A COMPARISON OF TRIPLE THERAPY DRUG REGIMEN DURATION FOR ERADICATION OF *HELICOBACTER PYLORI* IN ADULTS ATTENDING A NAIROBI GASTROENTEROLOGY CLINIC

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A Comparison of Triple Therapy Drug Regimen Duration for Eradication of *Helicobacter pylori* in Adults Attending a Nairobi Gastroenterology Clinic

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my mother, Ummie Mavumba who has been my greatest supporter, for her love,

encouragement and faith in me, during the period of this study.

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LIST OF ABBREVIATIONS AND ACRONYMS

cagA   Cytotoxin-associated gene A

CI   Confidence Interval

DPX   Di-N-Butyle Phthalate in Xylene

EAC1   Esomeprazole, amoxicillin, clarithromycin group 1

EAC2   Esomeprazole, amoxicillin, clarithromycin group 2

ELISA   Enzyme linked immunosorbent assay

H+/K+-ATPase   Hydrogen ion/potassium ion adenosine triphosphatase enzyme

H2   Histamine 2

IgG   Immunoglobulin G

JKUAT   Jomo Kenyatta University of Agriculture and Technology

KEMRI   Kenya Medical Research Institute

MALT   Mucosa-associated lymphoid tissue

NSAID   Non-steroidal anti-inflammatory drugs

PPI   Proton pump inhibitor

OR	Odds Ratio
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
UBT	Urea breath test
vacA	Vacuolating cytotoxin A

ABSTRACT

Helicobacter pylori (*H. pylori*) infection is a major cause of symptomatic gastritis and peptic ulcers in the Kenyan population. Persistence of *H. pylori* and its medical complications continue to be a major problem. Treatment duration is usually for one or two weeks. Studies in developed countries have shown equal or increased efficacy with two week triple therapy treatment, but no study has been done to establish this in Kenya. The main aim of this study was to compare efficacy of one and two week triple therapy for *H. pylori* eradication in adults at a Nairobi gastroenterology clinic. This was a single-blind randomized clinical trial conducted at the Centre for Clinical Research, Kenya Medical Research Institute, Nairobi - Kenya. Patients aged 18 years and above, referred to the gastroenterology clinic for endoscopy, were interviewed, asked to participate in the study and consent was acquired from them. 479 patients went through rapid urease testing on endoscopy of which 253 patients tested negative and 226 patients tested positive for *H. pylori*. The prevalence rate of *H. pylori* infection was 47%. 150 patients were *H. pylori* positive on rapid urease testing and were randomized to receive either esomeprazole 20 milligrams, amoxicillin 1 gram, and clarithromycin 500 milligrams twice daily followed by one week esomeprazole 20 milligrams plus amoxicillin and clarithromycin placebo twice a day (EAC1, n = 76), or esomeprazole 20 milligrams, amoxicillin 1 gram and clarithromycin 500 milligrams twice daily for two weeks (EAC2, n = 74). A cure check through stool

antigen testing (Meridian Bioscience) was performed 4 weeks after conclusion of therapy. Quantitative data was analyzed using SPSS computer program. Bivariate and multivariate analysis was done to measure the strength of association between the exposures and the outcomes. 40.2% of 112 patients, who completed follow up, were found to have *H. pylori* on stool antigen testing following endoscopy – a cure rate of 59.8%. *H. pylori* was eradicated in 66.1% of EAC1 compared to 53.6% of those in EAC2 of the per protocol analysis. There was no statistical difference noted (P>0.05). Both treatments were similarly well tolerated with no unexpected safety concerns. The main side effects were abdominal pain and headache for both treatment groups. There was no significant association between occurrence of a particular side effect and treatment groups (P>0.05). There was a significant association between *H. pylori* presence and return of symptoms at the end of the study on bivariate analysis (P<0.05). The final logistic regression model incorporated the following significant predictors (P<0.05): Age and Return of symptoms in comparison with *H. pylori* presence. For every unit increase in age, an individual was 5% less likely to test positive for *H. pylori*. The older the individual the less likely there were to test positive for *H. pylori*. An individual showing recurring symptoms was 3.31 times more likely to test positive for *H. pylori* compared to one without recurrence of symptoms. In the Kenyan population, the seven day treatment was as effective as the fourteen day treatment for *H. pylori* eradication. However, the seven day treatment was recommended for treatment of

H. pylori as it has good results and is cost-effective. Both treatments were well tolerated. It may be necessary to check antigen levels of *H. pylori* and drug resistance before treatment or retreatment for *H. pylori*. This will help determine the type of drugs to use and duration of treatment.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Helicobacter pylori (H. pylori), bacterial infection occurs worldwide in humans. It is a main etiological factor in chronic gastritis and gastro-duodenal ulcer disease in Kenya and across the world. It is closely related to gastric adenocarcinoma and low grade gastric lymphoma of mucosa-associated lymphoid tissue (MALT). Triple therapy (Proton-pump inhibitor and two antibiotics) is what is recommended for treatment and eradication of *H. pylori* in Kenya and most countries worldwide (Malfertheiner *et al.*, 2007). The duration of eradication therapy continues to be controversial. In many developed countries seven day therapy is considered enough for bacterial eradication. There is still an argument for increasing the duration of treatment to ten or fourteen days (Peterson *et al.*, 2000). In Kenya, however, treatment is given for mostly one week or sometimes two weeks depending on efficacy studies done abroad. There is no general consensus on what duration of treatment is appropriate for *H. pylori* patients in Kenya.

H. pylori is a microaerophilic, Gram-negative, spiral shaped bacillus discovered by Warren JR and Marshall BI in 1983 who connected it to gastritis (Warren and Marshall, 1983). Prevalence of *H. pylori* varies with geographical area and age. About 71% of patients with dyspepsia and 51% of asymptomatic persons have been

reported to have *H. pylori* infection in Kenya (Shmuely *et al.*, 2003). In children less than three years of age, prevalence of *H. pylori* infection in Kenya is about 45% (Langat *et al.*, 2006). Healthy and dyspeptic Nigerian adults, showed a prevalence of *H. pylori* infection of 80% versus 88% (Oluwasola *et al.*, 2002). Prevalence of *H. pylori* infection from both hospital patients and primary school children in South Africa was 50.6% (Samie *et al.*, 2007).

H. pylori was previously treated using drugs of the class of histamine receptor antagonists for example cimetidine to suppress acid, and one antibiotic. However, as better drugs were made and resistance was noted to increase with antibiotic monotherapy, there was a change from histamine receptor antagonists to use of proton-pump inhibitors; for example, omeprazole, lansoprazole, pantoprazole, esomeprazole and so on (Vanderhoff and Tahboub, 2002). Antibiotics were used in combination to reduce resistance. The most effective combination was found to be metronidazole and clarithromycin. However, with increasing metronidazole resistance this was replaced by amoxicillin (Lwai-Lume *et al.*, 2005). In Kenya, the most common combination being used is that of a proton-pump inhibitor with amoxicillin and clarithromycin, mainly for one week for eradication of *H. pylori*. Eradication rates in Kenya, with this treatment have not been established.

Developed countries, for example, Japan, China, North America and Western Europe are continuously developing guidelines for treatment of *H. pylori*. In Europe, guidelines for treatment of *H. pylori* recommend one week of therapy

(Malfertheiner *et al.*, 2002), whereas in the United States of America, 10 to 14 days of triple therapy (Peterson *et al.*, 2000, Howden and Hunt, 1998) is recommended with an eradication rate of about 80%.

In other countries such as Australia and Italy, in recent years, a decrease in efficacy of 1-week therapy has been reported (Katelaris *et al*., 2002, Maconi *et al.*, 2001). A longer duration of treatment could provide better eradication rates (Paoluzi *et al.*, 2006). Some studies have recently reported that prolonging the duration of triple therapy from 7 to 10 days does not improve eradication therapy (Vakil *et al.*, 2004, Calvet *et al.*, 2005, Gisbert *et al.*, 2005). Individual studies comparing treatment with a proton pump inhibitor and two antibiotics for one and two weeks, have generally shown little difference between the two regimens (Laine *et al.*, 1996, Louw *et al.*, 1998, Kiyota *et al.*, 1999, Moayyedi *et al.*, 1996). Overall few studies have directly compared one and two week triple regimens, and in Kenya, this study has not been done.

Persistence of *H. pylori* could be a cause of complications associated with this infection and these include chronic gastritis, peptic ulcer disease, gastric cancer and gastric MALT lymphoma (Ogutu *et al.*1998, Montalban *et al.*, 2001, Uemura *et al.*, 2001). Some studies currently show that *H. pylori* infection may cause iron deficiency anemia and idiopathic thrombocytopenic purpura (Malfertheiner *et al.*, 2007). In the battle for eradication of *H. pylori*, improper treatment should not be among the causes of persistence of *H. pylori*.

1.2 Problem Statement

H. pylori infection is a major cause of symptomatic gastro-duodenitis and peptic ulcer disease in the Kenyan population. Persistence of *H. pylori* and its medical complications for example gastric adenocarcinoma and lymphoma continue to be a major problem worldwide and its recurrence may eventually lead to drug resistance. Treatment of *H. pylori* is mainly by triple therapy that is use of two antibiotics and one proton pump inhibitor. Duration of treatment is usually for one or two weeks. There is no consensus on duration of treatment for *H. pylori* in Kenya. There has been no basis or guidelines to show whether one week or two week triple therapy treatment is effective in eradication of *H. pylori* in Kenya. Studies in developed countries have shown equal or increased efficacy with two week triple therapy treatment compared to one week triple therapy treatment, but no study has been done to establish this in Kenya.

1.3 Study Justification

H. pylori has been implicated in the etiology of most gastritis and duodenal ulcers and is believed to play a role in gastric cancer. *H. pylori*–related peptic ulcer disease significantly impacts patient quality of life and functional status. Patients with ulcers report being in poor health, incapable of major activity, restricted in

daily activity and unable to perform work or are confined to bed (Sonnenberg and Everhart, 1997).

The overall economic impact of *H. pylori* infection is staggering. In the United States of America, it is estimated that the direct costs of treating *H. pylori*–related diseases and associated complications and lost productivity is $3.0 to $5.6 billion annually (Sonnenberg and Everhart 1997, Imperiale *et al.*, 1995). In Kenya, there are no known figures, but considering the high prevalence of *H. pylori* infection, the economic burden could be similar.

Continued effective surveillance for this newly recognized pathogen is important to ensure prompt diagnosis, susceptibility testing and appropriate antimicrobial chemotherapy. This is crucial because gastrointestinal ulcers and cancers are increasingly becoming a problem in developing countries (Malfertheiner *et al.*, 2007).

Cure for *H. pylori* infection in patients with peptic ulcers is associated with a reduction in ulcer recurrence and may produce regression or resolution of low-grade gastric mucosa-associated lymphoid tissue lymphomas (Bayerdorffer *et al.*, 1995).

Knowledge of the appropriate duration of treatment for dyspeptic patients is important for mounting effective eradication therapy of *H. pylori* in Kenya. Cure for *H. pylori* will reduce persistence of the organism, concomitant complications and drug resistance, which is a major problem emerging in Kenya and the rest of

the world. Studies to find out what the appropriate duration of treatment of triple therapy regimens is, in Kenya, have not been done and this information is vital.

1.4 The Study Hypothesis

Null Hypothesis – There is no difference in efficacy of one week and two week triple therapy for *H. pylori* eradication.

1.5 Objectives

1.5.1 Broad Objective

To compare triple therapy drug regimen duration and its efficacy for *H. pylori* eradication in adults attending a Nairobi gastroenterology clinic.

1.5.2 Specific Objectives

1. To establish prevalence of *H. pylori* infection in patients suffering from dyspepsia before treatment.
2. To determine efficacy of one-week and two-week triple therapy drug regimen in eradication of *H. pylori.*
3. To determine the frequency of side effects of one-week versus two-week triple therapy.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction to *H. pylori*

H. pylori is a Gram-negative rod, which has 4-6 sheathed monoflagella on one side. It is microaerophilic, catalase negative and produces urease that enables it to survive in the hostile acidic environment of the stomach. *H. pylori* can persist for decades in this hostile environment (Park *et al.*, 2001). *H. pylori* reside on the surface of the gastric epithelium. The bacteria can be seen on Gram stain and/or by Haematoxylin and Eosin staining, but are more readily visualized by the Warthin-Starry silver stain or the Giemsa stain. They can be cultured in a microaerophilic environment for 4 to 5 days. Yield of culture is 70 to 90%. *H. pylori* has the ability to achieve a high degree of interstrain diversity of genomic DNA nucleotide sequences, while maintaining an overall genetic homology and phenotypic homogeneity amongst its strains. It is not clear if all strains of *H. pylori* are equally pathogenic; however the cytotoxin associated gene (*cagA*) and vacuolating cytotoxin A (*vacA*) subtypes of *H. pylori* have been shown to be important in the pathogenesis of inflammation and peptic ulceration (Gunn *et al.*, 1998).

2.2 Epidemiology of *H. pylori*

The exact mechanism *H. pylori* is acquired is unknown. It is found almost exclusively in humans, and sometimes in some non-human primates. It has rarely

been isolated from pet animals (Park *et al.*, 2001, Brown *et al.*, 2002). Some studies show that reservoirs for *H. pylori* may be divided into human and non-human reservoirs. Non-human reservoirs include: houseflies, dogs, cats and other mammals, water and raw vegetables (Grubel *et al.*, 1997, Fox, 1995). *H. pylori* is able to be transmitted from animals to humans and vice versa. This has been found to be especially common between humans and cats (Meng and Doyle, 1997). Houseflies have been noted to be vectors and can carry viable *H. pylori* on their bodies, in their intestinal tracts and in excreta (Grubel *et al.,* 1997). Prevalence of *H. pylori* infection varies widely by geographic area, age and socioeconomic status. Rates are higher in developing than in developed countries, with most infections occurring in childhood (Chung, 1998). *H. pylori* infection starts at a very early age, highest in the 2-3 year age-group, with risk of infection declining rapidly after 5 years of age (Rowland *et al.*, 2006). However, prevalence of infection has been noted to increase with age in the developed countries at a rate of about 0.3-1%, with this rate said to be higher in developing countries (Sultan, 2010). Up to 90% of the population is colonized in adult life in some countries, but majority of people remain healthy and asymptomatic. *H. pylori* causes chronic gastritis and has been associated with gastric ulcer, duodenal ulcer and gastric cancer. More than 95% of duodenal ulcer and 80% of gastric ulcer patients are infected with *H. pylori* (Walsh and Peterson, 1995). There have been conflicting results on effect of diet; smoking and alcohol on *H. pylori* infection. Most studies have found coffee use to enhance

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H. pylori infection and smoking and alcohol consumption to be protective against active *H. pylori* infection (Atsushi *et al.*, 2001, Brenner *et al.*, 1997). Other studies show that alcohol is associated with active *H. pylori* infection (Zhang *et al.*, 2009). Adequate nutritional status, especially frequent consumption of yoghurt, fruits and vegetables and of Vitamin C, appears to protect against infection with *H. pylori* (Brown, 2000). Other studies show that probiotics for example lactobacilli inhibit *H. pylori* growth and its ability to stick to parietal cells. Consumption of tea, both black and green and red wine is associated with a lower incidence of infection. Antibacterial foods for example garlic also prevent *H. pylori* growth. Foods to be avoided are those high in sugar which can be used by the bacteria that is chocolate, coffee, dairy products, red and processed meat, pickled products, salts and spirits. These have been associated with increased incidence of *H. pylori* infection (Tayomago, 2000). Inadequate sanitation, low social class, crowded or high-density living conditions seem to be related with a high prevalence of *H. pylori* infection (Brown, 2000).

