Prevalence of *Helicobacter pylori* and its relation to Cytotoxin-associated gene A status in HIV positive and negative haematology patients

By

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Declaration:

Here by I declare that the script submitted towards a M.Med.Sc. degree at the University of the Free State is my original and independent work and has never been submitted to any other university or faculty for degree purposes.

All sources I have made use of or quoted have been acknowledged by complete references.

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T. C. Abbott
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"The LORD is my strength and my shield; my heart trusts in him, and I am helped. My heart leaps for joy and I will give thanks to him in song."
Psalm 28:7

UNIVERSITY OF THE FREE STATE

ABSTRACT

Prevalence of *Helicobacter pylori* and its relation to Cytotoxin-associated gene A status in HIV positive and negative haematology patients

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Keywords: *Helicobacter pylori, HIV, CagA, Urea Breath test, haematology patients, water source, water storage, toilet facilities and boiling water.*

Review: *Helicobacter pylori* continues to be one of the most common bacterial infections in humans and is the major cause of duodenal and gastric ulcers. The presence of the Cytotoxin-associated gene A (*CagA*) and the 40 kb pathogenicity island (PAI), for which the gene *CagA* is a marker, is associated with a more severe clinical outcome of infection. Haematological diseases are of particular interest as a few of these have been seen to be directly related to *H. pylori* infection. The relationship between *H. pylori* and HIV infections in haematology patients, will help in determining the burden of disease within HIV positive patients. **Method:** The ^{14}C Urea breath Test (UBT) was used to determine *H. pylori* prevalence, and *CagA* seropositivity was determined using a rapid *CagA* ELISA kit. Furthermore, patients were asked to complete a questionnaire collecting data for various variables. **Results:** *H. pylori* prevalence of 46%, HIV prevalence of 29% and the prevalence of IgG antibodies to *CagA* was 37%. **Conclusion:** No correlation was found between HIV and *H. pylori* or HIV and *CagA*. *H. pylori* infection is most probably acquired during childhood.

Table of Contents

List of Pictures:

List of figures:

List of tables:

Abbreviations:

AIDS	Acquired Immunodeficiency Syndrome
Anti-*CagA*	Antibodies against Cytotoxin-associated gene A
CagA	Cytotoxin-associated gene A
CagA-PAI	Cytotoxin-associated gene island
CMV	Cytomegalovirus
COX-2	Cyclo-oxygenase-2
CTL	Cytotoxic T Lymphocytes
DNA	Deoxyribonucleic Acid
DPM	Disintegrations Per Minute
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
GIT	Gastrointestinal Tract
HIV	Human Immunodeficiency Virus
H. pylori	*Helicobacter pylori*
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
IFNGR1 Chain 1	of the interferon-γ (INF-γ) receptor
IgG	Immunoglobulin G
IFNGR1	Interferon gamma receptor 1
ITP	Immune thrombocytopenic purpura
JNK	Jun N-terminal Kinase
MALT	Mucosa-associated Lymphoid Tissue
MALToma	MALT Lymphoma
MHC	Major-histocompatibility-complex
OAL	Ocular Adnexa Lymphoid tissue
OD	Optical Density
PAI	Pathogenicity Island
PCR	Polymerase Chain Reaction
PCP	Primary Care Physicians
PPI	Proton Pump Inhibitor
RNA	Ribonucleic Acid
RUT	Rapid Urease Test
Th	T helper cells

Tox^{+}	Toxogenic
Tox^{-}	Non-Toxogenic
UBT	Urea Breath Test
USA	United States of America
VacA	Vaculating Cytotoxin gene

1 Introduction

Helicobacter pylori continues to be one of the most common bacterial infections in humans and is the major cause of duodenal and gastric ulcers (Suerbaum and Michetti, 2002). It has also been implicated in a number of other disorders outside the digestive tract including diseases of the skin, respiratory, cardiovascular and neurological systems (Tsang and Lam, 1999; Gasbarrini *et al*, 2003). However, these associations are controversial as they have only been reported by case studies, small pilot studies and *in vitro* experiments, (Franceschi *et al*, 2006; Goodman *et al*, 2006; Suzuki *et al*, 2006a; Franceschi and Gasbarrini, 2007).

Chronic inflammation due to *H. pylori* infection has been implicated in the pathogenesis of gastric cancer and mucosa associated lymph tissue (MALT) lymphoma, but the exact mechanisms remain poorly understood (Correa, 2003). A number of studies have also reported the presence of *H. pylori* in patients suffering from autoimmune diseases particularly immune thrombocytopenic purpura (ITP) (Gasbarrini *et al*, 1998; Cines and Blanchette, 2002; Provan and Newland, 2002; Rodeghiero, 2003; Franchini and Veneri, 2003; Huber *et al*, 2003).

The presence of the Cytotoxin-associated gene A (*CagA*) and the 40 kb pathogenicity island (PAI), for which the gene *CagA* is a marker, is associated with a more severe clinical outcome of infection (Blaser, 1998). Not all *H. pylori* strains are positive for this region and its presence is noted in different populations at different global locations (Takahashi *et al*, 2004). *CagA* status is suspected of being associated with certain disorders which result from a *H. pylori* infection, for example: in countries where the population has a high prevalence of *H. pylori* infection positive for *CagA*, eradication therapy has been shown to work well in treating ITP, (Takahashi *et al*, 2004).

Human Immunodeficiency Virus (HIV) infection is a big problem in the world today. By determining what the relationship is between *H. pylori* and HIV infections, follow-up studies determining the burden of disease amoung HIV patients will be possible. Considering that HIV is a huge factor affecting the community in the Free State

province, South Africa, this information will be priceless in contributing a better understanding of the relationship between these two infections in this community. Studies done elsewhere have suggested that there is a decrease in *H. pylori* prevalence due to immunologic factors in HIV patients but no such study has been done in the Free State community, South Africa (D'Elios *et al*, 2000).

The prevalence of *H. pylori* varies greatly between countries and amongst population groups within the same country due to differing socio-economic status (Malatey and Graham, 1994; Feldman, 2001). The prevalence amongst middle-aged adults is over 80% in many developing countries, as compared to the 20% - 50% in industrialized countries (Suerbaum and Michetti, 2002). Only one study has been conducted in the Free State province, South Africa, on the prevalence of *H. pylori*. This study was conducted by Pelser *et al* (1997), on children in the city of Bloemfontein in the Free State province, South Africa, showing prevalence as high as 84.2% in children between 10 and 15 years of age.

The choice of a diagnostic test should be dependent on the clinical circumstances, the pre-test probability of infection, sensitivity and specificity of the test, the cost effectiveness of the testing strategy, and finally the availability of the test (Vaira and Vakil, 2001). The urea breath test relies on the abundant *H. pylori*-derived urease activity in the stomach. The ^{13}C or ^{14}C labelled urea breath test, is based on detection of ^{13}C or ^{14}C labelled CO_2 in expired air as a result of *H. pylori* urease activity (Campbell and Suliva, 2002). It quantitatively detects active infection with a sensitivity and specificity of 94.7% and 95.7% respectively (Vaira and Vakil, 2001; Campbell and Sulliva, 2002). It is non-invasive, fast, relatively cheap and very effective (Howden, 1998; Arigbabu *et al*, 2004). The European *H. pylori* study group has recommended the use of UBT or stool testing in the initial diagnosis of *H. pylori* infection (Campbell and Suliva, 2002). Thanks to local availability and expertise, in this study we used UBT to identify infection.

The prevalence of *H. pylori* in a population is very important as associations between bacterial characteristics and the risk of developing disease have not yet been sufficiently outlined to guide the clinician in treatment decisions. Therefore, it is essential that the

prevalence within a specific community is known. These results will be needed to prevent and treat this infection in areas of the world where there is a high prevalence of chronic infection (Suerbaum and Michetti, 2002).

2 Literature review

2.1 *Helicobacter pylori* History

Although, the human gastric pathogen *Helicobacter pylori* (*H. pylori*), has been present for at least 3000 years (Egan and O'Morain, 2007), it was only first described by Marshall and Warren in 1984. It is one of the most common bacterial infections in humans today as it infects approximately half of the world's population (Suerbaum and Michetti, 2002). However, only 10 – 20% (Blaser, 1998) of these infected people develop symptoms of a clinically significant disease (Bravo *et al,* 2002).

H. pylori is categorised as microaerophilic, being a spiral shaped Gram negative bacterium which measures 0.5 microns in width and 3.5 microns in length (Peura, 2007). Since its initial isolation in 1983 (Marshall, 1986) substantial evidence has been gathered to prove that *H. pylori* is a major role player in peptic ulcer disease, acute and chronic gastritis, gastric adenocarcinoma, (Dunn *et al*, 1997), dyspepsia (Wang and Adair, 1999) and chronic immune thrombocytopenic purpura (ITP) (Sato *et al*, 2004). It is also a major predisposing factor in gastric lymphoma and MALT lymphoma (Marshall, 1986; Parsonnet, 1998; Suerbaum and Michetti, 2002).

Gastric cancer is the second most frequent cause of cancer-related deaths and the role of *H. pylori* infection is increasingly recognized (Suerbaum and Michetti, 2002). Chronic inflammation has long been suspected of playing a role in carcinogenesis, but although a number of hypotheses (Han and Peura, 2006) have been presented, the exact mechanisms by which inflammation causes cancer remains poorly understood (Correa, 2003).

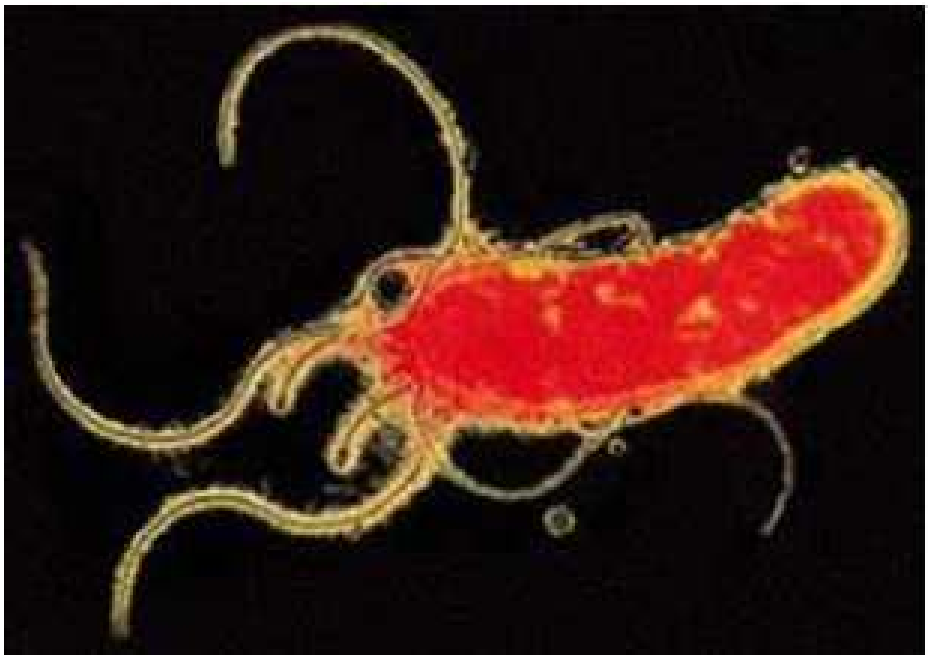
Despite the recommendation by the International Agency for research on Cancer of the World Health Organization that *H. pylori* be classified as a group I carcinogen many doctors remain sceptical about this association (International Agency for Research on cancer).

Other diseases and disorders have also been associated with *H. pylori* such as dysplasia and intestinal dysplasia and metaplasia, which may be intermediate steps in the development of gastric cancer (Kuipers and Meijer, 2000) as wells as iron deficiency anaemia (Valivaveettil *et al*, 2005) and Vitamin B12 deficiency (Dholakia, 2005).

Its role in diseases of the upper gastrointestinal tract is being investigated, (Suerbaum and Michetti, 2002) but there is still debate as to its relationship with gastroesophageal reflux disease (Axon, 2006).

However, the association between extragastric diseases and *H. pylori* are controversial, as only case reports, small pilot studies or *in vitro* data are available, (Franceschi *et al*, 2006; Goodman *et al*, 2006; Suzuki *et al*, 2006a; Franceschi and Gasbarrini, 2007).

As MALT lymphoma, ITP and iron deficiency are of particular interest these will be addressed in more detail.



Picture 1: Photo of *H. pylori* (http://www.bu.edu/bridge/archive/2004/04-02/photos/photonics.jpg)

2.2 Human Immunodeficeincy Virus and it relation to *Helicobacter pylori*

Studies have been done all over the industrialized world to try and determine the relationship between Human immunodeficiency virus (HIV) and *H. pylori* (Benz *et al*, 1993; Blecker *et al*, 1994b; Vaira *et al*, 1995; Cacciarelli *et al*, 1996; Fernando *et al*, 2001; Lichterfeld *et al*, 2002; Chiu *et al*, 2004), however, few studies have been done in Africa where HIV infection is much more prevalent. HIV infection predisposes to a multitude of opportunistic infections, many of them resulting in gastrointestinal symptoms (Sud *et al*, 2002). It is therefore not surprising that HIV seropositive patients frequently experience gastrointestinal tract (GIT) symptoms that cause considerable morbidity and are due to multiple aetiologies (AliMohamed *et al*, 2002). In HIV-positive as well as in HIV-negative patients, active chronic gastritis is predominately related to *H. pylori* infection (Benz *et al*, 1993). However, Vaira *et al* (1995) found that peptic ulcer disease is associated with *H. pylori* in HIV-1 infection. Abnormal biochemical and endoscopic findings were found to be common in HIV-positive patients with gastrointestinal symptoms (Sutherland *et al*, 1990), concluding that defects in carbohydrate absorption and ultrastructural changes may be responsible for some aspects of HIV enteropathy (Sutherland *et al*, 1990).

Chiu *et al* (2004) conducted a study in Japan which showed that AIDS patients had a lower prevalence of *H. pylori* infection but higher prevalence of Cytomegalovirus (CMV) infection (Chiu *et al*, 2004). The low prevalence of *H. pylori* infection and peptic ulcer disease in Acquired Immunodeficeincy Syndrome (AIDS) patients suggests a different role of *H. pylori* infection in peptic ulcer disease or even a different mechanism of peptic ulcerogenesis in HIV-positive subjects (Chiu *et al*, 2004).

The prevalence of *H. pylori* associated gastritis seems to be different in HIV positive and HIV negative patients (Benz *et al*, 1993). Research done in different countries has been contradicting, however, the majority of results show that there is a reduced prevalence of *H. pylori* in HIV-1 positive patients (Benz *et al*, 1993; Blecker *et al*, 1994b; Vaira *et al*, 1995; Cacciarelli *et al*, 1996; Lichterfeld *et al*, 2002; Fernando *et al*, 2001). A study done by AliMohamed *et al* (2002) showed a decrease in prevalence of *H. pylori* between

HIV seropositive patients and seronegative patients in Nairobi, Kenya. The seropositive patients have a lower prevalence by 10%, but this was found to be not statistically significant (AliMohamed *et al*, 2002). This was found to be similar in industrialised countries. Benz *et al* (1993) did a study in Germany and found that the prevalence of *H. pylori* associated gastritis in HIV positive patients was significantly reduced compared to HIV-negative controls. Vaira *et al* (1995) found similar results in Italy where *H. pylori* was found to be less common in HIV-1 positive patients than HIV-1 negative patients (Vaira *et al*, 1995).

Interestingly, it has been found that in patients with AIDS, the prevalence of *H. pylori* infection was reduced compared to HIV-1 positive patients without AIDS (Vaira *et al*, 1995). This is supported by a study initiated by Lichterfeld *et al* (2002) in Bonn. This study was done to determine whether the decreased frequency of *H. pylori* infections in HIV patients may have been associated with either the stage of the underlying HIV disease or concomitant drug regimens the patient had received. A significantly lower proportion of *H. pylori* infected individuals were observed among those HIV patients who had AIDS-defining diseases (Litcherfeld *et al*, 2002). A large yet statistically insignificant decrease of *H. pylori* infection prevalence was noted in HIV patients with an extensive decline of CD4 cell count (Lichterfeld *et al*, 2002). This suggests that the decreased *H. pylori* infection prevalence in HIV patients may be correlated to the stage of HIV-mediated immune suppression (Lichterfeld *et al*, 2002).

Fernando *et al* (2001) did a localized study of an urban African population correlating HIV status and *H. pylori* infection. The results, which showed a significant decrease in prevalence of *H. pylori* in the HIV seropositive patients, suggested that fully functional CD4 lymphocytes might be required for the genesis of gastroduodenal pathology. A study done by Cacciarelli *et al* (1996) in New York, USA, evaluated the prevalence of *H. pylori* and peptic ulcer disease in relation to absolute CD4 counts in HIV-seropositive patients with gastrointestinal symptoms. The prevalence of *H. pylori* in HIV-positive patients with CD4 count less than 200 is significantly lower than that found in HIV-negative patients. The trend for peptic ulcers was similar (Cacciarelli *et al*, 1996). These

results suggest a role of CD4 cell and immune function in sustaining *H. pylori* infection and *H. pylori*-related peptic ulcer disease (Cacciarelli *et al*, 1996).

Similarly, a study done by Fabris *et al* (1997) showed that in Italy the *H. pylori* prevalence rate was 55%; and no statistically significant differences were observed in HIV-infected subjects and those with overt AIDS. Interestingly, *H. pylori* appeared to be directly related to the peripheral CD4+ lymphocyte count, low CD4+ counts were associated with low-grade *H. pylori* infection (Fabris *et al*, 1997).

HIV positive patients without overt AIDS have increased serum levels of gastrin and pepsinogen C compared with HIV-positive patients with overt AIDS (Fabris *et al*, 2002). Subjects with hypoacidity are more likely to have positive *H. pylori* serology than subjects without hypoacidity (Shelton *et al*, 1998), indicating that a positive *H. pylori* serology was the most significant predictor of hypoacidity, accounting for an increase in gastric pH of 39% (Shelton *et al*, 1998).

The different immune responses induced by HIV infection have also been investigated to explain the low prevalence of *H. pylori* amongst HIV positive patients with overt AIDS. Despite the fact that most HIV-infected patients had IgG antibodies against other frequently encountered pathogens, none of them had a positive serology for *H. pylori*, compared to 19.2% of the control population (Blecker *et al*, 1994b). This difference was statistically significant (Blecker *et al*, 1994b).

In 1993 Benz *et al* suggested that the decreased susceptibility to *H. pylori* infection in HIV positive patients cannot be explained by the abnormal reactivity of their humoral or cellular immune response. However, Shirai *et al* (2001) showed that Th1 and Th2 cells play a central role in immune regulation during infection. In Japanese people *H. pylori* induces a Th1 cytokine response early (2 weeks) but a predominantly Th2 response later (6 weeks) in infection (Shirai *et al*, 2001). The switch is principally mediated by urease-specific CD4(+) T cells, and correlates with a loss of urease specific high-avidity Jun N-terminal Kinase (JNK) (+) Th1 and gain of low-avidity JNK(-) (possibly Th2) cells at the later stage of infection, (Shirai *et al*, 2001). This is concomitant with a 100-fold higher

colonisation level of *H. pylori* at 6 weeks than at 2 weeks, (Shirai *et al*, 2001). Differentiation of HIV gp 160-specific CD4(+) Th1 and CD8(+) cytotoxic T lymphocytes (CTL) into effector cells is impaired in 6-week infection, (Shirai *et al*, 2001). *H. pylori* infected mice immunized with vaccinia expressing gp 160, and serum IL-12 stimulated by vaccinia infection is barely detectable (Shiria *et al*, 2001). The *H. pylori* urease-mediated immune regulation in the switch from JNK(+) Th1 to JNK(-) Th2 phenotype, and the preceding low IL-12 response, are likely critical steps in the impairment of antiviral immunity (Shiria *et al*, 2001).

