compared to the other agents.[1,5] As the first choice drug, oxcarbazepine can be used as monotherapy at the lowest effective dose in the treatment of epileptic syndrome.[1] Careful planning and management of any pregnancy in women with epilepsy is essential to increase the likelihood of a healthy outcome for both mother and infant.[1,2] In our case, pregnancy had not been planned; antiepileptic drugs were used during the first 10 weeks of the pregnancy. After consultation among physicians within our departments, we recommended that she stop taking barbexaclone due to lack of data in the literature regarding its effects on pregnant women. In the light of the previous studies, we recommended oxcarbazepine as a monotherapy that could be taken throughout the remainder of her pregnancy. Oxcarbazepine seems to be safer than other available agents. Diet before conception and during organogenesis should contain adequate amounts of folic acid. This is valid also for pregnant women using antiepileptic drugs, but our patient did not elect to use folic acid upon our recommendation.

In the literature, this is the first case of an observation of barbexaclone exposure in a pregnant woman. We did not find any congenital anomaly in the baby. This single case is not enough to define the drug as "safe in pregnancy," but we wish to share this information with the other physicians.

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References

1. Morrow JI, Craig JJ. Anti-epileptic drugs in pregnancy: current safety and other issues. ***Expert Opin Pharmacother*** 2003; 4: 445-456.
2. Barrett C, Richens A. Epilepsy and pregnancy: Report of an Epilepsy Research Foundation Workshop. ***Epilepsy Res*** 2003; 52: 147-148.
3. Lindhout D, Omtzigt JG. Pregnancy and the risk of teratogenicity. ***Epilepsia*** 1992; 33 Suppl 4: S41-48.
4. Bokhari A, Connolly S, Coull BA, Harvey EA, Holmes LB, Bokhari A. Effects on toes from prenatal exposure to anticonvulsants. ***Teratology*** 2002; 66: 122-126. (Erratum in: Teratology 2002; 66: 348-349).
5. Bennett GD, Amore BM, Finnell RH, Wlodarczyk B, Kalhorn TF, Skiles GL et al. Teratogenicity of carbamazepine-10, 11-epoxide and oxcarbazepine in the SWV mouse. ***Pharmacol Exp Ther*** 1996; 279: 1237-1242.

No evidence of persistent *helicobacter pylori* infection in peripheral blood of patients with coronary heart disease

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Recently, it was proposed that coronary heart disease (CHD) may be associated with chronic *helicobacter pylori* infection.[1] The data, however, are conflicting since there are reports denying the link between the 2 conditions.[2] Similarly, there have been several claims of strong and significant correlation between chronic *H. pylori* infection and various vascular risk factors such as fibrinogen concentration and white cell count.[3] The presence of high concentration of immunoglobulin G antibodies to *H. pylori* has been associated with chronic gastric infection.[4] Association of chronic *H. pylori* infection with CHD was established on the bases of the evidence that antibodies against *H. pylori* were detected in sera of 76.6% of patients with CHD.[3] Since antibodies against *H. pylori* are also present in the sera of other wise normal healthy individuals and the significance of these antibodies in sera of patients with CHD and their association with CHD remains doubtful.[5] In order to investigate the association between persistent *H. pylori* infection and CHD this study was performed to detect the presence of *H. pylori* DNA in the peripheral blood of patients with CHD by polymerase chain reaction (PCR).

Sera from 20 male patients, with mean age of 54 ± 5, diagnosed as having CHD according to the World Health Organization criteria of non-fatal myocardial infraction, and 12 healthy blood donors with mean age of 56 ± 5 with no history of any significant illness in the past were tested. Ten ml of blood was collected by venipuncture in sterile tube, and was allowed to clot at 4^0C for 2-4 hours. Serum was separated from whole blood by centrifugation at 1200 x g for 20 minutes at room temperature, and stored at -20^0C until used. **Table 1** illustrates the structural design of primers and capture probe used in this study. The selection of primers and capture probe was based on the sequence *ure C*, and had been used in a previously published study.[6]

Serum sample was lysed using lysis buffer with *Proteinase K* and was precipitated in ethanol, which was followed by vacuum drying. In the first round

of amplification, DNA was added to a master mixture containing 0.4 mM of each primer and 0.2 mM of 4-deoxy nucleotide triphosphate (dNTP) in a PCR reaction buffer (Roche) and denatured at 94^{0}C for 10 minutes. The denatured product was then cooled on ice, and 2.5 U taq polymerase was added prior to 40 amplification cycles (94^{0}C for 1 minute, 55^{0}C for 1 minute and 72^{0}C for 1 minute). Following the same protocol as for the first round, the second round reaction (nested PCR) was performed using the previously amplified DNA and digoxigenin (DIG) labeled dNTPs (Roche). *Helicobacter pylori* NCTC 11637 DNA and sterile distilled water were incorporated in each run as positive and negative controls. Samples were run in duplicate and amplification was performed in a 9600 thermal cycler (Perkin Elmer). The cut off value was assessed as mean value ± 2 standard deviations as recommended by the manufacturer (Roche).

