ASSOCIATION BETWEEN HIV INFECTION AND GASTRIC CANCER IN AN AFRICAN POPULATION

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DISSERTATION

ASSOCIATON BETWEEN HIV INFECTION AND GASTRIC CANCER AN AFRICAN POPULATION

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DECLARATION

I, Violet Kayamba hereby certify that this dissertation is the product of my own work, and in submitting it for my Master of Medicine in Internal Medicine degree, further attest that it has not been previously submitted either wholly or in part for any other degree at this or any other university.

Signature……..

Date…….

CERTIFICATION OF COMPLETION

I, ……, having supervised and read this dissertation, I am satisfied that this is the original work of the author Dr. Violet Jolezya Kayamba and is ready for presentation to the examiners.

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CERTIFICATE OF APPROVAL

This dissertation of Dr. Violet Jolezya Kayamba has been approved as fulfilling the partial requirements for the award of Master of Medicine in Internal Medicine by the University of Zambia.

…….. Date: ……
External examiner

…….. Date: ……
Internal Examiner

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Internal Examiner

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Chairman

ABSTRACT

Introduction

There is a shortage of systematically collected data on gastric cancer in Zambia. An audit carried out as preliminary work showed that there was a change in the epidemiology of gastric cancer since the early 1980s, and it was hypothesised that this change was linked to HIV infection. The aim of this study was to evaluate a possible association between gastric cancer and HIV infection in patients seen at the University Teaching Hospital (UTH), Lusaka. Other well known risk factors such as infection with *Helicobacter pylori* (*H. pylori)*, presence of CagA, serum pepsinogen 1 to 2 ratios, smoking, alcohol intake and low income were also evaluated.

Methods

This was a prospective case-control study conducted over one year. Cases were patients with gastric adenocarcinoma confirmed by histopathology while controls were patients with no visible mucosal abnormality in the upper gastrointestinal tract. Two controls were enrolled for each case after matching for age and sex. The presence of HIV infection was determined by testing for HIV antibodies in each group and odds ratios (OR) was calculated to determine the presence of any association. The presence of antibodies to *H.pylori*, the virulence factor CagA and serum pepsinogen 1 and 2 levels were determined using ELISA. Also collected was data on other life style risk factors using an interviewer administered questionnaire. Results were analysed using STATA 10.

Results

A total of 38 cases and 76 controls were enrolled. There was no association between gastric cancer and HIV infection (OR 1.4, 95%CI 0.3-6.4; *P*=0.73). Smoking and alcohol were found to increase the odds of developing gastric cancer in both univariate and multivariate analysis (multivariate *P*=0.04 and *P*=0.02 respectively). Overall, 81% of the patients were found to be positive for *H. pylori* infection, with no significant difference between the cases and the controls (*P*=0.24). The presence of antibodies to CagA was also not different between the two groups (*P*=0.79). A small proportion of cases and controls had low serum levels of pepsinogen 1, (11% and 6% respectively) but this was not significantly different (*P*=0.45).

However, the presence of a low pepsinogen 1 to 2 ratio was more discriminating, with a higher proportion among the cases (*P*=0.009).

Conclusions

No association was found between HIV infection and gastric cancer in the patients seen at the endoscopy unit, UTH, Lusaka. Alcohol and smoking were shown to increase the odds of developing gastric cancer. Patients with gastric cancer have a lower ratio of pepsinogen 1 to 2, although there was no significant difference in the levels of pepsinogen 1, *H.pylori* infection or CagA between gastric cancer patients and healthy controls. In conclusion, the reason for the apparent change in epidemiology of gastric cancer has not been established and therefore, more work still needs to be done to answer this question.

DEDICATION

I dedicate this work to my late mother Emelia Haazele Busiku for teaching me the value of discipline and hard work.

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ABBREVIATIONS

ARV	- Antiretrovirals
BMI	- Body Mass Index
Cag A	- Cytotoxin-associated Gene A
CI	- Confidence Interval
CLO	- Campylobacter-like Organism
HIV	- Human Immunodeficiency Virus
CD4	- Cluster of Differentiation 4
EBV	- Epstein Barr Virus
ELISA	- Enzyme-linked Immunoabsorbant Assay
GLOBOCAN	-Global Cancer
IgG	- Immunoglobulin G
ZMK	- Kwacha
KS	- Kaposi's Sarcoma
MUAC	- Middle Upper Arm Circumference
NSAIDS	- Non-steroidal anti-inflammatory drugs
OR	- Odds Ratio
PG	- Pepsinogen
PPI	- Proton-pump inhibitors
UK	- United Kingdom
USA	- United States of America
UTH	- University Teaching Hospital

INTRODUCTION

Gastric cancer is a malignant tumour arising in the stomach. It is associated with evidence of invasive growth or metastasis to regional or distant organs.[1] It was first described as early as 3000BC in the hieroglyphic inscriptions and papyrus manuscripts of ancient Egypt. Napoleon Bonaparte died in 1824 due to gastric cancer. The first statistical analysis of incidence and mortality of gastric cancer was carried out in Verona, Italy from 1760 to 1839. Despite having been described for many centuries, it is still not clearly understood. The exact aetiology of the cancer has not yet been established, though some risk factors have been described.

To the author's knowledge, there are no systematic studies that have been done on gastric cancer in Zambia and no data on the incidence of the disease.[2] As a result, the disease burden of gastric cancer is not known. The epidemiology of gastric cancer in patients referred to the University Teaching Hospital appears to be changing, with younger patients being seen presenting with the disease.[3] This is not expected on the basis of current evidence as reported in the literature. Several secular trends might be operating to explain this observation, including the emergence of HIV, changing diet, changing life styles, and changes in referral patterns. If these trends are true, they may yield important clues to the aetiology of gastric malignancy.

The aim of this study was to test the hypothesis that HIV infection explains the emergence of gastric cancer in young adults. We also wanted to determine the contribution of *H. pylori* infection, its virulence gene cytotoxin-associated gene A (CagA), gastric atrophy (as assessed by the ratio of pepsinogen 1 to 2), and life style factors such as alcohol and smoking, to cancer risk in the same patient population.