There are a few contradicting research results. A study performed by Battan *et al* (1990) in New York, USA, showed that in AIDS patients, *H. pylori* infection and active chronic gastritis were as common as in other patients referred for upper gastrointestinal tract endoscopy and that cell-mediated immune deficiency does not appear to increase the risk of infection with *H. pylori* (Battan *et al*, 1990). There was also no difference in the prevalence of *H. pylori* infection between patients with and without AIDS in India (Sud *et al*, 2002).

Different strains of HIV may have different relationships with *H. pylori* infection, as it is known that some HIV strains found in Africa differ from those found in the rest of the world (Louw *et al*, 2001). This could be a major factor in different immune responses in the people of Africa. In conclusion, there are many factors concerning HIV infection and *H. pylori* which still need to be investigated.

2.3 Transmission routes of *Helicobacter pylori*

The source of *H. pylori* is unknown and its mode of transmission is controversial (Lee *et al*, 1991; Rolle-Kampezyk *et al*, 2004). Transmission routes, such as water, are important in developing countries as epidemiological studies have identified drinking water as a reservoir for the bacterium (Klein, 1991; Parsonnet *et al*, 1999; Rolle-Kampezyk *et al*, 2004). Development of coccoid forms of *H. pylori* have been seen in cultures (Goodwin and Worsley, 1993) and can explain the bacteria's ability to adapt to

hostile surroundings where they appear to be more resistant, enabling the bacterium to survive outside the human gut in water or faeces. (Peura, 2006).

The fact that the bacterium has been detected in dental plaques (Nguyen *et al*, 1993), faeces (Kelly *et al*, 1994) and saliva (Ferguson *et al*, 1993) suggests a faecal-oral transmission route. Person to person transmission has been suggested by deoxyribonucleic acid fingerprinting of isolates with similar patterns being observed within families (Bramford *et al*, 1993).

A number of factors have been seen to be associated with *H. pylori* infection such as, density of housing, overcrowding, sharing a bed and the lack of running water, (Webb *et al*, 1994; Hunt *et al*, 2001). Improving living conditions in early childhood would limit the chances of faecal-oral transmission and decrease the prevalence of infection with *H. pylori* (Sathar *et al*, 1997). This is supported by Zaterka *et al* (2007), where in a study done in Brazil it was concluded that improvement in living condition was an important factor in changing the risk factors related to *H. pylori* infection.

Interestingly, *H. pylori* has been found in a few domestic animals namely primates, cats and sheep (Handt *et al*, 1994; Fox, 1995; Dore *et al*, 2001). The mechanism by which these animals were originally infected is not clear. *H. pylori* isolates were found in both saliva and gastric juices in cats (Handt *et al*, 1994) and in milk and gastric tissue of sheep (Dore *et al*, 2001) suggesting that transmission to humans may occur.

Although infection during adulthood does happen, evidence has been presented that most infections are acquired during childhood, (Parsonnet, 1995; Pounder and Ng, 1995). Re-infection by *H. pylori* after successful eradication is possible however not common, the majority of re-infections are a re-occurrence of the original bacterial infection (Peura, 2006).

Picture 2: An example of a probable source of *H. pylori* infecton (http://watersecretsblog.com/archives/children%20dirty%20water.jpg)

With reference to the transmission routes and environments dicussed above, in this study the influence of running water, socioeconomic status and toilet facilities (both currently and during childhood) will be investigated in the local Free State community.

2.4 Helicobacter Prevalence in the world

As *H. pylori* is one of the most common bacterial infections in humans today, infecting approximately half of the world's population (Suerbaum and Michetti, 2002), a large number of prevalence studies have been performed. The prevalence varies greatly amongst countries and different population groups within the same country (Feldman, 2001) with socio-economic factors playing an important role (Malaty and Graham, 1994).

In industrialized countries, the prevalence of *H. pylori* was higher if there was more than one person per room in the family home during childhood, if a bed was shared, or if there was no hot water in the house (Mendall *et al*, 1992). As a general rule, the prevalence in developing countries is higher and infection occurs earlier than in industrialized countries (Pelser *et al*, 1997; Go, 2002)

The prevalence amongst middle-aged adults is over 80% in many developing countries, as compared to the 20 - 50% in industrialized nations (Suerbaum and Michetti, 2002). In the United States and Europe, 25 – 50% of the populations are infected while the prevalence in developing countries reaches 70 – 90%, with almost all individuals acquiring the infection before the age of 10 years (Dunn *et al*, 1997).

Epidemiological studies have shown that transmission occurs predominantly within families and most probably from mother to child, (Tindberg *et al*, 2001). The infection is acquired through oral ingestion of the bacterium and is mainly transmitted within families in early childhood (Feldman, 2001).

The importance of age and gender has been disregarded by some (Henriksen, 2001). Although one study clearly indicated a sex difference, as 41% of male patients and 13% of female patients had a duodenal ulcer when tested (Glupezunski *et al*, 1991) it is not certain whether this can be directly linked to *H. pylori* infection only. However, the importance of age distribution among patients with peptic ulcer disease in general cannot be excluded as a contributing factor in the skewing of data (Henriksen, 2001).

Studies have shown a correlation to race, however, this may be partially related to socio-economic factors (Smoak *et al*, 1994; Everhart *et al*, 2000). In a study performed in Brazil, Zaterka *et al* (2007), describe that an improvement in living conditions is an important factor in changing the risk factors related to *H. pylori* infection.

2.4.1 *Helicobacter pylori* prevalence in Africa

Prevalence rates, although consistently high, vary among African countries and show a much higher prevalence of *H. pylori* infection than in industrialized countries (Glupezunski *et al*, 1991; Lule *et al*, 1991; Holcombe *et al*, 1992; Louw *et al*, 1993; Gangaidzo *et al,* 1995; Pelser *et al*, 1997; Sathar *et al*, 1997; Becker *et al*, 1999; Chokunonga *et al*, 1999; Henriksen, 2001; Mc Farelane *et al*, 2001; Mitchell *et al*, 2002; Huang *et al*, 2003; Asrat *et al*, 2004; Mosane *et al*, 2004). Due to the association between *H. pylori* and gastric cancer, data for *H.* pylori prevalence has been collected for many of the studies from cancer registries in the various African countries (Chokunonga *et al*, 1999). However, there are few established population-based cancer registries (Chokunonga *et al*, 1999) and data obtained from these and other sources have recognized deficiencies (McFarelane *et al*, 2001).

In general, a high prevalence of *H. pylori* infection was also found in South Africa (Sathar *et al*, 1997). Louw *et al* (1993) showed that in South Africa the *H. pylori* prevalence differed between ethnic groups and that socio-economic differences do indeed have an influence on *H. pylori* prevalence within a population (Louw *et al*, 1993). In the Sowetan population, South Africa, 80% of children were infected with *H. pylori* by the time they reached 10 years of age (Sathar *et al*, 1997). An important study done in Umlazi, Durban, Kwa-zulu Natal, South Africa, showed a 50% seropositivity rate in infants under 6 months of age. Another study showed that 72% of mothers and 14% of children aged between 6 months and <15 years were *H. pylori* seropositive (Mosane *et al*, 2004). In the Bloemfontein area, Free State, South Africa, where the current study was performed, children between 2 and 5 years of age had a 48.5% prevalence; children from 5 to 10 years of age a 67.3% prevalence and children between 10 and 15 years of age an 84.2% prevalence for *H. pylori* (Pelser *et al*, 1997). The high prevalence of seropositivity at birth shown by Sathar *et al* (1997) indicated a high prevalence in the mothers and the subsequent drop in seropositivity shown in the other studies could be attributed to the natural regression of maternally acquired *H. pylori* IgG antibodies (Sathar *et al*, 1997). At age 10 years or older, children infected with *H. pylori* were around eight times more likely to have a seropositive mother (Mosane *et al*, 2004).

This study done by Mosane *et al* (2004) supports other studies in showing that an increase in prevalence correlated with an increase in age (Pelser *et al*, 1997). Similarly, Olivier *et al* (2007) showed that in the Venda population, South Africa, 84% of stomach biopsies tested positive for *H. pylori*. Despite this high prevalence in a survey conducted by Hunag *et al* (2003) the pathological role of *H. pylori* was of significantly less concern in South Africa than anywhere else.

To Ascertain whether treatment of people who have been long-term residents in Africa should be modified on return to their country of origin, Becker *et al* (1999) did a study on the prevalence of *H. pylori* in missionaries residing in developing countries. The results showed that long-term residents in developing countries have a relatively higher risk of *H. pylori* infection than in other countries (Becker *et al,* 1999). Eighty percent of adults in developing countries are predicted to be *H. pylori* positive (Suerbaun and Michetti, 2002). This may have implications for the diagnosis and treatment of gastrointestinal complaints in these people (Becker *et al*, 1999). This suggested that these patients may need to be treated with medication which is effective in the place where the organism was acquired (Becker *et al*, 1999).

2.4.1.1 The "African enigma"

The contradiction of a high prevalence of antibodies to *H. pylori* infection in the presence of a low occurrence of gastric cancer in developing countries has been termed the "African enigma" (Holcombe *et al*, 1992; Louw *et al*, 2001; Campbell and Suliva, 2002). The reasons for the "African enigma" are unclear but suggestions have been made relating to the hosts' immunological defences, environmental factors such as food and water, and the virulence of different strains of *H. pylori* (Louw *et al*, 2001; Bravo *et al*, 2002).

There has been considerable comparative research done on the genetics of *H. pylori* and strains found in industrialised countries and those found in developing countries (Bamford *et al*, 1993; Yamaoka *et al*, 1997; Covacci *et al*, 1999; Letley *et al*, 1999; VanDoorn *et al*, 1999; Salama *et al*, 2000; Louw *et al*, 2001; Campbell and Suliva, 2002;

Mitchell *et al*, 2002; Raeiszadeh *et al*, 2003; Thye *et al*, 2003). Louw *et al* (2001) suggesting that "rather than a lack of association between infection with *H. pylori* there are significant differences between the strains associated with gastric cancer and non-ulcer dyspepsia in African populations".

It has been discussed that the immune response of the host could be a factor determining whether the infection will be symptomatic or asymptomatic. The development of gastric atrophy requires a Th1 response in the gastric mucosa, which is associated with an IgG2 response to *H. pylori* antigens in the peripheral blood (Campbell *et al* 2002). Campbell *et al* (2002) proved that Gambian adults and children have a predominately Th2 response to *H. pylori* which may explain the lower incidences of gastric atrophy. However, the progression of chronic *H. pylori* gastritis is the same in Africa as in Europe and South America suggesting that the path of disease is not altered (Kuipers and Meijer, 2000). The mean age of African ulcer patients is, however, younger than those in industrialized countries. This as suggested by Henriksen (2001), can be explained by "a reduced life expectancy and early acquisition of infection" among Africans.

In some populations subjects are exposed to many antigens early in life, and the question should be asked whether the immune response to *H. pylori* infection might be altered in these populations (Mitchell *et al*, 2002). Future studies in developing countries determining the direct effect of common parasites and viral infections on host immune systems need to be further explored. For example: Helminth infections may have an effect on the immune response to *H. pylori* and disease development (Mitchell *et al*, 2002). There is a close association in epidemiology between *H. pylori* and the hepatitis A virus due to similar transmission routes of infection, which may also effect host response and disease development (Sathar *et al*, 1994). Furthermore, HIV seropositive patients show a decreased prevalence of *H. pylori* suggesting that fully functional CD4 lymphocytes may be required for the genesis of gastroduodenal pathology (Fernando *et al*, 2001). This again suggests that the host's immunological response to *H. pylori* may determine the pathogenicity of the bacterium, where the high HIV prevalence in Africa could be an explanation for the lower gastric ulcer and cancer

rates observed. Hatakeyama (2004) speculates that dietary habits and strain diversity in *H. pylori* could be the result of the difference between asymptomatic and symptomatic patients in Japan and Korea when compared to the African nations.

There are, however, more practical explanations for the "African enigma". Firstly, those who live in developing countries tend to have shorter lifespans than those living in industrialised countries and may simply die before any significant *H. pylori* morbidity has occurred (Asrat *et al*, 2004). Secondly, the availability of access to medical care in many parts of rural Africa is limited, with many patients not diagnosed. The results in a study conducted by McFarelane *et al* (2001) suggested that there is indeed under-diagnosis among those over 65 years of age - assuming that most of the clinical diagnoses are genuine stomach cancers. Thirdly, registration of death is often far from complete, and in some registries many cancer deaths are recorded as "unknown primary", indicating that the quality of data is likely to be poor with many poor and elderly patients dying at home without seeking medical services (Smith, 1992). An example of this can be seen in Kenya where there are few established cancer registries (Chokunonga *et al*, 1999) and data obtained from these sources have recognised deficiencies (McFarelane *et al*, 2001). Completeness may, however, be better in some urban African populations (Parkin *et al*, 2001). Lastly, cancer is often seen by the clinician in Africa at an advanced stage, when histological confirmation of *H. pylori* is of academic interest only, and a needless burden to the patient (McFarelane *et al*, 2001).

Another interesting conclusion which McFarelane (2001) and his co-workers came to was a seemingly higher rate of cancer found in populations living at higher altitudes. It has been suggested that the increased incidence in highland regions may be due to volcanic soils (Kitinya *et al*, 1988) or to an interaction between *H. pylori* and the immune response to malaria (Blaser *et al*, 1993). The effect might also be the result of better soils and climate at higher altitude promoting improved socio-economic status, and leading to better access to health care (McFarelane, 2001). A case-control study of gastric cancer conducted between 1968 and 1972 reported a relative risk of gastric cancer of 2.1 for subjects born 2000 m or higher above sea level, compared to 0.47 for subjects born between 1000 and 2000 m above sea level (Bravo *et al*, 2002).

The African situation needs to be taken seriously, not only because of the implications for the African nations but also because there are epidemiological lessons to be learned from Africa (Henriksen, 2001).

2.5 Genetics of *Helicobacter pylori*

Different genotypes of *H. pylori* have been observed in different locations around the world (Raeiszadeh *et al*, 2003). Identification of these genotypes may be important for understanding the clinical outcome of infection, the efficacy of antibiotic treatment, laboratory diagnosis, and possibly human migration patterns (Raeiszadeh *et al*, 2003). *H. pylori* bacteria are genetically diverse due to high rates of recombination and transmission from mother to infant, resulting in large differences among ethnic groups in the frequencies of alleles at loci associated with progression to disease (Spratt, 2003). This could over the long term lead to different human populations being colonised by distinct sets of *H. pylori* genotypes (Spratt, 2003). The genotype of a resident *H. pylori* isolate accumulates many very small replacements from genetically distinct co-infecting isolates, which rapidly diversifies its genome (Falush *et al*, 2001). High rates of recombination results in all *H. pylori* isolates having different genotypes but provide DNA replacements which are from the same population as resident *H. pylori* (polymorphisms) (Spratt, 2003).

Evidence is accumulating that genes of the bacterium *H. pylori* may provide information about the origins of their human hosts (Spratt, 2003). It is, however, the introduction of different sets of polymorphisms from other populations that may confound attempts to link *H. pylori* sequences to particular human populations (Spratt, 2003).

The likely importance of the host response in relation to *H. pylori* associated disease has been underlined by Alm and Trust (1999) who in a comparison of the whole genome sequence of two unrelated strains of *H. pylori* derived from patients with different disease profiles, showed the genetic make-up of these strains to be highly conserved. This led them to conclude that human host factors may play a significant and perhaps

unappreciated part in susceptibility to the clinical outcome of infection. Twin studies have shown a substantial genetic component in several lines of evidence indicating a genetic influence on the susceptibility to *H. pylori* (Mataly *et al*, 1994).

2.6 Virulence Markers of *Helicobacter pylori*

"The criteria for virulence factors include evidence of an association with disease or a disease surrogate such as severity of mucosal inflammation, epidemiology consistency, and biologic plausibility" (Lu *et al*, 2005). According to Lu *et al* (2005), because of the definition given above of a virulence factor, only "the cytotoxin-associated gene pathogenicity island, the outer membrane inflammatory protein, the duodenal ulcer-promoting gene, and possibly the blood group antigen adhesion" are the only factors to date that qualify as virulence factors (Lu *et al*, 2005).

However, other literature has made reference to the virulence markers associated with disease as the presence of the gene *CagA* (Cytotoxin-associated gene A) and the 40 kb pathogenicity island (PAI) for which the gene *CagA* is a marker, the s1/m1 and s1/m2 types of the vacuolating cytotoxin gene, *VacA*, and the types of the epithelial contact-induced gene, *IceA*. [*IceA1*, *IceA2B/C*, *IceA2D* and *IceA2E*] (Censini *et al*, 1996; Kidd *et al*, 2001). Although in a number of populations, disease-specific associations have been demonstrated for the above putative virulence factors, (Graham and Yamaoka, 2000), only *CagA* has been linked to a more severe *H. pylori* clinical outcome (Bhat *et al*, 2005).

It is important to remember that virulence determinants such as *VacA*, *CagA* and *Ice-A* are not present in all *H. pylori* strains (Covacci *et al*, 1999). Bravo *et al* (2002) suggest that virulence associated genes of *H. pylori* may partially explain the "African enigma", with other factors such as human genetic polymorphisms and diet also possibly playing a major role.

Henriksen (2001) proposed that repeated infections by different strains of *H. pylori* could increase the risk for accumulation of virulence factors and thus increase disease risk. Henriksen went further to say that it is important to treat these patients accordingly, not

hampered by misinterpretation of African epidemiology as there is no "African enigma" according to him.

A previous study on South African *H. pylori* isolates, has shown the universal presence of *CagA* but have differences in the *VacA* alleles which correlate with clinically significant disease (Kidd *et al*, 2001b). Analysis of *IceA* allelic types is also useful in South Africa (Kidd *et al*, 2001b). Certain combinations of virulence factors may therefore provide excellent negative markers for the development of disease (Kidd *et al*, 2001b).

2.6.1 Cytotoxin-associated gene A (*CagA*)

Cytotoxin-associated gene A (*CagA*) is a marker whose presence is associated with a more severe clinical outcome (Blaser, 1998; Bhat *et al*, 2005). Cytotoxin-associated gene A positive strains induce more severe inflammatory damage in the gastric mucosa, resulting in severe gastro-duodenal disease, as compared to *CagA* negative strains (Ali *et al*, 2005; Bhat *et al*, 2005). The association between *CagA* and disease states will need to be further explored, especially for patients who have diseases with a known link to *H. pylori* infection such as ITP (Suzuki *et al*, 2005).

