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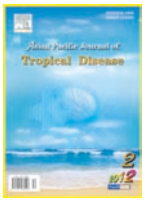

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Determination of efficacy, adverse drug reactions and cost effectiveness of three triple drug regimens for the treatment of *Helicobacter pylori* infected acid peptic disease patients

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ABSTRACT

Objective: To evaluate the clinical outcome and *Helicobacter pylori* (*H. pylori*) infection status by GLQI and PCR, before and after the triple (CMO, CAO or LTC) treatment regimen. **Methods:** Salivary samples of 225 patients of acid peptic disease were used to determine the infection status and GLQI score before and after treatment regimen. Information regarding direct/indirect costs and side effects were determined using validated questionnaires. **Results:** Infection status was determined by successful amplification of 16s r RNA and Cag T genes. Positive eradication rate was 72.7% in males and 70% in females in CMO treated, 88.13% in males and 87.5% in females in CAO treated and 84.44% in males and 96.67% in females in LTC treated patients. The highest improvement in the GIQOL score after the treatment regimen was visible in the LTC treated group with a change of 65.39, followed by CAO treated patients with a change of 49.73 and CMO treated patients with an improvement of 32.18. The average cost effectiveness ratio was found to be best in the CAO treatment regimen with a ratio of 9.43 followed by LTC treatment regimen with a ratio of 11.74 and CMO with a ratio of 49.13. Side effects like diarrhea, nausea, bad taste and metallic taste were significantly enhanced in CMO ($P<0.001$) when compared to CAO and LTC treatment regimens. **Conclusions:** The present investigation suggests that LTC is efficacious and bears less side effects, but CAO is most cost effective amongst the three treatment regimens. PCR assay can be scaled up for hospitals or clinics as a cost effective non invasive diagnostic test.

1. Introduction

Acid peptic disease is a disease characterized by abdominal pain, nausea, vomiting, loss of appetite, malena, hematemesis, early satiety, post prandial fullness and weight loss[1-3]. Complications include bleeding, perforation, obstruction in the digestive tract and sometimes cancer. It is the mucosal erosion equal to or greater than 0.5 cm. As many as 70%-90% of such ulcers are associated with *Helicobacter pylori* (*H. pylori*), which is a spiral-shaped, panmictic, gram negative, microaerophillic bacterium thriving in the acidic milieu of the stomach[4, 5]. Many studies have been conducted to elucidate the role of various factors contributing to the enhancement of susceptibility of individuals to *H. pylori*[3,6,7]. Transmission pathways have been elucidated to be mediated by water[8-11].

An array of treatment regimen is clinically prescribed to ameliorate *H. pylori* infection[5,12-15]. However, in India there is rampant prescription of the triple drug regimen to patients of acid peptic disease patients leading to widespread antibiotic resistance[16-19]. Moreover, the clinical outcome is not measured using validated instruments and there is a severe paucity of the data related with cost effectiveness of the various drug regimens[20, 21].

The objective of the present investigation was to evaluate the clinical outcome and *H. pylori* infection status by GLQI (gastrointestinal quality of life index) and PCR, before and after the treatment regimen. The study was designed to determine the positive eradication rate, the side effect profile and evaluate the average cost effectiveness ratio of the following anti *H. pylori* treatment regimens CMO (clarithromycin + metronidazole + omeprazole)/CAO (clarithromycin + amoxicillin + omeprazole) and LTC (lansoprazole + tinidazole + clarithromycin).

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2. Material and methods

2.1. Human ethics approval

The study protocol was approved by scientific and institutional human ethics committee and a formal written permission was obtained from the governing authorities of Tirupati Hospital, Khenat Hospital, Agarwal Hospital, Dhekane Clinic for the recruitment of patients from the outpatient departments of these hospitals.

