Brief Communication

Atypical cases of cholera due to serogroup O1 in the native population of Oman and its relation to non-O1/O139

Prakash K. Patel, MBBS, MD, Shyam Bawikar, MBBS, MD, Mercy John, MBBS, MD, Salah T. Al-Awaidy, MBBS, PG Dip.

Cholera is a major public health problem especially in developing countries and the seventh pandemic is still ongoing. In 2004, 56 countries officially reported 101,383 cases and 2,345 deaths to WHO and Africa accounted for 94% of these reported cases. *Vibrio cholerae* (*V. cholerae*) is an autochthonous inhabitant of marine and freshwater environments and also a facultative pathogen for humans. It is an acute bacterial enteric disease with high infection rate but has low disease rate. Important distinctions within the species are made on the basis of serogroups and production of cholera toxin (CT), which is responsible for severe diarrhea and potential epidemic spread. Only 2 sero-groups namely *V. cholerae* O1 and O139, have been considered as causative agents of cholera. All other strains namely, non-O1/O139 do not produce CT and are not usually involved in epidemics. These strains are frequently isolated from environmental sources and are associated with sporadic cases of gastroenteritis or extra-intestinal infections. Although occasionally non-O1/O139 strains can produce CT or other virulence factors; none of them have caused large scale epidemics. Diarrhea caused by *V. cholerae* is attributed to cholera enterotoxin (CT) codified by the ctx operon and regulated by a number of environmental factors and virulence protein genes such as toxT, toxR and toxS.1 *Vibrio cholerae* O1 may produce mild illness without causing outbreak and *V. cholerae* non-O1 produces enterotoxin similar to O1 causing cholera-like outbreak. A single factor cannot explain the entero-pathogenicity. However the ctx genetic element is increasingly associated with toxigenic *V. cholerae* O1 and O139 strains.1 In Oman, cholera is a notifiable disease since the beginning of communicable disease surveillance in March 1991. As early as 1999, 7 cholera cases were reported from Sohar, a coastal town in the North Batinah region. However, these cases were imported and amongst the non-nationals with no secondary transmission locally. *Vibrio cholerae* O1, Ogawa, biotype El Tor was isolated. In June 2000 the first indigenous cases of cholera were reported in Oman from Dhahira region. A descriptive study was conducted to investigate the atypical characteristic of the disease due to *V. cholerae* O1 and its probable relationship with non-O1/O139 strains from June to December 2002. A formal case-based epidemiological investigation for all the diagnosed cases was carried out upon notification. The stool samples for close family contacts and water samples were collected for examination. Stool samples were processed at the regional hospital laboratory using standard culture method. The stool samples were inoculated into Alkaline Peptone water and incubated for 6-8 hours, then further inoculated into TCBS selective media and incubated for 18-24 hrs at 37°C. The suspected colonies were sub-cultured in nutrient/blood agar and incubated overnight at 37°C. Presumptive *V. cholerae* identification was based on the positive oxidase test and reading from API 20E system as *V. cholerae*. Grouping was carried out by using polyvalent antiserum (Mast group, UK) by slide agglutination method and later on reconfirmed by the Central Public Health Laboratory, Muscat.

Total 26 cases of cholera were reported in Oman during the study period. *Vibrio cholerae* O1, El Tor was isolated from 12 cases while non-O1 strains from 14 cases. The median age was 17.5 years with a range of 2-48 years among the O1 positive patients. The female to male ratio was 1:1.4. The point prevalence of cholera O1 for the study period was 0.51/100,000 population. All the cases presented with mild symptoms of diarrhea with mild to moderate dehydration except in one imported case. The distribution of cases was focal namely restricted to only one Wilayat (District) of Dhahira region. The salient and atypical features observed were1 no explosive epidemic despite water contamination. The total numbers of cases were small, no clustering in time or place, mild clinical presentation, no fast spread and a wide range of incubation periods.2 The relation between the organism isolated from cholera patients and contacts could not be directly established. On the contrary some of the contacts were positive for non-O1.3 The organism isolated from the patients (O1) was different from the organism isolated from the environment (non-O1) except in one case where O1 was isolated from falaj water (a water stream).4 No significant exposure history could be elicited except in one imported case which had no relation to other cases.4 The degree of dehydration was minimal with no associated mortality.5 Multiple serotypes were isolated during the study period namely, *V. cholerae* O1, Ogawa from case no. 1-8, Inaba from case no. 9, 10 and 12, and Hikojima from case no. 11. The reasons for this atypical presentation of *V. cholerae* O1 in Oman probably are: 1) Persistence of the organism in a free, perhaps in an altered state in...
Atypical cases of cholera in Oman

