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The Role of Triple Therapy, Age, Gender and Smoking on the Genotoxic Effects of *Helicobacter pylori* Infection

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The role of age, gender and smoking on both the genotoxic effects of *Helicobacter pylori* and the efficacy of eradication therapy in a group of patients with gastritis was investigated. Gastritis was confirmed by endoscopy and biopsy, and the presence of *H. pylori* by urease testing. Pre- and post-treatment peripheral blood lymphocyte cultures were prepared from 17 patients and 25 metaphases per patients were analysed for sister chromatid exchange (SCE), a well-established technique for the evaluation of human exposure to toxic agents. Treatment with omeprazole, clarithromycin and amoxycillin triple therapy eradicated *H. pylori* in 94% of patients and significantly reduced the SCE frequency. Pre-treatment SCE frequency was found to be positively correlated with age. Female smokers tended to have higher post-treatment SCE frequencies than male smokers, and pre- and post-treatment SCE frequencies were higher in older males than in older females. Eradication therapy decreased the genotoxicity of *H. pylori*, but age in males and smoking in females may decrease treatment efficacy.

KEY WORDS: Eradication therapy; Genotoxicity; *Helicobacter pylori* infection; Age; Gender; Smoking; Sister chromatid exchange

Introduction

Helicobacter pylori is a Gram-negative, microaerophilic, spiral bacterium that infects the gastric mucosa and plays an important role in the aetiology of gastritis and peptic ulceration in half of the world's population. Colonization of the host is assisted by factors such as motility, urease production and adhesion mechanisms. It damages the gastric mucosa and induces inflammatory mediators because of disease-inducing properties, including the release of cytotoxins and urease. In addition, its long-term persistence promotes resistance to drug therapy, thereby increasing its ulcerogenic and carcinogenic effects.[1,2]

Helicobacter pylori was recently designated a carcinogen in humans by the International Agency for Research on Cancer, sponsored by the World Health Organization.[3 – 5] There is a strong relationship between *H. pylori* and gastric cancer, although *H. pylori* is not directly genotoxic or mutagenic. The carcinogenic effects of *H. pylori* are a result of its properties.

For example, the abundant ammonia produced as a result of the urease activity of the bacteria may promote cell division, *H. pylori* phospholipases may damage the epithelial cell membrane, and CagA strains, which have vacuolating cytotoxic activity, may impair the defence capabilities of the host and sensitize the epithelial cells to carcinogenesis.[4]

Sister chromatid exchange (SCE) analysis in peripheral blood lymphocytes is a well-established technique for the evaluation of human exposure to toxic agents.[6] This method measures the interchanges of DNA between replication products. Analysis by SCE is a very sensitive method of assaying DNA damage and, because many exchange-inducing agents are mutagens, carcinogens, or both, this method is used to test chemical agents with unknown carcinogenic potentials.[7]

Helicobacter pylori infection can be cured with antibiotics, and treating this infection can potentially prevent cancer. At the time of writing, however, there are no data to demonstrate that *H. pylori* eradication modifies an individual's cancer risk.[8]

This study aimed to investigate whether the risk of cancer is modified in patients with gastritis by determining their SCE frequencies before and after *H. pylori* eradication. The study also considered whether age, gender and smoking altered the effectiveness of the therapy.

Patients and methods

PATIENTS

Patients with both gastritis and *H. pylori* infection were enrolled in the study. Patients were included if they were positive for *H. pylori* by the urease test (CLO® test, Delta West, Australia), and had gastritis confirmed by histological examination of gastric biopsy specimens, were not on any medication, had not had gastric surgery, and had no symptoms or history of other pathology or disease. Patients gave verbal consent to participate in the study.

ANALYSIS AND TREATMENT

Age, gender and smoking habits were recorded. Peripheral blood lymphocyte samples were collected from the patients and cultured for cytogenetic analysis. Patients self-administered *H. pylori* triple therapy comprising omeprazole, 20-mg capsule, clarithromycin, 500-mg capsule, and amoxycillin, 1-g capsule, twice daily for 7 consecutive days. Seven days after the triple therapy had been completed, endoscopy, the urease test and gastric biopsy were repeated, and blood samples collected for each patient.

