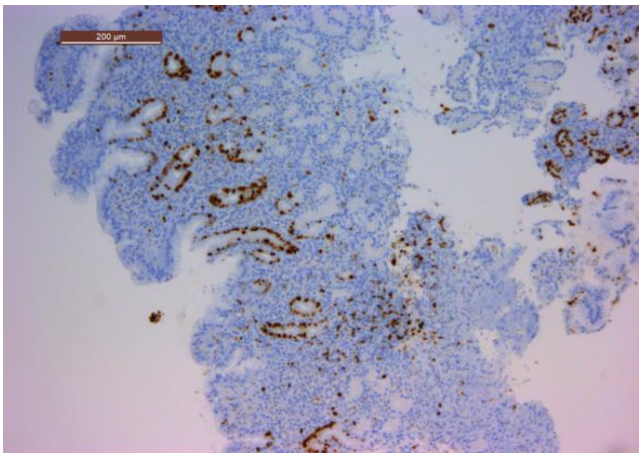

Figure 1: High Ki67 in gastric epithelia of Rural Africans residing in South Africa.

In Africa, the correlation between HP and GC seems to depend on the geographical location; being highest in East Africa and lowest in South Africa [10] (Table 1). Overall, the incidence in Africa is lower than all the other major continents (Table 2) [10]. With the progressive westernization of the African diet and habits, the risk of Africans getting GC is expected to change. It is important to focus more research on this field in order to prevent the possible outcome of these developing countries experiencing a public health 'double burden' of cancer in addition to that of infectious diseases.

African Region	Incidence (Male/female)	Mortality(Male/female)
North Africa	3.2	3.0
East Africa	4.7	4.6
Central Africa	4.0	4.8
South Africa	3.0	2.8
West Africa	3.8	3.7

** - /100,000*

Table 1: Incidence and Mortality of GC hased on African geography [10]*.

Continent	Incidence (Male/female)	Mortality(Male/female)
Africa	4	3.8
Asia	18.5	13.4
North America	4.2	2.1
South America	12.4	10.2
Europe	10.3	7.9
Australia	5.3	3.1

** - /100,000*

Table 2: Incidence and Mortality of GC based on Contient [10]*.

Factors Affecting Disease Progression

Host Genetic Predisposition

The general host immune response has been established as an important determinant of disease progression. Immune polymorphisms in IL1B, IL1RN [11], IL-8 [12], IL-10 [13,14], TNF α and IFN γ [15], has been shown to correlate with chronic HP infection and GC [16,17]. All these cytokines play different roles in the immune response; IL-1 modulates stomach acidity, IL-8 regulates the proliferation of the endothelial cells, IL-10 is an inflammatory down regulator and TNF α is an inflammatory up regulator. Most of the SNPs found were located in the promotor regions of these genes, thereby directly affecting gene expression [17].

The most important associations was found with IL1RN2, which was shown to be associated with an increased GC risk and IL1RN22, which was found to be associated with decreased GC risk. In Asians, IL1B-31 has been shown to be associated with reduced GC risk. IL10-1082G showed an increased GC risk in Asians and decreased GC in non-Asians [17]. In East Africans, IL-1 β -511 and IL-1 RN-L/L have been found to exist more in HP infected individuals suffering from Gastritis, PUD and GERD [18]. Overall, it is clear that there is scarce data on the association between genetic polymorphisms and HP disease prevalence in Africans.

HP Virulence Factors

Polymorphisms in several virulence genes of HP has been shown to be detrimental in the pathogenesis of the disease; they include cytotoxin-associated gene A antigen (CagA) and vacuolating cytotoxin (VacA) factor [19]. The cag pathogenicity island (cag PAI) is a 40-kb DNA insertion element that is flanked by a 31-bp direct repeats and encodes one of the most important HP proteins, CagA. At least, 18 different Cag genes have been shown to be components of the type IV cag secretion system, which transports proteins post processing and

modification into the host epithelial cells. The Cag pathogenicity island genes, unlike the vacA genes, do not exist in all HP strains. The CagA is the basis of the HP classification based on the genotype; CagA+ are either vacA s1 m1 or m2 genotype. While CagA- are vacA s2/m2 genotype [20]. Cag+ has been shown to be associated with an increased risk of gastritis and GC when compared to Cag- [19,21,22].

CagA becomes phosphorylated at the glutamateproline-isoleucine-tyrosine-alanine (EPIYA) motifs in the host cells by Src family kinases and this leads to a disruption of a variety of cellular proteins that have been implicated in carcinogenesis. The phosphorylation at the EPIYA motifs (A-D) has been shown to correlate with the geographical distribution of HP. Up to 70% of Western HP strains and almost all of the East Asian strains express CagA. The East Asian CagA strain (EPIYA, D) has been reported to be more virulent than the Western CagA (EPIYA A, B) and to be associated with more severe gastritis and GC [23-25]. The East Asian cagA is exclusively observed in East Asia, whereas the Western type cagA is widely distributed through Europe, South and central Asia, North and South American and regions in Africa [26].