The cytotoxin-associated gene pathogenicity island (also referred as to the Cag-PAI) is an approximately 40 kb cluster of genes (Censini *et al*, 1996; Kidd *et al*, 2001; Backert *et al,* 2004). The *CagA* protein by itself does not induce inflammation in experimental animals, and it is not clear which genes encoded by the pathogenicity island are responsible for the inflammation (Covacci *et al*, 1999). *Helicobacter pylori* strains containing the *CagA* pathogenicity island are associated with increased interleukin (IL)-8 production and inflammation, and an increased risk of a symptomatic outcome such as peptic ulcer disease or gastric cancer (Graham *et al*, 2004; Ali *et al*, 2005). The *H. pylori CagA* pathogenicity island encodes a secretory system that translocates *CagA* into epithelial cells, where it becomes tyrosine phophorylated and induces cytoskeletal rearrangements (Argent *et al*, 2004). Argent *et al* (2004) proved that *H. pylori* strains with more phosphorylation motifs induce higher levels of *CagA* phosphorylation in epithelial cells, induce more cytoskeletal changes, and are more likely to be associated

with gastric cancer. Hatakeyama (2006) also explains that *CagA* activities in the cells can promote the multiple genetic changes involved in malignant transformation and that further studies are needed in this area.

Another marker studied in populations in which *CagA* is ubiquitous is the length of the 3' portion of *CagA* with larger fragments appearing to be more closely associated with disease (Yamaoka *et al*, 1998). However, in a study conducted by Ali *et al* (2005) it was shown that partial deletions of the CagA-PAI appear to be sufficient to render *H. pylori* less pathogenic (Ali *et al*, 2005).

Cytotoxin-associated gene A positive strains were observed in South Africa at a frequency very close to that for strains from the United States (Letley *et al*, 1999). This was documented in a study conducted by Letley *et al* (1999) where 11 of 16 patients were *CagA* positive. Letley *et al* (1999) also investigated the relation between *CagA* positive strains in South African and Asian countries to find that, all the strains studied from Asia were *CagA* positive, as confirmed in a study conducted by Atherton *et al* (1996) (Letley *et al*, 1999).

2.6.2 Vacuolating cytotoxin gene (*VacA*)

The majority of *H. pylori* strains express the 95 kDa vacuolating cytotoxin gene (*VacA*), a secreted exotoxin (Suerbaum and Michetti, 2002). The toxin inserts itself into the epithelial-cell membrane and forms a hexameric anion-selective, voltage-dependant channel through which bicarbonate and organic anions can be released (Suerbaum and Michetti, 2002). Vacuolating cytotoxin gene is also targeted to the mitochondrial membrane, where it causes release of cytochrome *c* and induces apoptosis (Suerbaum and Michetti, 2002).. Vacuolating cytotoxin gene negative mutants can colonize in animal models, and strains with inactive *VacA* genes have been isolated from patients, indicating that *VacA* is not essential for colonization (Salama *et al*, 2001). However, *VacA*-negative bacteria were out-competed by wild type bacteria in a mouse model, indicating that *VacA* increases bacterial fitness in the model used (Salama *et al*, 2001).

Vacuolating cytotoxin gene alleles vary between toxogenic (Tox^+) and non-toxogenic (Tox^-) strains, the differences being most marked in the region encoding the signal sequence and the mid-region of the gene (Artherton *et al*, 1995). Vacuolating cytotoxin gene alleles of strains from the United states, Europe and Asia are mosaics consisting of any combination of three signal sequence types (s1a, s1b or s2) and two mid-region types (m1 or m2), with the exception of s2/m1 (Artherton *et al*, 1995). Vacuolating cytotoxin gene s1a alleles are associated with peptic ulceration more frequently than those with s1b or s2 alleles (Artherton *et al*, 1997). The mosaic structure of *VacA* could be explained by stepwise acquisition of stretches of DNA as single isolated events followed by clonal expansion or by acquisition of DNA and subsequent recombination between *VacA* alleles among *H. pylori* strains (Letley *et al*, 1999). Vacuolating cytotoxin gene diversity among *H. pylori* strains is clinically important because the scarcity of pathogenic strains could explain the "African enigma" (Holcombe, 1992).

Vacuolating cytotoxin gene subtypes have a particular geographic distribution (Van Doorn *et al*, 1999). Subtype s1a is predominant in populations of European ancestry (Van Doorn *et al*, 1999). Subtype s1b is predominant in Africa and is also very frequently found in Portugal, Spain, and Central and South America. In France, Italy, and the United States, the frequency of s1a and s1b genotypes is similar (Bravo *et al*, 2002).

The absence of the *VacA* s1a allele among isolates from black and mixed-race subjects from South Africa contrasts with the finding of s1a alleles among all Asian strains studied (Letley *et al*, 1999). The prevalence of type s1a *VacA* alleles among South African strains was also significantly less than that among strains from the United States, where a more even spread of strains with the three different signal types was found (Letley *et al*, 1999). In contrast to this finding for the *VacA* signal region, both *VacA* mid-region types were observed amongst South African strains, as was the case for strains from Asia and North America as well as a natural existence of a strain with *VacA* s2/m1 genotype (Letley *et al*, 1999). The finding that all combinations of *VacA* signal sequence and mid-region do occur naturally strongly supports the concept of

recombination occurring between *VacA* genes *in vivo* to create the mosaic gene structures observed (Letley *et al*, 1999).

2.6.3 Epithelial contact-induced gene (*IceA*)

A novel *H. pylori* gene, epithelial contact-induced gene (*IceA*), was identified following transcriptional up-regulation on contact with gastric epithelial cells (Peek *et al*, 2001). Epithelial contact-induced gene exists as two distinct genotypes, *IceA1* and *IceA2*, and only *IceA1* RNA is induced following adherence *in vitro* (Peek *et al*, 2001). The size of *IceA2B* and/or *IceA2C* is 229 bp and *IceA2D* is 334 bp (Kidd *et al*, 2001a). Gastric cancer isolates are distinguishable by *VacA s1*/*IceA1* compared with patients without diseases (Kidd *et al*, 2001a).

Although *H. pylori IceA1* demonstrated strong homology to a restriction endonuclease *nla*IIIR in *Neisseria lactamica* (Figueiredo *et al*, 2000), an *in vivo* carriage of *H. pylori IceA* strains has been reported to be associated with peptic ulceration and enhanced acute neutrophilic infiltration (Van Doorn *et al*, 1998). Results from a study done by Donahue *et al* (2000) showed that the *IceA1* gene does not encode a functional restriction endonuclease but probably acts as a transcription regulator. A study done by Kidd *et al* (2001) confirmed that *IceA1* does not encode a restriction enzyme and probably, as previously suggested by Donahue *et al* (2000), acts as a transcriptional regulator for downstream genes.

In contrast with *IceA1*, *IceA2* has no significant homology to known proteins and its structure reveals patterns of repeated protein cassets, (Kidd *et al*, 2001a). The genetic organisation and sequence structure of *IceA2* has been studied (Figueiredo *et al*, 2000), revealing five distinct *IceA2* subtypes. While *IceA2* strains are more prevalent among patients with asymptomatic gastritis and non-ulcer dyspepsia (Van Doorn *et al*, 1998), a statistically significant relationship between *IceA2* subtypes and disease has not yet been defined (Kidd *et al*, 2001a). The function of *IceA2* is unknown (Kidd *et al*, 2001a).

A study done in South Africa on the protein signal sequence showed secondary structures and topology for the South African *IceA2* variants (Kidd *et al*, 2001a). Of note were the observations that the *IceA2* proteins do not have a typical prokaryote signal sequence (from the signal P server), and the N terminus appears to be embedded within the cell membrane (Kidd *et al*, 2001a). The data also showed the presence of multiple *IceA* strains rather than mixed genotypes within a single strain in these cultures (Kidd *et al*, 2001a).

Kidd *et al* (2001a) also showed different distributions of strains between different disease symptoms. Epithelial contact-induced gene *2B* and *IceA2C* subtypes were found to be predominant in gastritis cases while *IceA2D* subtype is predominant in peptic ulcer disease cases. Although no differences in distribution were noted for patients with multiple *IceA2* subtypes (Kidd *et al*, 2001a), it is possible that the high prevalence of mixed strains in peptic ulcer disease patients may obscure any potential relationship between the allele and the disease (Kidd *et al*, 2001b). Strain difference in *IceA* genotypes may partially explain the differences in disease outcome associated with *H. pylori* infection in South African populations (Kidd *et al*, 2001a).

2.7 Diagnostic tests of *Helicobacter pylori*

The choice of a diagnostic test should be dependent on the clinical circumstances, the pre-test probability of infection, sensitivity and specificity of the test, the cost effectiveness of the testing strategy, and finally the availability of the test (Vaira and Vakil, 2001). *Helicobacter pylori* infection can be diagnosed by non-invasive methods or by invasive methods such as endoscopic biopsy of the gastric mucosa (Howden, 1998; Vaira and Vakil, 2001).

Invasive methods all require endoscopy and a gastric biopsy. These include histological examination, rapid urease testing (RUT), culture and polymerase chain reaction (PCR) DNA detection (Asrat *et al*, 2004).

Non-invasive methods include the urea breath test (UBT), serologic tests, stool antigen assays and chromotography (Suerbaum and Michetti, 2002; Asrat *et al,* 2004; Kaklikkaya *et al*, 2006). In a recent study, patients proved to be happy to provide a stool sample or to take part in a breath test as opposed to serological tests when informed that both the stool and breaths are more accurate than the serology tests (McNulty and Whiting, 2007).

2.7.1 Invasive tests

Endoscopy, histology, culture, rapid urease test and polymerase chanie reaction (PCR).

2.7.1.1 Endoscopy

Endoscopy is a less pleasant and the most expensive invasive test for *H. pylori* carrying risks of 0.008% mortality and 0.432% of morbidity (Quine, *et al*, 2000; Ricci and Vaira, 2007). Endoscopic *in vivo* histology using narrow band imaging, chromoendoscopy and confocal laser endomicroscopy have also been explored (Kiesslich *et al*, 2005; Uedo *et al*, 2006).

Narrow band imaging is where light rays are split, through narrow band filters, into red, green and blue rays (Ricci and Vaira, 2007). This results in light being reflected from the mucousal surfaces at different depths showing the surface microvasculature (Ricci and Vaira, 2007).

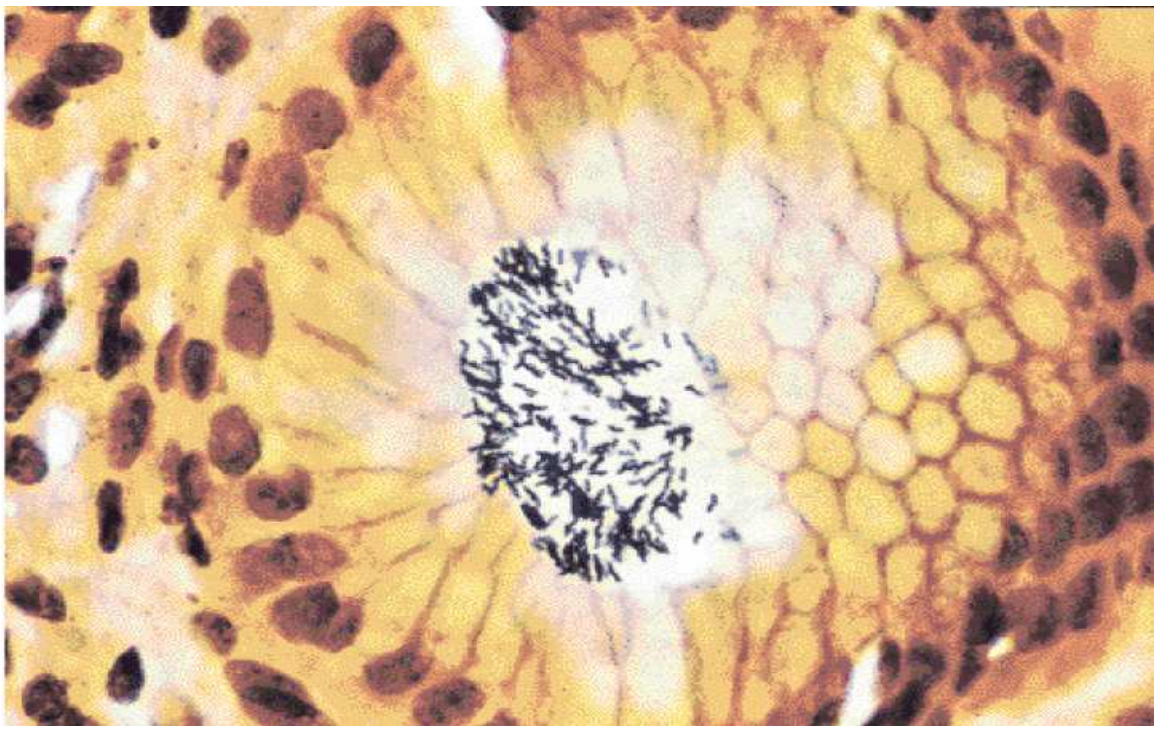
In conjunction with a contrast agent, such as acriflavin or fluorescein, a confocal laser endomicroscopy (A confocal laser microscope added to the tip of a video scope) can detect *H. pylori* infection, (Ricci and Vaira, 2007).

These techniques are however still being explored and were therefore not used for this study.

2.7.1.2 Histology

If a biopsy specimen is sampled, histological examination can be performed to not only confirm infection but to also identify the type of inflammation caused (Ricci and Vaira, 2007). This is done through staining (Ricci and Vaira, 2007).

Modified Giemsa staining has been favoured by many researchers because it is easy to perform, with a high sensitivity and is inexpensive (Wabinga, 2002; Ricci and Vaira, 2007). However, like many other stains, demonstration of the bacteria depends on its morphology (Wabinga, 2002). It has been argued in some circles that some of the organisms in the gastric mucosa may not be true *H. pylori* (Wabinga, 2002). The sensitivity of the modified Giemsa stain was 85% while the specificity was 89%. From these results the modified Giemsa staining method is recommended for diagnosis of *H. pylori* (Wabinga, 2002).



Picture 3: A histology slide of *H. pylori infection* (**http://www.pathguy.com/lectures/nejm_h_pylori.gif**)

2.7.1.3 Culture

Culture from biopsy specimens is a highly specific test for *H. pylori* (Ricci and Vaira, 2007) as it can be used to get important information on virulence factors (Krogfelt *et al*, 2005). The sensitivity of the test can, however, be jeopardised when insufficient biopsy samples are taken, when there is a delay in the transport of samples to the laboratory, the culture is exposed to an aerobic environment or through lack of experience of the laboratory (Ricci and Vaira, 2007).

Due to the challenges involved, culture testing is now largely only performed at research centres (Ricci and Vaira, 2007). However, with emerging antibiotic resistance in *H. pylori* strains there may be a need for culturing after first treatment failure (Ricci and Vaira, 2007).

2.7.1.4 Rapid urease test

The Rapid urease test (RUT) was designed to use the natural urease action of *H. pylori* as confirmation of its presence (Ricci and Vaira, 2007). The biopsy sample is tested for an increased pH using Phenol red (Ricci and Vaira, 2007). The increase in pH found in *H. pylori* positive patients is due to the urease action of the bacteria (Ricci and Vaira, 2007). The sensitivity can, however, be affected by the number of bacteria present in the biopsy. For the test to be considered positive at least 10^4 bacteria must be present (Ricci and Vaira, 2007). As it is possible for patients to harbour bacteria in numbers below this, false-negative results can arise (Ricci and Vaira, 2007). Tepes (2007) confirmed that using RUT, with two biopsy samples - one from the corpus and the other from the antrum - is reliable in evaluating *H. pylori* infection after treatment.

When compared with other invasive tests, RUT is cheaper and has comparable sensitivity and specificity (Ricci and Vaira, 2007). However, it should not be used post-treatment or in bleeding patients (Ricci and Vaira, 2007).

2.7.1.5 Polymerase Chain Reaction

Biopsy specimens, saliva, faeces and archival specimens can be used to identify *H. pylori* infection using PCR, (Bhat *et al*, 2005; Ricci and Vaira, 2007). Biopsy specimens are used for qualitative bacterial culture, from which mRNA is extracted for PCR analysis (Bhat *et al*, 2005).

Polymerase chain reaction analysis can also identify certain virulence factors, which may be useful for treatment (Bhat *et al*, 2005; Ricci and Vaira, 2007). Unfortunately, the presence of unknown factors in samples can cause ambiguities. This could be reduced by examination of single-colony isolates, however, this then increases the risk of overlooking strain variants unless numerous colonies are included (Kivi *et al*, 2005). False-negative results have been known to arise due to compounds in a biopsy having an inhibitory effect or where a very low number of bacteria are present, (Krogfelt *et al*, 2005).

Polymerase chain reaction testing is technically demanding and expensive compared to other invasive tests (Ricci and Vaira, 2007). As genotypic methods are limited by the availability of biological specimens (especially from asymptomatic individuals), and by the need to assess strain variants with serology (Yamaoka *et al*, 1998), the use of well-established serological tests is better supported (Kivi *et al*, 2005).

2.7.2 Non-invasive tests

Chromatography, serology, stool antigen assays and urea breath test.

2.7.2.1 Chromatography

This is a lateral flow chromatography test which utilizes a monoclonal anti-*H Pylori* antibody and can be bought in the form of a kit "immunoCard STAT! HpSA kit" (Meridian Bioscience, Europe) (Kaklikkaya *et al*, 2006). The lateral flow chromatography test, also

know as a chromatoFigurey test, has a 77.8% sensitivity, a 79.3% specificity, a 82.4% positive predictive value and 74.2% negative predictive value (Kaklikkaya *et al*, 2006). Research by Kaklikkaya *et al* (2006) supported this test stating that the test is "a rapid, simple, and helpful procedure not only to determine *H. pylori* infection but also to assess the success of eradication therapy" (Kaklikkaya *et al*, 2006).

Chromatography was not used for the current study due to financial limitations and the lack of experience with this test at the University of the Free State.

2.7.2.2 Serology

Serological testing is based on the detection of specific anti- *H. pylori* IgG antibodies in the patient's serum through Enzyme Linked Immunosorbent assay (ELISA) testing (Vaira and Vakil, 2001). Serologic testing has been shown to be an accurate tool for identification of *H. pylori* and is cheap and widely used (Becker *et al*, 1999; Huang *et al*, 2003).

Although the ELISA assay has sensitivity and specificity similar to those of the urea breath test, inconsistent results have been reported. The over-all accuracy of the assays averages 78% (range 68% - 82%), (Vaira and Vakil, 2001). Serological testing is of limited use in determining the success of therapy and is not reliable in young children (Suerbaum and Michetti, 2002). This is the cheaper assay to perform when looking at the non-invasive tests and is currently the most commonly used test by medical practitioners from North America and South Africa (Huang *et al*, 2003).

Mumtaz *et al* (2006) have confirmed the specificity and sensitivity of a new office-base serological test for the detection of current *H. pylori* infection with gastric histopathology. As described by Mumtaz *et al* (2006), this assay is a useful tool for rapid diagnosis of *H. pylori* in the out-patient setting (Mumtaz *et al*, 2006). However, it is not recommended as false-positive results have been known to arise when the prevalence of disease is low (Ricci and Vaira, 2007).

Cytotoxin-associated gene A has been identified as a marker whose presence is associated with a more severe clinical outcome in *H. pylori* infection (Blaser, 1998; Bhat *et al*, 2005). In a multivariate analysis Bhat *et al* (2005) showed that the presence of serum IgG antibodies to *CagA*, a virulence factor strongly linked to a more severe *H. pylori* clinical outcome, was strongly associated with measures of inflammation (Bhat *et al*, 2005). Thus the ELISA assay, using IgG anti-*CagA* antibodies, to determine *CagA* status of patients has become a standard testing method (Babu *et al*, 2005; Yilmaz *et al*, 2006; Jafarzadeh *et al*, 2007) with a sensitivity of 85% and specificity of 80 % (Yamaoka *et al*, 1998a).