LITERATURE REVIEW

Gastric cancer is one of the most epidemiologically significant cancers in the world. It is the fourth most common cancer and the second leading cause of cancer deaths worldwide, second only to lung cancer.[1, 4,5] Up to 95% of gastric cancers are adenocarcinomata. [6]

The incidence of gastric cancer varies in different regions of the world, with the highest being in Japan and Korea. [7,8] The incidence in Japan is as high as 69.2 per 100 000 in males and 28.6 in females. [9] In 2008, GLOBOCAN estimated the incidence in Eastern Asia to range between 18.3 and 42.4 per 100 000, while in North America and Western Europe incidences of 2.8 to 5.8 and 4.4 to 9.0 per 100 000 respectively. [5] Accurate data on the incidence of cancer in Africa are lacking and gastric cancer is no exception. It is estimated that the incidence ranges between 2.2 and 5.6 per 100 000. [5] Reported prevalence of gastric cancer is 2-3% of all malignancies in Nigeria, Sudan, Uganda and Zimbabwe. [10] In South Africa it was reported to be ranging from 3.3 to 11.5%. [11] The exact incidence of gastric cancer in Zambia is not known, making it difficult to estimate the disease burden. According to GLOBOCAN 2002, gastric cancer is the eighth leading cause of cancer mortality in Zambia, Southern Africa. [12]

Despite the fact that gastric cancer has been described for so many centuries, there is still a lot that remains unknown. Researchers have over the years brought out new information on the subject, and yet there are still many more questions that have remained unanswered. Gastric cancer has been shown to be influenced by many factors. [9] Individual risk factors for cancer cannot be ascribed as being the only cause but usually a set of these factors or components may be necessary for cancer to develop**.** [13] The marked geographical variation, time trends and the effect of migration on gastric cancer incidence suggest that lifestyle and environmental factors play an important role in its aetiology. [9]Within the same region, the incidence of gastric cancer tends to be higher among the lower socio-economic classes and increases progressively with age, with a peak between the ages of 50 and 70. [9] In a study by Kelly *et al.* (2008), at the University Teaching Hospital (UTH), Lusaka, Zambia, it was concluded that the average age of onset of gastric cancer is lower than in other parts of the world. Furthermore, the incidence in individuals younger than 45 years appeared to be

higher than in the USA or UK. [3] At a South African hospital in 1988, 5% of all the gastric cancer patients were found to be less than 35 years. [14] The explanation for this is unclear. Further work may reveal important clues to the aetiopathogenesis of gastric cancer.

A retrospective audit of endoscopy unit records at UTH was done and it revealed that, in 1980 and 1982 all patients with gastric cancer were above the age of 50, while in records for 2009, 26% of the patients were below the age of 45. A five year audit between 2002 and 2007 also showed that up to 25% of the patients at the same unit were young. This alarming observation might be explained by changes in referral pattern or better endoscopic equipment, or any number of alternative secular trends over the last 30 years, but we need to take seriously the possibility that it is real and reflects exposure to a major biological health hazard.

Some of the known risk factors of gastric cancer include infection with *Helicobacter pylori* [15, 16,17,18,19], smoking [20, 21], alcohol [22], and diet [9, 23]. Correa *et al.* in 1975, proposed a model for the progression of gastric cancer with the mucosa changing from normal to chronic gastritis, atrophy, intestinal metaplasia, dysplasia and finally malignancy. [24] *H. pylori* was designated as a human class 1 carcinogen in 1994. [25] According to Parkin, 2006, approximately two thirds of all gastric cancers worldwide are attributed to *H.pylori* infection. [26] The prevalence of the infection varies. Within a given population, the prevalence of the infection is closely linked to socio-economic factors such as low income, and poor education and living conditions during childhood, such as poor sanitation and overcrowding. [27, 28,29,30,31] Using Correa's model of gastric carcinogenesis, it is now known that *H. pylori* infection triggers the progressive sequence of gastric lesions leading to malignancy.[32]

The prevalence of *H. pylori* in the adult population in Lusaka is 81% [33] but it is not easy to see how it would cause cancer at a young age in this population rather than in other populations. Had *H. pylori* been the only factor in the pathogenesis of gastric cancer in Zambia, then the prevalence of gastric cancer would be very high. The risk of developing gastric cancer is not the same in all cases of *H. pylori* infection. The risk tends to be enhanced by infection with a more virulent strain of *H. pylori* carrying the cytotoxin-associated gene (CagA). [35,36,37] CagA positive strains are associated with higher risk than

CagA negative Strains.[36,38] CagA, therefore, has been accepted as a marker of carcinogenicity in *H. pylori* associated gastric cancer. *H. pylori* strains co-expressing CagA genes tend to worsen inflammation significantly.[39] Its presence is conveniently determined by measuring antibodies to the CagA protein. In a study by Held *et al*. 2004, it was shown that although the risk of patients with antibodies to CagA developing gastric cancer is very high, those with CagA negative infections still run a significantly greater risk than uninfected patients.[40]

In western countries, about 60% of *H.pylori* strains are CagA A positive, whereas in Japan, nearly 100% of the strains posses functional CagA. [41, 42] In a study done in Sudan in 1998, CagA seropositivity was found to be closely related to endoscopic gastroduodenal disease and the presence of more advanced histological lesions in the antrum. They, however, also found a high prevalence of CagA seropositivity in asymptomatic healthy individuals suggesting that other factors other than CagA predominate in ulcer pathogenesis in that population. [43]

Dietary factors also seem related, including types of food preparation, physical properties of some foods, and certain methods of food preservation (especially smoking, pickling, or salting). A study done by Kato *et al.* in 1992 showed that gastric cancer patients tended to consume more cigarettes, alcohol, rice, pickles and salted fish and less of fruits and vegetables, and to have more family histories of gastric cancer though this was not statistically significant.[23] Tredaniel J *et al.* in 1997 did a meta-analysis of 40 studies, and results showed that smoking cigarettes increases the risk of gastric cancer by 1.5-1.6 compared with non-smokers.[44] However, it still remains unclear exactly how these risk factors lead to the development of gastric cancer.

Other less common risk factors include radiation, pernicious anaemia, blood group A, prior gastric surgery and Epstein Barr virus (EBV) infection. It is also more often seen in people with a positive family history suggesting some genetic predisposition.[9]

In 1965, Lauren described two histological subtypes of gastric cancer, diffuse and intestinal. [45] Intestinal type usually arises in the distal part of the stomach and is found in elderly

patients. It is also more common in males and black patients while the diffuse type has a similar incidence in both sexes and is more frequent in younger individuals. Exogenous factors are believed to play a role in the development of intestinal type and it moves from chronic atrophic gastritis to cancer via metaplasia. The diffuse type is believed to develop more with some genetic influence. There is also a wider geographic variation with intestinal type while the diffuse type is more uniform across regions. [45, 46, 47]

The role of other infections in gastric cancer has not been well explored. EBV has already been referred to above. One such infection is the Human Immunodeficiency Virus (HIV). Sub-Saharan Africa is considered home to more than 60% of all HIV cases. [48] The first one in Zambia was described in 1984, [49]and since then, the infection has been spreading rapidly. Gastrointestinal symptoms are some of the most frequent complaints in HIV disease. [50] It is well documented that HIV infection causes some changes in the stomach environment. It was found to be associated with hypochlorhydria defined by a pH of more than 4.0, but this was not the case in those taking anti retroviral drugs (ARVs) suggesting that the effect of HIV on gastric pH is reversed by ARVs. [51]

HIV infected patients are also more susceptible to diarrhoeal diseases and less to worm infestations. [52] A study by Mach *et al.* showed that HIV infected patients with severe immunodeficiency had a lower prevalence of *H. pylori* and active chronic gastritis in the gastric antrum compared with the other HIV infected patients and controls. They also demonstrated that mycotic oesophagitis and mycotic colonization of the stomach were more frequent in patients with severe immunodeficiency.[53] In another study, HIV infected patients with gastrointestinal symptoms were found to have a lower prevalence of *H. pylori* infection and peptic ulceration. [54] This could suggest a different mechanism of peptic ulcerogenesis and a different role of *H. pylori* infection in chronic active gastritis. All these differences could be attributed to immunological changes caused by HIV infection which have not been well understood. It is therefore possible that these changes also predispose to the development of gastric cancer.

