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Kimang'a Nyerere
Aga Khan University

Shahin Sayed
Aga Khan University, shahin.sayed@aku.edu

Gunturu Revathi
Aga Khan University, gunturu.revathi@aku.edu

Peter Ojwang
Aga Khan University

Viviene Matiru
Jomo Kenyatta University

See next page for additional authors

Authors

Kimang'a Nyerere, Shahin Sayed, Gunturu Revathi, Peter Ojwang, Viviene Matiru, Smita Devani, Mahesh Shah, and Samuel Kariuki

Prevalence of gastric mucosal interleukin-1 polymorphisms in Kenyan patients with advanced gastric cancer

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To the Editor: *Helicobacter pylori* is the main cause of peptic ulceration, distal gastric adenocarcinoma, and gastric lymphoma.[1] Worldwide, gastric cancer is the second most common malignancy in men and women.[1] According to data from the Nairobi Cancer Registry, gastric cancer is the fourth most common malignancy in adult males and the fifth most common in adult females. However, this may not represent the true situation because of under-reporting of cases. In the development of gastric cancer, environmental factors such as smoking, diet and, in particular, infection with *H. pylori* are significant.[1] Based on epidemiological studies, the International Agency for Research on Cancer (IARC) identified *H. pylori* as a 'group 1 agent (definite carcinogen)'.[2] *H. pylori* infection can result in decreased acid secretion with subsequent mucosal atrophy and intestinal metaplasia.[1] Another precondition for mucosal atrophy is autoimmunity against parietal cells, which can mimic classic autoimmune gastritis with the presence of various autoantibodies in up to 40% of *H. pylori*-infected individuals.[1]

The occurrence of intestinal metaplasia, for which a relationship with gastric cancer is strongly suggested, has been demonstrated in approximately 60% of patients with *H. pylori* infection.[1] The metaplasia may then progress to gastric cancer, especially to tumours of the intestinal type.[1] Findings by Uemura *et al*. support the importance of these histological findings as a precancerous condition in *H. pylori*-associated gastritis.[3] However, only a minority of *H. pylori*-infected patients develop gastric cancer, which underscores the notion that the host genetic background could be of critical importance.

Data strongly suggest that the susceptibility to infection from *H. pylori* is mainly conferred by genes involved in inflammatory processes following colonisation with *H. pylori*.[1] Chronic gastritis is characterised by the release of pro-inflammatory cytokines such as interleukin-1β (IL-1β) or tumour-necrosis factor alpha (TNFα), which are potent inhibitors of gastric acid secretion.[1] Advanced-stage gastric cancer has been repeatedly associated with polymorphisms of the IL-1 gene cluster on chromosome 2q, which contains 3 related genes within a 430 kb region (*IL-1A, IL-1B,* and *IL-1RN*), encoding for IL-1α , IL-1β, and the endogenous receptor antagonist IL-1ra, respectively. It was hypothesised that genetic differences within these genes could influence the immune response against pathogens such as *H. pylori* and the development of premalignant histological alterations in the gastric mucosa.[1] In patients with advanced-stage gastric cancer, an increased frequency of the *IL-1B*-31C and *IL-1B*-511T alleles and the uncommon *IL-1B*-31C/*IL-1B*-511T haplotype was demonstrated. In addition, the *IL-1RN**2 allele and the homozygous genotype *IL-1RN**2/2 were found in increased prevalence in gastric cancers.[1] Subsequent studies confirmed these genetic associations.[1] El-Omar *et al*. genotyped patients with gastric cancer according to tumour localisation (cardia v. non-cardia) and oesophageal cancers (adenocarcinomas v. squamous cell carcinomas) for various polymorphisms of genes encoding for pro- and anti-inflammatory cytokines.[4] They described an increased risk for non-cardia gastric cancer in carriers of the *IL-1B*-511T allele, *IL-1RN**2 homozygotes, carriers of the TNF-A-308A allele and the haplotype *IL-10*-1082A/-819T/-592A. The cumulative risk depends on the number of high-risk alleles or genotypes per patient.[4] A previous study confirmed the risk increase for development of gastric carcinoma in carriers of multiple proinflammatory genotypes.[1] The alleles *IL-1RN**2 and *IL-1B*-511T are associated with increased synthesis of the proinflammatory cytokine IL-1ß, and the allele TNFA-308A results in an increased production of the proinflammatory cytokine TNF.[1]