2.7.2.3 Stool antigen assays

The stool antigen assay is an enzymatic immunoassay which detects the presence of *H. pylori* antigen in stool specimens (Vaira and Vakil, 2001). A polyclonal anti-*H. pylori* capture antibody adsorbed to microwells is the most widely used test but a monoclonal antibody test has been described and is under investigation (Vaira and Vakil, 2001).

Stool antigen tests for *H. pylori* provide an alternative to the urea breath test (UBT) with a sensitivity of 89% to 98% and a specificity of over 90% (Graham and Qureshi, 2001). Although being a more expensive test, the European *H. pylori* study group has recommended the use of stool testing or UBT in the initial diagnosis of *H. pylori* infection. Although UBT is the recommended test (Hirschl *et al*, 2005; Ito *et al*, 2005) the selection between the stool test and UBT will depend on the cost of the tests in individual countries, on convenience and on patient preference (Vaira and Vakil, 2001). However, due to the fact that stool tests require virtually no active cooperation on the part of the patient, it is the preferred test for paediatric patients (Suerbaum and Michetti, 2002; Hirschl *et al*, 2005)

2.7.2.4 Urea Breath test

The ^{13}C urea breath test (UBT), first described by Graham *et al* in 1987 is currently described as the most reliable non-invasive test for *H. pylori* infection and has been promoted as the "gold standard" for confirming eradication (Goddard and Logan, 1997; The European *H. pylori* Study Group, 1997; Ables *et al*, 2007). Unlike the other tests available, the breath test has also been recommended as the diagnostic test of choice in patients with a bleeding peptic ulcer, (Gisbert and Abraira, 2006).

The urea breath test relies on the abundant *H. pylori*-derived urease activity in the stomach. The ^{13}C or ^{14}C labelled urea breath test is based on detection of ^{13}C or ^{14}C labelled CO_2 in expired air as a result of *H. pylori* urease activity (Vaira and Vakil, 2001). The urea breath test quantitatively detects active infection with a sensitivity and specificity of 94.7% and 95.7% respectively (Howden, 1998; Vaira and Vakil, 2001).

The breath test can be performed by ingesting either the ^{14}C or ^{13}C solution based urea, ^{14}C or ^{13}C urea tablet or ^{14}C or ^{13}C urea capsule. Compared with the solution-based UBT, the breath test using encapsulated or tableted ^{14}C-urea and ^{13}C-urea may have disadvantageous features, in that: (i) the capsulated or tableted urea may take some time to dissolve and react to urease, and (ii) these formulations of urea may be passed out into the duodenum before dissolving (Ohara *et al*, 2004). The sensitivity, specificity and accuracy of the tablet-based method were 97.7%, 98.4% and 98%, respectively in a study conduced by Ohara *et al* (2004).

The difference between ^{13}C and ^{14}C detection is that ^{13}C is a non-radioactive isotope of ^{12}C and the results from the breath samples are counted in a mass spectrometer not a scintillation counter as for ^{14}C (Ohara *et al*, 2004).

Since certain people have indigenous bacteria with urease activity existing in the oral cavity (Ohara *et al*, 2004), when the ^{13}C test is performed and residual ^{13}C-urea is present in the oral activity, a non-specific peak of $^{13}CO_2$ ($\Delta^{13}C$) will appear in the initial phase and may lead to false-positive results (Lee *et al*, 2001). In Japan, urea breath-test

includes mouth rinsing with water immediately after the ingestion of ^{13}C-urea solution, to prevent false-positive results that are caused by oro-pharyngeal flora with urease activity (Ohara *et al*, 2004).

Arigbabu *et al* (2004) did a study in West Africa where the UBT was confirmed to be reliable enough to be used as a diagnostic test on its own. However, this relatively inexpensive test was less commonly used for diagnosing *H. pylori* infection, ranging from 0% in Africa to 39% in Europe, when compared to endoscopy and serology (Huang *et al*, 2003).

2.8 Infection of *Helicobacter pylori*

Helicobacter pylori infection may be symptomatic or asymptomatic (Sud *et al*, 2002). When an infection is symptomatic *H. pylori* colonises the human gastric mucosa leading to chronic superficial gastritis and creates a risk for peptic ulceration, gastric adenocarcinoma, and gastric lymphoma (Letley *et al*, 1999). *Helicobacter pylori* infection is chronic and may be lifelong in duration (Pelser *et al*, 1997).

After being ingested, the bacteria have to evade the anti-bacterial activity of the gastric luminal contents and enter the mucous layer (Mobley, 2001). Urease production and motility are essential for this step of infection. Urease hydrolyzes urea into carbon dioxide and ammonia, thereby permitting *H. pylori* to survive in an acidic milieu (Mobley, 2001). Furthermore, within *H. pylori* 's urease gene cluster is a specific gene, ureI, which encode for a pH dependant urea channel (Weeks *et al*, 2000). This enables *H. pylori* to maintain intracellular pH through internal movement of urea when the pH drops in the external surroundings (Peura, 2006).

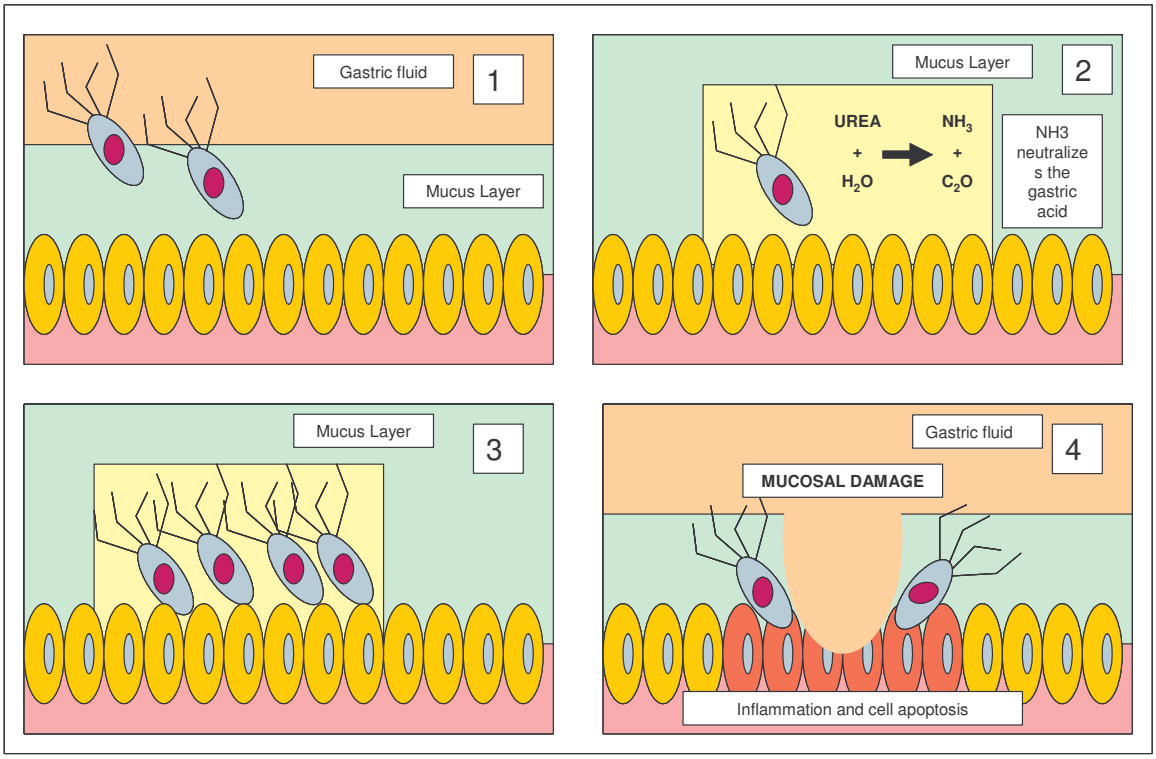
Helicobacter pylori infection induces a vigorous systemic and mucosal humoral response (Perez-Perez *et al*, 1988). In all symptomatic infected persons *H. pylori* causes continuous inflammation (Yamaoka *et al*, 1997). This is triggered primarily by the attachment of bacteria to epithelial cells (Yamaoka *et al*, 1997). The pathogen can bind to class II major-histocompatibility-complex (MHC) molecules on the surface of gastric

epithelial cells, inducing their apoptosis (Yamaoka *et al*, 1997). Host factors may also play a role, in that certain individuals may express more surface receptors making them more susceptible to *H. pylori* infection (Wadsrom *et al*, 1996).

As outlined by Konturek *et al* (2006), it is now widely accepted that *H. pylori* infection is the major cause of the inflammatory and atrophic changes in gastric mucosa. Over-expression of growth factors (e.g. gastrin), cyclo-oxygenase-2 (COX-2) and anti-apoptopic proteins including survivin and Bcl-2, lead to proliferation of mutated atrophic cells, excessive angiogenesis, inhibition of apoptosis and formation of gastric tumour (Harsch *et al*, 2001).

Helicobacter pylori gastritis with atrophy may provide a suitable environment within the gastric mucosa for the development of gastric cancer but it is also likely that other factors determine further progress towards dysplasia and cancer (McFarelane *et al*, 2000). The results of a study done by Louw *et al* (2001) argue against a simplistic association between *H. pylori* and gastric cancer. However, they lend some support to the hypothesis that the relationship between the organism and the development of gastric cancer may be more complex than the rather naïve concept of a simple association between infection and the development of disease (Louw *et al*, 2001).

Axon (2006) has hypothesized "why the complications of *H. pylori* infection and prevalence of *H. pylori* have changed over time". There is thought of a possible relation to putative increase in gastric acid secretion that may have taken place during the past 200 years (Axon, 2006)



Picture 4: The *H. pylori* infection process

2.9 *Helicobacter pylori and haematology diseases*

A number of disease have been linked to *H. pylori* (Hussell *et al*,1993, Gasbarrini *et al*, 1998; Barabino, 2002,; Cines and Blanchette, 2002; Crompton, 2002; Provan and Newland, 2002; Franchini and Veneri, 2003; Huber *et al*, 2003; Rodeghiero, 2003; Dubois, 2005; Lenze, 2006), of particular interest to this study are mucosa-associated lymphoid tissue (MALT) Lymphoma, Immune thrombocytopenic purpura (ITP) and iron deficiency anaemia.

2.9.1 Mucosa-associated lymphoid tissues (MALT) lymphoma

Mucosal surfaces and skin, are the most common entry sites of pathogens into the human body (Jønsson *et al*, 1999). In the first line of defence are secondary lymphoid tissues called mucosa-associated lymphoid tissues (MALTs) if pathogenic challenge occurs (Jonnson, 1999).

Apart from infections with external pathogens, the generation of MALT B-cell lymphomas can also be induced by autoimmune disorders (Jønsson *et al*, 1999) or chronic inflammations like *H. pylori*-induced gastritis (Lenze, 2006). A number of studies have shown an association of *H. pylori* infection with MALT lymphoma (MALToma), (Wotherspoon *et al*, 1991; Parsonnet *et al*, 1994; Eck *et al*, 1997; Stolte *et al*, 1997; Mazzucchelli *et al*, 1999), however this may be specific to unique strains only. A study has shown that "serum *CagA* IgG antibodies are more common in patients with MALToma than in the *H. pylori*-infected control group" (Eck *et al*, 1997).

Ongoing exposure of the lymphoid cells of the MALT to an antigen (chronic inflammation) may trigger excessive cell proliferation (Lenze *et al*, 2006). This in turn carries an increased risk of genetic defects (Lenze *et al*, 2006). Lenze *et al* (2006) explain that such genetic defects often induce the deregulation of apoptosis and at the end of a multi-stage process, might lead to malignant cells with uncontrolled cell growth (Lenze *et al*, 2006). *Helicobacter pylori*-induced gastritis induced tumour development on the basis of chronic MALT B-cell lymphoma (Wotherspoon *et al*, 1991). *Helicobacter pylori*-induced gastritis creates a CD4+ lymphocyte and B cell stimulation in the gastric lamina propria where these cells gather. The B cells proliferate and the T cells are activated leading to lymphoid follicle formation (Han and Peura, 2006). Although the exact mechanisms are still vague, it has been discovered that tumour-infiltrating T cells are important for the proliferation of MALT lymphoma tumour cells in the presence of *H. pylori* infection (Hussell *et al*, 1993). A hypothesis has been made proposing that the "antigen-presenting" cell interacts with the CD4+ T cells, these "activated" T cells, and B-cells then create an opportunity for unsuppressed proliferation resulting in a low-grade lymphoma (D'Elois *et al*, 1999).

This hypothesis is supported by the fact that eradication of *H. pylori* by antibiotic treatment, led to a complete remission of the associated gastric MALT lymphomas in most cases in a study conducted by Wotherspoon *et al* (1993). A study conducted by Lenze *et al* (2006) supported this evidence explaining that the remission of most gastric MALT lymphomas after eradication of *H. pylori*, links tumour cell proliferation to antigen-induced inflammation and the need for antigenic contact (Lenze, 2006). Lenze *et al*

(2006) went further to say that the majority of the tumour cell immunoglobulins displayed no binding to antigen in their study, isuggesting that the tumour Ig antibodies do not play a significant role in stimulation and proliferation of the MALT lymphoma tumour cells. Instead, activation of the tumour B cells might be mediated by other receptors participating in antigen-induced signalling (Lenze *et al*, 2006). However, in contradiction to other MALT – type lymphomas, it has been shown that *H. pylori* infection does not influence the clinical presentation of MALT-type lymphoma of the ocular adnexa (OAL) patients (Ferreri *et al*, 2006).

First-line antibiotic therapy for MALT lymphoma (MALToma) should be undertaken with caution as this is still considered experimental (Han and Peura, 2006). Not all patients undergo complete remission (Weber *et al*, 1994; Cooper *et al*, 1996), iand because of this it is recommended that tumour grade, depth and lymphadenopathy should be determined through multiple gastric biopsies and endoscopic ultrasound prior to antibiotic treatment, (Muller *et al*, 1995). In a new approach, it may be possible to identify patients (with a low-grade MALT lymphoma) who will be less likely to react positively to the *H. pylori* eradication therapy by confirming the presence of the BCL-10 gene mutation, and/or Nf-KB and/or t(11;18), (Alpen *et al,* 2000, Liu *et al*, 2001a; Lui *et al*, 2001b ; Starostik *et al*, 2002; Streubel *et al*, 2004 ; Martinelli *et al*, 2005).

Although, so much has been discovered, the exact mechanisms remain poorly understood (Correa, 2003), therefore continuous follow-up of patients who undergo *H. pylori* eradication as treatment is recommended. If the required response is not obtained with antibiotic therapy, or if relapse occurs alternative standard therapies should be considered (Sackmann *et al*, 1997).

2.9.2 Immune thrombocytopenic purpura (ITP)

Immune thrombocytopenic purpura (ITP) is an acquired bleeding disorder in which platelet destruction is mediated by anti-platelet antibodies (Tsutsumi, 2005). Unfortunately the mechanisms through which these anti-platelet antibodies develop, are still poorly understood (Higashi *et al*, 2004). The outcome of ITP is classified as acute or chronic depending on whether platelet counts return to normal within 6 months (Jaing *et al*, 2005; Jaing, 2006).

A number of studies have reported the presence of *H. pylori* in patients suffering from autoimmune diseases, particularly with ITP (Gasbarrini *et al*, 1998; Cines and Blanchette, 2002; Provan and Newland, 2002; Franchini and Veneri, 2003; Huber *et al*, 2003; Rodeghiero, 2003). Although there are studies which have found no statistically significant relationship between *H. pylori* infection and acute ITP, (Jaing, 2006) a larger number of other studies have found a correlation (Ando *et al*, 2004; Sato *et al*, 2004; Fujimura, 2005; Fujimura *et al*, 2005; Inaba *et al*, 2005; Suzuki *et al*, 2005; Tsutsumi *et al*, 2005; Veneri *et al*, 2005a; Veneri *et al*, 2005b). Therefore *H. pylori* eradication therapy has been used in an attempt to treat ITP and has proved to increase platelet counts in patients with chronic ITP (Ando *et al*, 2004; Sato *et al*, 2004; Fujimura, 2005; Fujimura *et al*, 2005; Inaba *et al*, 2005; Suzuki *et al*, 2005; Tsutsumi *et al*, 2005; Veneri *et al*, 2005a; Veneri *et al*, 2005b; Suvajdzic *et al*, 2006; Kodama *et al*, 2007).

In a randomised study no significant differences were observed in the complete remission and partial remission of ITP patients between *H. pylori* eradication therapy and proton pump inhibitor (PPI) monotherapy. However, in one study, *H. pylori* eradication therapy, although more suitable in cost performance than PPI monotherapy, was seen to be less effective (Zuniga-Noriega, 2006).

However, *H. pylori* eradication in ITP has led to speculation and further hypotheses such as, the treatment given for *H. pylori* eradication could be eradicating another bacterium which truly is the cause of ITP; or that eradication treatment could be causing a change in standing flora communities causing unwanted reactions; or that the drugs (associated

with immunomodulatory effects) used for *H. pylori* eradication could play a role (Asahi *et al*, 2006). However, a study conducted by Asahi *et al* (2006) showed that, at 12 and 24 weeks after *H. pylori* eradication, the anti-GPIIb/IIIa autoantibody response was suppressed indicating that *H. pylori* infection does play a role in anti-platelet autoantibody production.

2.9.3 Iron deficiency Anaemia (IDA)

Iron deficiency (ID) is one of the most common nutritional deficiencies in the world (Dallman, 1989). It is defined as a decrease in total body iron content, and can result in an impairment in erythropoiesis as well as immune, cognitive, and reproductive functions (Dallman, 1989). As described by Dallman in 1989, iron deficiency develops through three stages: 1) Iron depletion, 2) iron-deficient erythropoiesis and 3) iron deficiency anaemia (IDA) (Dallman, 1989).

Although not fully understood, current clinical and epidemiologic studies are implicating *H. pylori* infection as a communicable cause of iron deficiency (ID) and IDA (Crompton, 2002; Barabino, 2002; Dubois, 2005). Barabino *et al* (2002) hypothesized that gastritis increases the levels of neutrophil-derived lactoferrin, and since *H. pylori* has a lactoferrin-binding protein receptor, the infection would result in iron decreases related to bacterial turnover (Barabino, 2002).

In a large study involving 7462 participants from the United States of America (USA), Cardenas *et al* (2006) hypothesize that 13.6% cases of ID patients and 32.2% cases of IDA patients in the USA could be related to *H. pylori* infection. From this study it was concluded that *H. pylori* might cause microscopic bleeding and/or affect iron uptake and thus deplete iron stores in persons with ID and IDA, independently of ulcer disease. However, it was also noted that the associations between *H. pylori* infection and ID and IDA seem to be stronger among persons with a history of peptic ulcer disease, suggesting that ulcers probably play the larger role in that small subpopulation (Cardenas *et al*, 2006).

Cardenas *et al* (2006) also highlights the fact that the pathogenesis of iron deficiency is complex and possibly different in different age groups, "in children, iron loss seems likely, whereas in elderly persons with atrophic gastritis, low acid secretion and reduced gastric-juice ascorbic acid could result in impairment of iron absorption" (Cardenas *et al*, 2006). Nonetheless, this study has highlighted the fact that initiatives, in eradication therapy of *H. pylori* infection, are needed to further decrease the prevalence of iron deficiency and IDA (Cardenas *et al*, 2006). A number of other studies also support the proposal that *H. pylori* infection is associated with ID and IDA and that complete recovery of ID and IDA can be achieved using *H. pylori* eradication therapy (Kurekci *et al*, 2005; Valivaveettil *et al*, 2005).