A case report of a 33 year old homosexual HIV positive patient presenting with gastric adenocarcinoma was reported by Leatherwood JL, in 1991. [55] The tumour was rapidly

aggressive and quickly led to death. The author postulated that this presentation could have been due to suppressed immunity. Such a case report, though with very weak statistical power points illustrates that other scientists have also been wondering if there is indeed an association between HIV infection and gastric cancer. Kaposi's sarcoma and lymphomas are some of the well described malignancies whose lesions can also be found in the stomach, but these are not primarily regarded as gastric cancers. These well known HIV associated malignancies are more prevalent with lower immunity.

In Zambia, HIV infection is most prevalent between the ages of 15-45 years, [56] and 25% of the gastric cancer patients seen at the UTH endoscopy unit are also less than 45 years. It is possible that this change in the epidemiology could be due to co-infection with HIV, so this study was designed to investigate this association.

Gastric atrophy, a precursor of gastric cancer can be evaluated on biopsies obtained from the stomach. Gastric atrophy has been shown to increase the risk of developing gastric cancer. [23] Pepsinogen 1 to 2 ratios have been shown to correlate well with gastric atrophy. Serum pepsinogen consists of two biologically and immunologically distinct types, namely, pepsinogen 1 (PG 1) and pepsinogen 2 (PG 2). PG 1 is produced by chief and mucous neck cells in the fundic glands, while PG 2 is produced by these cells and also by cells in the pyloric glands as well as Brunner's glands in the duodenum. [57, 58, 59, 60] Serum pepsinogen levels reflect the functional and morphologic status of the gastric mucosa. As the fundic mucosa is reduced, PG 1 levels gradually decrease, whereas PG 2 levels remain fairly constant. [60, 61] As a result, a step wise reduction of the PG 1/PG 2 ratio is closely correlated with the progression from normal gastric mucosa to extensive atrophic gastritis; this ratio of more than 3 has a sensitivity of 93.3% and specificity of 87.7% for the diagnosis of normal fundic gland mucosa. [61, 62]

With all these unanswered questions regarding gastric cancer, it was justifiable to find out if there was an association between gastric cancer and HIV.

STATEMENT OF THE PROBLEM

The age of patients presenting with gastric cancer at the UTH is much lower than seen elsewhere [3]. An audit of the records at the endoscopy unit for 2009 was carried out and it showed that 25 % of patients with gastric cancer were below the age of 45 years. In contrast, the records of 1980-1982 from the same unit revealed that all the patients with gastric cancer were above the age of 50 years. This shows that there are younger patients presenting with gastric cancer now than in the past. With very little information on cancer available in the country currently, it is very difficult to explain this reduction in age at first presentation. There are several possible factors that could be attributed to this including changes in health seeking behaviour, lifestyle, diets, environmental factors, genetics or possibly infections. HIV infection has so far been shown to be associated with so many malignancies; it is possible that gastric cancer could be one of them. Most of the HIV associated cancers were in the past noted be more prevalent in younger age groups and this stimulated a lot of research. This could be the exact situation with gastric cancer at the moment and it is possible that the change in epidemiology of gastric cancer is associated with HIV infection.

STUDY JUSTIFICATION

Very little is known about gastric cancer in young adults at the UTH. This study was designed to establish whether indeed there was an association between gastric cancer and HIV as one of the probable explanations for the change in epidemiology. It was not very clear which risk factors were at play leading to the development of gastric cancer in these patients. Information on the number and age distribution of patient with gastric cancer was collected in a systematic way.

HYPOTHESIS

HIV infection is associated with gastric cancer in patients seen at the endoscopy unit at UTH.

OBJECTIVES

Main objective

To investigate the association between HIV infection and gastric cancer in patients seen at the endoscopy unit, UTH, Lusaka.

Specific objectives

1. To describe the age distribution of gastric cancer patients at the endoscopy unit, UTH.
2. To estimate the contribution of HIV infection to gastric cancer risk by finding the proportion of HIV infection in cancer patients compared to patients with normal gastric mucosa.
3. To evaluate the presence of gastric atrophy, *H.pylori* infection and pepsinogen 1 to 2 ratios in the patients enrolled, and to determine the proportion of cases and controls with exposure to smoking, alcohol and to poor socio-economic conditions.

ETHICAL CONSIDERATIONS

Permission to carry out the study was granted by the Research Ethics Committee of the University of Zambia (reference 008-02-10). The study subjects were required to give informed consent to participate in this study and they had the right to withdraw from the study at any given time without any compromise to the level of care. None of the patients underwent endoscopy purely for the purposes of the study and all patients were given HIV pre and post test counselling. Patient's information was treated with the highest confidentiality and the information obtained was solely for the purposes of the study. Cases and controls, as well as patients not recruited for any reason, were offered the highest standard of care available in UTH.

METHODOLOGY

Study design

The study was a prospective case-control study, running for one year from November 2010 to October, 2011. Both the cases and controls were selected from the endoscopy unit. Cases were defined as patients with gastric cancer confirmed by histopathology. Controls were patients with upper gastrointestinal symptoms but no visible mucosal abnormality.

Site

The study was conducted at the Endoscopy unit of the UTH, in Lusaka, Zambia.

Study population

These were patients presenting to the endoscopy unit at the UTH for oesophagogastroduodenoscopy. The UTH is a national referral hospital based in Lusaka, the capital city of Zambia and patients are referred to the institution from all over the country. Within the hospital, patients come from the out-patients clinics and in- patients from the various wards. Referrals also come from other departments within the hospital and from various medical institutions both in private and public sectors. Although patients of all ages and sexes are referred to the unit, only adults were considered for this study.

Inclusion criteria

Cases:

(i) Age 18 years or older

(ii) Histopathologically proven gastric cancer

(iii) Written consent

Controls

(i) Age 18 years or older; in same age band, (18- 30 years, 31 to 45 years, 46 to 60 years and more than 60 years), and of the same sex as the latest case

(ii) Symptoms of dyspepsia but no mucosal abnormality seen at endoscopy

(iii) Written consent

Exclusion criteria

1. All patients who did not meet the inclusion criteria were excluded from the study.

2. Patients who refused to have an HIV test

Sample size

The initial sample size calculations were based on rough assumptions, as in effect this was a pilot study. The calculations were based on the contribution of HIV to gastric cancer in young adults. Due to the fact that there was no past data available on gastric cancer and HIV infection, we set out to test the hypothesis that the majority (80%) of cases in young people are related to HIV and that the prevalence in controls (20%) would be the same as the general urban Zambian population[63]. Using this, we needed 16 in each group to have 90% power to detect a difference with 95% confidence. As young adults only represent 25% of the cases of gastric cancer, we planned to collect 4 times as many patients, giving a total of 64. To allow for margin of error, a total of 80 patients were to be enrolled in each group. However, while conducting the study, it became apparent that 80% assumption was too high, and that only 25% of the young adults with gastric cancer had HIV infection, and 21% of all the gastric cancer patients were actually young. Therefore, the sample size calculations were adjusted bearing in mind that 30 was the minimum number needed to express values as percentages for a pilot case-control study, and that 30 was also an adequate number from which one could draw conclusions.[64] 38 cases of gastric cancer were therefore sufficient for this purpose. In addition, all the cases of gastric cancer that presented to the unit and gave consent during the period November, 2010 to October 2011 were included in the study.