Patients and methods

The study used 41 archival paraffin-embedded gastric cancer tissue blocks obtained in 2007 from endoscopic biopsies and gastrectomies. Haematoxylin and eosin-stained slides were reviewed and the tumours classified according to the WHO

Department of Pathology, Aga Khan University Hospital, and Department of Medical Microbiology, Jomo Kenyatta University, Nairobi, Kenya
Kimang'a Nyerere, MSc

Department of Pathology, Aga Khan University Hospital
Shahin Sayed, MB ChB, MMed
Gunturu Revathi, MB BS, MD
Peter Ojwang, MB ChB, FRCPath, FCPath (SA)

Department of Medical Microbiology, Jomo Kenyatta University, Nairobi, Kenya
Viviene Matiru, BEd, MSc, PhD

Department of Medicine, Aga Khan University Hospital
Smita Devani, MB ChB, MRCP
Mahesh Shah, MB ChB, MMed

Kenya Medical Research Institute, Centre for Microbiology Research (CMR) Nairobi, Kenya
Samuel Kariuki, BVM, MSc, PhD

Corresponding author: S Shahin (shaheen.sayed@aku.edu)

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Center for the International Histological Classification of Tumors. DNA was extracted using the QIAamp DNA kit. The study was approved by the Aga Khan University (East Africa) Research and Ethics Committee. The *H. pylori* status was determined by polymerase chain reaction (PCR) using the ureA gene according to the method of Miyabayashi *et al.*[5] The ureA region was amplified using the following primers: 5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′. The *IL-1B* fragment was amplified using the following primers: 5′- TGGCATTGATCTGGTTCATC-3′ and 5′-GTTTAGGAATCTTCCCACTT-3′, while the *IL-1RN* intron 2 containing a penta-allelic 86-bp tandem repeats (VNTR) DNA oligonucleotides 5′-CTCAGCAACACTCCTAT-3′ and 5′-TCCTGGTCTGCAGGTAA-3′ flanking this region were used as primers.

Data analysis

Statistical analysis to determine the relationship between variables was performed using the chi-square test with *p*-values less than 0.005 considered as significant. Linear regression determined how independent factors predicted the outcome of dependent factors.

Results

We analysed 41 gastric biopsy samples that were preserved in formalin and embedded in paraffin wax, obtained over a year. All the biopsies were of adult Kenyan patients with advanced gastric cancer; 31 (76%) were of the intestinal type and 24% were of the diffuse 'signet ring' type; 26 (63%) were from male and 15 (37%) from female patients; their ages ranged from 36 years to 76 years, with 56% <50 years of age. We screened for the frequencies of genotypes and alleles of *IL-1B* and *IL-1RN* in these cancer tissues. Overall, 19 (46%) patients had *IL-1RN* while 18 (43%) had *IL-1B*. When analysed, the *IL-1RN* found in the Kenyan gastric cancer population only showed allele *IL-1RN* 1*4 (Fig. 1).

In contrast, when *IL-1B* was analysed by PCR-RFLP (restriction fragment length polymorphism) method, it gave products that either remained intact (allele 2; *IL-1B*-511*2) or were cut into two fragments of 190 and 114 bp (allele 1; *IL-1B*-511*1) (Fig. 2).