The route of transmission is usually person-to-person by oral-oral route or fecal-oral route. This is supported by the higher incidence of infection among institutionalized children and adults and clustering of *H. pylori* infection within families (Brown, 2000). *H. pylori* has been cultured from faeces and seems to survive in water in a form that is non-culturable and may be transmitted for

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example through vegetables grown with sewage water. Iatrogenic transmission of *H. pylori* following endoscopy has also been established.

2.3 Ecology of *H. pylori*

H. pylori has evolved with humans and it is difficult to classify it as a parasite or commensal (Ahmed *et al.*, 2009). It has a huge population size, rapid generation rate and high mutation rate allowing it to stay ahead of the host. It is highly evolved for it to survive and thrive in a special ecological niche, which is uninhabitable to almost all other infectious organisms (Sutton, 2001). It exists in a low pH such that gastric tissue protects itself by secretion of a thick mucus layer. Part of the evolution of *H. pylori* in this niche involves utilization of this mucus at least in part for protection against the harsh environment of the stomach (Sutton, 2001).

H. pylori produces a protein, *cagA* gene, which is associated with higher risk of acquiring peptic ulcer disease or stomach cancer than people with strains lacking it (Broutet *et al.*, 2001). Strains of *H. pylori* bearing the *cagA* gene cause more severe inflammation and tissue damage. *H. pylori* also bears the *vacA* gene that encodes a toxin that is stored in vacuoles. This protein turns off the infection fighting white blood cells, especially the T cells, in the stomach thus diminishing the human immune response to *H. pylori*. *H. pylori* strains both *m1* and *s1* variations produce the most damaging form of *vacA* toxin. Strains bearing this genotype of *vacA*, combined with *cagA* gene, are associated with the highest risk of

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stomach cancer. *CagA* is said to regulate the acid environment in order to increase its longevity in the stomach, but at the same time causes inflammation and disease of the stomach, hastening atrophic gastritis development and loss of their gastric niche (Blaser and Parsonnet, 1994, Blaser and Crabtree, 1996).

2.4 Pathogenesis of *H. pylori*

The organism's motility allows it to localize and live deep beneath the mucus layer closely adherent to the epithelial surface. Surface pH is close to neutral and any acidity is buffered by the organism's production of the enzyme urease. This produces ammonia from urea and raises the pH around the bacterium. Normal gastric epithelial cells that line the stomach are necessary for *H. pylori* persistence. *H. pylori* is not found in atrophied metaplastic epithelium (Sultan, 2010).

The organism is non-invasive, but stimulates chronic gastritis by provoking a local inflammatory response in the underlying epithelium due to release of a range of cytokines for example *vacA*, *cagA*, adhesins-for example, (*BabA$_2$*) - an adhesin which recognizes blood group antigen A, phospholipases and porins.

H. pylori exclusively colonizes gastric-type epithelium and is only found in the duodenum in association with patches of gastric metaplasia (Palmer *et al.*, 2002). In most people, *H. pylori* causes antral gastritis associated with depletion of somatostatin from D cells in the stomach, and gastrin release from G cells.

Subsequent hypergastrinemia stimulates acid production by parietal cells. Usually this has no clinical consequence. However, in a minority of patients, acid production is exaggerated, leading to duodenal ulceration (Palmer *et al.*, 2002).

The role of *H. pylori* in gastric ulcer pathogenesis is less clear, but may act by reduction of gastric mucosal resistance to attack from acid and pepsin. In 1% of infected people, *H. pylori* causes a pangastritis leading to gastric atrophy and hypochlorhydria. This causes bacteria to proliferate within the stomach, which may produce mutagenic nitrites from dietary nitrates predisposing to the development of gastric cancer (Palmer *et al.*, 2002).

Cross-reactivity between Platelet Associated Immunoglobulin G and *H. pylori cagA* gene suggests that molecular mimicry by *cagA* plays a key role in the pathogenesis of a subset of chronic Idiopathic Thrombocytopenic Purpura patients (Takahashi *et al.*, 2004).

2.5 Clinical presentation of *H. pylori* infection

H. pylori is often asymptomatic but may cause chronic gastritis and even peptic ulcer disease (Kusters *et al.*, 2006). The history of peptic ulcer disease is usually chronic with a natural history of spontaneous relapse and remission lasting for decades. Affected persons usually present with recurrent abdominal pain with three notable characteristics: localization to the epigastrium, relationship to food and episodic occurrence. Other symptoms include: burping, bloating, nausea, vomiting

and weight loss. Vomiting may occur in 40% of ulcer subjects. Persistent vomiting occurring daily is a complication suggesting gastric outlet obstruction (Palmer *et al.*, 2002).

In one third of patients, history may be less characteristic or completely 'silent', presenting for the first time with anemia from chronic undetected blood loss, or as an abrupt hematemesis or acute perforation. History is therefore, often a poor predictor of presence of an ulcer (Palmer *et al.*, 2002).

2.6 Management of *H. pylori* infection

2.6.1 Diagnosis of *H. pylori*

Many different diagnostic tests for *H. pylori* infection are available (Malfertheiner *et al.*, 2007). They can be divided into non-invasive and invasive tests or as non-endoscopic and endoscopic respectively, with each having its advantages and disadvantages based on a patient's clinical history and current presentation. They vary in sensitivity and specificity.

H. pylori detection by non-endoscopic methods such as blood antibody detection tests, urea breath tests (UBTs), and the recently approved assay for the detection of *H. pylori* antigen in stool specimens, is indicated in clinical situations in which endoscopy is not indicated. Unlike blood antibody detection methods, UBT and fecal antigen detection denote active *H. pylori* infection.

Breath-tests are best because of their accuracy, simplicity and non-invasiveness. However, they are expensive and unavailable especially in developing countries. The UBT is a measure of current *H. pylori* infection, relying on *H. pylori* urease to hydrolyze urea labeled with radioactive carbon (^{13}C or ^{14}C) and produce isotopically labeled carbon dioxide in the breath. There is the potential for false-negative UBT test results in individuals receiving antisecretory agents such as proton pump inhibitors (PPIs) or high-dose histamine 2 (H2) receptor antagonists, antimicrobial agents, or bismuth-containing compounds, which reduce *H. pylori* density (Laine *et al.*, 1998). Therefore, UBT should be avoided in those who have received bismuth or antibiotic drugs within the previous 4 weeks or antisecretory agents within the previous 2 weeks (Peterson *et al.*, 2000).

Other non-invasive tests include serology tests for *H. pylori* antibody. *H. pylori* antibody tests lack sensitivity and specificity and cannot differentiate current from past infection. Serology has a low diagnostic accuracy of 80-84% (Stenstrom *et al.*, 2008). Antibody tests to detect IgG antibodies to *H. pylori* are less expensive and more convenient to use than UBTs but are somewhat less accurate. Antibody tests can now be done in an office setting on finger stick whole blood specimens, with results obtained within 10 minutes. It is currently recommended that serology based office tests have no basis in management of *H. pylori* infection (Malfertheiner *et al.*, 2007). Antibody testing is commonly used in the evaluation of dyspeptic patients before endoscopy. Because antibody titers often take many

months to decrease after successful treatment and remain positive in many patients for years, blood antibody testing (for example, serologic testing) is less useful for the monitoring of post treatment *H. pylori* status (Peterson *et al.*, 2000).

Stool antigen testing has emerged as a rapid, non-endoscopic method of *H. pylori* detection (Peterson *et al.*, 2000). In the stool antigen test a simple sandwich enzyme linked immunosorbent assay (ELISA) is used to detect the presence of *H. pylori* antigens shed in the faeces. Studies have reported sensitivities and specificities similar to those of the ^{13}C-urea breath test (>90%). Stool antigen testing is highly sensitive and specific in the detection of *H. pylori* in patients with dyspepsia and in those who have completed an *H. pylori* eradication regimen (Peterson *et al.*, 2000). In the diagnosis of *H. pylori* infection, the stool antigen detection method is highly sensitive (80%-100%) and comparable to that of the UBT (84%-100%). A study showed stool antigen detection performed 4 weeks after completion of an *H. pylori* eradication regimen had a sensitivity of 90%, a specificity of 95%, and a negative predictive value of 98%, rates that were comparable to those obtained with ^{13}C UBT. Although the high rates of sensitivity and specificity with the stool antigen test in patients enrolled in the study (Vaira *et al.*, 1999) are promising, several other investigators found a high rate of false-positive results in patients tested 4 weeks after completion of anti–*H. pylori* treatment with the polyclonal stool antigen test but excellent results with the monoclonal antigen test (Gisbert and Pajares, 2004). The stool antigen test is appropriate when multiple specimens are tested as a batch.

However, it is necessary to store stool samples at $-20^{O}C$ before testing. The sensitivity of the stool antigen test decreased to 69% after 2-3 days at room temperature (Malfertheiner *et al.*, 2007).

Endoscopic or invasive methods involve assessing several gastric biopsy samples for the presence of *H. pylori* by histological examination, urease activity, or culture (Genta and Graham, 1994). Invasive tests include histology, which has a sensitivity and specificity of 95-98% (Moayyedi and Dixon, 1998, Megraud and Lehours, 2007). However, the process takes several days to process and false negatives may occur occasionally. Use of special stains such as Giemsa stain may make identification of the organism easier on histological examination.

Assessment of the biopsy sample for urease activity (rapid urease test) is a highly sensitive (approximately 90%) and specific (approaching 100%) method of *H. pylori* detection (Laine *et al..*, 1996). Rapid urease tests are also cheap and quick tests in terms of getting results. A limitation to the use of rapid urease testing is in patients receiving PPIs or high-dose histamine 2 receptor antagonists, which might decrease *H. pylori* density and consequently urease activity, thereby producing a false-negative result.

Microbiological culture is the theoretical gold standard and it defines antibiotic sensitivity (Destura *et al.*, 2004). It is however slow and laborious, needs specific and equally expensive equipment. Sensitivity is about 80 – 90%, lower than that of

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histological testing - 88 to 95% (Logan and Walker, 2001). Culturing of *H. pylori* is generally not used in establishing a primary diagnosis because of the potential for false negative results due to errors in specimen acquisition, storage, or transportation, and its time-consuming nature that is, requires up to 2 weeks for growth to occur (Cohen and Laine, 1997). Performance of the culture is useful for the determination of antibiotic resistance, especially in patients who continue to be positive for *H. pylori* after an initial treatment regimen (Peterson *et al.*, 2000).

2.6.2 Treatment of *H. pylori*

H. pylori eradication is the cornerstone of therapy for peptic ulcers, as this will successfully prevent relapse and eliminate the need for long-term therapy in majority of patients. All symptomatic patients who are *H. pylori* positive should be offered eradication therapy as primary therapy. Options for therapy for *H. pylori* eradication include a PPI, and two antibiotics (Malfertheiner *et al.*, 2002). PPIs include omeprazole, esomeprazole, lansoprazole, pantoprazole, among others. They act by halting the mechanism that pumps acid into the stomach (Vanderhoff and Tahboub, 2002). Antibiotic choice include those that act against both Gram positive and Gram negative, the most commonly used being amoxicillin, metronidazole, clarithromycin used in combination. A stomach protecting agent for example bismuth or misoprostol may also be added to this combination. Compliance, side effects and metronidazole resistance influences the success of therapy (Palmer *et al.*, 2002).

Common side effects of *H. pylori* eradication therapy include diarrhea in 30-50% of patients (Stentrom *et al.*, 2008). It is usually mild. *Clostridium difficile*-associated colitis can occur. Metronidazole has a metallic taste, and may cause vomiting when taken with alcohol. Other side effects include nausea, abdominal cramps, headache and rash (Palmer *et al.*, 2002).

According to European guidelines for treatment and eradication of *H. pylori*, a 'test and treat' approach is recommended in adult patients under the age of 45 years (the age cut-off may vary locally) presenting in primary care with persistent dyspepsia, having excluded those with predominantly gastro-esophageal reflux disease symptoms, non-steroidal anti-inflammatory drug (NSAID) users and those with alarm symptoms. Diagnosis of infection should be by urea breath test or stool antigen test.

The eradication of *H. pylori* is strongly recommended in all patients with peptic ulcer, including those with complications, in those with low-grade gastric MALT lymphoma, in those with atrophic gastritis and following gastric cancer resection. It is also strongly recommended in patients who are first-degree relatives of gastric cancer patients and according to patients' wishes after full consultation (Malfertheiner *et al.*, 2002).

It is advised that *H. pylori* eradication is considered to be an appropriate option in infected patients with functional dyspepsia, as it leads to long-term symptom

improvement in a subset of patients. It has been reported that the eradication of *H. pylori* is not associated with the development of gastro-esophageal reflux disease in most cases, and does not exacerbate existing gastro-esophageal reflux disease (Oberg *et al.*, 1999). Eradication of *H. pylori* prior to the use of NSAIDs reduces the incidence of peptic ulcer, but does not enhance the healing of gastric or duodenal ulcer in patients receiving antisecretory therapy who continue to take NSAIDs.

Treatment should be thought of as a package that considers first- and second-line eradication therapies together (Malfertheiner *et al.*, 2002). First-line therapy should be with triple therapy using a PPI or ranitidine bismuth citrate, combined with clarithromycin and amoxicillin or metronidazole. Second-line therapy should use quadruple therapy with a PPI, bismuth, metronidazole and tetracycline. Where bismuth is not available, second-line therapy should be with PPI-based triple therapy (McColl, 2010).

The choice of an alternative regimen should be based on the initial treatment regimen. For example, if triple therapy consisting of a PPI or ranitidine bismuth citrate, clarithromycin, and amoxicillin was used as first-line treatment, the second treatment course should consist of a PPI, metronidazole, bismuth subsalicylate, and tetracycline (The European *Helicobacter pylori* Study Group, 1997).

Triple treatment with a bismuth compound, tetracycline (or amoxicillin), and metronidazole is cheap and well investigated (De Boer and Tytgat, 2000, Soll, 1996). It may cause side effects that are usually not severe and have not led to non-compliance in trials. It reaches high cure rates in metronidazole sensitive strains after seven days but requires 14 days to eradicate a substantial percentage of resistant strains (De Boer and Tytgat, 1996, Lerang *et al.* 1997). This regimen is widely used in cost sensitive markets.

Bismuth triple therapy has mainly been surpassed by seven-day triple regimens using PPIs, for example esomeprazole or lansoprazole, with two antibiotics used twice daily (Lamouliatte *et al.*, 1997). The appropriate antibiotics are metronidazole (or tinidazole), amoxicillin, and clarithromycin. PPIs are most commonly combined with amoxicillin 1000 mg twice daily and clarithromycin 500 mg twice daily. This regimen has fewer side effects than other triple regimens (Huang *et al.*, 1997), but it is only moderately effective in clarithromycin resistant strains and secondary resistance is usually induced when treatment fails.

Regimens combining PPIs with metronidazole 400 or 500 mg and clarithromycin 250 mg or 500 mg twice daily are less effective in patients with primary metronidazole or clarithromycin resistance (De Boer and Tytgat, 2000). If treatment fails, the regimen may induce resistance against one or both of the antibiotics used in this regimen. Therefore, triple regimens that combine clarithromycin and metronidazole should not be used, as there is no valid empirical

back up regimen after failure. Treatment should start with a regimen based on clarithromycin with a back up regimen based on metronidazole unless resistance is above 15%, in which case the order should be reversed (De Boer and Tytgat, 2000).