the environment. The water sample survey from the environment was conducted in Dhahira region during the study period showed 36.5% (196/536) samples positive for non-O1 and none of the sample was positive for O1. In a study conducted in Malaysia, similar observations were made. 2. Existence of atypical non-toxigenic (NT) V. cholera O1 bacteria with low virulence which do not have epidemic tendency. Biochemically and serologically indistinguishable strains of V. cholerae belonging to the O1 serogroup which do not produce CT and which lack the toxin structural genes are currently termed as NT strains. Characterization of strains from an outbreak at the National Reference Center for Cholera in Calcutta (India) revealed the strains to be NT and these probably are the first strains of V. cholerae O1 to be termed as NT. Later on the isolation of the NT strains were reported from patients and environmental sources in several countries including Saudi Arabia. The NT strains of V. cholerae O1 have remained a scientific curiosity in the absence of a proper understanding of the mechanism of pathogenesis and paucity of knowledge of what the interplay of factors initiating acute secretary diarrhea. 3. Transmission of somatic antigen in the natural environment or in the host namely, non-O1 transformed into O1. Hybridization studies have revealed the existence of a homologous O antigen of O1 gene in a sample of non-O1, most likely occurring by spontaneous mutation of gene(s). The non-O1 strains can then produce an enterotoxin that is similar to the CT and are also able to generate CT gene under natural conditions due to change in the rfb-NAG genes. Evidence for horizontal transfer of O antigens, recombination events within the asd gene of V. cholerae has also been observed. 4. Possible role of environmental conditions (temperature, pH, osmolarity) in altering the toxigenicity and the virulence (amino acid of ctx AB Operon) of O1. The virulence-associated factors also depend on the source and the ecosystem. A molecular characterization study conducted in Malaysia showed CT gene in all clinical isolates and 82% of the environmental isolates. Thus it is evident that V. cholerae O1 as well as the non-O1 strains have significant public health implications. Non-availability of the receptors for the enterotoxin in the intestine or natural gut due to natural infection in Omant population needs to be further explored. One of the reasons for the different clinical manifestations is also the individual differences in the availability of intestinal receptors for V. cholerae or for their toxin but this has not been proven.

In conclusion probably the cholera due to the NT O1 organism is endemic in some parts of Oman and the existence of its possible link with non-O1 organism and the role of environmental conditions cannot be ruled out. More evidence is needed to arrive at a definite conclusion. Advanced studies on the isolates including molecular phage typing and characterization, amino acids structure, clonality and so forth may offer a clue to the relationship between O1 and non-O1 strains.

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From the Regional Epidemiologist (Patel), Directorate General of Health Services, Dhahira Region, Department of Communicable Disease Surveillance and Control (Bawikar, Al-Awaidy), National Epidemiologist, Muscat, Microbiologist (John), Ibr Regional Referral Hospital, Dhahira region, Ministry of Health, Sultanate of Oman. Address correspondence and reprint requests to: Dr. Prakash K. Patel, Regional Epidemiologist, PO Box 618, Postal code 511, Al Dhahira Region, Sultanate of Oman. Tel. +968 199003867. Fax. +968 2569220. E-mail: pakki_kp@hotmail.com

References


Colonization of cagA-positive Helicobacter pylori is significantly greater in infected human males than females. A possible factor in distal gastric cancer gender difference

Mohammed S. Al-Marhoon, MRCPath, PhD.
Sheila Nunn, BSc, PhD. Roger W. Soames, BSc, PhD.