In response to these studies, Mahalanabis *et al* (2005) conducted a supporting study showing that *H. pylori* eradication can result in recovery from ID and IDA in children with asymptomatic *H. pylori* infections (Mahalanabis *et al*, 2005). Mahalanabis *et al,* (2005) has, however, highlighted the difficulties in designing an adequate control strategy in a developing country population with a high rate of asymptomatic *H. pylori* infection. Mahalanabis *et al* (2005) described it as "expensive, unrealistic and of doubtful ethical justification" and therefore supports exploration of the use of different forms of iron and /or iron-absorption enhancers such as ascorbic acid (Mahalanabis *et al*, 2005).

2.10 Host immune response to *Helicobacter pylori*

Different infections patterns have been observed between industrialized and developing countries. The immune response to infectious agents leads to the expansion of particular CD4+ T helper (Th) cells subsets, Th1 and Th2 (Bellinghausen *et al*, 1999). Th1 cells are reported to produce interleukin (IL)-2, IL-12 and interferon gamma (INF-γ) and are associated with cell mediated immunity, while Th2 cells have been reported to secrete IL-4, IL-5, IL-6, IL-10 and IL-13 and are responsible for strong antibody responses including IgE-dependent allergic reactions of the immediate type (Bellinghausen *et al*, 1999).

The Th1 response is generally associated with intracellular micro-organisms including some bacteria, protozoa and fungi, whereas extracellular pathogens induce the Th2 response (Mitchell *et al*, 2002). In the industrialized countries during *H. pylori* infection, which is predominantly an extracellular infection, Th1 cells appear to predominate with IL-2, IL-12, TNF-α and IFN-γ being reported to be present in the gastric mucosa of *H. pylori* subjects (Delios *et al*, 1998). The Th2 cytokines IL-4 and IL-5 have been found to be virtually absent in *H. pylori* infected subjects in industrialized countries, although a number of studies have reported IL-10 to be present in the gastric mucosa of subjects with *H. pylori* related active gastritis, (Lindholm *et al*, 1998).

However, in developing African countries the response seems to be the opposite. Campbell *et al* (2002) showed that Gambian adults and children have a predominately Th2 response to *H. pylori* which may explain the lower incidences of gastric atrophy. A study done in Kenya in 2003 by Shmuely and co-workers showed that amongst persons with dyspepsia, the prevalence of *H. pylori* was consistently high for all ages, which yielded an unequivocal association between *H. pylori* infection and dyspepsia among persons 30 years of age or above. Mitchell *et al* (2002) suggested that because *H. pylori* is acquired early in life in developing countries it may alter the immune response to *H. pylori*. It has been suggested that in developing countries where parasitic infection is common, modulation of the immune response to *H. pylori* may occur due to infection with helminths and blood parasites (Fox *et al*, 2000).

A study done by Shiria *et al* (2001) in Japan (considered an industrialized country) may explain this phenomenon. The results contradicted previous results from industrialized countries showing the Th1 cytokine response is induced early (2 weeks) with predominantly Th2 responses later (6 weeks) in infection (Shirai *et al*, 2001). The switch is principally mediated by urease-specific CD4(+) T cells, and correlates with a loss of urease specific high-avidity Jun N-terminal Kiase (JNK), JNK(+) Th1 and gain of low-avidity JNK(-) (possibly Th2) cells at the later stage of infection (Shirai *et al*, 2001). This is associated with a 100-fold higher colonization level of *H. pylori* at 6 weeks than at 2 weeks that might tolerise high-avidity Th1 cells (Shirai *et al*, 2001). As key mediators of the T helper-1 (Th1) lymphocyte response, IFN-γ and its receptor are part of an

important signalling system in the immune response to pathogens (Shtrichman and Samuel, 2001). Studies in the mouse model of *Helicobacter felis* revealed that IFN-γ is involved in the pathogenesis of gastritis and immune protection (Sawai *et al*, 1999; Kamradt *et al*, 2000).

IgG subclass response to infection is considered to be a biomarker of the T helper cell response, IFN-γ having been shown in humans to promote the production of IgG2 subclass antibodies (Kawano *et al*, 1994). Measurement of the relative levels of anti- *H. pylori* IgG1 and IgG2 subclass antibodies in *H. pylori*-positive individuals from a industrialized country has shown a predominant IgG1 subclass response (Bamford *et al*, 1998).

In a comparative study done by Mitchell *et al* (2002) on adults and children in Soweto, South Africa, subjects were seen to have a predominately IgG1 response (IgG1/IgG2 ratio >1), while in the Australian and German subjects an IgG2 predominant response (IgG1/IgG2 ratio < 1) was observed. This adds to the evidence that the immune response in a population from a developing country sometimes differs significantly from that of industrialized countries. Previous studies, performed in developing countries, showed a Th1 cytokine profile and predominant IgG2 subclass response in patients with *H. pylori* infection (Bamford *et al*, 1998).

Thye *et al* (2003) believe that it is not antibody production but the natural history of *H. pylori* infection that is reflected by the phenotype of anti-*H. pylori* antibodies. In a study conducted by Thye *et al* (2003), IgG levels were found to be influenced by interferon gamma receptor 1 (IFNGR1) [the gene that encodes chain 1 of the interferon-γ (IFN-γ) receptor] polymorphisms. The data also suggested that IFNGR1 variants might also contribute to reduced clearance and mitigated gastric pathology of *H. pylori* in African populations. It has been suggested that asymptomatic *H. pylori* infection does not reflect a presymptomatic stage, but rather truly an asymptomatic infection (Blecker *et al*, 1994).

Virulence factors can also not be ignored when it comes to host immune responses. Serrano *et al* (2007) showed that in countries with a high *H. pylori* prevalence virulence factors and host immune response were connected.

In a multivariate analysis Bhat *et al* 2005 showed that the presence of serum IgG antibodies to *CagA*, a virulence factor strongly linked to a more severe *H. pylori* clinical outcome, was strongly associated with measures of inflammation (Bhat *et al*, 2005). The hypothesis that "colonization with an *H. pylori* population consisting predominantly of *CagA* positive strains introduces a substantially different host response than does a predominantly *CagA*-negative population" (Blaser *et al*, 1999) is therefore supported by Bhat *et al* (2005).

An IgA response has been associated with serious sequelae of *H. pylori* infection (Kosunen *et al*, 2005). In a study conducted by Kosunen *et al* (2005), irrespective of age, nearly all defined gastric diseases subjects had an *H. pylori* IgG antibody response (Kosunen *et al*, 2005). This study also looked at IgA response, where it was seen that the prevalence of IgA antibodies was highest in gastric cancer and gastric ulcer patients. Duodenal ulcer and chronic gastritis patients showed the next highest IgA prevalence, with the lowest prevalence being in the subjects who were tested without any information on possible gastric disorders (Kosunen *et al*, 2005).

Although the immune responses are different, the progression of symptomatic infection of chronic *H. pylori* gastritis is the same in Africa as in Europe and South America illustrating that the path of disease is not altered (Kuipers and Meijer, 2000).

2.11 Treatment of *Helicobacter pylori* infection:

The goal of *H. pylori* treatment is to totally eliminate the organism. This is usually not achieved with antibiotics alone (Suerbaum and Michetti, 2002). The infection is treated with a combination of acid-suppressive drugs and antibiotics (Howden, 1998). Triple or quadruple therapy with a combination of proton-pump inhibition and amoxicillin, tetracycline, nitroimadozole derivatives or clarithromycin is usually applied (Howden,

1998). Eradication therapy that effectively eliminates the bacteria also reverses the gastritis, (Morris *et al*, 1995). The treatment of asymptomatic infected persons is generally not recommended as it may cause some unwanted adverse effects (Lee and O'Morain, 1997).

Eradication is more difficult when first-line therapy has failed, usually because of poor patient compliance or the development of antibiotic resistance (Suerbaum and Michetti, 2002). A second-line of treatment is then prescribed (Suerbaum and Michetti, 2002).

First-line therapy includes proton-pump-based triple-therapy where 20 mg of omeprazole is given with either 1 g of amoxicillin and 500 mg of clarithromycin or 400 mg of metronidazole and 250 mg of clarithromycin (Suerbaum and Michetti, 2002). Otherwise a ranitidine bismuth citrate-based therapy can be applied where ranitidine bismuth citrate is administered as a dual therapy with clarithromycin for two weeks (Peterson *et al*, 1996).

Second-line therapy consists of a 10 – 14 day treatment course. The optimal strategy for retreatment after the failure of eradication has not yet been established (Suerbaum and Michetti, 2002). Culture of *H. pylori* with antibiotic sensitivity testing is usually recommended after the failure of secondary-line therapy (Bazzoli, 2001).

There has been a dramatic evolution in the history of *H. pylori* treatment, starting from a relatively single ineffective agent 16 years ago to the current most commonly used triple therapy (Huang *et al*, 2003). So-called triple therapy, combinations of one proton pump inhibitory agent with two antimicrobial agents for 7 to 14 days, have been extensively evaluated, and several regimens have been approved by the Food and Drug Administration (FDA) in the USA. Among all the triple therapies, a proton pump inhibitor, clarithromycin and amoxicillin is the most commonly used treatment combination for *H. pylori* infection (Huang *et al*, 2003). Bismuth-based triple therapies (Bismuth in association with metronidazole and tetracycline) is as effective as therapies based on proton-pump inhibitors or ranitidine bismuth citrate, even if the duration of treatment is reduced to 7 days (Houben *et al*, 1999). Triple-therapies using

clarithromycin, amoxicillin (or metronidazole) and a PPI twice a day for a week is the recommended first line therapy, (Wolle and Malfertheiner, 2007).

A one-day quadruple-course of treatment has also been shown to be effective (Treiber *et al*, 2002; Lara *et al*, 2003). This consists of bismuth subsalicylate, amoxicillin and metronidazole given 4 times, including a once off dose of lansoprazole, (Treiber *et al*, 2002; Lara *et al*, 2003). This treatment can also be considered a first line therapy (Wolle and Malfertheiner, 2007). Shorter courses of treatment have been investigated of 1 to 5 days and have demonstrated 89 – 95 % eradication rates, (Treiber *et al*, 2002)

Smoking has been shown to increase the treatment failure rate for *H. pylori* eradication, where the treatment failure for *H. pylori* in smokers in a study conducted by Suzuki *et al* (2006c) was about double compared to that in non-smokers (Suzuki *et al*, 2006c). Patients should, therefore be encouraged, at best, to stop smoking, or at least, to stop smoking during the treatment period.

Evidence that chronic gastric *H. pylori* infection is a factor in dyspepsia, peptic ulcer disease, gastric carcinoma and lymphoma has led to the suggestion that all serology-positive dyspeptic patients should be treated empirically with antibiotics to eradicate the infection even without endoscopic diagnosis (O' Keefe *et al*, 2001). The benefit of treatment to eradicate *H. pylori* in functional dyspepsia remains controversial (Laine *et al*, 2001). To manage un-investigated dyspepsia in industrialized countries, some authors recommend screening patients who are age 50 and above without severe symptoms with a non-invasive test for *H. pylori* and then treat those with positive results with *H. pylori*-eradicating drugs (Hunt *et al*, 1999). The study done by Shmuely and co-workers (2003) demonstrates that in Nakurur, Kenya, *H. pylori* infection is associated with dyspepsia, particularly in persons 30 years of age and above. Since, solid evidence exists that *H. pylori* eradication prevents the development and recurrence of gastric carcinoma and promotes regression of B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) of the stomach, the proposed test-and-treat strategy may be an efficient use of health resources in Kenya and perhaps other African countries (Montalban *et al*, 2001).

Resistance is a major determinant in the effect of anti-*H. pylori* treatment (Loffeld and Fijen, 2003). Resistance against metronidazole and clarithromycin is well known in industrialized countries (Loffeld and Fijen, 2003). Prior treatment with antibiotics for reasons other than *H. pylori* eradication and poor patient compliance are risk factors for inducing an antibiotic resistant strain in a population with a high prevalence of resistant strains (Loffeld and Fijen, 2003). A study done by Harries *et al* (1992) on the Malawian people revealed resistances to amoxycillin, tetracycline and metronidazole indicating that African patients may have to be treated differently to Europeans. This is probably due to the frequent use of nitroimidazole derivatives for infection with protozoa (Banatvala *et al*, 1994). Metronidazole resistance is invariably reported to be higher in women probably due to the use of these drugs for gynaecologic infections (European study group on Antibiotic susceptibility of *H. pylori*, 1992; Pilotto *et al*, 2000). Gholam-Hossein and Shohreh (2007) discuss *H. pylori* resistance as a probable cause of failure of *H. pylori* eradication and advise that culture and microbial susceptibility tests are needed in cases of first-line therapy failure.

The reported rise in antibiotic resistance can be explained by: (1) The use of different test methodologies (Loffeld and Fijen, 2003); (2) The inclusion of cultures from patients previously treated unsuccessfully with anti-*H. pylori* treatment (Loffeld and Fijen, 2003); (3) Poor patient compliance with treatment (Wolle and Malfertheiner, 2007); (4) Disease entities associated with *H. pylori* infection (Wolle and Malfertheiner, 2007); (5) Pharmacological activity of the drugs prescribed (Wolle and Malfertheiner, 2007); and (6) Natural resistance of *H. pylori* to the drug (Loffeld and Fijen, 2003; Pajares Garcia *et al*, 2007). In relation to the resistance build up, Woodford and Ellington (2007) explain that there is potential for mutational resistance to emerge for drugs not yet licensed and therefore feel that this should be investigated during the development process of the new drugs. Largely due to the common use of these drugs as antiparasitic drugs, a higher resistance to treatment has been seen in African, South American and Asian countries, (Pajares Garcia *et al*, 2007).

Strangely, the presence of *CagA* on a *H. pylori* strain has been linked to successful eradication (Suzuki, *et al*, 2006b). The reason is debatable, however, two main points to consider are (1) *CagA* positive strains induce more severe inflammatory damage in the gastric mucosa (Ali *et al*, 2005; Bhat *et al*, 2005), leading to an increased blood flow which could possibly help the diffusion of the antibiotics (Suzuki *et al*, 2006b), and (2) the variying growth rate between *CagA*-positive and *CagA*-negative strains, where *CagA* -positive strains proliferate more slowly and thus may be more easily eradicated as the numbers are smaller (Suzuki, *et al*, 2006b).

Interestingly, evidence suggested that ingesting lactic acid bacteria exerts a suppressive effect on *H. pylori* infection in both animals and humans (Wang *et al*, 2004). Supplementing with *Lactobacillus*- and *Bifidobacterium* containing yoghurt (AB-yoghurt) was shown to improve the rates of eradication of *H. pylori* in humans (Wang *et al*, 2004). Regular intake of yoghurt containing Bb12 and La5 bacteria effectively suppresses *H. pylori* infection humans (Wang *et al*, 2004).

Similarly, several plants have been tested *in vitro* for bactericidal and/or anti-adhesive effects on *H. pylori* (O'Mahony *et al*, 2005). O'Mahony *et al* (2005) has explained that the ingestion of plants with anti-adhesive properties could provide a potent alternative therapy for *H pylori* infection, which may overcome the problem of resistance associated with current antibiotic regimens (O'Mahony *et al*, 2005). Certain plants have been identified that can kill *H. pylori*, including: Tumeric (most efficient), cumin, ginger, chilli, borage, black caraway, oregano and liquorice (O'Mahony *et al*, 2005) Furthermore, extracts of tumeric, borage and parsley have been identified as being able to inhibit the adhesion of *H. pylori* strains to the stomach (O'Mahony *et al*, 2005). Nonetheless, resistance can develop to these plant extracts as well, as shown by O'Mahony *et al* (2005). However, if the plant extracts which inhibit the adhesion of *H. pylori* are used as a preventative, the likelihood of resistance developing in organisms is less likely than that for the plant extract used to eradicate *H. pylori* (O'Mahony *et al*, 2005). Due to uncertainties with regard to the effects of digestion on these herbal and botanical extracts, further studies are needed to investigate their efficiency *in vivo* (O'Mahony *et al*, 2005).

The last option for the control of *H. pylori* prevalence is that of prevention rather than treatment. Ford *et al* (2005) showed that despite the initial cost involved in screening and treating subjects in a community, there may be significant reductions in total dysepsia-related health costs if this is performed (Ford *et al*, 2005).

Due to the extremely high prevalence of *H. pylori* infection in the general population in many countries, a proper approach to the management of *H. pylori* is of crucial importance (Howden, 1998). The current major international and regional management guidelines have been prepared, in most cases, by specialists (Howden, 1998).

Studies have shown considerable differences between gastroenterologists and primary care physicians in beliefs and attitudes regarding the relationship between *H. pylori* infection and upper gastrointestinal diseases (Fendrick *et al*, 2003). This has led to significantly different approaches to patient management (Fendrick *et al*, 2003). It is important, however, to remember that different therapeutic approaches to *H. pylori* infected patients by some physicians may be due to the limited availability and/or access to resources (Huang *et al*, 2003). Suzuki *et al* (2007) suggested that in countries such as Japan where *H. pylori* is strongly associated with gastric cancer the necessary action may be to totally eradicate *H. pylori* from the entire population.

The primary-care physicians from South Africa seem to be less concerned about the role of *H. Pylori* and it is not clear if this may be related to the so-called "African enigma" (Huang *et al*, 2003). Interestingly, when asked about the causal relationship between the infection and upper gastrointestinal diseases, all South African respondents consider that *H. pylori* infection is related to duodenal and gastric cancer, suggesting a degree of confusion among South African primary-care physicians about the pathological role of *H. pylori* infection (Huang *et al*, 2003). The lack of association between *H. pylori* serology and upper gastro-intestinal disease in black South Africans indicates that serological investigation cannot substitute for endoscopic management of black Africans with dyspepsia, and that anti-*H. pylori* therapy cannot be administered alone (O'Keefe *et al*, 2001).

The high incidence, worldwide, of infection with *H. pylori* points to a clear need for a vaccine with the ultimate immunization target population being children as *H. pylori* is typically acquired in childhood (Graham *et al*, 2004). Vaccine studies in animal models have shown that the concept is viable and vaccines against *H. pylori* are in development (Czinn and Nedrud, 1999; Del Giudice, 2001; Del Giudice *et al,* 2001).

3 Aims of study

1) To determine and compare the prevalence of *H. pylori* in the HIV negative and positive adult patients of the academic hospital complex of the Free State province.

2) To determine the presence of *CagA* positive *H. pylori* by detection of anti-*CagA* immunoglobulin G (IgG) in *H. pylori* positive patients.

3) To determine and compare the prevalence of *CagA* positive *H. pylori* in the HIV negative and positive adult patients of the academic hospital complex of the Free State province.

4) To determine the relationship of both *H. pylori* and *CagA* (of *H. pylori* positive patients) statuses with demographic data including, but not limited to, age, sex, socio-economic status.

5) To determine the relationship of both *H. pylori* and *CagA* (of *H. pylori* positive patients) statuses with variables that may be important to infections including, but not limited to, water sources, water transportation and storage, if needed and toilet facilities.

4 Materials and methods

4.1 Study design

This was a comparative study to determine differences in prevalence of *H. pylori* infection, between HIV positive and HIV negative patients among haematology patients from the academic hospital complex in the Free State community, South Africa.