The amplified DNA was then hybridized to a specific capture probe that was complementary to the inner part of the amplification product. Before the hybridization this specific capture probe was labeled with biotin to allow immobilization of the hybrid (DNA-probe) to a streptavidin-coated microtiter plate surface. The bound hybrid was detected by an anti-DIG peroxidase conjugate using colorimetric substrate 2,2'-Azino-d(3-ethyl-Benz Thiazoline)-6-sulfonic acid (ABTS). Briefly, as indicated by the manufacturer, 40 ml of denaturation DNA solution was added to 20 ml of amplification product. After 10 minutes of incubation at room temperature, hybridization solution containing biotinylated probe at an optimal concentration was added to a volume of 500 ml. Two hundred microliters of this mix was added to the appropriate wells. Hybridization took place at 50^{0}C under mild agitation for one hour. After hybridization, the plates were washed and 200 ml of anti-DIG-peroxidase solution was added. The plates were incubated at 37^{0}C for 30 minutes and then washed prior to the addition of 200 ml of ABTS substrate solution to each well. After 30 minutes of incubation at 37^{0}C, the OD_{405} was read in Titertek Multiskan Plus (Labsystems, Finland). A blank (ABTS substrate solution), a negative detection control (water), a positive labeling control, a PCR -enzyme-linked immunoabsorbent assay (ELISA) blank-negative control, and a PCR positive control (*H. pylori* DNA) were tested at the same time. **Table 2** shows the results of the PCR-ELISA of 20 patients with CHD and 12 normal controls. No evidence for the presence of DNA was found in sera of either patients with CHD or normal controls. Since all the OD_{405} obtained were below the cut off value OD_{405} 0.2 nm. Despite reports linking *H. pylori* infection with CHD, this study failed to demonstrate the presence of *H. pylori* DNA in the peripheral blood of patients with CHD. Detection of *H. pylori* DNA was prompted by the fact that the presence of antibodies against *H. pylori* in peripheral blood has been linked with the presence of *H. pylori* gastric infection. On the contrary, CHD association with the antibodies against *H. pylori* in peripheral blood is not strong and there is no deference in serum antibody titers between CHD patients and normal controls.[3] Moreover, reliance on antibody detection for present infection has drawbacks. Serum antibody titer elevated long after successful treatment for *H. pylori* and their presence in the peripheral blood may not necessarily indicate a persistent *H. pylori* infection.[4] Detection of *H. pylori* DNA in the peripheral blood would therefore be more useful approach to obtain a confirmed evidence of the existence of *H. pylori* in peripheral blood. In a similar study using nested PCR, no evidence of *H. pylori* infection in the peripheral blood of patients with CHD was found which was in

Table 1 - Primers and probe used in the identification of *ure C* (glmM) gene of *H. pylori* in the nested PCR-ELISA technique.

Primers and probe	Sequence of the *ure C* (glmM) gene	Position
Round 1		
Primer 1	5'-AAG CTT TTA GGG GTG TTA GGG GTT T-3 F1	784 - 808
Primer 2	5'-AAG CTT ACT TTC TAA CAC TAA CGC-3' R1	1054 - 1085
Round 2		
Primer 1	5'-CTT TCT TCT CAA GCA ATT GTC-3' F2	829 - 849
Primer 2	5'-CAA GCC ATC GCC GGT TTT AGC-3' R2	1012 - 1032
Capture probe	5'- AGA ATT GAA GCA TTG CGC GAT TGG GGA TAA GTT TGT GAG CGA AT-3'	904 - 946

F - forward amplification (original template), R - reverse (complementary) amplification,
PCR-ELISA - polymerase chain reaction - enzyme-linked immunoabsorbent assay, H. pylori - helicobacter pylori

Table 2 - Polymerase chain reaction - enzyme-linked immunoabsorbent assay screening sera of patient with CHD and healthy normal controls for the presence of *H. pylori* DNA.