Helicobacter pylori (H. pylori) has been designated a group one (definitive) carcinogen by the World Health Organization. The intestinal type distal gastric cancer is twice as common in males as females. Infection with cytotoxin-associated gene A positive
Colonization of cagA-positive *H. pylori* is significantly greater in infected human males than females

(cagA+) is associated with an increased risk of distal gastric cancer. The aim of this study is to explain the gender difference in the incidence of gastric cancer. Ninety-nine patients were randomly recruited from endoscopy clinics in the North of England without prior knowledge of their *H. pylori* status. The study was approved by both the Leeds Health Authority clinical research ethics committee and the York research ethics committee. Patients were included in the study if they were not taking acid suppressive drugs, non-steroidal anti-inflammatory drugs or receiving treatment for *H. pylori*, but excluded if they were known or found to have peptic ulcer disease or gastric cancer. There were 42 (42%) males and 57 (58%) females with mean ages of 46 ± 11 for the males and 51 ± 15 years for the females. Four biopsies were taken from each patient for culture of *H. pylori*, polymerase chain reaction (PCR), and histology (antrum and body). *Helicobacter pylori* infection was assessed by histology, culture, and PCR. Patients were considered to be *H. pylori* positive if 2 or more of the above tests were positive. Identification of *H. pylori* cagA+ strain was carried out by PCR. The DNA extraction and PCR were performed using standard protocols. Each gastric biopsy taken, was placed into a sterile cryogenic tube (Nalgene, 1.2 ml) containing one ml transport and storage medium (37 g l-1 brain heart infusion, 10 % v/v glycerol, and 2.5 % v/v fetal calf serum). The tube was immersed in liquid nitrogen for transport. Direct biopsy weight was determined using an analytical balance (Mettler AC 100, Switzerland). The biopsy was placed in a sterile homogenization tube (Anachem, 1.5 ml) and homogenized using a disposable pellet pestle (Anachem K-749520-0000) operated by a hand held electric homogenizer (Cordless motor, Anachem K-749540-0000) to make a tenfold dilution (10−1) of the homogenate by the addition of 9 µl of phosphate buffer saline (PBS) solution for each mg of biopsy weight. Five serial dilution tubes (Eppendorf, 1.5 ml) each containing 90 µl PBS were prepared. The serial dilution was made by adding 10 µl of the initial homogenate (10−1) to the first tube to make a dilution of 10−2 and then 10 µl was transferred from the first tube to the next progressing the dilutions from 10−3 to 10−6. For each biopsy, 4 culture plates were inoculated, 2 selective Vancomycin, Polymixin E, Amphotericin, Trimethoprim (V-PAT) media (3 mg l-1 Vancomycin; 6 mg l-1 Colistin (Polymixin E); 1 mg l-1 Amphotericin; and 6.5 mg l-1 Trimethoprim lactate) and 2 non-selective heated blood agar (HBA) media. From the initial homogenate (10−1 dilution), 10 µl was pipetted onto each of the V-PAT and HBA plates and spread out using a flamed sterilized metal loop. A further 2 plates (V-PAT and HBA) were divided by a marker pen into 8 sections then 10 µl in duplicate were pipetted from dilutions 10−3 to 10−6 on each plate without spreading. The culture plates were incubated under microaerophilic conditions (10% CO₂, 6% O₂, 84% N₂ and 95% humidity) at 37°C for 3-7 days. The culture plates that showed the characteristic *H. pylori* colonies following incubation were studied further by Gram stain and the Urease test to confirm the presence of the organism. The culture plates (V-PAT and HBA) that had been inoculated with dilutions of 10−3 to 10−6 were used to assess the viable count. After incubation, the dilutions with discrete countable colonies were counted by eye and the average of the duplicate dilution calculated. The number of bacteria present in the original homogenate was calculated according to the dilution factors made. The viable count is expressed in colony forming unit (c.f.u. mg-1) of biopsy weight. Results were analyzed using SPSS software version 10.1 and expressed as mean ± SE (standard error of the mean). Statistical significance was tested using Analysis of Variance (ANOVA).