Procedure

Recruitment

All patients coming to the endoscopy unit for suspected gastric cancer were approached and the study was explained to them. Patients willing to participate signed consents forms that were made available for them. If a gastric lesion suspicious of a cancer (including any gastric ulceration) was seen during the procedure, appropriate biopsies were obtained in the usual way and the patient was designated as a case. However, status as a case was only confirmed if the pathology report confirmed the malignancy. For each case, controls were enrolled. These were patients presenting with dyspepsia and of the same sex and age band as the case. As with the cases, the study was explained to them and a written consent was signed. Status as a control was only confirmed if the endoscopy was normal, six biopsies were obtained, two from gastric fundus, two from gastric antrum and two from the body for

histology (looking for gastric atrophy). Tissue samples were sent to the histology laboratory for analysis by histopathologists. For some patients, an extra biopsy was obtained for the simple urease, (CLO) test. Some patients who gave consent were not actually enrolled if they did not meet the above criteria, and this was clearly explained to them.

Blood samples were only collected from the patients who qualified for enrolment after endoscopy. 10 ml of blood was collected for the HIV test, CD4 count, *H. pylori* and Cag A serology and to measure the levels of pepsinogen 1 and 2. These samples were taken to the laboratory and stored at $-80^{o}C$ until the analysis. Following best practice, pre-test counselling for HIV was carried out by trained counsellors prior to collecting the blood. A questionnaire was then administered to both cases and controls, with which information about demographic data, symptoms, any relevant past medical and surgical history was collected.

Patients were asked to come for review after two weeks and all the results were communicated to them and their chosen relatives. It should be emphasized that this is part of routine medical practice and was done at no extra cost to the patient. Effort was taken to make sure that the results of all the patients were acted upon fully and that a high standard of available care was subsequently delivered to these patients. Urgent surgical consultations were organized for all the patients with gastric cancer. Before informing the patient on their HIV status, appropriate post-test counselling was carried out by trained counsellors. Patients who refused to have an HIV test (n=2) were excluded from the study. Those found to be HIV positive were referred to the UTH Centre of Excellence, which is an outpatient clinic set for the management of HIV positive patients.

The presence of HIV infection was determined by the presence of antibodies at the virology laboratory, UTH using Unigold for screening and Determine as a confirmatory test.

Serology for H.pylori, CagA, PG 1 and 2

For the *H. pylori*, CagA, and pepsinogen 1 and 2 assays, commercially available kits were used (Biohit Gastro Panel ELISA kits for *H. pylori*. pepsinogen 1 and pepsinogen 2). To determine the presence of CagA IgG antibodies, kits produced by Genesis Diagnostics were

used. In all cases, instructions provided in the package insert were followed, and all standards and controls were used.

For H.pylori;

1. Frozen serum was allowed to thaw to room temperature (20-25^{o}C), and diluted with the provided diluent buffer at a ratio of 1 to 200.
2. 0.1 mls of each the diluted samples and the provided control solutions were placed in their respective wells on a micro plate coated with partially purified *H.pylori* bacterial antigen, and incubated for 30 minutes at 37^{0}C and then washed with a washing buffer.
3. A conjugate solution was then placed into the wells and the incubation and washing repeated.
4. 0.1 mls of the substrate solution was then pipetted into each well, and incubation was done for 30 minutes at room temperature.
5. A reaction stop solution was then added to each well, and the mean absorbance measured at 450nm within 30 minutes.
6. A sample was considered positive if the calculated enzyme immunounit value was greater than or equal to 30.

For PG 1;

1. Frozen serum was allowed to thaw to room temperature (20-25^{o}C), and diluted with the provided diluent buffer at a ratio of 1 to 10.
2. 0.1 mls of each the diluted samples and the provided control solutions were placed in their respective wells on a micro plate coated with high affinity, monoclonal anti-human PG 1 IgG_1 incubated for 60 minutes at 37^{0}C and then washed with a washing buffer.
3. A conjugate solution was then placed into the wells and the incubation and washing repeated.
4. After the second wash, 0.1mls of the substrate was added to each well and incubated at room temperature for 30 minutes.
5. 0.1 mls of a stop solution was then added and the absorbance measured at 450 nm within 30 minutes.
6. PG 1 was considered to be low if the concentration was less than 30 micrograms per

litre.

For PG 2;

1. Frozen serum was allowed to thaw to room temperature (20-25^{O}C), and diluted with the provided diluent buffer at a ratio of 1 to 10.
2. 0.1 mls of each the diluted samples and the provided control solutions were placed in their respective wells on a micro plate coated with high affinity, monoclonal anti-human PG 2 IgG_1 incubated for 60 minutes at room temperature and then washed with a washing buffer.
3. A conjugate solution was then placed into the wells and the incubation and washing repeated.
4. After the second wash, 0.1mls of the substrate was added to each well and incubated at room temperature for 30 minutes.
5. 0.1 mls of a stop solution was then added and the absorbance measured at 450 nm within 30 minutes.
6. A PG 1 to 2 ratio of less than 3.0 was considered to be low.

For CagA;

1. Samples were thawed at room temperature and diluted with the provided diluent at a ratio of 1 to 200.
2. 0.1 mls of each diluted samples and the provided standards were pipietted into the wells on a micro plate pre-coated with recombinant CagA protein and incubated at room temperature for 30 minutes.
3. The wells were then washed using the provided buffer and 0.1 mls of conjugate dispensed in each well. Incubation was then done for 30 minutes at room temperature and then washed again.
4. 0.1 mls of TMB substrate was added to each well and incubated for 10 minutes. Then, 0.1 mls of a stop solution was added.
5. The optical density was read at 450nm in each micro plate.
6. Optic densities above 0.642+/- 0.03 were considered to be positive, while those below this range were negative. Values lying within this range were considered to be indeterminate.