Triple and quadruple therapy has been suggested as the best options in the eradication of *H. pylori* (Sun *et al.*, 2010, Malfertheiner *et al.*, 2007). An alternative regimen is 10-day sequential therapy, involving a PPI plus amoxicillin for 5 days followed by a PPI plus clarithromycin and tinidazole for 5 more days. This regimen was reported to achieve an eradication rate of 93%, as compared with a rate of 77% with standard triple therapy in some trials (Jafri *et al.*, 2008). However in other trials, the eradication rate among patients randomly assigned to receive sequential therapy was only 84%, indicating a need to confirm its efficacy before it is used widely (Sanchez-Delgade *et al.*, 2008). Others argue that a longer length of treatment (14 days versus 10 days) results in better eradication rates (CDC, 2006).

Newer drug combinations include levofloxacin, with amoxicillin or clarithromycin, combined with a PPI have been recommended as second line treatment regimens especially if treatment fails or there's resistance especially to metronidazole or clarithromycin (Gisbert, 2008). However, eradication rates for this therapy are conflicting with certain areas having low eradication rates most likely from levofloxacin resistance. This combination has also been recommended to be given for longer periods, that is, 10 to 14 days as this gives higher cure rates (Gisbert,

2008). The table below shows a summary of regimens used in the treatment of *H. pylori* infection (McColl, 2010).

Table 2.1 Regimens used to treat *Helicobacter pylori* infection (McColl, 2010).

Standard initial treatment (use one of three options)	Type of medication used	Duration
Triple therapy	- amoxicillin, 1 g twice a day†, clarithromycin, 500 mg twice a day, PPI, healing dose twice a day*	7-14 days
Quadruple therapy‡	-tetracycline, 500 mg four times a day, metronidazole, 250 mg four times a day§, tripotassium dicitratobismuthate, 120 mg four times a day, PPI, healing dose twice a day*	10–14 days
Sequential therapy	- amoxicillin, 1 g twice a day and a PPI, healing dose twice a day*	Days 1–5
	- clarithromycin, 500 mg twice a day, tinidazole, 500 mg twice a	Days 6–10

	day§ and a PPI, healing dose twice a day*	
Second-line therapy, if triple therapy involving clarithromycin was used initially (use either)		
Triple therapy	- amoxicillin, 1 g twice a day, metronidazole, 500 mg (or 400 mg) twice a day§, PPI, healing dose once a day*	7–14 days
Quadruple therapy, as recommended for initial therapy		

Note: ‡ Quadruple therapy is appropriate as first-line treatment in areas in which the prevalence of resistance to clarithromycin or metronidazole is high (>20%) or in patients with recent or repeated exposure to clarithromycin or metronidazole.

* Healing doses of proton-pump inhibitors (PPIs) include esomeprazole 20 mg, rabeprazole 20 mg, pantoprazole 40 mg, and lansoprazole 30 mg, all taken twice daily. † If the patient has an allergy to amoxicillin, substitute metronidazole (at a dose of 500 mg or 400 mg) twice per day.

§ Alcohol should be avoided during treatment with metronidazole or tinidazole, owing to the potential for a reaction resembling the reaction to disulfiram with alcohol use.

Before prescribing a second course of therapy, it is important to confirm that the infection is still present and consider whether additional antimicrobial treatment is appropriate. Successful eradication should always be confirmed by UBT or an endoscopy-based test if endoscopy is clinically indicated, for example, to confirm ulcer healing after treatment. Stool antigen test is the alternative if UBT is not available (Malfertheiner *et al.*, 2002). Further attempts at eradication are indicated in patients with confirmed peptic ulcers. If the patient was being treated for uninvestigated dyspepsia with unlikelihood of ulcer, further eradication therapy is unclear (McColl, 2010).

For those who are still colonized after two treatments, the choice lies between a third attempt with quadruple therapy (bismuth, PPI and two antibiotics) or long-term maintenance therapy with acid suppression. Continuous maintenance treatment should not be necessary after successful *H. pylori* eradication. For the minority who require maintenance treatment the lowest effective dose should be used (Palmer *et al.*, 2002). In case peptic ulcer perforation or hemorrhage is noted,

or complications such as gastric outflow obstruction; these are indications for surgery in peptic ulcer disease.

In the United States guidelines for *H. pylori* eradication, it was decided that dual medication regimens should not be used for therapy (Peterson *et al.*, 2000). Despite the results of numerous European studies (Lind *et al.*, 1996, Misiewicz *et al.*, 1997) that suggest that 7 days of triple therapy is sufficient, clinical trials (Laine *et al.*, 1996) performed in the United States have found that the highest rates of cure are associated with treatment durations of 10 to 14 days. Therefore, it was recommended that twice-daily triple therapy with a PPI or ranitidine bismuth citrate, clarithromycin, and amoxicillin for 10 to 14 days is an appropriate therapy (Peterson *et al.*, 2000).

Probiotics are not effective in *H pylori* eradication, but some studies have shown a decrease in gastritis severity or in bacterial density with these agents (Lesbros-Pantoflickova *et al.*, 2007). Other studies have shown a reduction in adverse effects with probiotics (Lesbros-Pantoflickova *et al.*, 2007). There is increasing evidence that they may be used as adjunctive therapy however further research is necessary before these can be recommended for routine use.

2.7 Follow-up of *H. pylori* therapy

Patients who remain positive for *H. pylori* after completion of an effective anti–*H. pylori* regimen should be assessed with regard to treatment compliance. However,

many patients may continue to experience symptoms or remain positive for *H. pylori* despite full treatment compliance (Fendrick *et al.*, 1999).

Options for treatment include acid-inhibitory therapy, endoscopy to check for underlying ulcer or another cause of symptoms and repeat use of the non-invasive test and treat strategies. Other causes should be considered for example biliary or pancreatic disorders. If another course of therapy is administered to eradicate *H. pylori* infection, adherence to therapy and resistance to the drugs clarithromycin and metronidazole should be considered especially in populations where there is established resistance (Fischbach and Evans, 2007).

Patients who do not respond to the initial treatment course may be referred to a gastroenterologist for further workup. These patients often require endoscopy with biopsy and culture for the determination of antibiotic resistance. Further therapy should then be based on the resistance patterns detected. Evaluation of patients after completion of *H. pylori* therapy is helpful in determining the patient's future clinical course. Ulcer recurrence after treatment is much lower (4-6%) in cured patients, and higher (59-60%) in those who continue to harbor *H. pylori* (Hopkins *et al.*, 1996).

Patient symptoms after *H. pylori* therapy do not always correlate with eradication success or failure. Relief of dyspepsia does not always suggest *H. pylori* cure, although some studies have found that persistence of certain ulcer-related

symptoms that is, nausea, epigastric discomfort, and ulcer pain, are predictive of continued infection (Vakil *et al.*, 2000, Phull *et al.*, 1996). Conversely, continued symptoms do not always denote treatment failure. Therefore, follow-up and confirmatory testing might be useful in determining a patient's response to treatment and risk of ulcer recurrence (Vakil *et al.*, 2000).

Non-endoscopic methods (for example, UBT) are recommended for confirming *H. pylori* eradication after completion of therapy. It should be performed no sooner than 4 to 6 weeks after completion of *H. pylori* therapy. Use of PPIs or high-dose H2 receptor antagonists should be discontinued for at least 2 weeks before administration of the UBT. The stool antigen detection test is a reliable method of confirmatory testing when performed 4 weeks after treatment. However, some studies (Calvet *et al.*, 1999) have found a high rate of false-positive results using stool antigen detection 4 weeks after treatment.

Antibody testing is less useful in the immediate evaluation of post treatment response because high levels of antibodies to *H. pylori* remain for variable and extended periods (Kousunen *et al.*, 1992). However, for an individual more than a year after therapy, seroconversion is a reliable indicator of successful eradication (Feldman *et al.*, 1998). If endoscopy is clinically indicated (that is, to confirm ulcer healing) after treatment, the clinician should obtain multiple biopsy specimens from the gastric body and antrum for histological examination and urease testing to exclude persistent infection.

Patients have a desire to know their *H. pylori* status after completion of an *H. pylori* eradication regimen. However, economic factors might limit the utility of performing confirmatory testing in all patients. Follow-up testing to confirm eradication should be performed routinely in all patients with a confirmed diagnosis of new ulcer disease or a documented history of complicated ulcer disease. Patients with a reported history of uncomplicated ulcer disease not documented by endoscopy or radiography and those with a history of dyspepsia should have follow-up testing if symptoms recur. Confirmatory testing may also be performed in patients in whom their unknown *H. pylori* status is causing excess worrying or loss of sleep (Peterson *et al.*, 2000).

2.8 Choice of Esomeprazole and Clarithromycin in the triple therapy regimen

Esomeprazole is the S-isomer of omeprazole, and it provides greater inhibition of acid secretion than omeprazole and all other PPIs (Lind *et al.*, 2000, Röhss *et al.*, 2001). Esomeprazole is a PPI that suppresses gastric acid secretion by specific inhibition of the H+/K+-ATPase in the gastric parietal cell. Esomeprazole blocks the final step in acid production, thus reducing gastric acidity. Several multicenter trials have shown that esomeprazole in combination with clarithromycin and amoxicillin is effective as first line therapy of *H. pylori* infection (Laine *et al.*, 2000, Veldhuyzen van Zanten *et al.*, 2000). Eradication rates for esomeprazole-based therapy are about 95% compared to pantoprazole-based therapy, which ranges from 80 to 85% (Ping-I *et al.*, 2005).

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Esomeprazole based triple therapy is the most commonly used regimen in Kenya today and that is the reason this study is based on this regimen to compare its duration of treatment, which is usually for one week, with two-week therapy to find out if there is any difference in efficacy in the eradication of *H. pylori*.

Resistance to antibiotics is important as it leads to treatment failure. Prevalence of *H. pylori* to clarithromycin is between 5-20% in Europe. Resistance to clarithromycin is caused by previous consumption of macrolides (Megraud, 2004).

Metronidazole resistance in *H. pylori* strains vary geographically. Studies show *H. pylori* resistance to metronidazole varies from 20-40% in Europe, while in developing countries the resistance as high as 50-80% (Torres *et al.*, 2001, Debets-Ossenkopp *et al.*, 1999). Metronidazole is used extensively for treating parasitic diseases in tropical countries and probably why there is more resistance in developing countries (Megraud, 2004).

Metronidazole resistance in Kenya varies, with a study recording 100% resistance, while resistance to clarithromycin was 6.4% (Lwai-Lume *et al.*, 2005). In a recent Kenyan study, clarithromycin showed no resistance and metronidazole showed a low prevalence of 4.6% resistance to *H. pylori* (Kimang'a *et al.*, 2010). The low resistance of *H. pylori* to clarithromycin was the reason for using clarithromycin in our study.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Site

The study was performed at the Kenya Medical Research Institute (KEMRI), Center for Microbiology Research and the Center for Clinical Research Nairobi, Kenya.

Patient recruitment and follow up was done at the endoscopy unit at the Centre for Clinical Research.

3.2 Design Overview

This was a prospective randomized single-blind placebo-controlled study. At baseline, patients referred to the local gastroenterology clinic for endoscopy were asked to participate in the study if found to be *H. pylori* positive. They were evaluated for inclusion and exclusion criteria and provided written informed consent. Patients were randomly assigned to a treatment group. Follow-up evaluations were used to assess the eradication rate of *H. pylori* infection, treatment adherence and side effects.

3.3 Study Population

Between December 2007 and December 2009, consecutive patients with dyspepsia 18 years of age and above, who had *H. pylori* and had never received treatment for

it, and had been referred to a gastroenterology clinic for endoscopy, were asked to participate in the study. No special recruitment techniques (such as advertisements or letters sent to primary care physicians) were used.

Written consent was acquired from them. It was explained to them that both treatments work well, but the process was to find out, which was the more effective drug regimen. They were also told that eradication failure was possible. Those with eradication failure would be given rescue therapy.

3.3.1 Inclusion Criteria

- Presence of at least two of the following symptoms; upper abdominal pain or discomfort, bloating, nausea, vomiting or early satiety.
- Persistent or recurrent symptoms occurring at least three times per week during six or more months in the year(s) preceding the study.
- Age 18 years and above.
- Written informed consent.
- *H. pylori* positive on rapid urease test.

3.3.2 Exclusion Criteria

- Previous treatment for *H. pylori* less than 4 weeks prior to endoscopy.
- Allergy to penicillins or macrolides.
- Significant liver or kidney disease.
- Severe cardiac or pulmonary disease.

- Suspected or confirmed malignancy.

- Concurrent reflux oesophagitis.

- Active upper gastrointestinal bleeding.

- History of gastric surgery except uncomplicated appendectomy, cholecystectomy or hernia repair.

- Pregnancy or breast-feeding.

- Patients using antibiotics in the month before inclusion, bismuth-containing compounds during the 3 months before inclusion, or PPIs, H2 receptor antagonists, misoprostol or sucralfate in the 2 weeks before the pre-entry endoscopy.

- Patients receiving regular treatment with NSAIDs more than or equal to 5 days a week, for at least 2 weeks during the month before the start of the study.

3.4 Study Procedure

After explaining to the potential patient about the study, the investigator or local physician assessed symptoms and collected relevant demographic data for purposes of recruitment to the study (Appendix 1).

3.4.1 Description of the endoscopy procedure

Endoscopy is a diagnostic procedure, involving introduction of a long plastic tube with a lens at the tip and camera-like part at the base, into the duodenum via the

mouth. Biopsy specimens were taken during upper gastrointestinal endoscopy before the start of the study treatment. Two biopsy specimens one from the corpus and one from the antrum, were taken for the rapid urease test (Esokit HP Test). For histological assessment of *H. pylori* infection, two biopsy specimens were taken from the corpus and two from the antrum.

3.4.2 The Rapid urease test procedure

Biopsy specimens were tested for urease production. The rapid urease test kit (Esokit HP Test, Cambridge Life Sciences) consisted of a twin well cartridge, containing urea, phenol red and buffer salts in tablet form and an ampule of buffer. The cartridge lid was opened and each well was filled to a marked line with buffer. The lid was then closed and the tablet dissolved by gentle shaking. The biopsies taken at endoscopy were put in the wells. The lid was closed and the kit labeled with the patient's details – name, age, sex, study number and date.

If the urease enzyme of *H. pylori* was present in biopsy specimens, the rise in pH associated with the hydrolysis of urea caused a color change from yellow to pink/red. The color change usually occurred within 30 min to 24 h from insertion of biopsy specimen depending on *H. pylori* bacteria density. The kit was stored at room temperatures (20 -22˚C) for 24 to 48 h after which the color changed and the biopsies were read and discarded.

Patients were asked to come the following day for rapid urease results and allocation of treatment.

3.4.3 Histology procedure of biopsy specimens

Biopsy specimens were fixed in 10% formal saline. These specimens were sent to a pathologist for histopathological analysis to determine histopathological morphology and presence of *H. pylori*. To do this, specimens were stained separately with Haematoxylin-eosin stain to identify tissues, Giemsa stain to look for comma or S-shaped bacilli organisms which would indicate a positive result for *H. pylori* and observed under a microscope under high magnification.

The biopsies were fixed with 10% formal saline overnight for preservation and to prevent post-mortem changes. The biopsies were processed and prepared for sectioning using paraffin wax processing technique in the following procedure. Biopsies were placed in 70% alcohol I for one hour, then placed in 80% alcohol II for one hour, then placed in 95% alcohol I for one hour, then 95% alcohol II for one hour, then absolute alcohol I for one hour, then placed in absolute alcohol II for one hour in the dehydration process. Biopsies were then placed in a mixture of chloroform and absolute alcohol for one hour, then placed in chloroform I for one hour, then placed in chloroform II for one hour, then placed in wax I overnight, then placed in wax II for one hour and finally in wax III for one hour. Tissues were then blocked in paraffin wax and allowed to cool in the fridge for one hour in

preparation for sectioning. Three micrometer-thick sections were obtained, fixed on a microscope slide, labeled and put in the oven at 56°C for one hour for the wax to melt and for the sections to adhere firmly onto the slides. Sections were removed from the oven, allowed to cool to room temperature and stained using Haematoxylin and Eosin, and Giemsa staining.