2.2. Study design and Patient recruitment

The study was designed and conducted as an interventional, multicentric, open labeled, parallel group, longitudinal study. 75 patients of acid peptic disease being prescribed each of the treatment regimens[(CMO: clarithromycin (250 mg), metronidazole (400 mg), omeprazole (40 mg)) /[CAO: clarithromycin (500 mg), amoxicillin (1000 mg), omeprazole (40 mg) /[LTC: lansoprazole (30 mg), tinidazole (500 mg), clarithromycin (250 mg)] were recruited in the study from the outpatient departments of above mentioned hospitals and grouped into three respective cohorts comprising of 75 patients each. Salivary samples were collected and analyzed before beginning the treatment. It was ascertained that only those individuals who showed presence of *H. pylori* (virulent and non virulent) in the saliva were recruited in the study. All individuals signed an informed consent in local language (Marathi) or English in order to be included in the study. The patients suggestive of acid peptic disease were enrolled from the outpatient departments of above mentioned hospitals located at various parts of Pune city. The patients were examined by gastroenterologists and their symptoms were recorded using a questionnaire bearing the details of the symptoms suggestive of acid peptic disease. Only the patients presenting clinical symptoms of acid peptic disease and positive *H. pylori* salivary test were recruited in the study. The study population consisted of men and women of more than 18 years of age. It was ascertained that none of the participants of the study had consumed proton pump inhibitors, H2 blockers or antibiotics in the last one month of saliva sampling.

2.3. Collection of saliva

Unstimulated saliva was collected in pre-sterilized eppendorf microcentrifuge tubes according to previously reported method[22] and stored at −20 ^{0}C until DNA isolation was performed.

2.4. Determination of infection status

The infection status was determined using polymerase chain reaction technique[23, 24]. Cag T gene (301 base pairs) and 16s r RNA gene (534 base pairs) specific to *H. pylori* was amplified using the DNA of *H. pylori* isolated from saliva of patients.

2.5. Preparation of genomic DNA for PCR

DNA isolation from salivary samples was performed according to phenol chloroform CTAB (cetyl trimethyl ammonium bromide) method[22, 25]. All the steps were performed in aseptic conditions to minimize contamination. Genomic DNA was preserved at −20^{0}C until amplification was performed.

2.6. PCR amplification

2.6.1. Assay of sensitivity

The limit of detection of the PCR assay was determined by preparation of 10-fold serial dilution, from 50 nanogram to 1 fentogram of the isolated genomic DNA from *H. pylori* strain ATCC 26695 in sterile water for injection. An aliquot of each dilution was amplified by PCR and the amplicons visualized on 1.5% agarose gel stained with ethidium bromide. Sensitivity of this PCR assay was determined based on the maximum dilution of genomic DNA in which the primers were able to amplify their corresponding gene sequences.

2.6.2. Assay of specificity

DNA isolated from an entirely sequenced *H. pylori* reference strain DNA (ATCC 26695) was used as a positive control. The specificities of the PCR method was evaluated for three different bacteria obtained from NCIM (National Centre for Industrial Microbes): Staphylococcus aureus NCIM 2079, Escherichia coli NCIM 2345 and Bacillus subtilis NCIM 2063.

2.7. Amplification of genes of H. pylori

DNA isolated from the salivary sample of each individual was subjected to PCR thermal cycles using specific *H. pylori* primers to amplify 16s r RNA and Cag T gene to determine the virulence in the *H. pylori* samples[23]. The primer used for 16s r RNA gene was F 5′- TAA GAG ATC AGC CTA TGT CC-3′ and R 5′-TCC CAC GCT TTA AGC GCA AT-3′. The primer used for cag T gene was F5′-ATG AAA GTG AGA GCA AGT GT-3′ and R 5′-TCA CTT ACC ACT GAG CAA AC-3′. The amplicons were of 534 and 301 base pairs for 16s r RNA and cag T genes respectively. At each amplification step, *H. pylori* DNA isolated from strain ATCC 26695 was used as a positive control, while sterile water for injection instead of DNA served as a negative control. The PCR products were analyzed by agarose gel electrophoresis unit (Bangalore Genei, India) and all the gel photographic registries were performed using a gel documentation system (Alpha Innotech Inc. USA).

2.8. Determination of costs

The cost of the respective regimens prescribed by the physician, cost of consultation, cost of commutation, cost of PCR test were recorded for each patient being prescribed a particular regimen before the beginning of the dosage regimen[26, 27]. After the completion of the regimen at the follow up visit, the costs incurred to manage the side effects of the regimen, consultation fees and travelling costs were determined for each patient in each regimen. Thereafter from the pooled data, direct and indirect costs were calculated for each patient. The summation of direct and indirect costs was the total cost.

2.9. Calculation of average cost effectiveness ratio (ACER)

Average cost effectiveness ratio was calculated by dividing total cost incurred per patient by average change in the GLQI score per patient[28].