Data analysis

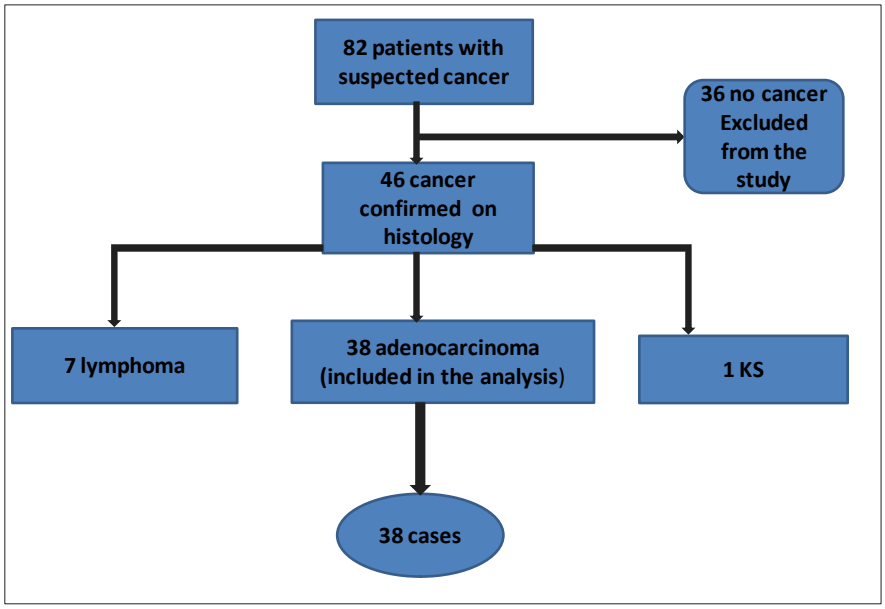
The data was entered into an Excel spread sheet, coded and anonymised, and analysed using STATA 10. For continuous variables showing a non- Gaussian distribution, the Kruskal-Wallis test was used to compare the two groups. For categorical variables, the Fisher's exact test was used as the numbers were relatively small. Odds Ratios with 95% confidence intervals, and P values were derived to define the frequency of risk factors in cases and controls. A probability value less than 0.05 was considered statistically significant. Stepwise logistic regression was used to assess the relative contributions of different exposure variables to the risk of developing gastric cancer.

RESULTS

Patient enrolment

A total of 82 patients with suspected gastric cancer at endoscopy were initially enrolled. The suspicion was based on the presence of a gastric ulcer with raised edges or an obvious tumour mass. On histology, however, 38 had confirmed adenocarcinoma, 7 had gastric lymphoma and 1 had gastric Kaposi's sarcoma (KS). The remaining samples did not show any evidence of malignancies. For the analysis, only patients with confirmed adenocarcinoma were included. None of the patients with gastric cancer declined to give consent for participation in the study.

Figure 1: Flow chart showing patient enrolment



Case-control matching

Controls were selected for these confirmed cases, after matching them for age and sex. An attempt was made to select two controls for each case, but it was discovered that there were very few elderly patients above the age of 60 presenting with dyspepsia but no evidence of any pathology at endoscopy. Therefore, 38 perfectly matched controls were enrolled and another 38 who were matched either for age or for sex. The two groups were analysed separately and compared. There was no statistical difference between the two results, and therefore, using either way of matching; the results would be the same. In order to increase the power of the study, the larger pool of patients was therefore used.

Table 1. Basic characteristics of Cases and Controls

CHARACTERISTIC	CASE n=38 (%)	CLOSELY MATCHED CONTROLS n=38 (%)	ALL CONTROLS n=76 (%)	P
SEX (males)	23 (60)	23 (60)	35 (45)	0.104
(females)	15 (40)	15 (40)	41 (55)	
AGE BAND				
18-29	1 (3)	1 (3)	3 (4)	
30-45	7 (18)	7 (18)	22 (28)	0.088
46-60	6 (16)	6 (16)	23 (29)	
>60	24 (61)	24 (61)	30 (38)	
MARITAL STATUS				
Single	0 (0)	3 (8)	4 (5)	0.370
Married	30 (79)	26 (68)	54 (69)	
Widowed	5 (13)	7 (18)	17 (22)	
Divorced	1 (3)	1 (3)	1 (1)	
separated	2(5)	1 (3)	2 (3)	
FORMAL EDUCATION				
None	8 (21)	6 (16)	7 (9)	
Primary	11 (29)	11 (29)	28 (36)	0.370
Secondary	15 (40)	9 (24)	23 (29)	
Tertiary	4 (1)	12 (32)	20 (26)	
MONTHLY INCOME (ZMK)				
<500,000	11 (30)	7 (18)	16 (21)	0.070
500,000-1,000,000	4 (11)	5 (13)	14 (18)	
>1,000,000	4 (11)	11 (29)	24 (31)	
Irregular	18 (48)	15 (40)	24 (31)	
BMI (mean)	20.9	31.8	28.6	<0.001*
MUAC (cm)	24.7	28.7	28.2	0.001*

(*) denotes significant P value

Basic characteristics of the patients

Demographic characteristics were obtained for all the patients enrolled. There was no statistical difference between the ages and sexes of the cases and controls, so it was well

justified to make valid comparative analyses between the two groups. Gastric cancer was found to be more prevalent among male than females, with a ratio of 2:1. One of the aims of this study was to determine the age distribution of gastric cancer patients and it was found that up to 21% were below the age of 45 years, revealing a significant number of young gastric cancer patients in Zambia. Comparing the age groups according to the sex also yielded no significant difference between the two groups (*P*=0.81). The youngest case was a 28 year old female and the oldest was a 93 year old male. Other demographic features including, marital status, formal education and monthly income, showed no difference between the cases and the controls. As expected, Body Mass Index (BMI), was found to be significantly lower among the cancer cases than the controls, (*P*<0.001). The mid-upper arm circumference (MUAC), also showed a significant difference between the cases and the controls, (*P*=0.001).

Table 2 Medical History of the Cases prior to presentation (n=38)

SYMPTOMS	n (%)	MEDICATION PRIOR TO PRESENTATION	n (%)
None	0	**None**	23 (61)
Abdominal pain	29 (76)	**Antibiotics**	4 (11)
vomiting	6 (16)	**PPI**	3 (8)
Symptoms of anaemia	9 (24)	**Haematinics**	3 (8)
Abdominal swelling	2 (5)	**NSAIDS**	2 (5)
Weight loss	2 (5)	**H2-blockers**	1 (3)
Constipation	3 (8)	**Drugs not listed above**	2 (5)
Body weakness	1 (3)		
Dysphagia	3 (8)		
GI bleeding	6 (16)		