The *H. pylori* gene was present in only 6 of the 41 samples. This was expected because, when *H. pylori* has been present for many years and the stomach becomes atrophic and hypochlorhydric, the intragastric environment is no longer optimal for *H. pylori*. Competing faecal-type organisms are then able to colonise the stomach. Furthermore, *H. pylori* is able to colonise only normal gastric cells, and not those that have differentiated into intestinal metaplasia. Therefore, *H. pylori* disappears from the stomach when atrophy and intestinal metaplasia supervene. In patients who are most likely to develop gastric cancer (those with extensive intestinal metaplasia and atrophy) and those already with advanced
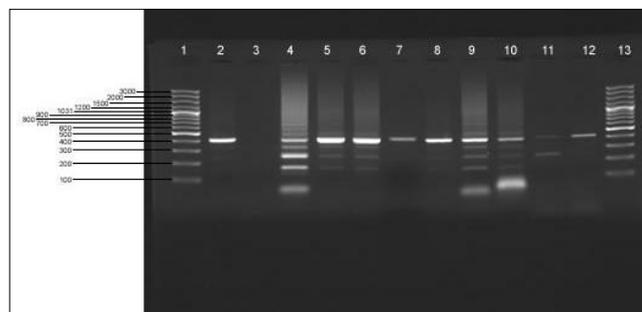

Fig. 1. Gel electrophoresis with ethidium bromide staining demonstrating allele IL-1RN *1*4. Lane 1 and lane 13, molecular maker (Gene ruler 100bp DNA ladder plus). Lane 2,* H. pylori-*positive control. Lane 3 negative control, lanes 4 - 12 represent DNA from the stomachs of selected cancer patients obtained from paraffin-embedded tissues.*
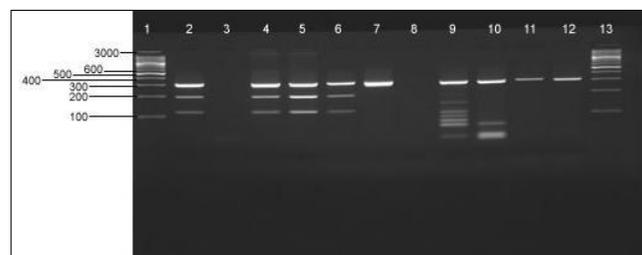

Fig. 2. RFLP analysis of interleukin 1B (IL-1B)*. A single bp polymorphism at -511 in the promoter region of the* IL-1B *was analysed by the PCR-restriction fragment length polymorphism method. Lane 1 and lane 13, molecular maker (Gene ruler 100bp DNA ladder plus). Lane 2,* H. pylori-*positive control. Lane 3 negative control. Lanes 4 - 12 represent DNA from the stomachs of gastric cancer patients obtained from paraffin-embedded tissues. Lanes 4 - 6, 2 bands representing allele 1 (*ILB-511*1). Lanes 7 and 9 - 12, 1 band fragment of allele 2 (*IL-1B-511*2).

cancer, as in our cases, the serology is often negative and the genes will almost always not be detectable by PCR.

No significant relationship was found between age and either *IL-1RN* or *IL-1B* in gastric carcinoma from Kenyan patients; the chi-square test for both was not significant: *p*=0.9 for *IL-1RN* and *p*=1.0 for *IL-1B*, against a significance level of 0.3 and 0.2 respectively. This indicates that these variables are probably independent of each other. Similarly, there was no relationship between gender and the occurrence of either *IL-1RN* or *IL-1B*. Linear regression also showed that neither age nor gender influenced the expression of *IL-1RN* or *IL-1B*.

The situation differs, however, concerning the relationship between *IL-1RN* and *IL-1B*. The large chi-square statistic (8.021) and a small significance level (0.005) indicate that it is unlikely that these variables are independent of each other. Therefore, we concluded that there is a relationship between the occurrence of *IL-1RN* and *IL-1B*. Linear regression showed an R-value of 0.45, which indicates that a great deal of variance is shared by *IL-1RN* and *IL-1B*. Analysis of variance (ANOVA) obtained from the regression F=9.5 appears to indicate that the predictor variable *IL-1RN* is not equal to the dependent factor *IL-1B*, and that the presence of *IL-IRN* could be used to predict the presence of *IL-1B*.