In the Haematoxylin and Eosin staining method, processed tissues were placed in xylene I for 10 minutes, then in xylene II for 10 minutes, then dipped in alcohol I ten times, then dipped in alcohol II ten times, then run under tap water 10 times. Tissues were then placed in Harris haematoxylin stain for 10 min, then dipped in tap water 10 times, then dipped in 1% acid alcohol ten times, then rinsed in tap water by dipping ten times. The tissues were blued by dipping in Scot's tap water 10 times, then dipped in tap water 10 times to wash, then counterstained with 1% aqueous eosin for 5 min, then dehydrated in alcohol I by dipping 10 times, then dipped in alcohol II ten times, then dipped in alcohol III 10 times. Tissues were then cleared in xylene I by dipping 10 times, then dipping in xylene II ten times. Tissues were mounted with DPX chemical, and DPX allowed to dry before examining under the microscope. Results showed tissue with blue nuclei and pink cytoplasm.

In demonstrating *H. pylori*, the Giemsa stain was used as follows. Tissues were placed in xylene I for 10 min, then in xylene II for 10 min, then dipped in alcohol I ten times, then dipped in alcohol II 10 times, then dipped in tap water 10 times

before rinsing in distilled water. Sections were flooded with 1% filtered aqueous Giemsa stain for 10 min, and then rinsed with tap water. Sections were then drained and air dried for 10 min, then cleared in xylene. Tissues were mounted in DPX and the DPX allowed to dry before examining for *H. pylori* under the microscope. Blue rods were expected to be seen if *H. pylori* was present (Bancroft and Stevens, 1982).

3.4.4 Allocation of treatment (Sampling method)

150 *H. pylori* positive consenting patients were given a computer generated randomized number allocating them to a treatment group. The allocation number was concealed in an opaque envelope, which contained a number that would correspond to the numbered blister packs. The envelope was opened when the patient met the inclusion criteria and provided informed consent.

Patients were blinded to treatment groups and were randomly allocated to receive a 14-day regimen. One group received one week of 20 mg esomeprazole, 1 g amoxicillin and 500mg of clarithromycin, twice a day (EAC1). Patients in the EAC1 group were treated for an additional week with esomeprazole 20 mg, twice daily and amoxicillin and clarithromycin placebos twice daily. Another group (EAC2) received esomeprazole 20 mg, 1g amoxicillin, and 500 mg clarithromycin, twice daily for two weeks.

Note: For those who did not meet the above criteria or did not wish to be part of the study, they would continue to be seen by their attending doctor at the gastroenterology clinic, and treated as usual.

3.4.5 Follow up of patients

Patients were seen at the clinic on day 5 or day 6 of treatment for collection of information, in the form of clinical interview, on dyspeptic symptoms, and possible treatment related adverse events. Contact information was given on the data collection forms. Patients came for review at KEMRI on day 15 or day 16 to assess resolution of presenting symptoms and drug related adverse effects. This information was recorded on the provided follow up forms (Appendix 3). Patients were seen on day 42 for evaluation of clinical symptoms and issuance of stool poly pot for stool antigen testing. Data collection forms were important in order to make sure patients were within inclusion and exclusion criteria, to keep data and to follow up the patients. Forms for side effects were given so the patient could document any side effects felt on each day for monitoring purposes, and it would be easier for the patient to remember (Appendix 2). Participants were informed of these forms in the consent form (Appendix 4/5).

3.4.6 Stool antigen testing procedure

Stool samples were collected in plastic polypot containers, carried in a cooler if necessary, transported to the lab and stored at -20˚C to -80˚C, until tested. Those

unable to give stool at site were allowed to go home with the polypot and bring stool within 24 h (antigen viability). Those with watery stools were excluded. The test used the enzyme-linked immunosorbent assay technique (ELISA) to detect *H. pylori* antigen. This test involved the following: - antibody to *H. pylori* was already fixed to the wells of a microtitration plate which came with the stool antigen kit. Soluble microbial antigen (in stool) bound to this antibody. Washing was done to remove excess stool antigen. Antibody conjugated to an enzyme for example horseradish peroxidase was added. Conjugated antibody bound to the antibody-antigen complex. Washing was done to remove excess conjugated antigen. A chromogenic substrate for example hydrogen peroxidase joined to an indicator was added. The enzyme in the conjugated antibody hydrolyzed the substrate, producing a color reaction. Color was read visually or spectrophotometrically. This was done using a built-in control, which came with the kit, for quality control.

The infection was considered to be successfully eradicated if results were negative. Those who were positive after treatment were given treatment for another week and stool antigen test was repeated after 4 weeks.

Those who had severe side effects from treatment, or diarrhea at the time of stool antigen testing were withdrawn from the study. Those who incurred side effects from the study regimen were asked to stop treatment for at least one week, and a repeat treatment was given for one week, unless they were allergic to the study

medication in which case alternatives were sought. Those who had a recurrence of symptoms after treatment were prescribed alternative treatment or advised to see a gastroenterologist.

Note: On follow up, those participants who defaulted or did not return for follow up were contacted through their contact addresses, or their relatives' contact addresses or phones. Adherence was defined as consumption of more than 90% of the prescribed drugs. Causality of side effects was assessed using temporal relationship of the symptom to the start of therapy. Any symptom beginning after treatment was assumed to be drug-related. All new symptoms and exacerbations of pre-existing symptoms were considered treatment-related and were included in the analysis.

3.5 Data Management

3.5.1. Data Storage

The data was entered and kept in a research workbook, computer Microsoft Word and Excel/Access software. A flash disc or diskette was used for back up. Hard copies, like data collection forms and consent forms were stored in files safely and privately.

3.5.2   Sample Size Determination

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) statistical package version 11.

The primary efficacy variable was the eradication/clearance rate of *H. pylori*. With 90% power, 52 patients were needed in each group. This sample size was calculated using normal approximation to the binomial distribution at a significance level of 0.05 (Fisher, 1922). Allowing for a 10 % dropout rate, 114 patients were required to be enrolled. Using the formula for sample size to compare two proportions the minimum sample size was determined as follows (Casagrande *et al.* 1978):-

$$n = [Z_{1-\alpha/2} \sqrt{2P(1-P)} + Z_{1-\beta} \sqrt{[P_1(1-P_1) + P_2(1-P_2)]}]^2 / (P_1-P_2)^2$$

$$P = (P_1 + P_2)/2$$

n = sample size for one arm

α = significance level

$1-\beta$ = power of the study

At 95% confidence $Z_{1-\alpha/2}$ = 1.96 for α=0.05

At 90% power $Z_{1-\beta}$ = 1.28 for β=0.10

P_1= Assumed clearance rate for group 1(50%)

P_2= Clearance rate for group 2 (80%)

n = 52 (Sample size for one group)

Sample size for two groups = 104

The differences between the proportion of the eradicated infections and the 95% confidence intervals for the two treatments, was calculated. The level of significance was assessed by using the Fisher exact test (Fisher, 1954). For all the other variables, the chi-square and t-test were used as appropriate and P values less than 0.05 were considered significant.

Data from all randomized patients who had taken all study medication underwent statistical analysis. Data from those patients who had taken at least 75% of the study medication, except those who were lost to follow-up and those with major protocol violations that could have influence on treatment outcome also underwent statistical analysis. Protocol violations included using disallowed drug during the study, if assessment of *H. pylori* status was performed too early, or if *H. pylori* status was unknown due to missing data.

A chi-square test or Fisher's exact test, as appropriate were performed to compare demographic characteristics and eradication rates between treatment groups. The P value of < 0.05 was considered significant.

3.6 Ethical considerations

The study was performed according to good clinical practices, good laboratory practices and followed the Declaration of Helsinki (Ethics Forum, 2002). Permission to carry out the study was obtained from the graduate school, JKUAT, KEMRI Scientific Committee and Ethics Review Committee. Patients were enrolled into the study only after voluntary informed consent (Appendix 4, 5). All information about the patients was handled with utmost confidentiality and only used for intended purposes. There were no risks to patients for in addition to routine endoscopy the patient provided only a stool sample. The patients got results for all the tests undertaken. Subject confidentiality was kept and data coded and kept safely.

Participants, (that is, adults) with dyspepsia, who were *H. pylori* positive, were asked to give written informed consent. Gastroenterologists, hospital and lab staff followed normal hospital procedure for performing of endoscopy, rapid urease testing and stool testing for *H. pylori* antigens. In the study, any procedures outside normal hospital procedures were not performed. Nevertheless, informed consent was sought for these procedures.

The possible risks to the participants included discomfort and retching during the endoscopy procedure could occur. The endoscopy tube could be uncomfortable when swallowed into the mouth. However, this feeling was reduced by giving of

pre-operative local anesthesia with anticholinergic drugs. Biopsies taken did not harm the patient except for minimal risk of bleeding from biopsy sites. Participants could have side effects from the antibiotic drug regimen for *H. pylori* eradication. Participants directly benefitted by knowing their *H. pylori* status and getting treatment for the same free of charge.

Data on duration of treatment helped to establish the appropriate duration for use of antibiotics in the triple therapy regimen and efficacy of the drug regimens in the eradication of *H. pylori*. This was important in reducing relapses of *H. pylori* infection and to slow down antimicrobial resistance.

Informed consent of the participants was sought before endoscopy to allow assessment of their *H. pylori* status through rapid urease testing. In addition, consent was sought to use histology samples and stool samples taken from the patient, for the study and for possible further research at KEMRI.

All participants' specimens were identified by codes. Data did not contain names of individual subjects. No subject was identifiable by name in the report or publication of the results of the study.

The study did not involve use of vertebrate animals. Laboratory tests were done at the Immunology lab at Kenyatta National Hospital. All materials, reagents and apparatus were maintained and calibrated according to their standard operating procedures. The samples and tests done were documented and recorded promptly

and accurately. Any change in raw data was made without obliterating the previous entry and/or countersigned. All materials and documents in the study were retained and stored in secure archives.

3.7 Outcomes and application

The primary outcome of the study was eradication of *H. pylori* infection.

Secondary outcomes were to establish prevalence of *H. pylori* infection in patients undergoing endoscopy, to determine efficacy of drug regimen, and to determine the frequency of self-reported side effects. These outcomes could be applied in the Ministry of Health guidelines for treatment of *H. pylori* and be of use to general physicians alike.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic characteristics of patients

From a total of six hundred and two patients who came for endoscopy and fit criteria for *H. pylori* infection screening, four hundred and seventy nine patients were interviewed and examined using the rapid urease test of which two hundred and fifty three patients tested negative for *H. pylori* and two hundred and twenty six patients tested positive for *H. pylori*. One hundred and twenty three patients did not undergo the rapid urease test for various reasons, mainly because they had esophageal varices or were suspected for gastric cancer. The prevalence rate of *H. pylori* infection therefore was 47%.

A total of 150 patients with mean age 38.7 ± 15.1 years ranging from 18 to 85 who were *H. pylori* positive consented to be part of the study (**Figure 4.1**). Among them 46.0% were males while 54.0% were females. Among the 150 interviewed patients 2.7% were aged less than 20 years, majority of them (58.7%) were between 20 to 39 years, 12.0% between 40 to 49 years, and 13.3% between 50 to 59 years, while 13.3% of the patients were aged 60 and above.
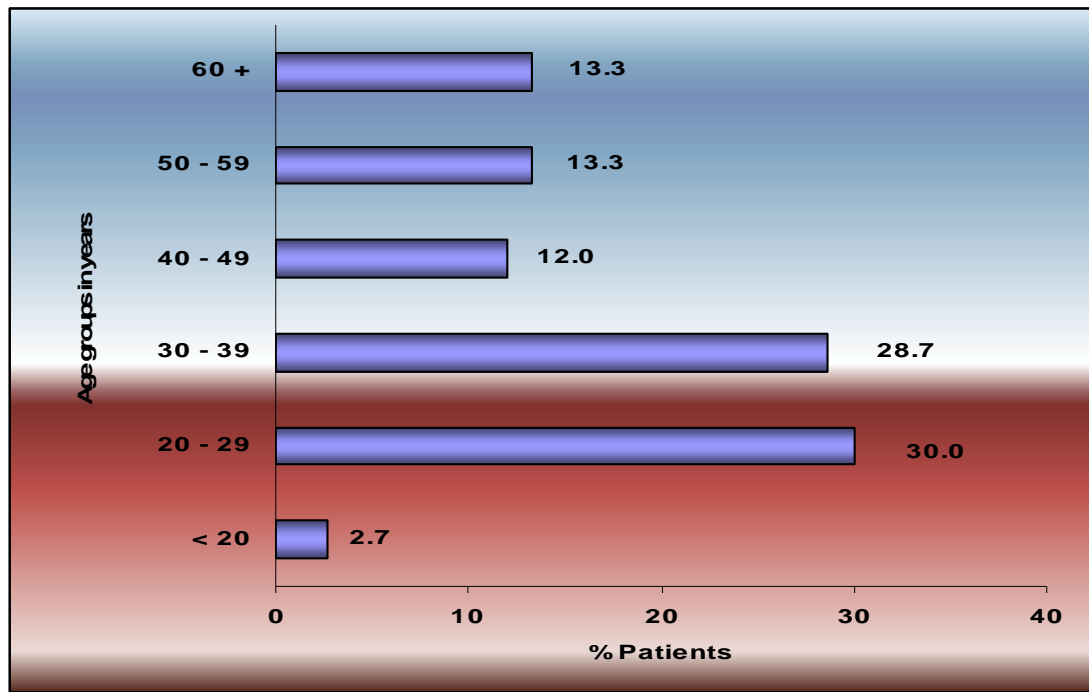
Figure 4.1: Distribution of study participants by age in years

4.1.1 Relationship between selected demographic characteristics and treatment groups

50.7% of the patients were put on drug regimen EAC 1 (esomeprazole, amoxicillin, clarithromycin given twice daily for one week followed by esomeprazole and placebo for one week) treatment while 49.3% were put on EAC 2 (esomeprazole, amoxicillin, clarithromycin given twice daily for two weeks) treatment.

Analysis of selected demographic characteristics by treatment groups revealed that there was baseline equivalence between the two treatment groups. None of the characteristics was significantly associated with the treatment groups ($P > 0.05$).

Distribution of each of the characteristics was fairly similar in both treatment arms. This was an important finding since the characteristics could hardly influence the outcome of the results after intervention. **Table 4.1** shows distribution of selected demographic characteristics on each treatment group.

Table 4.1: Distribution of selected demographic characteristics by treatment groups

Variables/ Categories	EAC 1 n=76	%	EAC 2 n=74	%	P value
Sex					
Males	34	44.7	35	47.3	0.753
Females	42	55.3	39	52.7	
Age					
< 20	1	1.3	3	4.1	
20 – 29	21	27.6	24	32.4	
30 – 39	24	31.6	19	25.7	0.121
40 – 49	5	6.6	13	17.6	
50 – 59	11	14.5	9	12.2	
60 +	14	18.4	6	8.1	

n = number of cohorts

Analysis of age between the treatment groups showed comparable results. **Table 4.2** shows mean summary of the analysis and demonstrates median age comparison between the two groups. Mean age between the treatment groups was not statistically different (P= 0.124). Mean age for patients in group EAC 1 was 40.6 years compared to 36.9 years for those in group EAC 2.