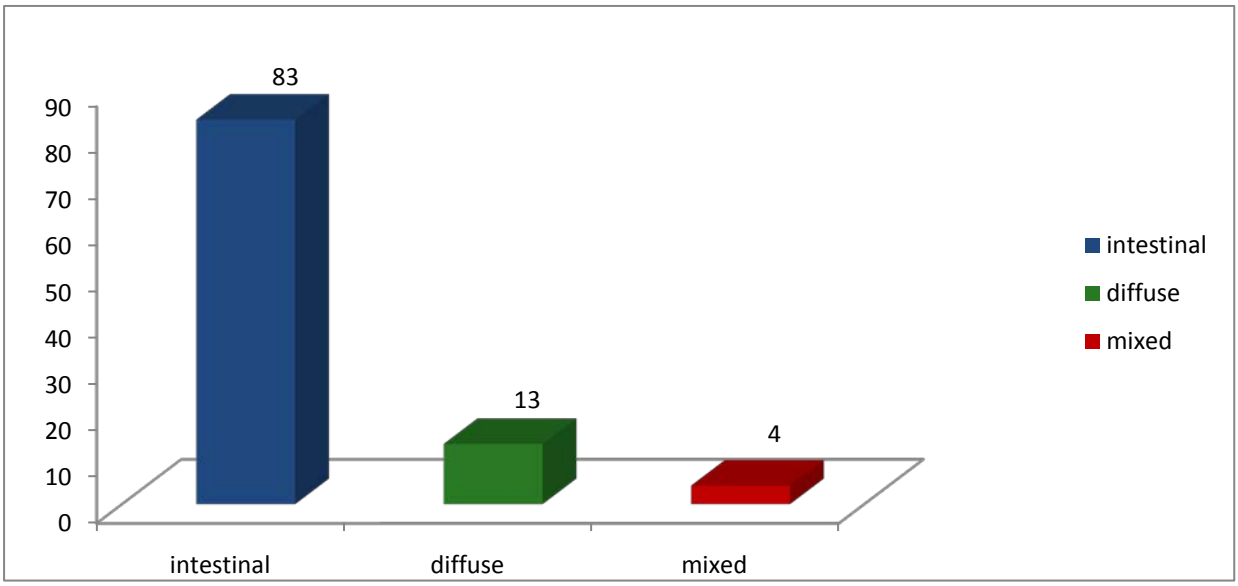
Clinical presentations

All the patients with confirmed gastric cancer were symptomatic. Data on these symptoms was collected in order to understand the clinical presentation of gastric cancer patients. The most common complaints were abdominal pain, vomiting, gastrointestinal bleeding and symptoms of anaemia, with some patients having more than one of these symptoms. Up to 39% (15) of the cases were on some form of medication prior to presentation.

Histological classification of Gastric cancer

The types of gastric adenocarcinoma were determined on histology. The majority, 82% had intestinal type of cancer and only 4% had the mixed type. This showed that environmental risk factors largely influenced the gastric cancer cases that were enrolled. The diffuse type of gastric cancer which is largely influenced by genetic factors was found in 13 % of the cancer cases, correlating well with the fact that only one of these cases gave a positive family history of gastric cancer.

Figure 2, Histological types of Gastric Cancer



Primary hypothesis – the influence of HIV infection

The comparison between the proportion of HIV infection among the cases and the controls was the main aim of this study. 11% of the cases were HIV positive, and this was not significantly different from the controls with 8% being positive (*P*=0.72) is shown in table 3. Had there been any association between gastric cancer and HIV infection, then the proportion of HIV infected patients among the cases would have been significantly more than among the controls. Therefore, these results demonstrate no association between HIV infection and gastric cancer. Even among the patient below 45 years, there was no significant difference in the prevalence of HIV infection among the cases and the controls.

Table 3: HIV infection among cases and controls

HIV INFECTION	CASES n=38 (%)	CONTROLS n=76 (%)	ODDS RATIO (CI)	P VALUE
Positive	4 (11)	6 (8)	1.4(0.3-6.4)	0.72
Negative	34 (89)	74 (92)		

Life style risk factors

Another aim of this study was to evaluate the contribution of life style risk factors in the development of gastric cancer. Alcohol and smoking were found to increase the odds of developing gastric cancer, with significant P values of less than 0.001 in both cases. Low income did not show any significant difference and thus was found not to increase the odds of developing gastric cancer. A stepwise logistic regression was done to check for any interaction between these risk factors. Alcohol and smoking still remained significant with P values of 0.02 and 0.04 respectively. Included in the analysis were patients who were currently smoking or taking alcohol and those who had stopped.

Table 4: Life style risk factors for gastric cancer

VARAIBLE	CASES n=38	CONTROLS n=76	UNIVARIATE OR (95%CI)	P	MULTIVARIATE OR (95%CI)	P
SMOKING						
EVER SMOKED	13	6	6.2 (1.9-21.9)	<0.001*	3.5 (1.1-11.4)	0.04*
CURRENT SMOKER	8	2	10.1(1.2-101)	0.002*		
LOW INCOME	17	32	3.1 (0.8-14.5)	0.07		
ALCOHOL						
EVER DRINKING	20	15	4.7 (1.8-11.9)	<0.001*	3.1 (1.2-7.8)	0.02*
CURRENT DRINKER	11	7	4.1(1.3-13.8)	0.01*		

(*) denotes significant P value

Biological risk factors

Biological risk factors of gastric cancer were also evaluated. However, as only two ELISA plates were available, only 88 patients were included in this part of the analysis, 37 cases and 51 controls. Of these, 81% were found to be positive for *H.pylori* antibodies, with no significant difference in the proportion among the cases and the controls (*P*=0.25). These results reflect the presence of *H.pylori* antibodies at particular that time and do not include the cases in which the antibodies could have been cleared. Of those with *H. pylori* antibodies detected, the presence of antibodies to CagA proteins was determined, and there was no significant difference detected between the two groups (*P*=0.79). Low pepsinogen 1 levels were found in very few patients, less than 15% in both groups, but the ratios of pepsinogens 1 to 2 were significantly different (*P*=0.009), with lower levels among the cases. An attempt was made to include all the risk factors in one logistic regression model but it broke for the following reasons; the data set for the serological factors was not complete as only 88 were looked at instead of 116, and using this reduced data set some factors had reduced numbers in some cells for example no current smokers among the controls. A larger data set was therefore, needed in order to run such an inclusive logistic

regression. In addition, some serological factors were dropped off from the regression model due to collinearity. Only the pepsinogen 1:2 ratio differed between cases and controls (Table 5)***.***

Table 5: Biological risk factors for gastric cancer.

VARIABLE	CASES n=37 (%)	CONTROLS n=51 (%)	UNIVARIATE OR(CI)	P
H. PYLORI **Positive**	29 (73)	45 (88)	0.5 (0.1-1.8)	0.25
CagA (28 cases and 45 controls) **Positive**	19 (68)	31 (69)	0.8 (0.3-2.6)	0.79
PEPSINOGEN 1 **Lower than normal**	4 (11)	3 (6)	1.9 (0.3-13.8)	0.45
PEP 1:2 RATIO **Lower than normal**	18 (41)	9 (18)	3.9 (1.3-11.7)	0.009*

(*) denotes significant P value

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The performance of the urease test compared to serological testing for* H. pylori*.

Initially, the simple urease test (CLO) was used for detection of *H. pylori* infection. However, it was abandoned after 22 cases as it seemed not to be working and it is expensive. The data having been collected allowed a comparison with the serological test in order to estimate its sensitivity and specificity of the CLO test in these patients. The sensitivity was found to be 56%, with a specificity of 75%. The positive and negative predictive values were found to be 91% and 27 % respectively. This is illustrated in table 6.

Table 6: Comparison of the simple urease test and serology for detection of H. pylori

	Antibodies against *H.pylori* infection	
CLO TEST	**POSITIVE**	**NEGATIVE**
positive	10	1
Negative	8	3

DISCUSSION

It has been long believed that gastric cancer is rare in Africa, including Zambia and indeed to this day there are no clear data available on its exact incidence. This study, though hospital based has helped shed light on the fact that the problem of gastric cancer exists and more work needs to be done on the subject. As expected, gastric cancer is more common among males than females. This correlates well with what has been described in literature.[5] A fifth of the gastric cancer patients were young (less than 45 years old). HIV infection has not been found to be associated with gastric cancer and hence it does not explain the change in epidemiology that has been observed at the UTH, Lusaka. Risk factors found to be associated with gastric cancer include alcohol, smoking, and low PG 1:2 ratios. In addition, the predominant type of gastric cancer is the intestinal type, whose pathogenesis is largely influenced by environmental factors.