The factors investigated include, *CagA* status in *H. pylori* positive patients, *CagA* status in *H.pylori* positive patients in relation to HIV status, age, sex, race, socio-economic status, water source (both current and in childhood), transportation of water (both current and in childhood), water storage (both current and in childhood), boiling of water before consumption (both current and in childhood), toilet location (both current and in childhood) and toilet facilities

4.2 Target population

The target population was all haematology patients visiting the academic hospital complex, either in ward 29 or 30, at National hospital; as well as all patients visiting the haematology clinic based at Universitas hospital.

The original study design included all hopitalised patients in the academic hospital complex. However on further investigation it was found that most patients are on prohibited medication or HIV tests were not performed. The group of haematology patients however, had fewer patients using prohibited medication and in most cases an HIV test was performed. For this reason it was decided to focus on the haematology patients.

4.3 Sample selection

Haematology patients were selected from both the wards and clinics of the academic hospital complex of the Free State province, South Africa. In the selection of patients it was ensured that all the inclusion and exclusion criteria were met.

A blinded screening process was used to protect patient privacy and to excel the patient screening process. The attending medical practitioners were approached by the researcher who described the nature of the study and presented a list of inclusion and exclusion criteria for their review. The practitioner was then asked to identify patients who may be eligible to take part in the study **without disclosing** any patient information. This was done verbally by the medical practitioner answering non-specifically "Yes, the patient does qualify" or "No, this patient does not qualify".

All the patients, who the attending medical practitioners confirmed as eligible for the study, were then individually consulted to obtain informed consent. The informed consent form (Appendix 1) was signed by each patient after the particulars of the study were presented to them and they decided to take part in the study. The informed consent form was presented in three different native languages, English, Afrikaans and SeSotho, covering the three major languages spoken in this area.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

1) Haematology patients or patients visiting the haematology clinic.

4.4.2 Exclusion criteria

1) Patients who were younger than 18.

2) Patients who have not been tested for HIV infection and who were unwilling to be tested.

3) Patients who did not understand the requirements of the study and therefore could not give informed consent or were unwilling to give informed consent.

4) Patients who had not fasted for at least 6 hours before testing.

5) Patients who were unable to blow into the hyamine solution.

6) Patients who were currently being treated with antibiotics or bismuth, as well as patients who had been treated with these 30 days prior to testing.

7) Patients who were currently being treated with proton pump inhibitors as well as patients who had been treated with these two weeks prior to testing.

8) Patients who had undergone any recent upper gastrointestinal tract surgery.

9) Patients who were known to suffer from peptic ulcer disease.

By abiding by these exclusion criteria methodological or measurement errors were minimised.

4.5 Measurement

Patients who agreed to take part in the study and signed the informed consent form, gave permission for the researcher to (a) extract information from their file to determine whether or not the patient fulfilled the requirements of the study (b) take breath samples for the ^{14}C-Urea breath test and (c) to take a blood sample for *CagA* analysis.

All patients enrolled into the study were asked to complete a questionnaire (Appendix B). Information such as, but not limited to, living conditions and socio-economic status were recorded here.

The specific diagnosis of each patient was obtained from the records in the patient file and discussed further with his/her attending docter. Simlarily, the HIV status of the patient was obtained from the patient file. Only patients who had an HIV status were screened for this study. No HIV testing was performed for this study. Socioeconmoc status was also obtained from the patient file. This was recorded by the administration staff from the academic hospital complex.

Collection of the ^{14}C-Urea breath test samples was done by the researcher, while the analysis of the samples was done by an expert in the Department of Nuclear Medicine, University of the Free State.

The anti-*CagA* IgG ELISA was performed by the researcher in the Department of Haematology and Cell Biology, University of the Free State.

4.5.1 ^{14}C-Urea breath test

The ^{14}C-Urea breath test was performed to determine the presence of *H. pylori* infection. This test has been validated by the Department of Medical Physics, University of the Free State (Jansen *et al*, 2001).

4.5.1.1 The Diagnostic requirements for the ^{14}C-Urea breath test

1) Three scintillation flasks (small glass bottles) with 2 ml hyamine solution in each were prepared for each patient. The solution comprised of 1 ml hyamine to bind the Carbon dioxide captured, 1 ml ethanol to preserve the sample and 1 drop phenolphthalein to use as an indicator to see when the solution had captured at least 1 mmol Co_2.

2) Three straws.

3) A carbon-14-urea capsule with 1 mg urea marked with 37 kBq (1uCi) ^{14}C.

4.5.1.2 The patient procedure

1) Before the capsule was ingested the patient had to blow into the first hyamine solution with one of the straws until the colour changed from purple to colourless. The colour change confirmed that 1 mmol CO_2 had dissolved into the hyamine solution.

2) The patient then ingested the ^{14}C capsule with 20 ml warm water. Warm water was used in order to encourage the digestion of the capsule.

3) After 3 minutes another 20 ml warm water was consumed. The second consumption of warm water was to further enhance the digestion of the capsule.

4) After 10 minutes the first post-dosage test was done. The patient blew into the second scintillation solution, through a straw, until the colour changed from purple to colourless. A time window of 10 minutes was used in order to accommodate the digestion process. After 10 minutes digestion of the capsule would have started exposing the bacteria, if any, to the urea.

5) After a further 20 minutes the final post-dosage test was done. The patient blew into the third scintillation solution, through a straw, until the colour changed from purple to colourless.

4.5.1.3 The processing of the breath samples

The standardisation of the *H. pylori* breath-test for the academic hospital complex, Free State, South Africa, was performed by the Department of Nuclear Medicine, University of

the Free State. All standards used for this study are those which were determined in the standardisation study and are currently used in the academic hospital complex.

1) 10 ml Read Value scintillation fluid (Beckman Coulter So. Africa (Pty), Ltd.; Halfway House, 1685, Johannesburg, Republic of South Africa) was added to each of the scintillation flasks which were sealed well and shaken. Read value scintillation fluid was added in order to initiate the Fluorescence of the ^{14}C for *H. pylori* positive patients.

2) The sample stood for 1 – 2 hours after the addition of scintillation fluid, preferably until the next day. If the background sample had a value of 50 – 300 disintegrations per minute (DPM), it stood longer to exclude the effects of background radiation. Some background radiation was expected thus the background (or control sample) was used as an indication as to when most radiation not linked to the ^{14}C test had depleted. Thus, if a sample showed a reading of 50 – 300 DMP it was allowed to stand longer in order to release more of the background radioactivity.

3) The samples were counted in a scintillation counter. The amount of radioactive flourescence was determined by the scintillation counter.

4.5.1.4 The determination of results

DPM was calculated with the following formula:

DMP = Sample DMP – Background DMP

The background sample contained no C^{14} as the capsule was only consumed after this sample was taken, and served as the control sample.

The standard references were:

- ≤ 199 DPM = negative for *H. pylori*.
- > 199 DPM = positive for *H. pylori*.

4.5.2 Anti-*CagA* ELISA

IgG antibodies to the *CagA* protein were detected by using a rapid anti-*CagA* IgG ELISA kit **(Product code: GD33; Genesis, Cambridge, U.K.)** according to manufacturers instructions.

The anti-*CagA* ELISA was performed in one batch using the serum of the all the patients who tested positive for *H. pylori* according to the breath test. Venous blood was drawn and serum was removed from centrifuged whole blood and stored at - 80°C until used.

Serum samples were diluted 1:200 in the sample diluent buffer and incubated for 30 minutes with the recombinant *CagA* protein immobilised on microtitre wells.

After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase was added to the wells. This antibody binds to the patients' antibodies that are bound to the recombinant *CagA* protein.

The unbound conjugate was washed away and a solution containing TMB (a substrate for horse radish peroxidase) was added to the wells and the reaction was stopped with 0.25 M sulphuric acid.

Standard samples containing 0.625, 12.5, 25, 50 and 100 U/ml were used to set-up a standard curve from which the IgG antibodies in the patients' serum was could be read.

A positive and negative control were included in the test. The optical densities of the standard, controls and samples were measured using a microplate reader at 450 nm. The optical density is directly proportional to the antibody activity in the sample.

4.6 Analysis

The data analysis was done by the Department of Biostatistics, University of the Free State. Results are summarized by frequency, percentages (categorical variables) and means, standard deviations or percentiles (numerical variables). Comparisons were done by 95% confidence interval for differences in percentage, adjusting for confounders such as age if necessary.

4.7 Ethical aspects

Written informed consent forms were signed by all participants. The accompanying details of each patient are being kept confidential and the results have been presented next to a patient name code. This has been done in accordance with the Helsinki declaration of 1975, as revised in 1983. The original protocol and all protocol amendments were submitted to the Ethics Committee, Faculty of Health Sciences, University of the Free State and ethics approval was granted (ETOVS nNR 56/05).

5 Results

5.1 Sample selection

Patients were recruited from 27 June 2006 until 22 January 2007 in the haematology ward at National hospital.

The clinic recruitment started on 09 October 2006 and ran until 22 Januray 2006 in the haematology clinic.

5.1.1 National Hospital Haematology Ward 29 and 30 Patient Recruitment

For the first year of the study only hospitalized patients were screened. Although there were roughly 18 patients to be screened at a time in the ward, only one or two, if any, patients would qualify in any given week. The enrolment was slower than expected as many patients were excluded on grounds of medication more often than expected. The medications which were the most common cause of screening failure were antibiotics and proton pump inhibitors.

The attending medical practitioner was contacted telephonically twice a week, Tuesday and Thursday mornings. He/she would screen all of the available patient files in each haematology ward against the inclusion and exclusion criteria of the study to determine whether a patient was eligible for the study or not. The eligible patients were consulted the afternoon of the screening by the researcher and tested the next morning before morning tea (± 04h30 am).

As the researcher was not directly involved at the screening site, it was not possible for screening pages to be filled out for each patient screened. Thus, the number of screening failures could not be accurately calculated for the ward.

Because of the lower than expected enrolment rate, it was decided to broaden the study and include the haematology clinic patients.

5.1.2 Haematology Clinic Recruitment

A total of 131 patients were screened and recruited at the clinic over a 4 month period. Thirty-eight of these patients were enrolled into the study. The screening documentation for each patient screened (pass or fail) was recorded at each clinic visit, thus a full record of all patients screened was available.

The screening rate of the clinic recruitment as reflected in Figure 1 showed a high screening rate at each visit yet a low enrolment of patients into the study.

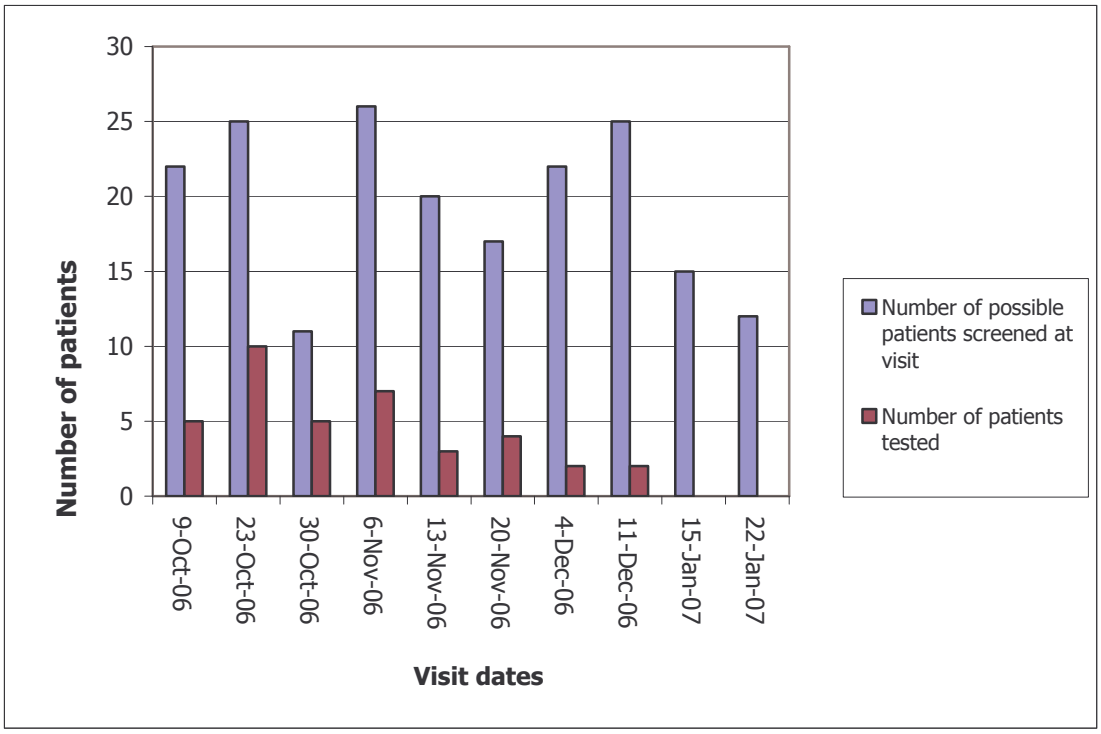


Figure 1: Number of patients screened versus number of patients enrolled into the study.

At the first 4 visits (9 Oct 2006, 23 Oct 2006, 30 Oct 2006 and 6 Nov 2006) the low enrolment rate was primarily due to (1) patients not having been tested for HIV and patients refusing to be tested for HIV and (2) patients not being fasted.

From clinic visit 5 (13 Nov 2006) onwards (20 Nov 2006, 4 Dec 2006, 11 Dec 2006, 15 Jan 2006 and 22 Jan 2006), the primary reason for the low enrolment rate was largely due to (1) patients having already been enrolled in the study on previous visit occasions (Patients generally return to the clinic on a monthly or bimonthly schedule) (2) patients not being fasted and (3) patients having been screened before.

For all visits, another reason for the low enrolment rate was patient's not giving informed consent after consultation.

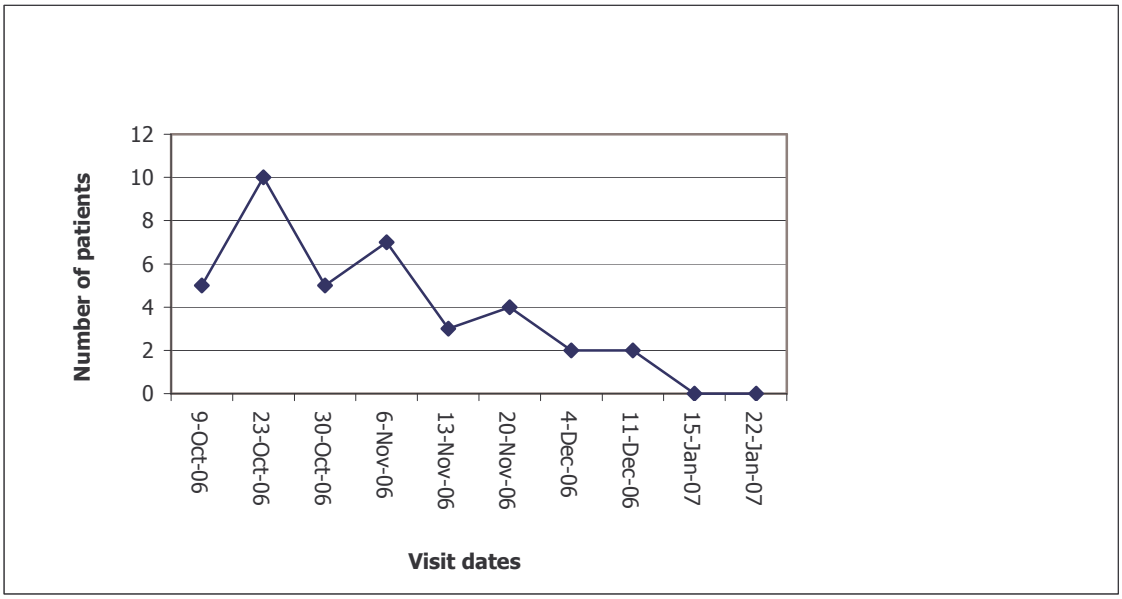


Figure 2: Number of patients tested per visit, for the clinic.

From Figure 2 it can be clearly seen that enrolment of patients at the haematology clinic had reached saturation point on 15 January 2007, where for both that visit and that of 22 January 2007. No patients were enrolled. For this reason and financial constraints and timeline limitations recruitment was terminated at this point.

5.2 Sampling group

A total of 68 patients were tested for *H. pylori*, 30 ward patients and 38 clinic patients. A total of 59 patients were included in the study analysis. One patient was unable to

complete the breath test, another confessed to being a minor after the breath samples were taken and 7 patients refused HIV testing after giving informed consent.

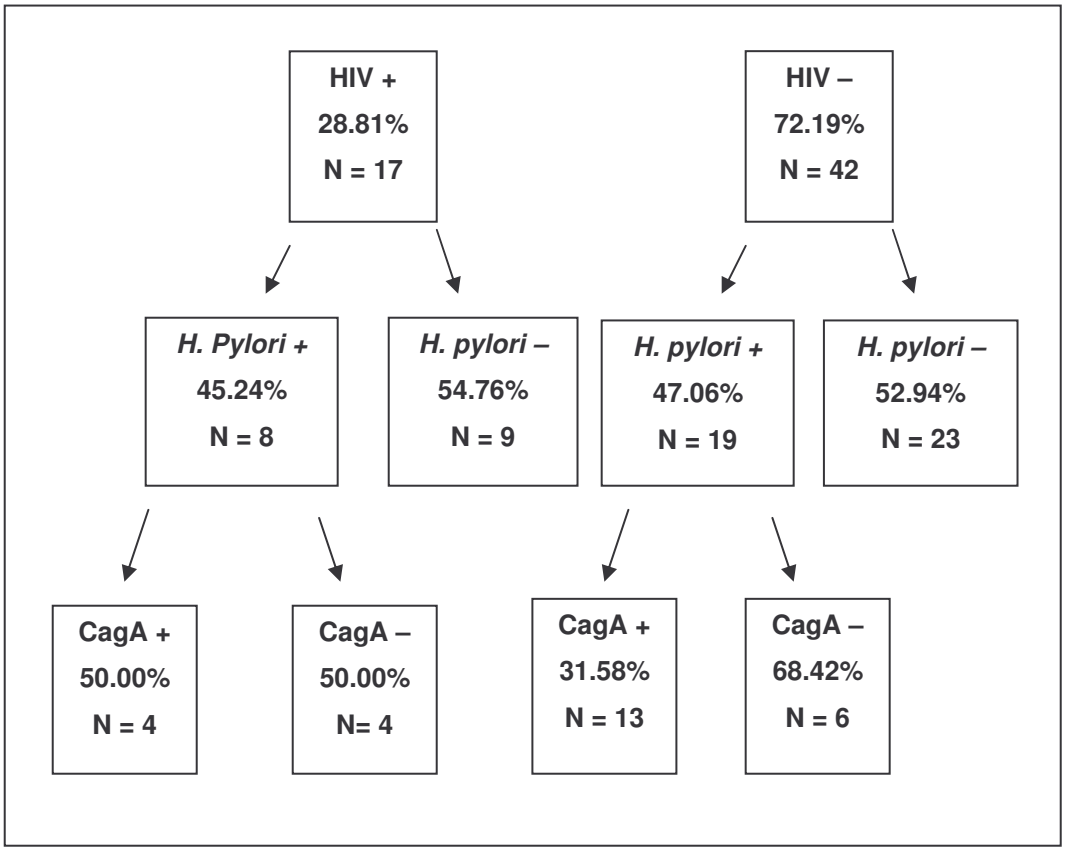


Figure 3: Patient numbers for *H. pylori* prevalence

Figure 3 summarises the main findings for this study, which will be presented in the following sections.