2.10. Determination of the clinical outcome

A trained pharmacist interviewed each volunteer and completed a detailed questionnaire. The individuals were questioned regarding the presence and the frequency of symptoms referable to the upper gastrointestinal tract including indigestion, heartburn, sour stomach, and filled in the details of the GLQI[29]. GLQI was filled up before the beginning and after the completion of the regimen. The questions regarding the side effect profile and the details about the fees of consultation and the travelling cost were recorded for each patient. The side effect profiles of the three regimens were statistically compared. The average GLQI scores for each drug regimen was determined before and after the treatment and statistically compared.

2.11. Determination of side effects

A questionnaire in English or local language (Marathi) bearing the details regarding the side effects were filled by each patient after the completion of the treatment. The questionnaire consisted of options depicting the presence of following symptoms: dyspepsia, diorhoea, nausea, abdominal pain, stool abnormality, dizziness, headache, cough, bad taste, metallic taste.

2.12. Statistical methods

The statistical analysis was carried out to examine the association between the various study variables with saliva PCR positivity for *H. pylori* using Fischer exact test. The statistical analysis was performed using SPSS version 16.

3. Results

3.1. Infection status and eradication rate

Infection status was determined by successful amplification of 16s r RNA and Cag T genes, corresponding to an amplicon of 301 and 534 base pairs using salivary samples of patients (Figure 1A and 1B). Positive eradication rate in CMO treated patients was 72.7% in males and 70% in females. 17.3% males and 30% females tested positive for *H. pylori* saliva test after the treatment regimen. Positive eradication rate in CAO treated patients was 88.13% in males and 87.5% in females. 11.87% males and 12.5% females tested positive for the bacterium in their saliva after the completion of treatment regimen. Positive eradication rate in LTC treated patients was 84.44% in males and 96.67% in females. 15.56% males and 3.33% females tested positive after completion of the triple drug regimen. The highest improvement in the GIQOL score after the treatment regimen was visible in the LTC treated group with a change of 65.39, followed by CAO treated patients with a change of 49.73 and CMO treated patients with an improvement of 32.18. The average cost effectiveness ratio was found to be best in the CAO treatment regimen with a ratio of 9.43 followed by LTC treatment regimen with a ratio of 11.74 and CMO with a ratio of 49.13 (Figure 1 and Table 1).

Table 1.
Positive eradication rate among the acid peptic disease patients treated with CMO, CAO and LTC treatment regimens.

Treatment	Men (*n*=130)	Women (*n*=39)
CMO (*n*=75)	40/55 (72.7%)	14/20 (70%)
CAO (*n*=75)	52/59 (88.13%)	14/16 (87.5%)
LTC (*n*=75)	38/45 (84.44%)	29/30 (96.67%)
P value	0.0891	0.0264
Chi square	4.835	7.269
df	2	2

Table 2.
Various side effects experienced by the patients after the completion of the CMO, CAO and LTC treatment regimens.

Characteristics	CMA (*n*=75)	CEA (*n*=75)	LTC (*n*=75)	P value	Chi square	df
Dyspepsia	11	9	5	0.3101	2.342	2
Diorhoea	50	17	9	<0.0001	56.31	2
Nausea	54	25	19	<0.0001	38	2
Abdominal Pain	13	13	7	0.3852	1.908	2
Stool abnormality	7	7	9	0.8239	0.3874	2
Dizziness	7	7	9	0.8239	0.3874	2
Headache	7	9	7	0.8239	0.3874	2
Cough	9	7	9	0.8353	0.3600	2
Bad Taste	60	25	21	<0.0001	55.37	2
Metallic Taste	58	29	25	<0.0001	34.60	2

Table 3.
Measurement of the quality of life of the patients using GQLI and calculation of ACER (average cost effectiveness ratio) of CMO, CAO and LTC treatment regimens

Sr. No.	Drug	Mean GIQOL score before treatment (n=75)	Mean GIQOL score after treatment (n=75)	Change in mean GIQOL Score (n=75)	ACER
1	CMA	44.69	76.87	32.18	(1581.02/32.18) = 49.13
2	CAO	44.51	94.24	49.73	(469/49.73) = 9.43
3	LTC	44.41	109.8	65.39	(768/65.39) = 11.74

Table 4.
Direct and indirect costs of the CMO, CAO and LTC treatment regimens(Rs.).