As in previous audits done at the endoscopy unit at the UTH, gastric cancer is a problem even among the young. The explanation for this has remained unclear as the risk factors found during this study do not seem to differ much from what described elsewhere. As expected the cases were found to have lower BMI and MUAC values than their controls. What is interesting is that the mean BMI among the cases was 20.9 which is normal. Possible explanations are that either these patient's BMIs were very high before the onset of the cancer, or these patients presented quite early with the disease or that the rate of fall of BMI is not as dramatic. This study was however, not designed to answer this question.

The clinical presentation of patients with gastric cancer is variable though there are some 'alarm' symptoms which should make one suspect gastric cancer (anaemia, dysphagia, evidence of GI bleeding or weight loss). Most of the cases in this study presented with non-specific abdominal pain, either on its own or with other symptoms. This would justify screening for gastric cancer in all patients presenting with unexplained abdominal pain, vomiting and anaemia in patients presenting to the UTH. It is also interesting to note that 3 (8%) of the patients presented with constipation as their main symptom. One had vomiting and constipation, the other vomiting weight loss and constipation and the last one abdominal pain and constipation. This result echoes the diversity of gastric cancer

presentation, and the clinician needs to be very alert in order to suspect gastric cancer in such patients.

The overall prevalence of HIV infection found in this study was lower than the national prevalence of 14 %. This can be explained by the fact that the average age of the patients enrolled was 57 years and in Zambia, the prevalence of HIV infection in that age group is much lower than 14%. [56] In this study, proportion of HIV infection among the young patients less than 45 years was found to be 25%. This correlates well with the demographic and health survey Zambia of 2007,[56] in which the prevalence of HIV infection in the age group 18 to 45 was found to be between 12 to 26 %, which goes further to illustrate the point that HIV prevalence differs according to the age group being considered. If HIV was the sole explanation to the changing epidemiology of gastric cancer in Zambia, then the proportion of patients with the infection would have been significantly higher among the cases. Smoking and alcohol intake were found to be associated with gastric cancer on both univariate and multivariate analysis. Information was obtained on which patients were still smoking or taking alcohol at the time of enrolment and which patients had stopped. However, the type or the duration of exposure to these risk factors was not evaluated in this study. Low socio-economic status has been shown to increase the risk factor of gastric cancer. [9] In this study, income was used as a measure to estimate the social status. Unfortunately, close to half of the respondents had irregular incomes and could not therefore state what their exact income was. Of those who responded, there was no significant difference between the income of the cases and the controls on both univariate and multivariate analysis.

The prevalence of *H. pylori* infection in Zambia is well known to be high [33] and if this was the only factor at play, it would be expected that the prevalence of gastric cancer to be very high as well. Overall, we have found it to be 81%, without any significant difference between the cases and the controls. It appears to be slightly lower in the cases which can be explained by the fact that after development of gastric cancer, the stomach environment changes becoming less acidic and this is unfavourable for the growth of the bacteria. In addition, *H. pylori* clearance tends to occur with the progression to cancer and the infection may not always be present when the cancer is diagnosed, which contributes to the

underestimation of the association between the infection and cancer in studies with retrospective case- control designs. [65, 66, 67] A more accurate and valid estimate of the risk of gastric cancer in infected individuals can be obtained through prospective studies. [68] We used serum IgG levels to determine exposure to *H. pylori*; this test tends to become negative 18 months after clearing the infection. Therefore, it is not surprising that the prevalence among the cases is slightly lower. In addition, up to 11% of the cases had received antibiotics prior to presentation which could have cleared the *H. pylori* infection. A direct correlation between *H. pylori* prevalence and gastric cancer rate is not always observed at an ecological level, there are some countries that present low gastric cancer incidence despite the high frequency of infection. [69] It has also been observed that in these settings, the cancer precursor lesions like intestinal metaplasia are less frequent than the given high prevalence of intestinal metaplasia. [70]

With such a high prevalence of *H. pylori* infection in our patients, the next question was that of Cag A, which is the virulence factor. It would be logical to expect that Cag A, is more prevalent in the cases than controls. However, it was found that there is no significant difference between the two groups, something which would warrant further investigations to explain. Fernando *et al*. in 2001 found no significant difference in the prevalence of *H. pylori* infection between patients with or without gastroduodenal lesions from an urban population in Lusaka. They also found no evidence that CagA was associated with the development of gastoduodenal pathology. [33] These findings relate well with what was found in this study suggesting that there might be other factors at play in the pathogenesis of gastroduodenal diseases including cancers.

Low serum pepsinogen 1 levels have been known to correlate with gastric atrophy, but recently, low pepsinogen 1 to 2 ratios have been shown to correlate even better. In this study, both cases and controls showed similar proportions of patients with low pepsinogen 1 levels. However, there was a significant difference with the pepsinogen 1 to 2 ratio, with the cases having a much higher proportion. As gastric atrophy is believed to be a precursor lesion to gastric cancer, these results suggest that pepsinogen 1 to 2 ratios might be a better serological marker of gastric atrophy in our patients, and this finding fits with Correa's model. An interesting observation is that 18% of the controls had serological evidence of

gastric atrophy. This suggests that there are some unexplained factors at play that cause this atrophy in otherwise healthy individuals and it calls for further research.

The intestinal type of gastric cancer is mostly influenced by environmental factors. The majority of the cases in this study had this type of gastric cancer. This clearly shows the need to look in to the environment risk factors in this population as preventing them would reduce the number of gastric cancer patients. Only one of the gastric cancer patients gave history of having a relative diagnosed with the cancer. Much as this could be erroneous due to recall bias, it does give a clue as to how infrequent family history of gastric cancer is in the patients diagnosed with the disease.

There are several ways of making the diagnosis of *H. pylori* infection, among them is are the simple urease (CLO) test and serology for antibodies to *H.pylori.* Bearing in mind that both tests have some limitations, the sensitivity and specificity of the CLO test was estimated. The sensitivity was found to be very low. This is because the CLO test detects the presence of the bacteria itself, and if the patient was treated with antibiotics, it would be negative, despite the patient having been exposed to the infection. However, the serology will remain positive for longer. The specificity was 75%, which is also much lower than what would be expected.

These results appear to refute the hypothesis that HIV infection is implicated in the development of gastric cancer, but other explanations need to be found.

Study Limitations

This study however had some limitations. It was a hospital based study and the controls were selected from the endoscopy unit which has the potential to bring in selection biases. However, the prevalence of HIV infection in these controls correlated well with the expectation in this community and therefore, use of community based controls would have not changed the conclusions. This being a pilot study, the numbers used were quite small, but even then, conclusion can still be drawn from the findings but with less confidence.

Dietary factors, being one of the probable risk factors for gastric cancer in this population were not explored. Collecting data on diet could have yielded some valuable information. The duration of the exposure to the risk factors was not determined, so it remains unclear how these exposures lead to the development of the cancer, as this study was not designed to explore that.