The pathogenesis of CagA has been attributed to both the phosphorylated and unphosphorylated forms. The phosphorylated CagA interacts with tyrosine phosphatase and affects the Ras/Erk pathway which regulates downstream both the NFkB dependent genes and the Elk-1 dependent genes; both groups playing crucial roles in the immunomodulation of the inflammatory response [21,27]. The crk and csk/Src on the other hand have been shown to play a role in the morphological changes of HP related to actin cross linking. Non-phosphorylated CagA targets at least seven different host intracellular pathways. They are Elk-1 dependent genes, NFkB dependent genes, NFAT dependent genes Wnt/β-catenin dependent genes PAR1 kinase, ZO-1/JAM and C-Met [21,27]. These pathways overall also affect the immunomodulatory, morphology, cell migration and cell growth [21,27]. CagA has also been shown to play a role in the degradation of p53, an important tumor suppression protein that is often referred to as 'the guardian of the genome' [28].

VacA is a 140 kDa protein that undergoes post translational modification before being released as the active end product causing alteration in the host cell endosomal maturation, and subsequent epithelial cell vacuolation. Similar to Cag, VacA shows variations based on strains [29,30]. Overall, VacA suppresses the T-cell responses to HP, protecting the bacterium from autophagy [31,32]. VacA has been to shown to play a role in the process of ulceration by binding to receptor-type protein tyrosine phosphatase (RPTPβ) disrupting cell adhesion and proliferation [33].

In a previous attempt to understand the role of CagA and VacA in the pathogenesis and progression of GC in South Africa, Louw et al. found that the absence of vacA subtype s2 and the presence of an extended cagA-3′ length might be suggestive of developing GC in HP infected patients [34]. In a study in West Africa, mixed infection with cagA positive and cagA negative strains, was shown to be more prevalent in the study group with non-ulcerative disease, when compared to the group with gastric diseases [35]. Further work and larger studies are crucial to further assess and understand the effect of genetic polymorphisms and HP genotypes in the African setting. Unfortunately, the exact HP cag strains in rural Africans have not yet been sequenced.

Stomach Microbiome

Recent developments in the measurement of the gut microbiome have shown that HP is not alone in its ability to survive in the acid environment of the stomach. Consequently, interactions between HP and other microbes may also explain variations in the pathogenicity of HP [36,37]. Many scientists believe that HP is a normal component of the stomach microbiota because research has shown that it coexisted with humans since man migrated out of Africa 60,000 years ago [38,39]. This co-evolutionary existence might explain partially the reason why HP has been shown to have such a wide genetic diversity. Sequencing of the gastric microbiome has demonstrated more than 130 bacterial phylotypes which can be organized in up to thirteen different phyla [39,40]. Overall, the human gastric microbiota can, like the rest of the gut microbiota, be divided into 5 major phyla, including Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Fusobacteria [40,41].

The effect of HP infection on the gastric microbiome has not yielded consistent results [40, 42-45]. Significant differences have been found in the microbiome of HP positive and negative individuals by Maldonado-Contreras et al. [43]. The HP positive subjects had higher Proteobacteria (not including HP) and Acidobacteria, whereas, HP negative patients had higher prevalence of Actinobacteria and Firmicutes [43]. On the other hand, gene sequencing done by another group showed no differences in mircobiome composition based on HP existence [40].

The potentiating role of HP in gastric carcinogenesis was shown experimentally by Lee et al. in transgenic INS-GAS mice [46]. Transgenic INS-GAS mice overexpress gastrin leading to increased parietal cell number and overall gastric acid production as early as 1-3 months of age. By 4-6 months, the mice develop hypochlorhydria, gastric atrophy, metaplasia and dysplasia. Eventually, by 7-9 months of age, all the INS-GAS mice develop GC. Introducing HP into the gut of these animals orally, lead to a higher rate of GC than the controls. Treating the mice with an antibiotic against HP delayed GC progression in these animals [46]. The interaction between HP and the stomach mi-

crobiome in INS-GAS mice was also found to be an important factor affecting the rate and severity of gastritis; the complexity of the commensal flora was found to be associated with less HP induced gastritis [47]. Overall, it is clear that this field is rapidly advancing; understanding the role of HP in the gastric microbiome of patients from different geographical settings will probably lead to better understanding. Further research is desperately needed to better understand the normal composition and function of the human gastric microbiome, and how it protects us from environmental challenges.