Fourty-two HIV negative adult patients and 17 HIV positive adult patients from the Academic Hospital Complex of the Free State Province, South Africa, were tested for *H. pylori* infection with the ^{14}C-Urea breath test. Blood was drawn from every patient, however only the blood samples from those patients testing positive for *H. pylori* were used for *CagA* analysis (Figure 3). Blood was drawn the same day that the ^{14}C-Urea breath test was done.

5.3 HIV prevalence in haematology patients

From figure 4 it is clear that the majority of patients were HIV negative.

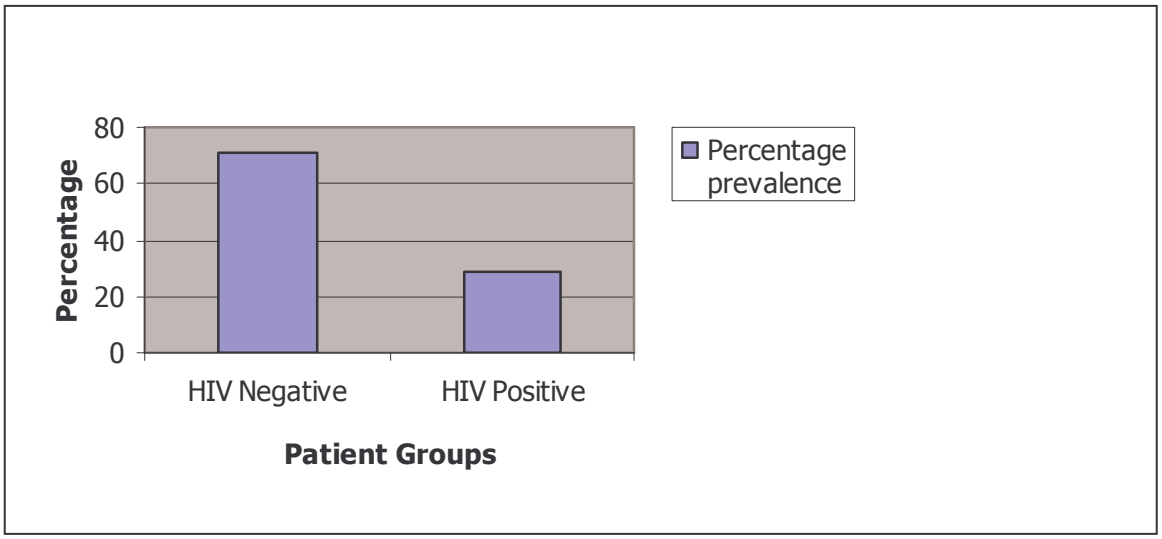


Figure 4: HIV prevalence in haematology patients

The prevalence of HIV positive patients amongst the Sampling group was 28.8% (95% Confidence interval [CI], 19% - 41%). The difference in prevalence between the HIV positive and HIV negative patients was statistically significant (P = 0.0018), (Figure 4).

5.4 *H. pylori* prevalence in haematology patients

Figure 5 illustrates a predominantly *H. pylori* negative patient group.

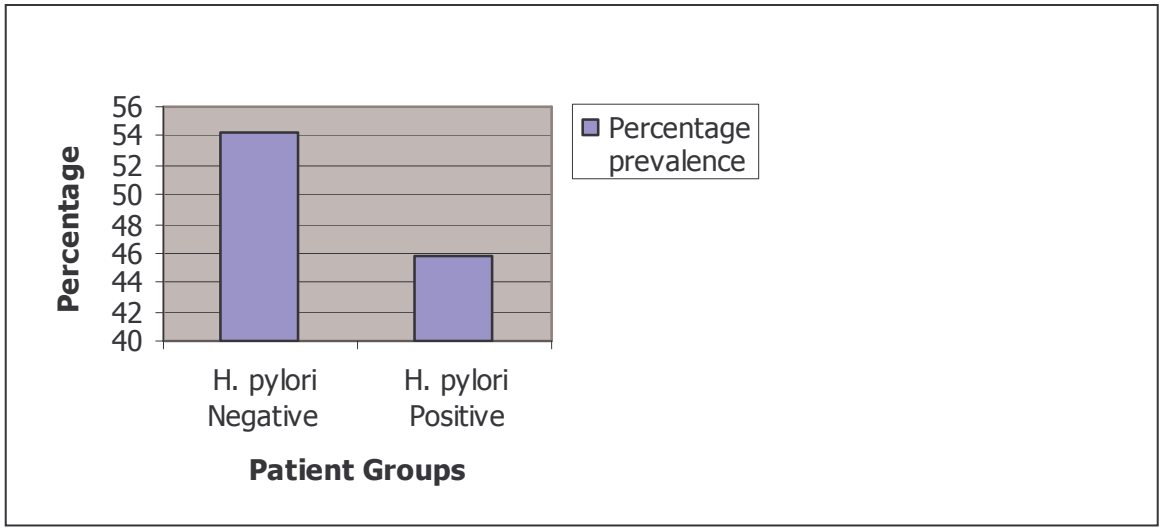


Figure 5: Prevalence of *H. pylori* positive and *H. pylori* negative patients

The prevalence of *H. pylori* positive patients amongst the sampling group was 46% (95% Confidence interval, 34% - 58%). The difference in prevalence amongst the *H. pylori* positive and negative patients was not statistically significant (P = 0.6025), (Figure 5).

5.4.1 *H. pylori* status and HIV status

Table 1 represents the patient numbers with respect to HIV status and *H. pylori* status.

Table 1: *H. pylori* prevalence in HIV positive and negative patients

Patient group	N	*H. pylori* positive patients	*H. pylori* negative patients
HIV Positive	17	47.06 %	52.94 %
HIV Negative	42	45.24 %	54.76 %

The relationship between HIV positive and HIV negative patients with regards to *H. pylori* status is illustrated in Figure 6.

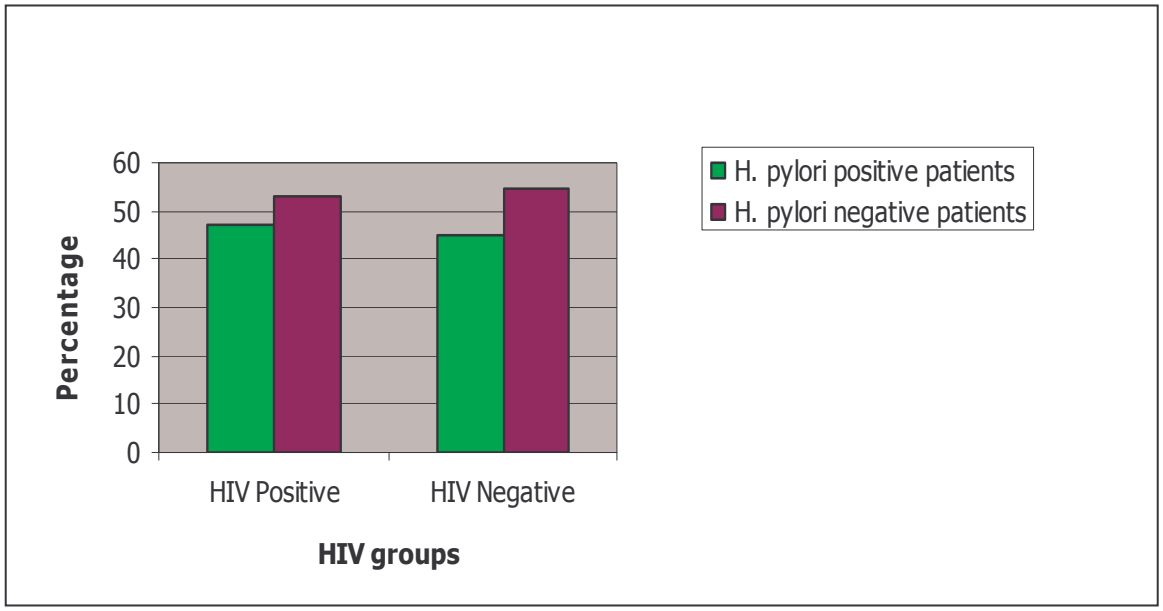


Figure 6: HIV status vs *H. pylori* status

From Figure 7 it can be seen that there was no significant difference in *H. pylori* status between the HIV positive and the HIV negative patients (P= 0.8988).

A 47 % prevalence of *H. pylori* was seen in HIV positive patients (95% CI, 23 – 72 %). The prevalence of *H. pylori* amongst HIV negative patients was marginally less at 45 % (95% CI, 30 – 60 %), (Figure 6).

5.5 *CagA* status

Only the *H. pylori* positive patients were tested for IgG antibodies to *CagA*.

To establish the accuracy of the ELISA a standard curve was calculated (Figure 7).

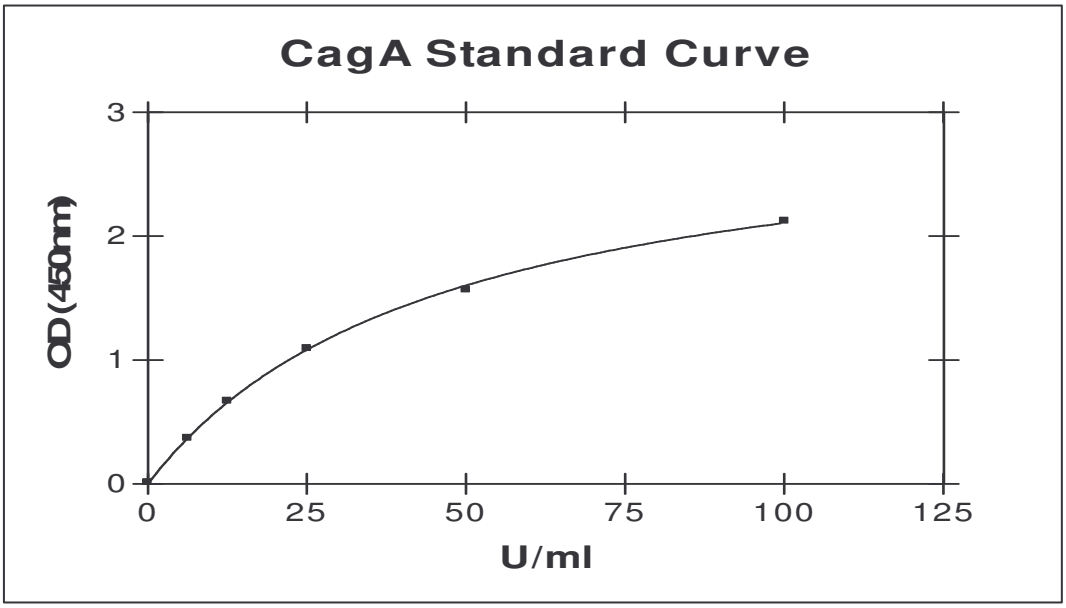


Figure 7: Standard curve for the detection of IgG antibodies to *CagA*

Figure 7 clearly shows the accuracy of the results in relation to the standard control solutions, as clear curve has formed as the "positivity" of the stand solutions increase. Therefore the results can be considered accurate.

In the *H. pylori* positive patient group, 37% were anti-*CagA* positive (95% CI, 22% – 59%; p = 0.2482). This is however only 17 % of the total sampling group (95% CI, 10% - 29%; p = 0.0001).

Figure 8 illustrates the anti-*CagA* prevalence amongst *H. pylori* positive and negative patients.

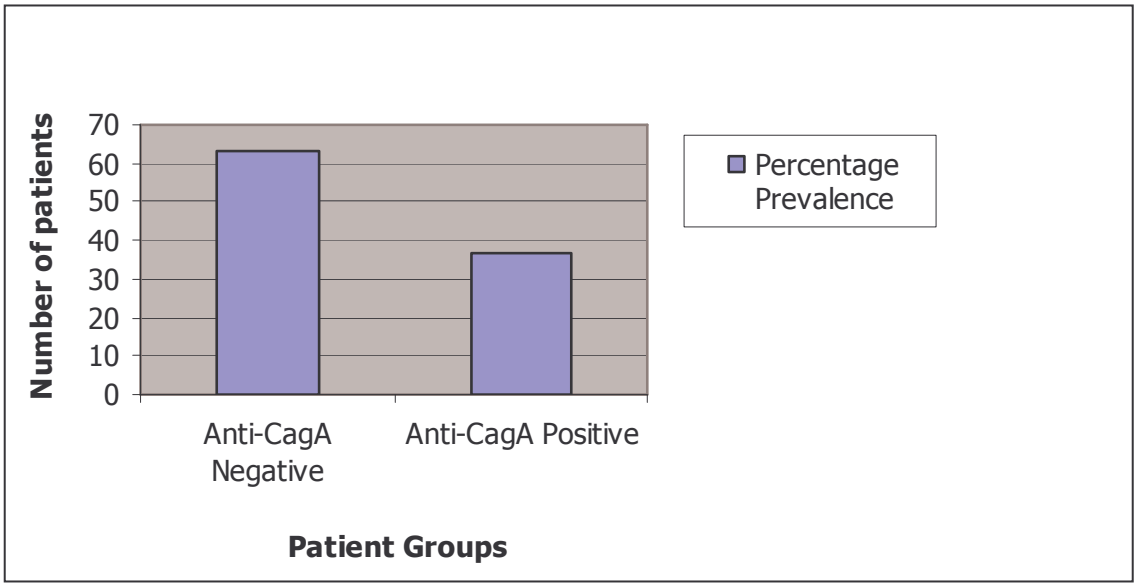


Figure 8: Prevalence of *H. pylori* positive patients versus anti-*CagA* status

There was no statically significant difference in the number of anti-*CagA* positive and anti-*CagA* negative patients who were *H. pylori* positive patients (p = 0.2482).

5.5.1 *CagA* status and HIV status

Table 2 represents the patient numbers with respect to HIV status and anti-*CagA* status.

Table 2: Anti-*CagA* prevalence in HIV positive and negative patients

Patient group	N	Anti-*CagA* positive Patients	Anti-*CagA* negative Patients
HIV Positive	8	50.00 %	50.00 %
HIV Negative	19	31.58 %	68.42 %

Figure 9 illustrates anti-*CagA* prevalence amongst HIV positive and negative patients

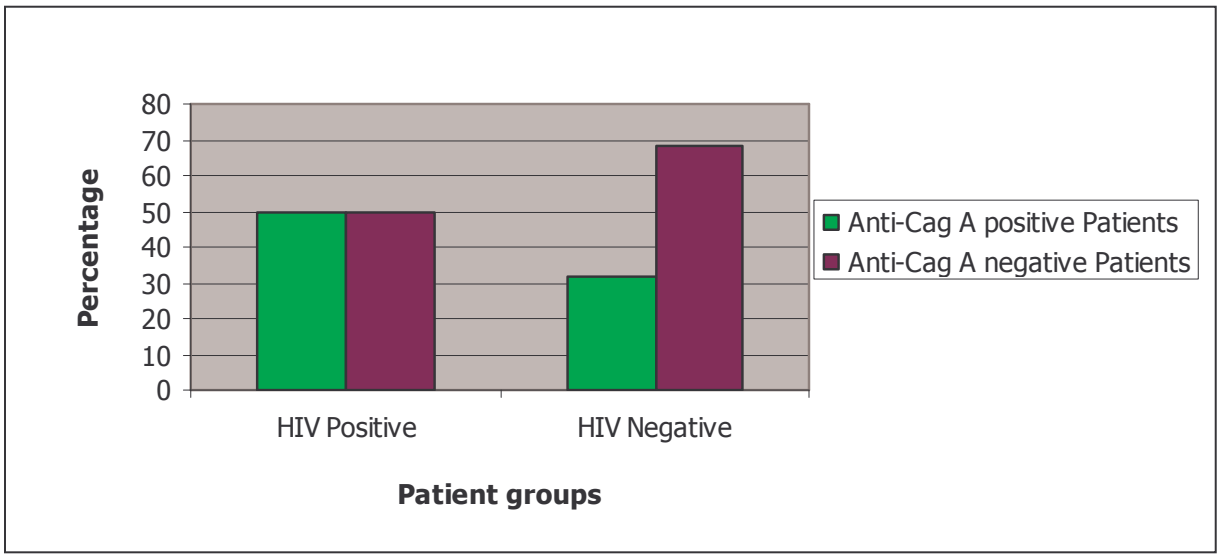


Figure 9: HIV status versus *CagA* status of patients included in study

There was no difference between anti-*CagA* negative and positive patient numbers in the HIV positive group. However for the HIV negative group, a difference was observed, Figure 9. This difference was however, not statistically significant (P = 0.3654).

5.6 Socio-economic status

The socio-economic status was collected from the patient files. The patients were graded according to the system used at Universitas and National Hospital (table 3).

Table 3: Socio-economic status grading as determined by Universitas hospital.

Individual/ Family income	H0	H1	H2	H3	H4MA	PP
Total annual income for Single person	Unemployed	R 1 – R 35999	R 36000 – R 71999	+ R 720000	Medical Aid	Private patient
Total annual income for family	Unemployed	R 1 – R 49999	R 50000 – R 99999	+ R 100000	Medical Aid	Private patient

Table 4 shows HIV, *H. pylori* and anti-*CagA* status with respect to socio-economic status.

Table 4: Socio-economic status in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients

Patient group	N	HO	H1	H2	H3	H4MA
HIV positive	17	23.53 %	64.71 %	0 %	0 %	11.76 %
HIV negative	42	33.33 %	57.14 %	2.38 %	0 %	7.14 %
H. pylori positive	27	44.44 %	44.44 %	0 %	0 %	11.11 %
H. pylori negative	32	16.75 %	71.88 %	3.13 %	0 %	6.25 %
Anti-*CagA* positive	10	40.00 %	50.00 %	0 %	0 %	10 %
Anti-*CagA* negative	17	47.08 %	41.18 %	0 %	0 %	11.76 %

Amongst the HIV, *H. pylori* and anti-*CagA* patient groups it was observed that the majority of the patients have an H1 grading (Table 4). This is an income of R 1 – R 35999 for an individual or R 1 – R 49999 for a family (Table 3).

The H0 grading was second highest, patients with no income (Table 4) and the grading of H4MA, patients with Medical aid coverage (Table 3) was third. Very few patients were graded to H2 and none to H3.

Figure 10 illustrates socio-economic status in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients.