For cytogenetic (SCE) analysis of the pre- and post-treatment blood samples, 0.3 ml of peripheral blood was cultured in 5 ml technetium-199 medium supplemented with 20% fetal calf serum, 1% L-glutamine, 2.5% phytohaemagluttinin and penicillin–streptomycin solution for 72 h. After 24 h, 10 μg/ml 5-bromodeoxyuridine was added and at the end of 72 h, routine harvest was performed. The slides were kept for 7 days at room temperature before staining for SCE with 0.3 M di-sodium hydrogen phosphate dihydrate buffer solution with 2% Giemsa's stain. The pH of the solution was adjusted to 10.4 with ammonia. For each patient, 25 metaphases were scored.

STATISTICAL ANALYSIS

The Wilcoxon, partial correlation and Pearson correlation tests were used to analyse the results. $P < 0.05$ was considered statistically significant.

Results

Seventeen patients took part in the study (seven females and 10 males; age range, 14 – 53 years; mean, 36.8 years), seven of whom were smokers. None was receiving any other medication. The age, gender, smoking habits, and pre- and post-treatment SCE frequencies are shown in Table 1. *H. pylori* was eliminated in all but one patient (94%).

TABLE 1:
Characteristics of patients with gastritis and *Helicobacter pylori* infection treated with omeprazole, clarithromycin and amoxycillin: age, gender, pre- and post-treatment sister chromatid exchange (SCE) frequencies, and smoking habits

Sex	Age	Pre-treatment SCE frequency[a]	Post-treatment SCE frequency	Smoking status
F	39	7.36	7.12	NS
M	33	10.16	6.24	S
M	22	6.68	6.05	NS
M[b]	43	7.72	4.24	NS
M	34	9.56	5.60	NS
M	40	7.64	6.20	NS
M	44	11.76	4.20	NS
M	44	11.80	6.08	S
M	23	5.44	6.68	S
M	32	5.76	4.00	S
F	14	8.48	5.40	NS
F	42	11.00	4.80	S
M	45	9.80	4.20	NS
F	34	10.00	9.60	S
F	50	10.20	5.00	S
F	34	7.68	5.48	NS
F	53	9.20	8.40	NS

NS, non-smoker; S, smoker.
[a]The number of SCEs in normal human cells ranges from 2 to 20 with a mean frequency of between 5 and 8.
[b]This patient tested positive for *H. pylori* after treatment.

Table 2 shows that the SCE frequency, a measure of the damage and repair processes of DNA, was significantly reduced after the triple therapy in patients with gastritis ($P < 0.01$). The mean SCE frequency decreased from 8.94 to 5.84.

Patients whose pre-treatment SCE frequencies were high tended to have higher post-treatment SCE frequencies than patients whose pre-treatment SCE values were low ($r = –0.66$; $P < 0.01$). Treatment effectiveness, as measured by a decrease in the SCE frequency, was thus dependent on the SCE frequency before treatment.

There was a positive correlation between age and pre-treatment SCE frequency ($r = 0.59$; $P < 0.05$) and, in men, increasing age appeared to be associated with a reduction in the effectiveness of the treatment. In older patients, both the pre- and post-treatment SCE frequencies of men tended to be higher than in women. For men, the analysis of age and pre-treatment SCE frequencies gave values of $r = 0.69$ and $P < 0.05$, and the analysis of age and post-treatment SCE frequencies gave $r = –0.85$ and $P < 0.01$.