Table 4.2: Mean age of patients by treatment groups

Treatment groups	n	Mean	SD	Median	Minimum	Maximum
EAC 1	76	40.6	16.1	35	19	85
EAC 2	74	36.9	13.9	34.5	18	85
Total	150	38.8	15.1	35	18	85

4.1.2 Number of withdrawn patients and those patients lost to follow up

Treatment in both groups was administered for two weeks. By the end of the study 4.0% of the recruited patients withdrew from the study and another 21.3% were lost to follow-up amounting to 25.3% attrition **Figure 4.2. Figure 4.2** shows the study flow chart for patients recruited for the study.

```
┌─────────────────────────────┐
│ 150 Patients with H. pylori │
│ enrolled for the study      │
└─────────────────────────────┘
              │
              │        ┌──────────────────────┐
              ├────────│ 6 Patients withdrew  │──────▶ Exit
              │        │ from the study       │
              │        └──────────────────────┘
              │
              │        ┌──────────────────────┐
              ├────────│ 32 Patients lost to  │──────▶ Exit
              │        │ follow-up            │
              │        └──────────────────────┘
              │
┌─────────────────────────────┐
│ 112 Patients followed to the│
│ end of the study            │
└─────────────────────────────┘
```
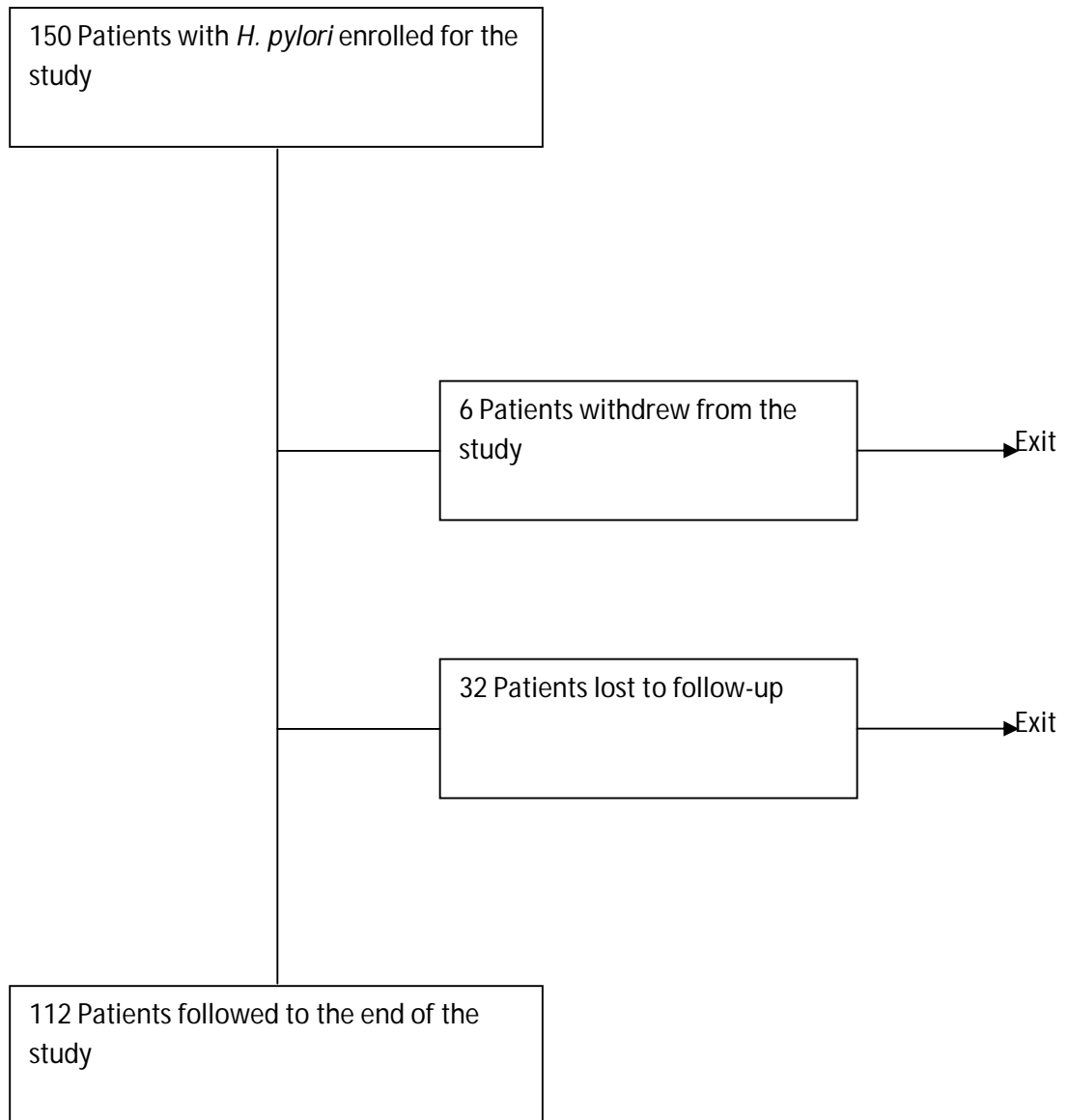
Figure 4.2: **Flow chart for patients recruited for study**

The study took a total of one year and three months up to the end of follow up of all the patients. Four patients withdrew because of allergic reactions to the medicine and two patients withdrew because they were unable to complete treatment as they were to go for surgery for other conditions. Thirty two patients were lost to follow

up mainly because of loss of contact, inability to come back for follow up for example travel upcountry or abroad, patient's own decision not to continue with follow up, among other reasons.

4.2 Distribution of patients with duodenal ulcers on endoscopy

Among the 150 study participants, results on endoscopy were available for 104 patients (**Figure 4.3**). Seventeen patients (16.3%) were diagnosed to have duodenal ulcers. There was no significant difference in distribution of duodenum ulcer between the two groups (P=0.996). 16.4% of the patients on EAC 2 experienced duodenal ulcers compared to 16.3% of those that received EAC 1.
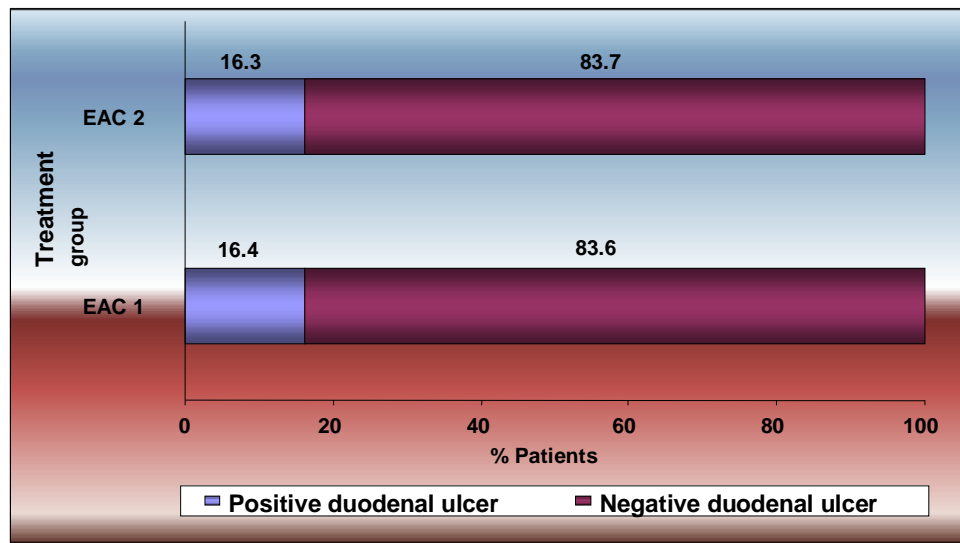
Figure 4.3: Percentage of patients with and without duodenal ulcer compared to treatment group

4.3 Presence of *H. pylori* at the end of the study

At the end of the study, presence of *H. pylori* was determined. Among 112 patients that were followed successfully, 40.2% were still positive for *H. pylori*; an eradication rate of 59.8%

Association between presence of *H. pylori* and gender was not statistically significant (OR= 1.37, 95% C.I= 0.64 – 2.92, P=0.419). However a higher percentage of females (43.9%) tested positive for *H. pylori* compared to 36.4% of the males at the end of the study (**Table 4.3**).

Table 4.3: Occurrence of *Helicobacter pylori* in relation to gender at the end of the study

	Males		Females		P value
H. pylori status	n	%	n	%	
H. pylori present	20	36.4	25	43.9	
H. pylori absent	35	63.6	32	56.1	0.419
Total	55	100	57	100	

Analysis of occurrence of *H. pylori* across the two treatment arms revealed some deviation (**Figure 4.4**). A patient on EAC 1 was 1.7 times more likely to become negative for *H. pylori* compared to one that was placed on EAC 2. However there

was no significant association between the occurrence of *H. pylori* and treatment groups (P=0.177, OR= 1.7, 95% C.I for OR= 0.8 – 3.6).
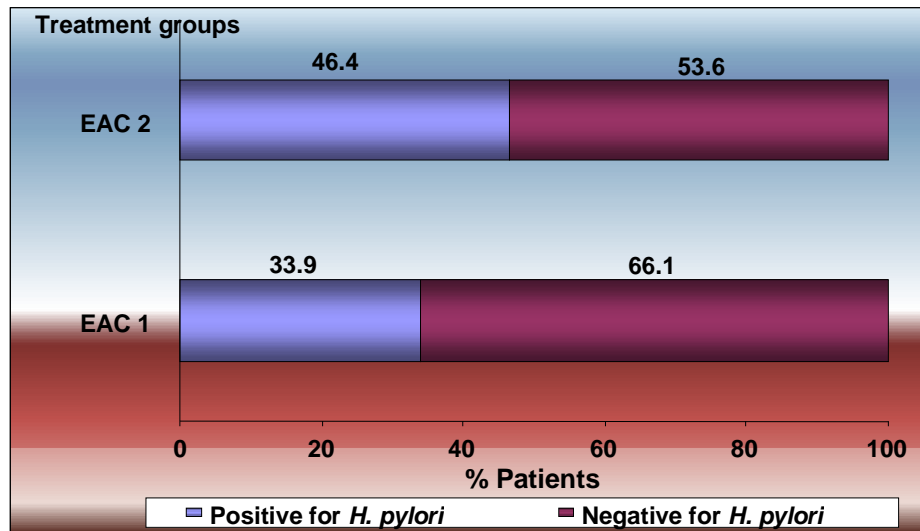
Figure 4.4: *Helicobacter pylori* **occurrence in relation to treatment group at the end of the study**

The relationship between *H. pylori* and age was not statistically significant (P=0.291). However, there was a gradual decrease in *H. pylori* with increase in age (**Figure 4.5**). Patients aged between 20 – 29 years had the highest occurrence of *H. pylori* (52.5%). The occurrence reduces to 39.2 in age 30 – 39 years, 36.4% in age 40 – 49 years, 33.3% in age 50 – 59 years to 11.1% in age 60 and above. Interesting, a slight deviation from the trend was observed among those aged less than 20 years.
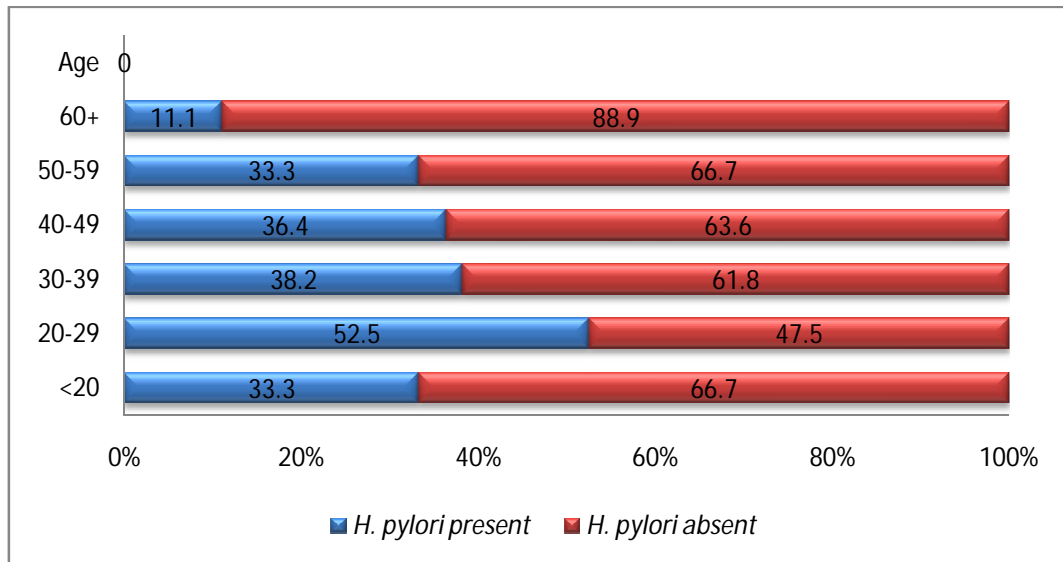
Figure 4.5: Occurrence of *Helicobacter pylori* by age at the end of the study

4.4 Drug side effects of the triple therapy regimen

Drug side effects occurred among 30.7% of the 150 patients enrolled in the study **Table 4.4**. The most occurring condition was abdominal pain reported by 14.7% of the patients, followed by headache (10.7%), taste disturbance (9.3%), Nausea & vomiting (8.0%), Diarrhea (4.7%), Constipation (4.0%), and Skin rash (2.7%). Eighteen patients (12.0%) reported other side effects.

Table 4.4: Reported side effects of the triple therapy regimen

Side effect	n = 150	%
Abdominal pain	22	14.7
Headache	16	10.7
Taste disturbances	14	9.3
Nausea and vomiting	12	8.0
Diarrhea	7	4.7
Constipation	6	4.0
Skin rash	4	2.7
Others*	18	12.0

NB: Others*- Included other side effects, for example, increased hunger, general body weakness or fatigue, backache and heartburn.

Drug side effect combination among the 30.7% reported cases varied considerably (**Table 4.5**). While 69.3% suffered no side effects, 12.0% suffered from only one side effect, 8.7% suffered from two, 4.7% from three, 4.0% from four while 1.3% suffered from five side effects.

Table 4.5: Reported triple therapy side effects that occurred singly and in combination

Drug side effect	n =150	%
No side effect	104	69.3
1 side effect	18	12.0
2 side effects	13	8.7
3 side effects	7	4.7
4 side effects	6	4.0
5 side effects	2	1.3

Occurrence of drug side effects across the two treatment arms revealed insignificant deviations (**Table 4.6**). There was no significant association between occurrence of a particular side effect and treatment groups (P> 0.05). Minimal distribution differences were observed some showing high proportions in EAC 1 and some in EAC 2.