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Discussion

Instrumental in the development of *H. pylori*-related gastric carcinogenesis are some pro-inflammatory cytokines such as interleukin 1 beta (IL-1β) and interleukin 1 receptor antagonist (IL-1ra). The interleukin-1 (*IL-1*) gene cluster on chromosome 2q contains 3 related genes within a 340-kilobase (kb) region, *IL-1A, IL-1B* and *IL-1RN*, which encodes the pro-inflammatory cytokines IL-1α and IL-1β as well as their endogenous receptor antagonist IL-1ra respectively.[6] IL-1β is up-regulated in the presence of *H. pylori* and is important in initiating and amplifying the inflammatory response to this infection. IL-1β is also a potent inhibitor of gastric acid secretion. The *IL-1RN* gene has penta-allelic 86-bp tandem repeats (VNTR) in intron 2, of which the less common allele 2 (*IL-1RN**2) is associated with a wide range of chronic inflammatory and autoimmune conditions. *IL-1RN**2 is associated with enhanced IL-1β production.[6] Carriers of *IL-1RN* 2/*2 and *IL-1B*-511 T have been reported to be at increased risk of hypochlorhydria and gastric cancer.[6]

We tested the hypothesis that *IL-1* genetic polymorphisms are circulating in the Kenyan population and that they influenced the presence of *IL-1B* in *H. pylori*-associated gastric cancer. Intron 2 of the *IL-1RN* gene was detected and amplified by PCR. Homozygous genotype *IL-1RN*1/*4 was found to be present. We also observed the *IL-1B* gene with heterozygous genotype *IL-1B*-511*1 and *IL-1B*-511*2 in our cases of advanced gastric cancer. This is in contrast with reports from elsewhere in the world, where *IL-1RN* 1/*2 has been detected with increased frequency.[7]

Il-Ran Hwang *et al.* found that carriers of *IL-1B*-511 T/T genotypes, denoting a single band of 155 bp or *IL-1RN**2, had higher mucosal IL-1β cytokine levels compared with non-carriers.[6] They also observed that carriers of *IL-1RN* 1/*2 had higher mucosal IL-1β levels than those of *IL-1RN* 1/*1 consistent with an effect of this polymorphism on mucosal IL-1β. Also, *IL-1RN* 1/*2 carriers have atrophy scores approximately 1 point higher than *IL-1RN* 1/*1 carriers, which implies that in *IL-1RN* 1/*2 carriers atrophy is accelerated such that the mucosa are functionally 20 years older than the gastric mucosa of *IL-1RN* 1/*1 carriers.

IL-1RN*2 allele occurs in the Kenyan population at a much higher frequency (46.3%), in contrast with reports on Western and Asian populations.[7] For example, the frequency of the *IL-1RN**2 allele in East Asian countries was lowest (3 - 6%), while that of the Thai population was 12%. The frequency in Western populations was reported as 22 - 24%.

Our frequency of the *IL-1B*-511 allele was lower (18%) than that reported in the Thai population (34%) but falls within the threshold of other populations (15 - 25%).[7] These results are consistent with the notion that in any population, the dominant factor or combination of factors in relation to gastric cancer risk among *H. pylori*-infected individuals will vary and must be examined directly. Only 6 of our cases tested positive for *H. pylori* by gene analysis despite the fact that Ogutu *et al*.[8] reported a prevalence of 80.5% in Kenyan adults presenting with dyspeptic symptoms and normal endoscopic findings. This anomaly can be explained by the fact that all our cases involved advanced carcinomas, and *H. pylori* is known to disappear as atrophy and intestinal metaplasia supervene over a prolonged period of infection.[9]

In conclusion, this pioneer study of genotype polymorphisms for interleukin 1 in the Kenyan population provides evidence that individuals with *IL-1B* and *IL-1RN* polymorphisms associated with *H. pylori* infection have an elevated risk of developing gastric cancer. Since it was a pilot study, the observed significant association could be a chance observation because of lack of complete clinical information on the patients whose tissues were used. Our findings require confirmation by prospective analysis of a larger sample, looking at other related co-factors.

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