Of the 99 patients studied, 69 patients were *H. pylori* negative (HP-) and 30 *H. pylori* positive (HP+): 10 cagA+, 18 cagA-, 2 undetermined. In the HP+ group 97% (29/30) had positive culture growth for *H. pylori*. Colonies of *H. pylori* from primary culture on selective and non-selective media at 37°C are circular (1-2 mm), convex and translucent in appearance. In the present study, 9% (9/99) of patients had atrophic gastritis of which 67% (6/9) were infected with cagA+ strains. There was no significant difference (p = 0.666) in *H. pylori* colonization density between cagA+ and cagA- infected patients (2.6 ± 1.3 × 10⁶ c.f.u. mg⁻¹, n = 9 for cagA+, and 3.1 ± 1.1 × 10⁶ c.f.u. mg⁻¹, n = 18 for cagA-). Also, there was no significant difference (p = 0.147) in *H. pylori* colonization density between infected males and females (3.6 ± 1.3 × 10⁶ c.f.u. mg⁻¹, n = 17 for males, and 1.7 ± 0.6 × 10⁶ c.f.u. mg⁻¹, n = 12 for females). However, *H. pylori* colonization density in males infected with cagA+ (4 ± 2.1 × 10⁶ c.f.u. mg⁻¹, n = 5) was significantly greater (p = 0.04) than cagA+ infected females (7.1 ± 3.2 × 10⁶ c.f.u. mg⁻¹, n = 4) (Figure 1). In contrast, in cagA- patients there was no significant difference (p = 0.363) in bacterial density between males and females (3.9 ± 1.9 × 10⁶ c.f.u. mg⁻¹, n = 10 for males, and 2.1 ± 0.9 × 10⁶ c.f.u. mg⁻¹, n = 8 for females).

The present study shows that 67% (6/9) of patients infected with cagA+ had atrophic gastritis, which indicates the high association between cagA+ infection and severe gastric lesions. Kuipers et al reported a high association between cagA+ infection...
Colonization of cagA-positive *H. pylori* is significantly greater in infected human males than females.

and atrophic gastritis (62%) compared with cagA-infection (32%). Atherton et al.\(^4\) have shown that cagA+ patients have a significant higher bacterial density than cagA- patients. This finding was not supported in the present study, however, the present study has shown that *H. pylori* colonization density in males infected with cagA+ was significantly greater than cagA+ infected females. One possible explanation why cagA+ organisms have previously been shown to have a higher colonization density than cagA- organisms is their possession of better survival mechanisms in the gastric mucosa by strongly binding to surface epithelial cell receptors. The gender difference in the colonization density between human males and females infected with cagA+ strains may point towards cagA+ infection in males having a different pattern of behavior than in females, the reasons for this are not clear. However, Fox et al.\(^5\) in their study of INS-GAS mice, demonstrated a gender difference in the susceptibility of gastric tissue to *H. pylori* infection, as males had more severe hyperplasia, dysplasia, and epithelial cell proliferation than females. Since gastric cancer is more common in cagA+ infected patients compared with the uninfected, and the intestinal type distal gastric cancer is twice as common in males as females, it is speculated that the greater colonization density of cagA+ strains in infected males may explain why distal gastric cancer is more common in males. The underlying mechanisms responsible for this gender difference in susceptibility are not known. This raises the question as to whether the combination of cagA+ infection and male sex increases the risk of gastric cancer. This needs further investigation in humans to substantiate the present study, which is based on a small number of cagA+ patients.

In conclusion, the combination of cagA+ infection and male sex in humans may increase the risk of distal gastric cancer as a consequence of higher cagA+ colonization density in males. This needs further investigation.

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