Patient samples	Age	od (nm)	Healthy controls	Age	od (nm)
1	61	0.170	1	59	0.152
2	57	0.118	2	54	0.134
3	59	0.136	3	63	0.164
4	64	0.131	4	61	0.169
5	56	0.144	5	54	0.146
6	48	0.162	6	49	0.132
7	55	0.157	7	62	0.170
8	62	0.163	8	55	0.150
9	49	0.148	9	56	0.149
10	52	0.136	10	51	0.167
11	48	0.133	11	48	0.134
12	53	0.141	12	63	0.165
13	62	0.140			
14	50	0.133			
15	56	0.142			
16	59	0.153			
17	48	0.170			
18	54	0.170			
19	49	0.129			
20	56	0.139			
Mean ± SD	54 ± 5	0.137± 0.01		56 ± 5	0.153 ± 0.01

PCR-ELISA - polymerase chain reaction - enzyme-linked immunoabsorbent assay, od - optical density
CHD -coronary heart disease, SD - Standard deviation

agreement with the findings of this study.[5] The absence of *H. pylori* DNA in the peripheral circulation of patients with CHD observed in this study therefore it shows that *H. pylori* is not present in peripheral blood of patients with CHD. Markers have reported suggesting the presence of *chlamydia pneumoniae* in coronary atheroma of patients with CHD. It would therefore be quite relevant to examine the atheromas for the presence of *H. pylori* infection to establish the link between *H. pylori* infection and CHD.[6] This study was performed using a simple practical, reliable, and sensitive colorimetric hybridization assay for the detection of amplified *H. pylori* DNA. The assay was a combination of a sensitive DNA hybridization reaction and a colorimetric protocol, similar to those of conventional enzyme immunoassays, widely used in routine clinical microbiology laboratories. The urease gene region *ure C* (glmM) of *H. pylori* was targeted, which is considered to be a suitable target site for quantitative PCR due to its high level of expression. Moreover, the *ure C* (glmM) has also been shown to be more sensitive and specific for detection of *H. pylori* infection.[6] Failure to detect *H. pylori* DNA among CHD patient specimens in this study by using a highly reliable, sensitive and specific technology supports the notion that in CHD patients there is no evidence of persistent *H. pylori* infection at least in the peripheral blood.

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References

1. Pieniazek P, Karczewska E, Duda A, Tracz W, Pasowicz M, Konturek S. Association of Helicobacter pylori infection with coronary heart disease. ***J Physiol Pharmacol*** 1999; 50: 742-751.
2. Ossewaarde J, Feskens E, De Vries A, Vallinga C, Kromhout D. Chlamydia pneumoniae is a risk factor for coronary heart disease in symptom-free elderly men, but Helicobacter pylori and Cytomegalovirus are not. ***Epidemiol. Infect*** 1998; 120: 93-99.
3. Patel P, Mendall M, Carrington D, Strachan D, Leatham E, Molineaux N et al. Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors. ***BMJ*** 1995; 311: 711-714.
4. Hirschl A, Rotter M. Serological tests for monitoring Helicobacter pylori eradication treatment. ***J Gastroenterol*** 1996; 31: 33-36.
5. Danesh J, Koreth J, Collins R, Ranjit Arnold J, Balarajan Y, McGee J et al. Fast-track communication: is Helicobacter pylori a factor in coronary atherosclerosis? ***J Clin Microbiol*** 1999; 37: 1651.
6. Kerr JR, Al-Khattaf A, Barson AJ, Burnie JP. An association between sudden infant death syndrome (SIDS) and Helicobacter pylori infection. ***Arch Dis Child*** 2000; 83: 429-434.

Suicide after treatment of chloroquine-resistant falciparum malaria with quinine

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Occasionally, unusual patterns of clinical manifestations of falciparum malaria involving the central nervous system are seen in Sudan.[1] Chloroquine is still regarded the first line treatment for falciparum malaria, although more than 70% resistance on the drug was reported in Khartoum and in Eastern Sudan.[2]

A 27-year-old male presented to New Halfa Teaching Hospital, Sudan complaining of fever, sweating, headache, vomiting, and backache for 10 days. He completed the full course of chloroquine 7 days before reporting to the hospital. His weight was 58 kg, fully conscious, with pulse rate of 95/minute, blood pressure of 110/70 mm HG, temperature of 38.7^{0}C, with clear chest and there