		Direct Costs (per patient)					Indirect cost (per patient)	
Sr. No.	Drug	Physician consultation per patient	Cost of 1 day treatment per patient	Drugs to treat side effects per patient	Average cost of travelling to hospital (per patient)	Diagnostic costs (PCR salivary test)	Average expense on hospital enrollment and personals (per patient)	Total cost of 7 day regimen (per patient)
1	CMA	150	180.86	40	60	15	50	1581.02
2	CAO	150	22	7	60	15	50	469.00
3	LTC	150	69	2	60	15	50	768.00

3.2. Adverse effects

In the side effect profile side effects like diarrhea, nausea, bad taste and metallic taste were significantly enhanced ($P < 0.001$) whereas other side effects like dyspepsia, abdominal pain, stool abnormality, dizziness, headache, cough were not significantly different among the three triple drug regimens (Table 2).

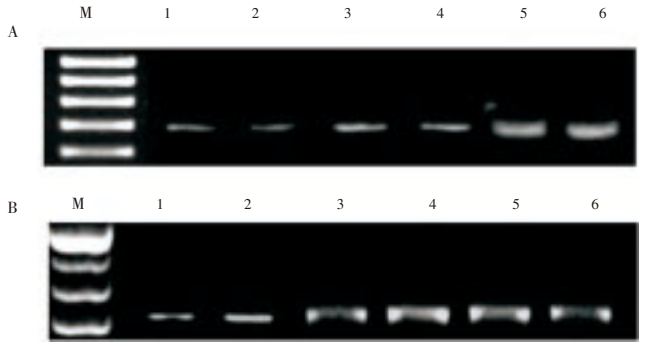

Figure 1. Successful amplification of *H. pylori* specific genes 16 s rRNA gene (534 base pair) (A) and cag T gene (301 base pair) (B).

3.3. Average cost effectiveness ratio

In the CMO treated group of 75 patients, the consultation cost of physician was Rs. 11250; cost of 1 day treatment was Rs. 13564; cost of the drugs required to treat the side effects was Rs. 3000, cost of travelling to the hospital was Rs. 4500, cost of the PCR salivary test to detect *H. pylori* was Rs. 1125, cost of hospital enrollment and personals was Rs. 3750, leading to a total cumulative cost of Rs. 118576.5. The average cost per person has been depicted in the Table 3. In the CAO treated group of 75 patients, the consultation cost of physician was Rs. 11250; cost of 1 day treatment was Rs. 1650; cost of the drugs required to treat the side effects was 525, cost of travelling to the hospital was Rs. 4500, cost of the PCR salivary test to detect *H. pylori* was 1125, cost of hospital enrollment and personals was Rs. 3750, leading to a total cumulative cost of 35175. The average cost per person has been mentioned in the Table 4. In the CMO treated group of 75 patients, the consultation cost of physician was Rs. 11250; cost of 1 day treatment was Rs. 5175; cost of the drugs required to treat the side effects was Rs. 150, cost of travelling to the hospital was Rs. 4500, cost of the PCR salivary test to detect *H. pylori* was Rs. 1125, cost of hospital enrollment and personals was Rs. 3750, leading to a total cumulative cost of Rs. 57600. The average cost per person has been depicted in Table 4.

4. Discussion

Peptic ulcer disease is precipitated due to an imbalance between aggressive and defensive factors in the gastro duodenal mucosa. *H. pylori* infection, NSAIDs, acid secretary abnormalities, spicy food is the principal factors that disturb this equilibrium[30-32].

A number of treatment options are available for the healing of ulcers[33, 34]. These include antacids, H2 Receptor antagonists and Proton pump inhibitors. Eradication of *H. pylori* infection accelerates the rate of healing of duodenal and gastric ulcers. The in vivo activity of antibiotics is variable; hence combinations of two antibiotics plus a PPI are used to ensure complete eradication. The efficacy of these regimens is approximately 90%. A common pharmacotherapeutic approach is to use a metronidazole- or clarithromycin- based triple-therapy regimen as first-line therapy. Eradication of *H. pylori* involves a triple drug regimen consisting of clarithromycin, metronidazole, omeprazole/clarithromycin, amoxicillin, omeprazole/lansoprazole, tinidazole and clarithromycin[35-39]. These combinations are prescribed by the gastroenterologists once *H. pylori* infection is confirmed in the patient of acid peptic disease. The treatment regimen lasts for 7 days and comprises of any of the three regimens[40, 41]. Many studies have been carried out to evaluate the comparative safety and efficacy of these regimens in US, UK and Europe[42-48].