Table 4.6: Reported triple therapy side effects compared to treatment group

Drug side effect	EAC 1 (n=76)		EAC 2 (n=74)		P value
	n	%	n	%	
Abdominal pain	10	13.2	12	16.2	0.597
Headache	9	11.8	7	9.5	0.636
Taste disturbances	8	10.5	6	8.1	0.611
Nausea and vomiting	6	7.9	6	8.1	0.962
Diarrhea	5	6.6	2	2.7	0.442
Constipation	3	3.9	3	4.1	0.973
Skin rash	1	1.3	3	4.1	0.363
Others	9	11.8	9	12.2	0.952

Analysis of drug side effects, singularly or in combination revealed no significant association with treatment arms (P=0.644) (**Figure 4.6**). 32.4% of the patients on EAC 1 suffered from one or multiple side effects compared to 28.9% of the patient on EAC 2.
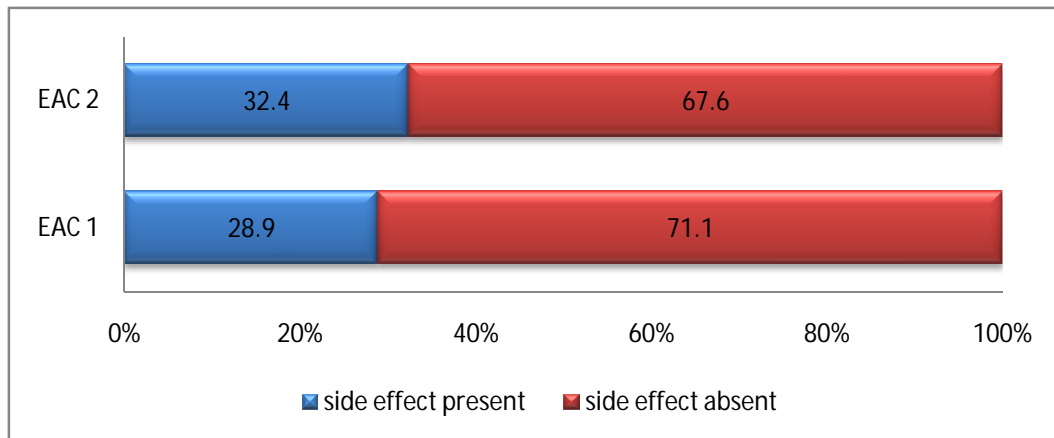
Figure 4.6: Triple therapy side effects in relation to treatment groups

Analysis of drug side effects, singularly or in combination revealed no significant association with occurrence of *H. pylori`* (P=0.786) (**Figure 4.7**). 38.5% of the patients with at least one side effect tested positive for *H. pylori* at the end of the study compared to 41.1% of those without any side effect.
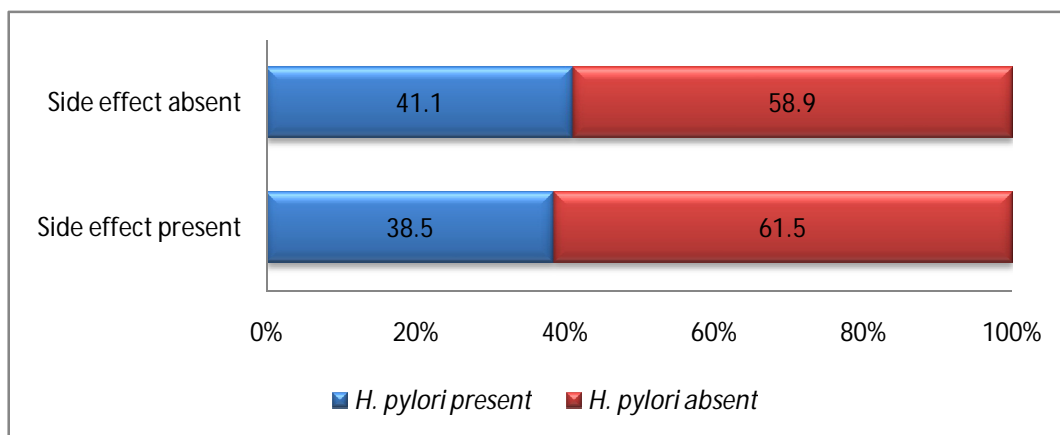
Figure 4.7: Drug side effect in relation to *Helicobacter pylori* presence

4.5 Recurrence of symptoms after *H. pylori* treatment

Among 118 patients whose data on recurrence of symptoms was available, 43.2% reported to experience recurrence of symptoms while 56.8% did not experience any recurrence of symptoms. The most occurring symptom was abdominal pain reported by 26.7% of the patients. The rest were abdominal bloating reported by 8.7%, and belching reported by 1.3% of the patient (**Table 4.7**).

Table 4.7: Recurrence of symptoms as reported by the study participants

Symptoms	n	%
Abdominal pain	40	26.7
Abdominal bloating	13	8.7
Belching	2	1.3

Distribution of those who experienced recurrence of symptoms was not statistically different between the two treatment groups (P=0.327) (**Figure 4.8**). Among those who received treatment EAC 1, 38.6% experienced recurrence of symptoms as compared to 47.5% of those that received EAC 2 treatment.
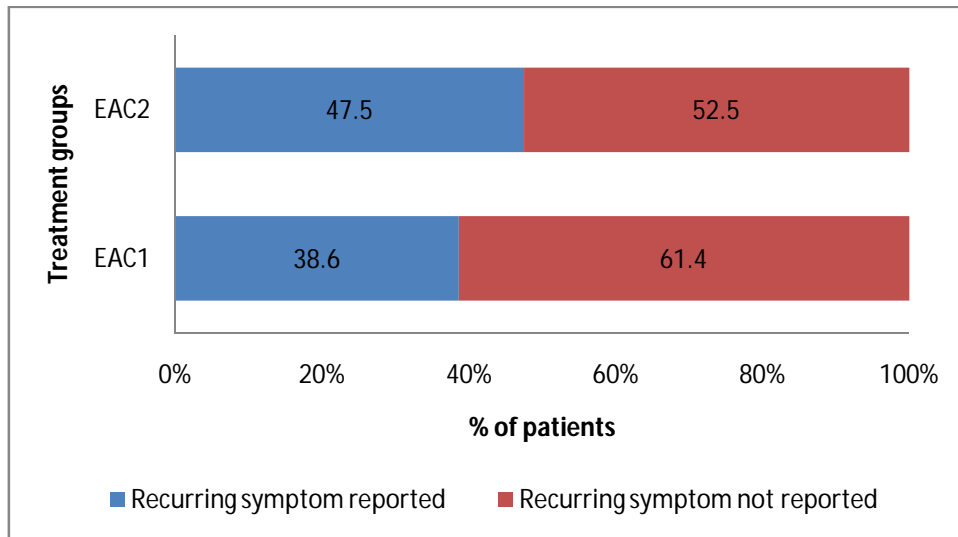
Figure 4.8: Recurrence of symptoms in relation to treatment group

Relationship between recurring symptoms and presence of *H. pylori* at the end of the study was statistically significant (OR=2.3, 95% CI= 1.1 – 5.1, P= 0.031) (**Figure 4.9**). Majority of the patients who reported recurrence of symptoms (52.2%) tested positive for *H. pylori* compared to 31.8% of those that did not report any form of recurrence of symptoms. An analysis of this outcome revealed that, a patient that reports a form of recurring symptom is 2.3 times more likely to have *H. pylori* compared to one that does not report any form of recurring symptoms.
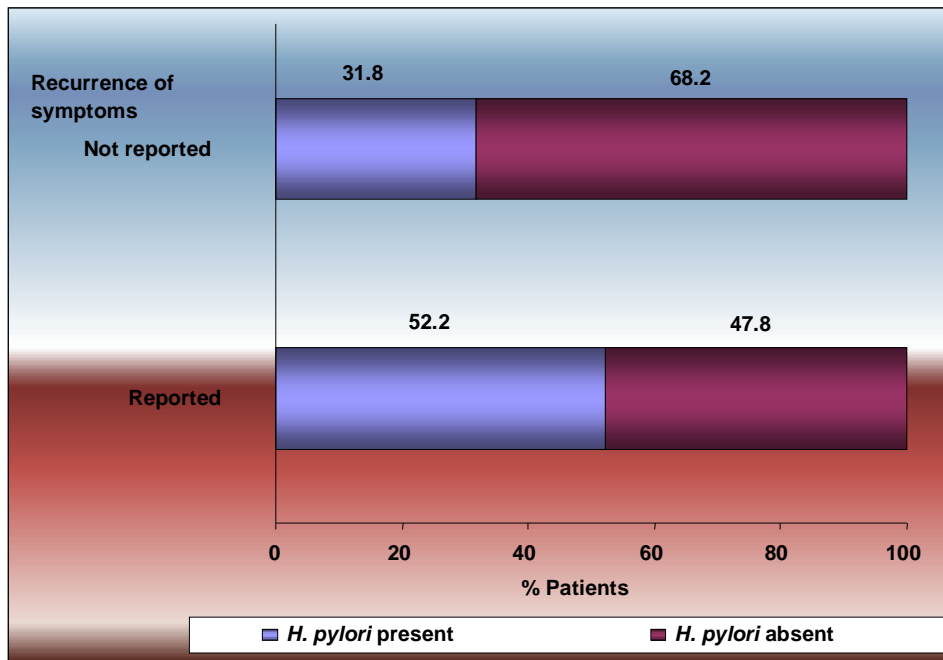
Figure 4.9: Presence of *Helicobacter pylori* in relation to recurrence of symptoms

4.6 Presence of *H. pylori* in patients with duodenal ulcer after treatment

After treatment, 54.5% of the patients who experienced duodenum ulcer tested positive for *H. pylori* as compared to 36.9% of those that were negative for duodenal ulcer. There was no significant difference in relationship between duodenal ulcer and *H. pylori* presence (P=0.326) (**Figure 4.10**).
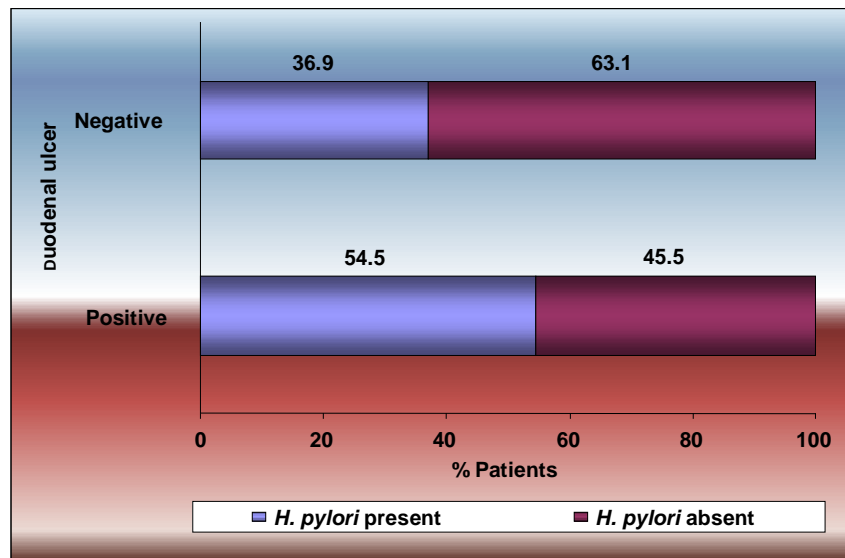
Figure 4.10: Presence of *Helicobacter pylori* after treatment in patients with and without duodenal ulcer

4.7 Multivariate analysis

Binary logistic regression was used to model non-clearance of *H. pylori* pathogens using seven variables, namely; Age (Continuous), Sex, Alcohol use, Smoking, Side effects, Recurrence of symptoms and Duodenal ulcer.

The resulting outcome model is shown in **Table 4.8**. Two predictor factors to non-clearance of *H. pylori* were identified that is age, and recurrence of symptoms.

Table 4.8: Logistic regression predicting non-clearance of *Helicobacter pylori*, age and recurrence of symptoms

Variables	ß	S.E. (ß)	Adjusted OR (95% C. I)	P value
Age	-0.05	0.02	0.95 (0.91 – 0.99)	0.022
Recurrence of symptoms	1.20	0.51	3.31 (1.21 – 9.05)	0.020

Table 4.8 shows beta coefficient (β), standard error of beta (S.E. (β)), adjusted odds ratio and P value for each of the factors significantly associated with non-clearance of *H. pylori* infection. Adjusting for other factors, age and recurrence of symptoms were significantly associated with non-clearance of *H. pylori* by the time the study was concluded.

There was significant association between occurrence of *H. pylori* and age (OR=0.95 (0.91 – 0.99), P=0.022). For every unit increase in age, an individual is 5% less likely to test positive for *H. pylori*. The older the individual the less likely there are to test positive for *H. pylori*. Similarly, the relationship between recurrence of symptoms and occurrence of *H. pylori* was statistically significant (OR=3.31 (1.21 – 9.05), P=0.020). An individual showing recurring symptoms was 3.31 times more likely to test positive for *H. pylori* compared to one without recurrence of symptoms.

CHAPTER FIVE

5.0 DISCUSSION

5.1 The prevalence of *H. pylori* before treatment

Of the four hundred and seventy nine patients interviewed, two hundred and twenty six patients tested positive for *H. pylori*. This study therefore found a prevalence of 47% on rapid urease testing following endoscopy before treatment in symptomatic patients who mainly suffered gastritis, duodenitis or peptic ulcer disease. A study in Kenya in 2005 found *H. pylori* positivity in 69% of patients with dyspepsia using the rapid urease test after endoscopy was done (Lwai-Lume *et al.*, 2005). The prevalence of *H. pylori* among endoscopy patients in Nigeria was as high as 78.5% (Mustapha *et al.*, 2007). The prevalence of *H. pylori* done in another study in Kenya (Shmuely *et al.*, 2003) found that there was a 51% prevalence of *H. pylori* in asymptomatic patients and 71% prevalence in symptomatic patients after *H. pylori* blood antibody testing. This prevalence however related to the general population using a different method of testing. The low percentage prevalence may be due to the increased use of antibiotics and PPIs as treatment for gastritis by clinicians in Kenya, or the method of testing for prevalence of *H. pylori*. However, the rapid urease test, which was what was used in this study, has been established as an accurate and inexpensive means of diagnosis of *H. pylori* in patients undergoing endoscopy before treatment, with its

reliability diminishing post-treatment especially after use of PPI therapy (Chey *et al.* 2007).

Prevalence of *H. pylori* in relation to age and gender at the beginning of the study was not significant (P>0.05). Findings in this study show there was a higher prevalence in the 20 - 29 age-bracket, of 30% which was slightly higher than in Singapore (Chong *et al.*, 2008) where the results of *H. pylori* prevalence in endoscopy patients was 26.9%, highest in the 30-39 age-bracket, and the percentages were higher in those with dyspepsia and peptic ulcer disease. Reasons for this difference in prevalence in certain age groups in the study were not completely known though it was postulated that lower prevalence may be due to improved living and hygienic conditions.

Prevalence affects the diagnosis of *H. pylori*. The *H. pylori* testing and treatment approach is likely to be more beneficial than other strategies in infected patients, and the impact of this strategy is likely to be small if the infection is not very prevalent (Talley *et al.*, 2005). In addition, cost-effectiveness studies suggest that a choice of noninvasive testing should be based on the prevalence of infection in the community. In intermediate prevalence situations, the stool antigen test or urea breath test dominate (Rhew *et al.*, 2000). The higher costs of these tests are offset by their accuracy. Accordingly, it is clear that determining the prevalence of *H. pylori* infection in the community is fundamental to deciding the most cost-effective strategy for managing patients with uninvestigated dyspepsia. The urea

breath test is not widely available in Kenya, but the stool antigen test is available. Both tests require discontinuation of PPI for two weeks because the drugs used inhibit urease, leading to false negative results (Gatta *et al.*, 2004). With regard to patient follow-up after *H. pylori* eradication, urea breath test remains the preferred test. If unavailable, a laboratory-based stool test – preferably using monoclonal antibodies – could be used. The timing of this follow-up should be at least four weeks after the end of the eradication treatment (Malfertheiner *et al.*, 2007).