Diet

Epidemiological research has shown that high red meat, heme iron [48] and salt may lead to an increased risk of HP infection. Recent experimental evidence using human samples [49] and animal models [50] also support this. Recent work by Poplawski et al. showed that that DNA damage induced by heterocyclic amines present in red meat was significantly higher in HP infected gastric cells when compared to healthy ones [49]. In Mongolian gerbils, the infection with HP in association with high salt in diet lead to all the animals developing GC, when compared to WT-infected/regular-diet animals that showed ≈58% GC development [50].

The antioxidants content in diet might be a significant factor reducing HP pathogenicity [48, 51]. These results seem interesting, our results have shown that the African diet which is rich in fiber and antioxidant does not confer any protection from HP infection [8]; taking into account the reduced overall risk of GC and PUD in rural Africans, the high antioxidant content of the diet might be protecting these individuals from gastric diseases despite the high HP infectivity. Fresh fruits and vegetables have been shown to be associated with a reduced risk of GC [22]. Our results [8] seem to accord with those of Epplein et al. [52], which showed that higher carotene levels might be a factor that decreases HP associated GC. We found the rural African diet is richer in carotene when compared to the normal western diet [8]. Most studies assessing GC, HP and diet used case-control models; this makes driving causative conclusions impossible. In addition, most studies used serological assessment of HP, which does not always correlate with the disease [9, 22]. Furthermore, the protective role of fresh fruits and vegetables in GC is an important confounding factor that needs to be addressed in future studies before concluding that these factors decrease HP related GC in specific.

Parasitic Infections

One of the possible explanations for the enigma; include the co-infection with helminthes [53], which has been shown to reduce the extent of HP gastritis most likely due to an enhanced systemic immunity in response to the intestinal infection. In a Colombian study, infection with the intestinal parasite Ascaris and Toxoplasma was shown to correlate with decreased HP infection as evidenced by the IgG and IgE responses [54]. Similar results were observed in a Chinese cohort, where decreased HP IgG titres and CagA seropositivity was seen when the patients were co-infected with the intestinal parasite, Schistosoma Japonicum [53]. In addition, in an Egyptian study, intestinal schistomiasis caused by Schistosoma mansoni was associated with less severe HP gastritis as confirmed by multiple approaches including endoscopy [55]. These important observations and others were also supported by experimental evidence by Martin et al. who showed that the immunological response to filaria resulted in changes in the extent of gastritis in the gerbil model [56]. Similar study designs have not been replicated yet in African cohorts.

Other Factors

The lack of efficient medical recording, low life expectancy, unavailability of endoscopic services and the vagueness of GC presentation all added to the difficulty of unravelling the enigma. The low life expectancy and the late age onset of GC seemed convincing enough to some researchers to doubt the existence of the enigma, however the early infection onset and the extent of the infection was probably missed. In a previous attempt to understand the enigma, we investigated the association between HP sero-positivity, endoscopic assessment and clinical outcome in rural Africans. The results showed no association between sero-positivity and HP gastritis, PUD or GC. Patients with non-ulcer dyspepsia showed similar seropositivity to others with gastric diseases. Our results confirmed that in African cohorts HP serological testing does not correlate with clinical outcome and that endoscopic assessment is a crucial tool that should be implemented in the assessment of HP in Africans [9].

1. Different interaction between HP and gastric microbiome.
2. Consumption of diet rich in fiber and antioxidants.
3. Co-infection with intestinal parasites.
4. Prevalence of different less pathogenic strains of HP in rural Africans.
5. Lack of efficient medical recording and statistics in Africa.
6. Lack of efficient diagnostic tools and/or personnel for HP and upper GI diagnosis.
7. Early mortality related to other infectious causes such as Malaria.

Table 3. Possible Explanations for the African Enigma.

Conclusion

Despite the fact that more than 20 years have passed since the African enigma was first described, the causes of the enigma remains unresolved; although there are some possible explanations (Table 3). Whilst HP certainly plays a role in peptic ulceration and GC, large sections of the world's populations have HP colonization throughout their lives, but never get these diseases. The fact that HP is present in most if not all rural Africans suggest that the microbe is part of the normal gastric microbiota, and that it only potentiates disease when host or environmental conditions change. Thus general HP eradication programs of asymptomatic populations might result in more harm than good, as the associated disturbances in the gut microbi-

ome, which can last years after discontinuation of therapy, are likely to prove more harmful to the host. Better understanding of the gastric microbiota and the HP strains prevalent in the southern region of Africa and in rural Africans in particular might lead to better understanding of the relationship between HP, PUD and GC.

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