Conclusions

In conclusion, this study showed that was no association between HIV infection and gastric cancer in patients seen at the endoscopy unit, UTH, Lusaka. A fifth of the gastric cancer cases were found in young patients, less than the age of 45 years. Smoking, alcohol and low income increase the odds of developing gastric cancer. While the cases and controls showed no difference in the levels of serum pepsinogen 1, presence of H. pylori infection and CagA, there was a significant difference between in the ratios of pepsinogen 1 to 2 ratios in the two groups. And finally, the predominant type of gastric cancer in these patients is of the intestinal type.

Recommendations

After analysing the results obtained from this study, the following recommendations have been drawn;

To fellow researchers,

1. More research needs to be done in order to understand the epidemiology of gastric cancer in this African population, and to bring out any other risk factors at play.

To the Ministry of Health

2. To improve country wide cancer registries as this will enable collection of more accurate data on the incidence of gastric cancer in Zambia
3. More endoscopy units to be set up in health centres outside Lusaka and more staff to be trained so as to improve the diagnosis of gastric cancer.
4. Increase public awareness on gastric cancer and put in programmes that will educate people on the risk factors of gastric cancer.

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APPENDIX 1

DATA SHEET

DATE: _____ ID number _____

Name _____

Sex 1_ male
2_female

Age ___ years

Marital status: 1_ single 2_ married 3_ widowed 4_ divorced 5_separated 6_co-habiting

Occupation _____ incl. none/housewife

Type of work 1_formal 2_informal 3_none

Level of education attained 0 none 1 primary 2 secondary 3 tertiary

What are your symptoms at the moment? (To enable us help the patient there and then in case there are any symptoms requiring urgent attention)

	Duration (weeks)
1 _____	_____
2 _____	_____
3 _____	_____

Do you smoke tobacco? 1_no 2_yes

If yes, how many cigarettes per day? 1_ less than five 2_five to ten 3_ more than ten to

fifteen 4_ more than fifteen to twenty 5_more than twenty.

Do you drink alcohol 1_no 2_yes

If yes, is it: 1_ every day 2_ more than three days a week 3_once a week 4_rarely 5_ none

Do you know your HIV status? 1_no 2_yes

What is it? _____

If positive: When did you first get this result? _____

Have you ever been on anti-retrovirals? 1_ no 2_yes

If yes, type, duration? _____

What other medications are you currently taking? Also indicate when you started, dose and frequency

Family history of gastric cancer 1_ no 2_ yes

At what age was it diagnosed?

APPENDIX 2

INFORMATION SHEET

You are invited to take part in a study looking at the association between cancer of the stomach and HIV infection. This study is being done as part of the requirement for a masters degree in internal medicine. Information about this study is outlined in this document. Should you need clarification on any of the information below, please feel free to ask for assistance.

Who is doing the study?

Dr Violet Jolezya Kayamba is the principal investigator under the supervision of Dr Paul Kelly and Dr. Soka Nyirenda. The study is being conducted at the endoscopy unit of the University Teaching Hospital.

What is the purpose of the study?

The aim of this study is to find out if there is any association between cancer of the stomach and HIV infection. Cancer can affect any part of the body including the lungs, breast, and liver but this study is specifically on the stomach. Cancer of the stomach usually occurs in elderly patients that are above the age of 50 years but we have noticed that at the endoscopy unit, younger patients are being found with the cancer. It is not very clear why this is the case, and we therefore have decided to find out. As you are about to have an endoscopy, we would like to ask you to consider participating in this study.

What procedure is going to be done?

We are going to take a look at your stomach using the special endoscopic instruments for which you have been referred. Before introducing the instruments, we will numb your throat and give you a pain killer in order to keep you as comfortable as possible.

If you have something which has the appearance of a cancer we will include you in the study, and possibly if your stomach has a completely normal appearance (this is not certain as we expect many more to be normal than have cancer). If you are included we will collect a blood sample (10 mls about two teaspoons full). We will also ask you a few questions

about you previous health problems. During the endoscopy, additional tiny samples of stomach tissues will be collected to see if your stomach is thinner than usual, and if you have an infection which can cause problems in the stomach. If you agree, we will be very grateful as it may help us understand why this cancer is becoming more common in young people than we thought, and this may help us work out how to prevent it.

What are we going to do with the samples we take?

Tissue samples collected from the stomach will be sent to the laboratory for evaluation. One sample will be tested for the presence of the bacteria called *Helicobacter pylori*. The blood samples will be tested for HIV, for the same Helicobacter pylori infection and for further evidence if thinning of the stomach lining (which is called atrophy).

What are the possible benefits to you?

We will see you again after two weeks to make sure that you have been appropriately followed up for whatever we found today, and we will discuss the results of these tests. It is also possible that during a research study the examination of the stomach may be more thorough than usual as we will give you sedation, and this does make the procedure more sensitive.

What are the possible disadvantages to you?

The main disadvantage of this research protocol is that it will prolong the endoscopic examination by about 2-3 minutes and the minor discomfort of a blood sample being taken. Otherwise there are no major disadvantages.

Confidentiality

Your details will be recorded on a form which will be locked away in an office here in the UTH. Your details will be entered on a computer but only in coded form and your name will not be included, only your enrolment number. Any information and results obtained will remain absolutely confidential, and other family members or work colleagues will not be granted access to this information.

The study is voluntary.

You do not have to participate in the study if you do not want to, and even if you refuse to participate in the study, you will be provided with the best care available and there will be no discrimination of any sort. If you do agree, you are also free to change your mind at any time. This research study has been approved by the Research Ethics Committee of the University of Zambia and their contact details are given below.

Contact details of the Principle Investigator: Dr Violet Jolezya Kayamba, Department of Internal medicine, University Teaching Hospital, and Lusaka. (Phone 0977 254 854).

Contact details of Research Ethics Committee: Dr Esther Munalula-Nkandu, REC office, Department of Anatomy, Ridgeway Campus, nationalist Road, Lusaka (phone 0211 256067)

APPENDIX 3

CONSENT RECORD SHEET

I confirm that I understand the information I have been given about the study. I agree to participate in the study. I confirm that I am joining the sturdy of my free will and that I can withdraw at any time without affecting the care available to me. I understand what will be required of me.

Name of Participant: ……

Signed: ……..or thumb print……....

Date: ……..

Name of Witness: ……..

Signed: ……or thumb print……..

Date: ……

I confirm that I have explained the information fully and answered any questions

Signed by Investigator: ……

Name: ……...

Date: ……

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

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15 April, 2010
Ref.: 008-02-10

Dr Paul Kelly
Tropical Gastroenterology and Nutrition Group
School of Medicine
University of Zambia
LUSAKA

Dear Dr Kelly,

RE: SUBMITTED RESEARCH PROPOSAL: **"GASTRIC CANCER IN ADULTS IN ZAMBIA: AN EXPLORATORY CASE-CONTROL STUDY"**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee meeting on 24 February, 2010 where changes/clarifications were recommended. We would like to acknowledge receipt of the corrected version with clarifications. The proposal is now approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- Obtain authority to export samples for quality control purposes from the Ministry of Health.
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Dr James Munthali
A/CHAIRPERSON

Date of approval: 15 April, 2010 **Date of expiry**: 14 April, 2011