5.2 *H. pylori* presence before treatment in relation to duodenal ulcer

Seventeen out of 104 patients (16.3%) were diagnosed to have duodenal ulcers. There was no significant difference in distribution of duodenal ulcer between the two groups (P=0.996). 16.4% of the patients on EAC 2 experienced duodenal ulcers compared to 16.3% of those that received EAC 1. However, there was no significant difference in relationship between duodenal ulcer and *H. pylori* presence (P=0.326). In previous Kenyan studies, duodenal ulcer following upper gastrointestinal endoscopy was 29.2%, and this was more prevalent in the younger age group that is those less than 50 years old, and all of them had *H. pylori* infection (Ogutu *et al.*, 1998). In another study duodenal ulcer was 7.8% of all cases who came for upper gastrointestinal endoscopy (Lodenyo *et al.*, 2005). Duodenal ulcer is frequently found in patients with dyspeptic symptoms and it is important to rule out *H. pylori* infection on endoscopy usually through rapid urease

testing. It is also important to establish through endoscopy the presence of peptic ulcers as this will influence treatment and follow up.

54.5% of patients who had duodenal ulcers found on endoscopy prior to treatment were also more likely to have a recurrence of *H. pylori* infection after treatment compared to 36.9% of patients who did not have duodenal ulcers. This may mean that those who had persistent *H. pylori* after treatment and had duodenal ulcer were more likely also to have a persistence of their duodenal ulcer. *H. pylori* has been shown to contribute to development of duodenal ulcers (Olbe *et al.*, 2000). A Kenyan study on endoscopic patients with gastritis, found that 27% of patients had duodenal ulcers compared to 2% with gastric ulcers although their relation with *H. pylori* despite being present was not significant (Kalebi *et al.*, 2007). Though there was no statistical significance, the results show that the most common cause of gastritis and peptic ulcer disease is *H. pylori* infection. In conclusion there is a need to make sure that *H. pylori* is eradicated in patients with duodenal ulcers, as persistence causes recurrence of duodenal ulcers and its complications for example peptic ulcer bleeding (Laine *et al.*, 1998). Hence the importance of the test and treat strategy especially for patients with duodenal ulcers.

5.3 Prevalence of *H. pylori* in relation to age and gender after treatment

Prevalence of *H. pylori* after treatment was less in males at 36.4% as compared to 43.9% in females. There was no significant association between *H. pylori* presence

and gender of the patient (P=0.419). Most studies report no difference between the genders in *H. pylori* prevalence (Javed *et al.*, 2010). A study of United States military recruits did not find gender to be a significant predictor of seropositivity (Evans and Brachman, 1998). Some studies have shown a moderately elevated risk of *H. pylori* infection and its related diseases among men (Evans and Brachman, 1998). However, this association has not been observed worldwide.

There was a significant association between *H. pylori* presence and age on logistic regression (P<0.05). For every unit increase in age, an individual was 5% less likely to test positive for *H. pylori*. The older the individual the less likely they were to test positive for *H. pylori*. *H. pylori* was seen to be at its peak in patients aged 20 to 29 years which then decreased with age. *H. pylori* infection is known to be acquired in childhood with most young adults having *H. pylori* especially in the developing countries. In another study done in Kenya, the prevalence of *H. pylori* infection in the adult segment was 54.8%; and 73% in children which shows a higher prevalence in children as compared to adults (Kimang'a *et al.*, 2010). The results differed slightly with other studies where prevalence usually increases with age (Hussein, 2010).

Prevalence of *H. pylori* infection increases with age in all population groups. An exception to this is seen in the advanced elderly, particularly in developing countries. Serosurveys suggest that the very old may have a lower prevalence of *H. pylori* than persons more junior (Evans and Brachman, 1998). It has been

speculated that this is due to advanced pre-neoplastic lesions that occur with older age. Since *H. pylori* can only survive adjacent to gastric epithelium replacement of gastric mucosa by metaplastic intestinal epithelium might result in loss of infection (Evans and Brachman, 1998).

5.4: Eradication of *H. pylori*

Eradication rates were measured by finding out the number of patients who had been cured after treatment with both drug regimens by testing for *H. pylori* through the stool antigen test which is non-invasive but is still recommended for testing of *H. pylori* post-treatment (Malfertheiner *et al.*, 2007). *H. pylori* presence at the end of the study after stool antigen testing was 40.2% of the 112 patients who completed the study that is a clearance rate of 59.8%, 36.4% of those still with *H. pylori* being males and 43.9% being females. The study compared two drug regimens that is one week triple therapy of esomeprazole, amoxicillin and clarithromycin, followed by one week of placebo (EAC1), and two weeks of triple therapy on the same combination of drugs (EAC2). The eradication rate for EAC1 was 66.1% compared to EAC2 which was 53.6% which was not statistically significant. This was not the expected outcome. Bivariate and multivariate analysis did not show any specific association for this result. It was expected that the eradication rates would be higher and the longer the duration of treatment the better the cure rate.

Many studies have investigated *H. pylori* eradication through various meta-analyses. There is no doubt that triple therapy that is a PPI and two antibiotics (amoxicillin associated with clarithromycin or metronidazole) is the most preferred first line therapy regimen in clinical practice (Malfertheiner *et al.*, 2007). Drug regimens for *H. pylori* have changed with time all in an effort to increase efficacy of eradicating *H. pylori*. In an attempt to improve cure rates of the triple therapy regimen it was assumed that increasing its duration from one to two weeks would yield better results. However, results in efficacy of the two week triple therapy vary worldwide. *H. pylori* eradication rates following triple therapy are decreasing substantially in several countries (Lee *et al.*, 1999), with eradication rates as low as 25% in a recent study (Altintas *et al.*, 2004).

Variance may be due to bacterial virulence, environmental factors and antibacterial resistance, peculiar for each country. Other individual factors for example patient's age, presence of duodenal ulcer and treatment duration have been linked to therapy efficacy (Broutet *et al.*, 2003).

In this study, low eradication rates could have been due to compliance issues maybe because of length of time or side effects of medicine, although side effects were noted in both regimens and did not affect completion of treatment. Patient adherence was also self-reported and despite pill count, adherence would be better regulated if patients were in an in-patient setting where one could directly observe the patient. However, the major reasons may be antibacterial resistance or other

environmental factors which were not included in the study. Bacterial eradication following a failed initial standard triple therapy is notoriously difficult to achieve (Huang and Hunt, 1999). A seven day triple therapy has had disappointing cure rates especially in patients harbouring a high bacterial density (Scaccianoce *et al.*, 2006). New approaches to curing *H. pylori* for example the simple attempt to increase duration of triple therapy has been conflicting with some cases reporting success and others giving disappointing results (De Francesco *et al.*, 2004)

Some studies have shown a failure of two week triple therapy in terms of lower eradication rates than usually reported but usually slightly higher or equal to one week triple therapy eradication rates (Gumurdulu *et al.*, 2004). If several 14 day therapy regimens have failed to significantly increase *H. pylori* eradication rate, this shows that triple therapy failure is due to an intrinsic weakness of such a schedule rather than duration of therapy (Scaccianoce *et al.*, 2006). Low eradication rates suggest that antibiotic resistance or the genetic difference of the micro-organism might be in effect (Altintas *et al.*, 2004). A recent study in Kenya showed no *H. pylori* resistance to clarithromycin or amoxicillin (Kimang'a *et al.*, 2010). Older studies in Kenya showed that susceptibility of the *H. pylori* isolates was 93.6% for clarithromycin and 95.4% for amoxicillin (Lwai-Lume *et al.*, 2005). Recently, virulence factors of *H. pylori*, such as *cagA* and *vacA*, are reported to be major factors determining the cure rates. Individuals infected with strains with *cagA*-negative and *vacA s2* genotypes have significantly increased risk of

eradication failure of *H. pylori* infection (Sugimoto and Yamaoka, 2009). Further studies locally are required to verify these suggestions.

5.5 *H. pylori* infection persistence after treatment

There was no significant association between treatment group and return of symptoms (P=0.327). Presence of *H. pylori* after treatment had a positive association with recurrence of symptoms (P=0.031). There was also a significant association between *H. pylori* presence and recurrence of symptoms on logistic regression (P<0.05). Those who had symptoms of *H. pylori* infection, for example abdominal bloating, pain or belching after treatment, were 2.3 times more likely to have a relapse of *H. pylori* infection. This was more in the EAC2 regimen, which could indicate an issue with compliance or drug resistance.

Not many studies have been done assessing recurrence of symptoms with the different treatment groups, though recurrence of symptoms has been mentioned in certain studies (McColl, 2010). Symptoms related to *H. pylori* may also be found in patients with non-ulcer dyspepsia who may not have *H. pylori* infection hence confusing the picture even more but also making it important to distinguish if *H. pylori* is indeed a cause. The possibility that symptoms may be due to a different cause (e.g., biliary tract, pancreatic, musculoskeletal, or cardiac disease or psychosocial stress) should routinely be considered. If another course of therapy is administered to eradicate *H. pylori* infection, the importance of adherence to the

treatment regimen should be emphasized, since poor adherence may underlie the failure of initial therapy (McColl, 2010).

Recurrence of symptoms may be due to antibiotic resistance or patient non-compliance (Vakil, 2005). Hence the need to test the patient accordingly for *H. pylori* and offer appropriate treatment and considering use of endoscopy to rule out *H. pylori* resistance to certain antibiotics. Clarithromycin resistance is not as common as metronidazole. Primary amoxicillin resistance is very rare (Poon *et al.*, 2002). Successful eradication of *H. pylori* is affected more by the presence of resistance to clarithromycin than to metronidazole (Hsu *et al.*, 2001). In studies, dual therapy with the combination of PPI and clarithromycin showed higher resistance than with triple therapy using PPI and clarithromycin and amoxicillin, suggesting these regimens containing amoxicillin may prevent selection of secondary clarithromycin resistance (Laine *et al.*, 2000). The prevalence of clarithromycin resistant *H. pylori* is low but appears to be increasing. Point mutations in the 23s rRNA gene are responsible for the resistance. Because of this eradication rates may be reduced and treatment failure rates may rise (Hua-Xiang *et al.*, 1999)

5.6 Side effects of the EAC drug regimen

There was no significant association between treatment group and side effects of triple therapy regimen (P=0.644). There was no significant association between *H.*

pylori presence and drug side effect either singularly or in combination (P=0.786). The most common side effects experienced in both regimens were abdominal pain at 14.7%, headache at 10.7% and taste disturbance at 9.3%. Other side effects were nausea and vomiting at 8%, diarrhoea at 4.7% and skin rash at 2.7%. Most patients were able to finish their medication despite these side effects. A Cochrane review reported that the most common adverse effects of therapy for *H. pylori* were diarrhea in 8%, altered taste in 7%, nausea and vomiting in 5%, skin rashes in 2%, headache in 4%, abdominal discomfort or pain in 5%, and stomatitis in 2.5% of patients treated for *H. pylori* (Ford *et al.*, 2006).

Up to 50% of patients have side effects while taking *H. pylori* treatment. Side effects are usually mild, and fewer than 10% of patients stop treatment because of side effects. For those who do experience side effects, it may be possible to make adjustments in the dose or timing of medication. Some of the treatment regimens use a medication called metronidazole or clarithromycin. These medications can cause a metallic taste in the mouth. Alcoholic beverages for example beer and wine should be avoided while taking metronidazole; the combination can cause skin flushing, headache, nausea, vomiting, sweating and a rapid heart rate. Bismuth, which is contained in some of the regimens, causes the stool to become black and may cause constipation. Many of the regimens used cause diarrhea and stomach cramps (Crowe *et al.*, 2011). The patient may need to be alerted on the side effects of the medicine in order to enhance compliance.

5.7 Limitations of the study

The study had limitations. Patients were referred for endoscopy and may not be representative of patients with dyspepsia seen in primary care settings. In addition, the sample size was modest, which may result in relatively wide confidence intervals.

Environmental factors and antibacterial resistance was not included in the data and hence I was not able to study these associations with *H. pylori* prevalence. Antibiotic resistance may have played a role in the low eradication rates. Other limitations include the fact that compliance and adherence monitoring was based on self-reporting, and that I was unable to use the same test for testing for *H. pylori* that is either rapid urease test or stool antigen testing due to availability, financial and ethical reasons. However, both rapid urease testing and stool antigen testing are used in testing *H. pylori* with high sensitivity and specificity. Most of the findings are consistent with other studies with the prevalence being slightly lower, but this provides a guide for prevalence of active *H. pylori* infection following rapid urease testing (Lwai-Lume *et al.*, 2005, Ogutu *et al.*, 1998).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- The study showed that the prevalence of *H. pylori* in patients undergoing endoscopy is still relatively high at 47%, although prevalence has been higher in other studies.

- In Kenya, a 7 day triple therapy regimen is as effective as 14 day therapy in treatment of *H. pylori* with both being well tolerated with few side effects.

- The study showed that *H. pylori* infection peaked at the 20-29 age group then reduced with age. Prevalence of *H. pylori* usually increases with age throughout the age groups; therefore this result may indicate that with the increased use of triple therapy and PPIs this trend is changing or that other factors related to age may be reducing *H. pylori* density.

- Eradication rates for the triple therapy regimen were lower than those in other studies in Kenya and worldwide at 59.8%.

- Recurrence of symptoms for example abdominal bloating and heartburn, after treatment means you are more likely to have *H. pylori* infection. This may be due to re-infection or resistance of *H. pylori* to certain antibiotics and therefore the test and treat strategy remains relevant.

- Side effects of both the one week and two week triple therapy regimens were few and well tolerated. However, side effects anticipated should be explained to the patients to ensure they do not stop medication and enhance patient compliance.

- Compliance of the patient is a main factor determining success, and the seven day regimen is short and simple given twice daily with good cure rates.

In conclusion, there is no difference in efficacy between one week and two week triple therapy drug regimen, used for eradication of *H. pylori*.

6.2 Recommendations

Studies should continue to be done on both duration and new antibiotic regimens on the treatment of *H. pylori*. Prevalence and environmental factors in different areas of Kenya should be studied to determine overall prevalence and interactions with various environmental factors using a larger number of patients, and in a controlled in-patient hospital setting to establish adherence and compliance to medication which will provide a true picture of drug efficacy.

A seven day treatment regimen is still recommendable in our setting as it has a lower pill burden and is cost-effective, and is likely to be more tolerated because of the reduced duration of time for the patient to experience side effects.

If one has recurrence of symptoms after treatment for *H. pylori*, it should be recommended that they repeat testing for *H. pylori* in order to consider treatment or change of antibiotics.

It is important to isolate strains of *H. pylori* and determine their resistance to different antibiotics in the Kenyan setting. Considering the low eradication rates, and patterns of antibiotic resistance a treatment scheme specific to Kenya would be suitable, this could guide on treatment in future. This should be done yearly or after every two years, as is done in other developed countries which base their guidelines on such studies.

The uncertainty of patients' compliance and the increasing rate of antibiotic resistance to clarithromycin and metronidazole strongly support the need for confirming the eradication for all treated patients, given the availability of a simple and accurate non-invasive test that is stool antigen testing. Apart from stool antigen testing, another useful test is the rapid urease test, which should be recommended to gastroenterologists as a quick test to find out *H. pylori* infection when necessary during endoscopy. On the basis of this study, due to the prevalence, the choice of noninvasive test in Kenya should be either the urea breath test or stool antigen test, both of which have been shown to be accurate for the initial diagnosis of *H. pylori* infection and for confirming eradication (Talley *et al.*, 2005). This assures quick diagnosis and effective treatment of the patient.

Presence of duodenal ulcers should prompt for testing of *H. pylori* and if positive, treatment commenced for the same. Test and treatment strategy for *H. pylori* is relevant in our setting and considering the high prevalence and recurrence of *H. pylori*, the cost of testing and treatment should be reduced to make it more affordable to patients.