However, such studies have seldom been conducted in India. A few studies in India demonstrated the resistance of *H. pylori* to nitroimidazoles[17].

It is worth noting that these medications are rampantly prescribed which lead to antibiotic resistance in the bacteria. Due to resistance to metronidazole and clarithromycin, using these two agents together in the initial treatment of *H. pylori* is not recommended[49]. However, such therapies are in practice in India, where the epidemiological data regarding the *H. pylori* infection status is scarce. The prevalence of infection and resistance is unknown in India and few studies have been reported which report the comparative efficacy of various triple drug regimens[17]. The facets of cost effectiveness and health outcomes of these regimens have not been explored. This is a pioneer study to draw a correlation between the health economics and outcomes research of anti *H. pylori* triple drug regimens being prescribed in India. It is evident from the results that the LTC treatment regimen bears the best positive eradication rate followed by CAO and CMO. This observation could be attributable to two aspects. The results depict that the side effect profile of the LTC regimen was much better than CAO and CMO. Similar findings have been reported in other parts of world[50, 51]. The virulence factor depicted by cag T was present even after one week regimen of triple therapy in a few patients in each group.

The number of patients demonstrating virulent *H. pylori* after completion of regimen were maximum in the CMO treated group followed by CAO treated and LTC treated group. This shows CMO therapy was not sufficient to ameliorate the virulent strains of *H. pylori*. The cost of the hospital admission, travelling and consultation of the physician was same for the patients of all the groups. Previous studies conducted in India have proven that nitroimidazoles are not the treatment of choice in Indian patients[17] but recently there has been a paucity of studies comparing the efficacies of the various treatment regimens in India.

Another facet of the investigation was the use of PCR assay to determine the infection status of the patients using PCR assay. It is an attractive alternative to other non invasive tests like C13 Urea breath test (UBT) or ELISA. C13 UBT is costly and ELISA provides the investigator with varying results due to the residence of the antibodies in the saliva even after eradication of the bacterium. In India, due to infrastructural deficiencies in most of the healthcare settings, UBT and ELISA are difficult to install. However, a gradient PCR thermal cycler is easily available in most of the laboratories. The consumable costs of C13 UBT and ELISA tests are very high when compared to salivary PCR test[51]. The cost of sample for each PCR assay stands at Rs. 7.50 per sample. The patient compliance in salivary determination is better than ELISA as it requires blood to be drawn from the patient. ELISA from salivary samples is also possible and is being employed in some hospital settings[52]. But salivary or serum ELISA may detect *H. pylori* specific antibodies which may reside in the blood or saliva long after the bacterium has been eradicated. C13 UBT involves ingestion of radiolabelled urea which is not possible to install at all the hospital set ups. There are other tests available such as western blot for the detection of *H. pylori* which provides the information regarding serological make up of *H. pylori* infection but require costly reagents. These shortcomings can be overcome in salivary PCR assay which is a cost effective, sensitive and specific test suitable for a clinical setting in India. However, the salivary PCR assay needs to be optimized for larger clinical settings and employment of skilled staff is indispensable for successful implementation of this technique in clinics. The major advantage of PCR over these diagnostic tests is its ability investigator with a complete picture of the virulent state of the *H. pylori* infection. The presence of the various genes corresponding to the cag pathogenecity island can be determined easily just by varying the respective primers or even subjecting the bacterial DNA to a multiplex PCR[53] to elucidate the entire genetic makeup which is not feasible with other diagnostic options. Moreover, the modern techniques like RFLP (restriction fragment length polymorphism) which requires similar set up and sample as PCR may provide the information regarding mutations prevalent in the bacterium and may help in the judicious prescription of the medicines and alleviate rapid resistance of *H. pylori* to antibiotics.

The present investigation suggests that LTC is efficacious and bears less side effects, but CAO is most cost effective amongst the three treatment regimens. PCR assay can be scaled up for hospitals or clinics as a cost effective non invasive diagnostic test.

Conflict of interest statement

We declare that we have no conflict of interest.

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