TABLE 2:
Comparison of the pre- and post-treatment sister chromatid exchange (SCE) frequencies in all patients receiving *Helicobacter pylori* eradication therapy with omeprazole, clarithromycin and amoxycillin, and the smoker and non-smoker subgroups

	SCE frequency						
	Pre-treatment			Post-treatment			
Group	Mean	SD	Median	Mean	SD	Median	*P*-value
All patients (n =17)	8.94	2.01	9.56	5.84	1.52	5.60	< 0.01
Smokers (n = 7)	9.19	2.53	10.16	6.06	6.08	1.82	< 0.05
Non-smokers (n = 10)	8.59	1.51	8.10	5.68	5.54	1.34	< 0.01

Female smokers tended to have higher post-treatment SCE frequencies than male smokers (r = –0.80; P < 0.05), and post-treatment SCE frequencies were significantly reduced both in the smokers and non-smokers (P < 0.05, smoker group; P < 0.01, non-smoker group; Table 2). A comparison of the percentage variation in SCE frequencies, pre- and post-treatment, between the groups of smokers (29%) and non-smokers (31%) was not significant (Table 3).

Discussion

Helicobacter pylori is a carcinogen in humans, although it is not thought to cause gastric cancer directly. It may, however, provide a suitable environment, by causing chronic gastritis and intestinal metaplasia, for neoplastic changes. *H. pylori* infection leads to changes in many factors, such as the vitamin C content of gastric juice, the levels of reactive oxygen metabolites in the tissues and epithelial cell proliferation, that are important in the pathogenesis of gastric cancer. Eradication of *H. pylori* may therefore help prevent gastric cancer.[5]

Sister chromatid exchanges are seen in most normal mammalian eukaryotic cells, including those of humans. The number of SCEs in normal human cells varies between different cell groups, ranging from 2 to 20 with a mean frequency of 5 – 8.[9] SCE analysis is a sensitive index of the damage and repair processes of DNA, and there is an elevated frequency of SCE in cells exposed to various mutagens and carcinogens *in vitro*. Elevated frequencies of SCE in bone marrow cells and peripheral blood lymphocytes of patients with leukaemia, breast cancer and lung cancer have also been reported.[10] These findings have provoked much interest in the

TABLE 3:
Comparison of the percentage variation in the sister chromatid exchange (SCE) frequencies before and after *Helicobacter pylori* eradication treatment with omeprazole, clarithromycin and amoxycillin between the smoker and non-smoker subgroups

	Variation in SCE frequencies (%)						
	Smokers (n = 7)			Non-smokers (n = 10)			
Mean	SD	Median		Mean	SD	Median	*P*-value
–0.29	0.29	–0.39		–0.31	0.21	–0.32	> 0.05

relationship between SCE and a predisposition to cancer or cancer status.[11] This study has shown that SCE frequency was reduced significantly after triple therapy with omeprazole, clarithromycin and amoxycillin.

A study in China reported significant differences in the SCE frequencies between a group of gastric cancer patients and a control group, and between a group of patients with chronic atrophic gastritis and a control group.[12]

The frequency of SCE can also vary in relation to a large number of factors such as age, sex, genetics and environmental factors, including smoking, alcohol intake, radiation, chemical exposure and culture conditions during sample preparation.[11] Cigarette-smoke condensates produce many dose-related lesions in the cellular DNA of cultured human lymphocytes as measured by SCE induction.[13] This investigation found that smoking in females and increasing age in males appear to reduce the efficacy of eradication treatment. Recent evidence from both *in vivo* and *in vitro* studies strongly suggests that oestrogens are epigenotoxic carcinogens. They do not act as mutagenic or DNA-damaging agents directly, but cause inhereditable changes by an unknown alternative mechanism. Progesterone also has similar effects, causing excess chromosomal gaps and breaks, and a synergistic effect is seen with progesterone, oestrogen and human chorionic gonadotrophin. Both prolactin and follicle-stimulating hormone produce excess chromosomal gaps and breaks. These reports clearly demonstrate that a variety of female hormones may play a role in modifying or enhancing DNA damage.[14]

In this small study, the genotoxic effects of *H. pylori* prior to and following eradication therapy were analysed with SCE frequency analysis, a cytogenetic method. SCE frequency was found to be reduced significantly after eradication therapy in a group of gastritis patients infected with *H. pylori*. Age in males and smoking in females may be risk factors that decrease effectiveness of the treatment.

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