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APPENDICES

Appendix 1

Data Collection Form

Full Name_____   Initials _____

Age _____   Sex _____ Study No. _____

Symptoms _____
_____
_____

Duration of symptoms _____
_____

History of allergies (drug/food) _____
_____

History of medical illness/malignancy _____
_____

History of surgery/bleeding disorder _____
_____

History of medication use _____
_____

Last menstrual period/Last Delivery _____
_____

Parity and current history of contraception_____
_____

History of alcohol/smoking _____
_____

Endoscopic findings on day 0 _____
_____

Histology findings on day 0 _____
_____
_____

Rapid urease on day 0 _____
_____
_____

Stool antigen report on day 42 _____
_____
_____

Participant's Contact information:
Telephone (residence) _____
Telephone (work) _____
Address (residence) _____
Address (work) _____
Area of residence _____
Name of workplace _____

Participant's relatives (type of association) for example father
_____
Telephone (residence) _____
Telephone (work) _____
Address (residence) _____
Address (work) _____
Area of residence _____
Name of workplace _____

Appendix 2

Record of side effects from Day 1 to Day 14 (If any).

DAYS	SIDE EFFECTS
1	1)_____ 2)_____
2	1)_____ 2)_____
3	1)_____ 2)_____
4	1)_____ 2)_____
5	1)_____ 2)_____
6	1)_____ 2)_____
7	1)_____ 2)_____
8	1)_____ 2)_____
9	1)_____ 2)_____
10	1)_____ 2)_____
11	1)_____ 2)_____
12	1)_____ 2)_____
13	1)_____ 2)_____
14	1)_____ 2)_____

Appendix 3

Follow up form (day 6, day 15, day 42)

Tick day of follow up:
      Day 6 ___   Day 15 ___   Day 42 ___

1. Have you had any problem or side effects on taking of the drugs? If yes, of what nature?

   _____
   _____
   _____
   _____
   _____
   _____
   _____

2. How many pills have you taken so far? If not as expected, which days did you skip taking the pills and why?

   _____
   _____
   _____
   _____
   _____
   _____
   _____

3. Have you had any return of symptoms since you ceased taking the medication, 4 weeks ago? (for day 42)

   _____
   _____
   _____
   _____

4. Do you have any issue you would like to raise?

   _____
   _____
   _____
   _____
   _____

Appendix 4

CONSENT

Study Title: A comparative study to determine the efficacy of a triple therapy drug regimen used for two weeks, in the eradication of *Helicobacter pylori (*organism known to cause gastritis and peptic ulcer).

Investigator
Dr. Mavumba P. S., P. O. Box 67406-00200, Nairobi.
Telephone: +254-0722998757

Invitation to take part – Consent Explanation
You are being invited to take part in a research trial conducted by Dr. Soraya Mavumba, for her Masters degree being offered at JKUAT in association with KEMRI (ITROMID).

Description of the Study

You have come for an endoscopic examination. This diagnostic procedure done in theatre, involves introduction of a long plastic tube with a lens at the tip and camera-like part at the base, into the stomach via the mouth. This instrument enables the doctor to detect disease and unpleasant conditions that affect the upper part of the digestive tract. The procedure is harmless but uncomfortable. A local anesthetic will however be sprayed in your mouth and throat in order to reduce the discomfort.

During examination, small pieces of tissue about the size of a medium sized pinhead or grain of finger millet will be removed from the stomach lining. The tissues will be tested for bacteria *H. pylori,* and examined histologically that is under a microscope. This bacterium is known to cause ulcers or sores in the stomach.

If you are found to have these bacteria, you will be requested to participate in a study that will compare the effectiveness of a drug regimen used to treat *H. pylori*, given for either one or two weeks. To participate in the above study, you will need to have fulfilled certain requirements, for example, you will need to be an adult found to have the *H. pylori* bacteria.

You will be randomized into two groups. Both groups will receive medicine for two weeks and neither the doctor nor you will know which regimen you will be

using. Currently, the medicine is given for one week but I would like to know whether the outcome in treatment would be better when given for two weeks.

To show your willingness to participate voluntarily in the study, you will sign a consent form; a copy of which will be given to you. If the consent form is not understood, we will try to explain in a language familiar to you. A witness that is endoscopy nurse/personnel will be present to explain to you the procedure before endoscopy is done.

During the period of taking your medicine, a field worker will visit you at a place of your convenience with prior arrangement on Day 5 or Day 6. You will be required to come to the clinic for review on Day 15 to follow up on your progress and Day 42 for assessment of cure from your condition. You will be required to produce a stool specimen on Day 42 of follow up. Outcome of your treatment will also be communicated to you after stool analysis. If the infection has not cleared, an alternative treatment will be given to you.

Participation is voluntary and you are free to withdraw from the study at any time without loss of benefits.

Benefits and risks to participants
Participants will directly benefit by knowing their *H. pylori* status and getting treatment for the same free of charge.

Possible risks include discomfort and retching during the endoscopy procedure. The endoscopy tube may be uncomfortable when swallowing into the mouth. However, this feeling is reduced by giving of pre-operative local anesthetic with/without anticholinergic drugs.

Stomach biopsies taken will not harm the patient except minimal risk of bleeding from biopsy sites. As with any drug, it is possible that you could experience some undesirable reactions such as nausea, vomiting, diarrhea, skin itchiness, and skin rashes. All these reactions are reversible on stopping medication. You will be required to fill daily, a diary of your symptoms or any undesired reactions.

If you have any queries, please contact the investigator on the telephone and address given above. You can also contact the Secretary, Scientific and Ethical Research Committee at the following address.

Secretary Scientific Research committee
KEMRI
P. O Box 54840, Nairobi, Kenya.

CONSENT FORM

Please make sure any questions have been answered and that you understand the study. Participation is entirely voluntary. If you decide to take part in this research study, a copy of this signed form will be given to you.

Subject's Initials: _____

Subject's Name: _____

Subject's Age: _____

i) I confirm that I have read and understood the information sheet [ ]

dated _____ for the above study and have had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free

to withdraw at any time, without my medical care or legal rights being affected. [ ]

I agree to take part in the study. [ ]

Date: _____

Subject's Signature/Thumbprint (right):

_____

Name of Investigator: _____

Signature: _____   Date: _____

Name of Witness: _____

Signature of Witness:_____

Date: _____

Appendix 5

IDHINI YA KUSHIRIKI

Aina ya Utafiti

Utafiti unaofanywa ni wa kuchunguza iwapo madawa matatu maalum yanayotumika kwa muda wa wiki mbili, yataweza kumaliza bakteria *H. pylori*, inayosababisha ugonjwa wa vidonda vya tumbo(alsa).

Mtafiti ni:
Dakta Mavumba P.S
Sanduku la Posta: 67406-00200
NAIROBI
Nambari ya Simu: + 254-0722-998757

Mwaliko Kwa Washiriki
Unaalikwa kushiriki katika majaribio ya utafiti unaofanywa na Dakta Soraya Mavumba kwa ajili ya shahada yake ya uzamili (Masters) katika chuo kikuu cha JKUAT kwa ushirikiano na taasisi ya utafiti wa matibabu ya KEMRI.

Kuhusu Utafiti Wenyewe
Umekuja kwa uchunguzi wa tumbo, unaofanywa thieta. Katika uchunguzi huu, kijipira kirefu chembamba kitatiwa tumboni mwa mshiriki kupitia mdomoni na kitabeba kamera ndogo sana itakayomwezesha daktari kuchunguza maradhi yaliyomo tumboni. Utaratibu huu hauna madhara yoyote ila huenda mtu akahisi maumivu kidogo. Hata hivyo, dawa ya ganzi itanyunyizwa mdomoni na kooni ili kupunguza maumivu hayo.
Katika uchunguzi huu, vijinyama vidogo sana, mfano wa tembe ya mawele, vitatolewa kutoka tumboni ili vichunguzwe na pia kutizamwa kwa kutumia darubini, kuona kama vinabeba bakteria *H. pylori*, inayosababisha ugonjwa wa vidonda vya tumboni yaani alsa.

Ukipatikana na bakteria hii, utaombwa kushiriki katika utafiti ambao lengo lake ni kulinganisha uwezo wa mchanganyiko wa aina fulani za madawa ya kutibu bakteria *H. pylori*, unaotolewa kwa wiki moja au mbili. Ili kushiriki kwa utafiti huu, itakubidi utekeleze maagizo fulani, moja wapo uwe mtu mzima mwenye miaka kumi na minane au zaidi, na kupatikana kwa hizi bakteria. Mtagawanywa katika makundi mawili. Makundi yote mawili watapata madawa kwa wiki mbili. Daktari wala mshiriki, hawatajua mchanganyiko upi wa madawa utakaotumiwa. Kwa sasa hivi, dawa hii hutumiwa kwa wiki moja lakini ningetaka kujua kama matokeo yatakuwa bora iwapo itatumiwa kwa wiki mbili.

Ili kuonyesha kukubali kwako kushiriki utatia saini fomu ya idhini kuonyesha kwamba umeelewa unachotakiwa kufanya na kwamba umekubali kujitolea kufanya hivyo halafu utapewa nakala ya fomu hiyo. Ikiwa huelewi fomu ya idhini utaelezwa kwa makini kwa lugha unayoifahamu. Afisa wa matibabu atakueleza utaratibu mzima utakaofuatwa kabla ya uchunguzi wowote kufanywa.

Wakati unatumia madawa haya, afisa wa nyanjani atakutembelea pahali palipo rahisi kwako, mkiwa mumepanga mbeleni, siku ya tano (5) au siku ya sita (6). Halafu utatakiwa kumuona daktari kwa kliniki siku ya kumi na tano (15) ili aweze kuchunguza jinsi dawa inavyofanya kazi, na siku ya arobaini na mbili (42) ili uchunguzwe kama tatizo ulilokuwa nalo limetibiwa kwa kutumia dawa uliyopewa. Utahitajika kupeana choo chako kupimwa bakteria *H. pylori*. Matokeo ya kutibiwa kwako yatawasilishwa kwako baada ya choo chako kupimwa. Ikiwa tatizo litakuwa bado halijaisha, basi utapewa aina nyingine ya dawa.
Kushiriki kwako ni kwa hiari yako, na unaruhusa kutoka kwa utafiti huu wakati wowote bila kupoteza faida zozote kwako.

Faida kwa Washiriki
Washiriki watafidika moja kwa moja kwa vile watajua kama wanayo bakteria hii inayosababisha alsa au la na vile vile kupata matibabu ya bure iwapo watagunduliwa kuwa nayo. Licha ya faida hii, upo uwezekano kwa mshiriki kupata maumivu kidogo wakati wa kutiwa kijipira tumboni hasa wakati wa kukimeza. Hata hivyo, maumivu haya yatapungua sana kutokana na dawa ya ganzi itakayonyunyizwa kabla.

Vijinyama vidogo vya tumboni vitakavyotolewa wakati wa utaratibu huu, havitaleta madhara yoyote yale ila upo uwezekano wa kutoka damu kidogo. Kama kawaida ya dawa yoyote ile, huenda pia mshiriki akapata matatizo madogo kama vile kichefuchefu, kutapika, kuharisha, kuwashwa kwa ngozi na harara. Lakini haya yote yataisha mara tu mshiriki atakapoacha kutumia dawa. Vile vile mshiriki atatakiwa kuorodhesha mabadiliko yoyote atakayohisi au athari zozote atakazoziona kila siku.

Ikiwa una maswali yoyote kuhusu utafiti huu, tafadhali wasiliana na matfiti Daktari Soraya Mavumba ukitumia anwani na nambari ya simu uliyopewa. Pia waweza kuwasiliana na katibu wa kamati ya KEMRI inayosimamia utafiti huu ukitumia anwani ifutayo.

Secretary Scientific Research Committee.
KEMRI
P.O BOX 54840, Nairobi, Kenya.

FOMU YA IDHINI YA KUSHIRIKI

Tafadhali hakikisha kuwa umejibu maswali yote na kwamba umeyaelewa vema maelezo kuhusu utafiti huu. Kushiriki kwako ni kwa hiyari na kwa kujitolea. Ukiamua kushiriki katika utafiti huu, utapewa nakala ya fomu hii uliyoitia saini.

Herufi za mwanzo za Majina Yako_____

Majina Kamili_____

Umri _____

i) Nathibitisha ya kwamba nimeyasoma na kuyaelewa maelezo niliyopewa kuhusu utafiti huu

mnamo tarehe _____ na nimepata fursa ya kuuliza maswali.

ii) Naelewa kwamba kushiriki kwangu katika utafiti huu ni kwa kujitolea na kwa hiari yangu na kwamba niko huru kujiondoa wakati wowote nitakapo pasi na haki zangu za matibabu au kisheria kuathirika.

iii) Nakubali kushiriki katika utafiti huu.

Tarehe _____

Saini au alama ya kidole gumba cha mshiriki
(kulia)_____

Jina la Mtafiti   _____

Saini   _____

Tarehe   _____

Jina la shahidi   _____

Saini ya shahidi   _____

Tarehe   _____

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200 NAIROBI, Kenya
Tel: +254 (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax +254 (020) 2720030,
E-mail: director@kemri.org; info@kemri.org Website: www.kemri.org

ESACIPAC/SSC/2535   27th March, 2008

Soraya Popo Murumba

Thro'

Director, CMR
NAIROBI

forwarded
03.04.2008

REF: **SSC No.1317 (Revised) – A comparison of triple drug regimen duration for eradication of helicobacter pylori in adults attending a Nairobi gastroenterology Clinic**

I am pleased to inform you that the above mentioned proposal in which you are the PI, was approved for implementation by the KEMRI Scientific Steering Committee (SSC), during its 142nd SSC meeting held on 26th November 2007 and has since been forwarded to the Ethical Review Committee (ERC) for consideration.

The SSC however, advises that work on this project can only start when ERC approval is received.

C. Mwandawiro, PhD
SECRETARY, SSC

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KENYA MEDICAL RESEARCH INSTITUTE

ESACIPAC/SSC/6359   29th March, 2010

Soraya Mavumba

Director, CMR   *Forwarded*   7/4/10
NAIROBI

REF: SSC No.1317 (Amendment) – A comparison of triple therapy drug regimen duration for the eradication of helicobacter pylori in adults attending a Nairobi gastroenterology clinic. PI: Soraya Mavumba (CMR)

I am pleased to inform you that the above mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its 165th meeting held on 2nd March 2010 and has since been approved for implementation by the SSC.

The proposal has been forwarded to the ERC and we advise that work on this project can only start when ERC approval is received.

Sammy Njenga, PhD
SECRETARY, SSC

KENYA MEDICAL RESEARCH INSTITUTE

KEMRI/RES/7/3/1   August 6, 2010

TO: DR. SORAYA MAVUMBA,
             PRINCIPAL INVESTIGATOR

THROUGH: DR. SAMUEL KARIUKI,   *Forwarded 11/8/10*
             THE ACTING DIRECTOR, CMR,   *Dr C. B4 AgD*
             NAIROBI

RE: SSC PROTOCOL NO. 1317 (*REQUEST FOR 1ST AMENDMENT*):
             A COMPARISON OF TRIPLE THERAPY DRUG REGIMEN
             DURATION FOR THE ERADICATION OF *HELICOBACTER
             PYLORI* IN ADULTS ATTENDING A NAIROBI
             GASTROENTEROLOGY CLINIC

Thank for the covering letter that elucidates the nature of the amendment.

The Committee was of the view that the proposed amendment to 1) change the number of patients from 140 to 114 and of the time which was overtaken by events 2) change of design overview from double blind to single blind 3) change calculation of sample size as recommended by statistician does not alter the risk/benefit status of the study and are granted approval for implementation.

You are required to submit any further amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

Yours sincerely,

RCTKithinji

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

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