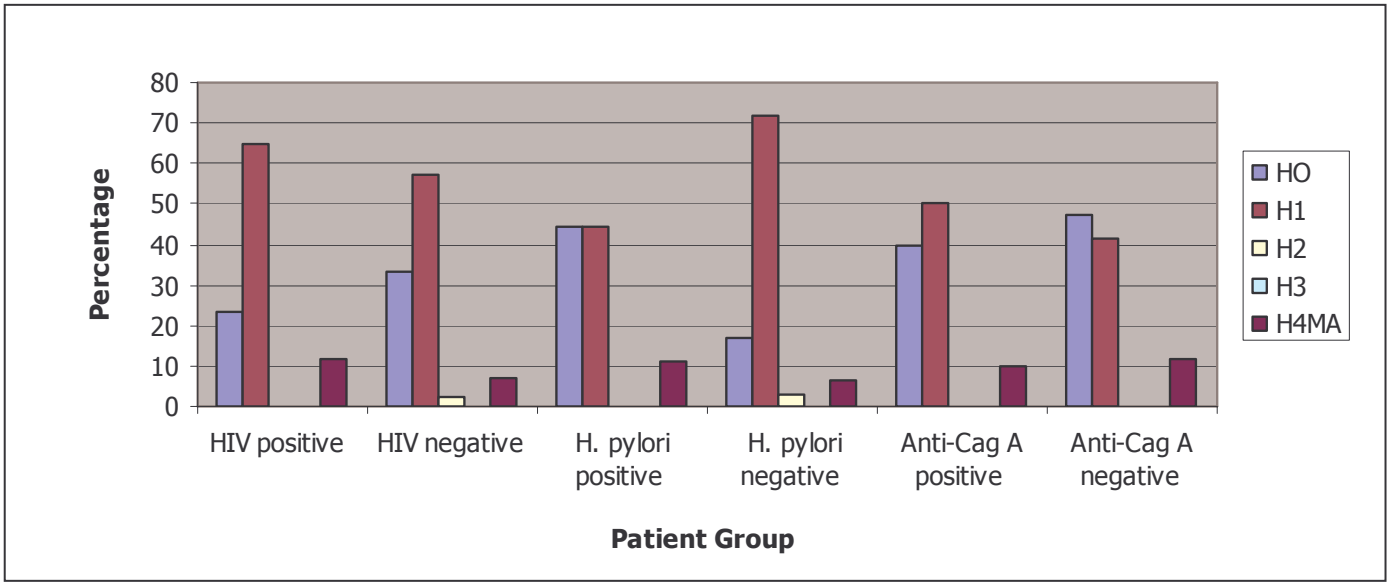


Figure 10: Socio-economic status in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients

Small differences in socio-economic status were found in the HIV patient groups, however no difference was found to be statistically significant (p = 0.7703). Similarly the anti-*CagA* patient group also showed no statistically significant difference between positive and negative patients in terms of socio-economic status (P = 1.0000.0). The *H. pylori* positive group showed no difference in prevalence between H1 socio-economic status and HO socio-economic status (Prevalence of 44.44 % for both), however the *H. pylori* negative group showed a great difference in the percentage between patients with an HO socio-economic status those with an H1 socio-economic status (16.75 % H0 status and 71.88% H1 status). This difference in socio-economic status between *H. pylori* positive and *H. pylori* negative patients was very close to being statistically significant with a p value of 0.0741.

A similar trend can be seen in patients who have known HIV and *H. pylori* status and known HIV and anti-*CagA* status (Table 5). A majority of the patients were graded as H1, H0 or H4MA. Very few patients were graded as H2 and none as H3 (Figure 10).

Table 5 shows socioeconomic status in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Table 5: Socio-economic status in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Patient group	N	HO*	H1*	H2*	H3*	H4MA*
HIV positive *H. pylori* positive	8	37.5 %	62.5 %	0 %	0 %	0 %
HIV positive *H. pylori* negative	9	11.11 %	66.67 %	0 %	0 %	22.22 %
HIV negative *H. pylori* positive	19	47.37 %	36.64 %	0 %	0 %	15.79 %
HIV negative *H. pylori* negative	23	21.74 %	73.91 %	4.35 %	0 %	0 %
HIV positive anti-*CagA* positive	4	0 %	100 %	0 %	0 %	0 %
HIV positive anti-*CagA* negative	4	75 %	25 %	0 %	0 %	0 %
HIV negative anti-*CagA* positive	6	66.67 %	16.67 %	0 %	0 %	16.67 %
HIV negative anti-*CagA* negative	13	38.46 %	46.15 %	0 %	0 %	15.38 %

* Please refer to table 3 for definitions of gradings

Figure 11 illustrates socio-economic status in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

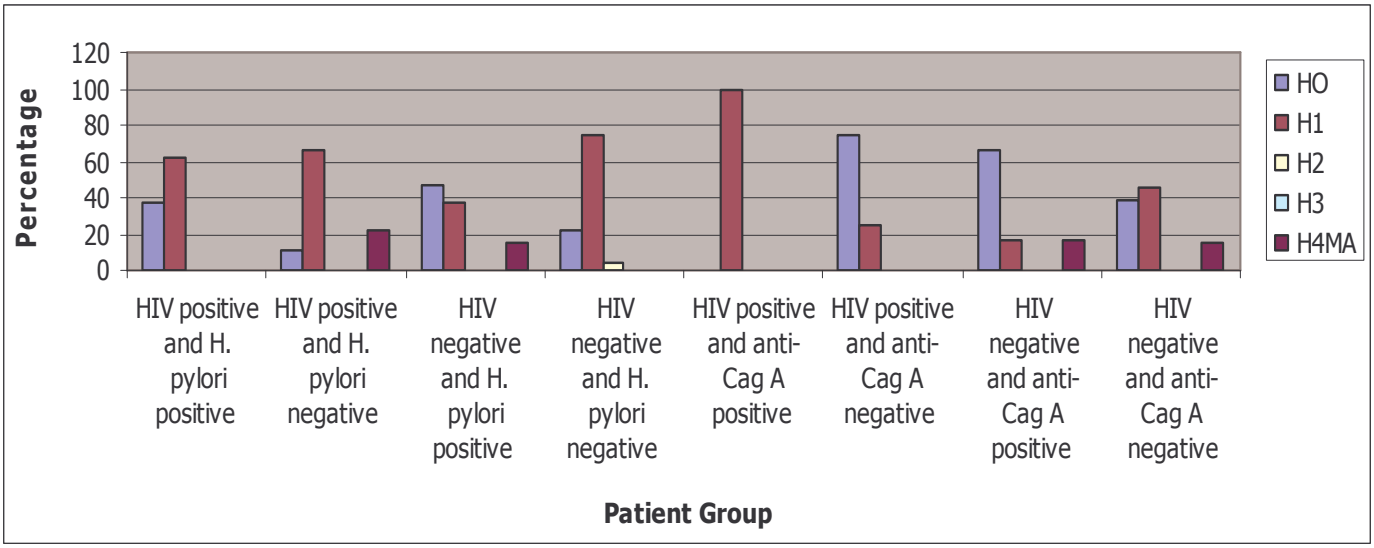


Figure 11: Socio-economic status in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

In the HIV positive patient group, no statistical difference could be found between *H. pylori* positive and negative patients (P = 0.3158), Figure 11. However, in the HIV negative group the differences between the *H. pylori* positive and negative patients were statistically significant (P = 0.0127), Figure 11. The HIV negative *H. pylori* positive group showed more H0 gradings amongst patients, and the HIV negative *H. pylori* negative group showed more H1 gradings amongst patients. Due to the low patient numbers in the HIV status versus anti-*CagA* status group, statistical significance was not calculated for age in this group.

5.7 Age

Age was collected on the patient questionnaire. The oldest patient taking part in the study was 82 years old and the youngest was 18 years old, Table 6.

Table 6: Age in relation to HIV status, *H. pylori* status and Anti-*CagA* status of patients

Patient group	Median	Minimum	Maximum
HIV positive	33	23	66
HIV negative	55	18	82
H. pylori positive	54	20	82
H. pylori negative	46	18	77
Anti-*CagA* positive	57	23	82
Anti-*CagA* negative	54	20	73

In the HIV positive patient group the median age was 33 (Table 6). However, for the HIV negative patients the median age was higher at 55 (Table 6). This difference is statistically significant (P = 0.0004).

In the *H. pylori* positive patient group the median age was 54 (Table 6). However, for the *H. pylori* negative patients the median age was 46 (Table 6). This difference was not statistically significant (P = 0.4651).

In the anti-*CagA* positive patient group the median age was 57 (Table 6). However, for the anti-*CagA* negative patients the median age was 54 (Table 6). This difference was not statistically significant (P = 0.7440), Figure 6.

Table 7 shows the age for patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Table 7: Age in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Patient group	Median	Minimum	Maximum
HIV positive *H. pylori* positive	30	24	44
HIV positive *H. pylori* negative	38	24	66
HIV negative *H. pylori* positive	63	20	82
HIV negative *H. pylori* negative	52	18	77
HIV positive anti-*CagA* positive	28	23	36
HIV positive anti-*CagA* negative	31	23	44
HIV negative anti-*CagA* positive	67	50	82
HIV negative anti-*CagA* negative	55	20	73

The median age of the patients who were HIV positive *H. pylori* positive (30) was lower than that of the patients who were HIV positive *H. pylori* negative (38), (Table 7). The difference in age between these two patient groups was statistically significant (P = 0.0342).

The median age of the patients who were HIV negative *H. pylori* positive (63) was higher than that of the patients who were HIV negative *H. pylori* negative (52), (Table 7). The difference in age between these two patient groups was not statistically significant (P = 0.0707). With larger patient numbers this may prove to be statistically significant.

In patients who were HIV positive anti-*CagA* positive the median age was 28, whereas for the patients who were HIV positive and anti-*CagA* negative, the median age was 31, (Table 7).

In patients who were HIV negative anti-*CagA* positive the median age was 67, where as for the patients who were HIV negative and anti-*CagA* negative, the median age was 55, (Table 7). However, due to the low patient numbers in the HIV status versus anti-*CagA* status group, statistical significance was not calculated for age in this group.

5.8 Sex of patients

There were 20 male patients and 39 female patients included in the study. The sex ratio in the HIV, *H. pylori* and anti-*CagA* groups is listed in table 8. All patient groups showed a majority female sampling group (Table 8).

Table 8: Sex status in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients

Patient group	N	Female (%)	Male (%)
HIV positive	17	76.47	23.53
HIV negative	42	61.90	38.10
H. pylori positive	27	66.67	33.33
H. pylori negative	32	65.63	34.38
Anti-*CagA* positive	10	80.00	20.00
Anti-*CagA* negative	17	58.82	41.18

Figure 12 illustrates sex status in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients.

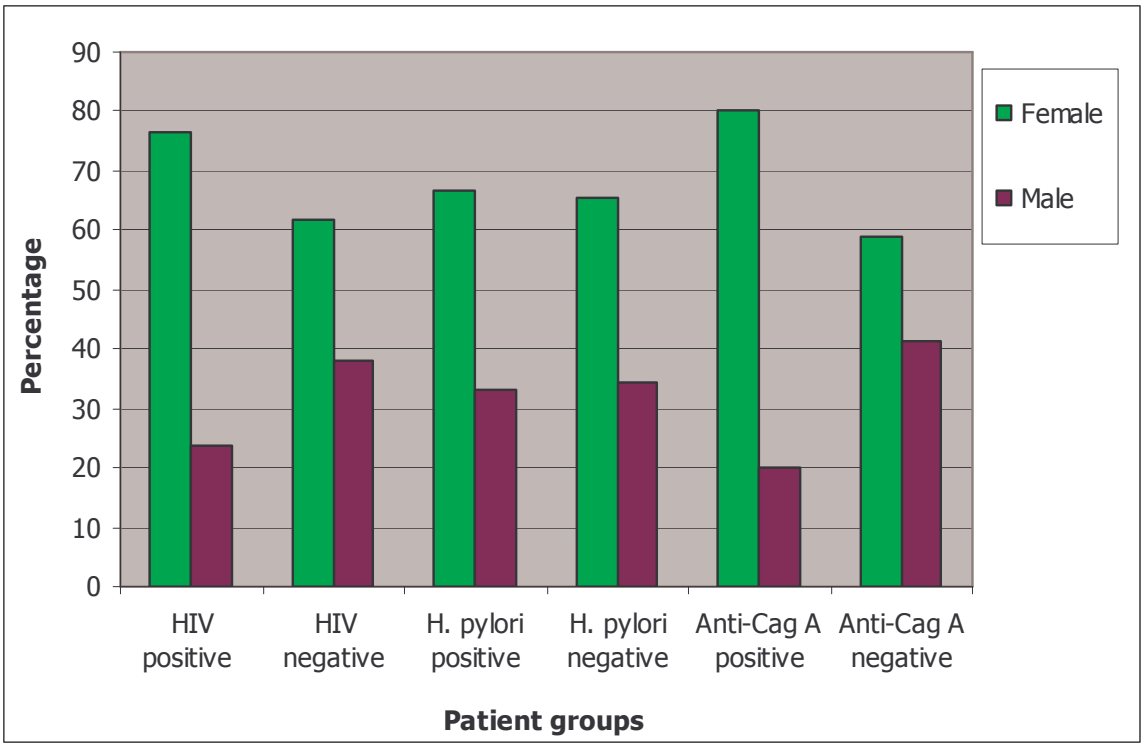


Figure 12: Sex status in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients

The difference between HIV positive and negative patients with relation to sex was not statistically significant (P = 0.2844), (Figure 12). The difference between *H. pylori* positive and negative patients with relation to sex was not statistically significant (P = 0.9329), (Figure 12). The difference between anti-*CagA* positive and negative patients with relation to sex was not statistically significant (P = 0.4059), (Figure 12).

Table 9 shows sex in patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Table 9: Sex in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Patient Groups	N	Female (%)	Male (%)
HIV positive *H. pylori* positive	8	75.00	25.00
HIV positive *H. pylori* negative	9	77.78	22.22
HIV negative *H. pylori* positive	19	63.16	36.84
HIV negative *H. pylori* negative	23	60.87	39.13
HIV negative anti-*CagA* positive	4	100	0
HIV negative anti-*CagA* negative	4	50.00	50.00
HIV positive anti-*CagA* positive	6	66.67	33.33
HIV positive anti-*CagA* negative	13	61.54	38.46

Figure 13 illustrates sex in relation to patients with known HIV and *H. pylori* status.

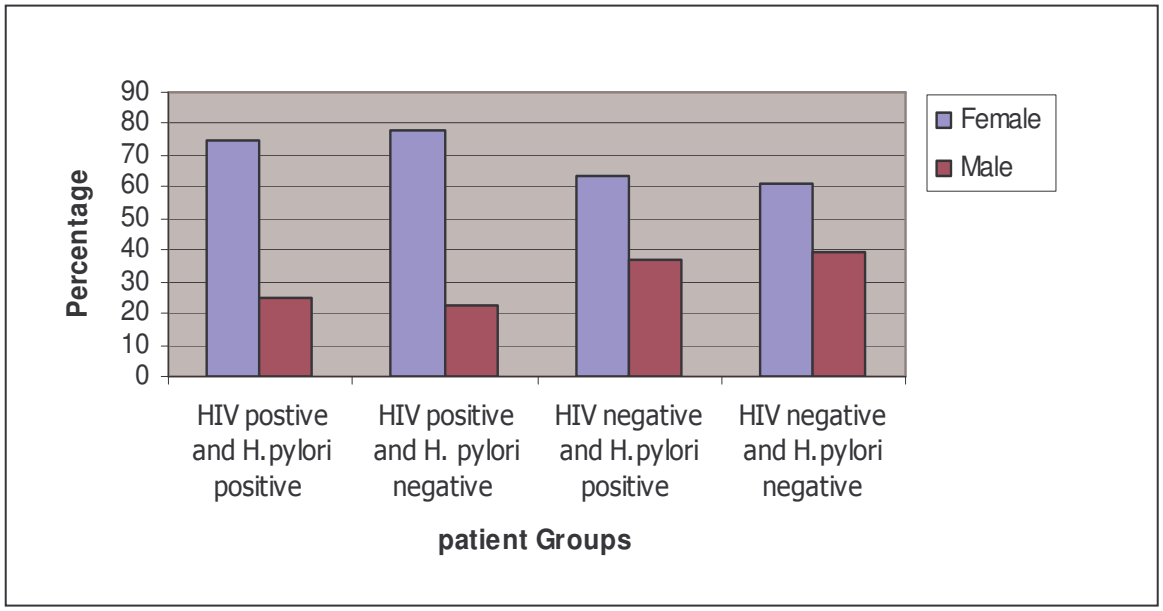


Figure 13: Sex in relation to patients with known HIV and *H. pylori* status.

The difference in sex ratio between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative was not statistically significant (P = 0.4235), (Figure 13).

Similarly, the difference in sex ratio between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative was not statistically significant (P = 0.8792), (Figure 13).

Figure 14 illustrates sex in relation to patients with known HIV and anti-*CagA* status.

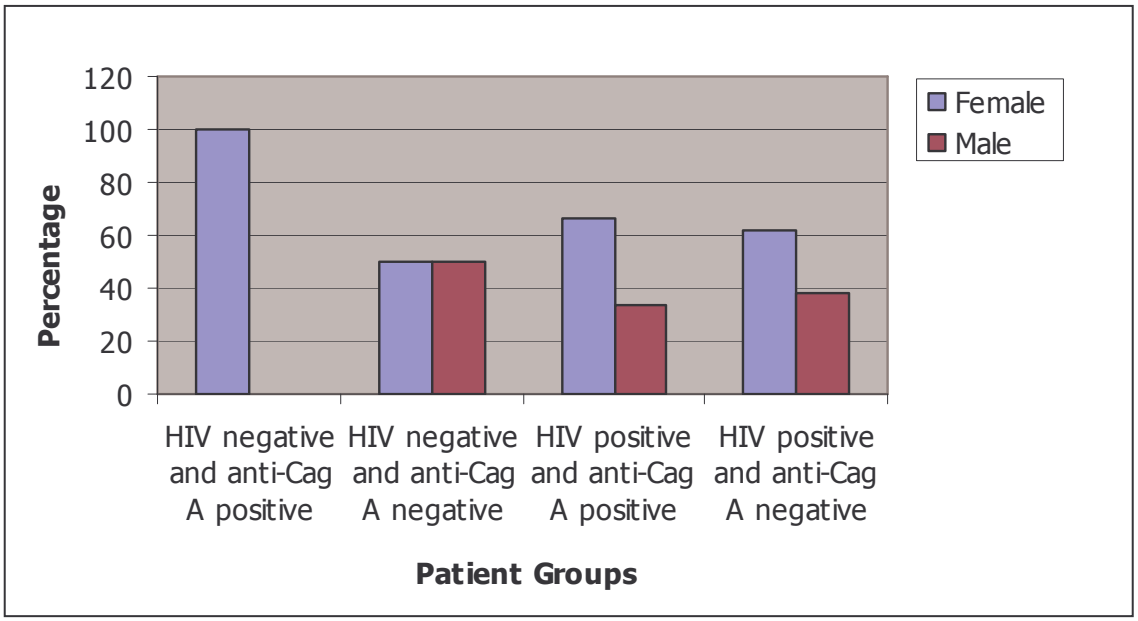


Figure 14: Sex in relation to patients with known HIV and anti-*CagA* status.

The patients who were HIV negative anti-*CagA* positive were all women, where as the patients who were HIV negative anti-*CagA* negative showed a 50% distribution of female and 50% distribution of male.

The patients who were, HIV positive and anti-*CagA* positive and the patients who were HIV positive anti-*CagA* negative, showed very small, non-significant differences. Due to the low patient numbers in the HIV status versus anti-*CagA* status group, statistical significance was not calculated for sex in this group.

5.9 Race of patients in the study

The sampling group consisted of 44 black patients, 9 white patients and 6 coloured patients. Table 10 shows the distribution of race amongst the HIV, *H. pylori* and anti-*CagA* patient groups.

Table 10: Race in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients

Patient group	N	White (%)	Black (%)	Mixed race (%)
HIV positive	17	5.88	82.35	11.76
HIV negative	42	19.05	71.43	9.52
H. pylori positive	27	11.11	77.78	11.11
H. pylori negative	32	18.75	71.88	9.38
Anti-*CagA* positive	10	0	80.00	20
Anti-*CagA* negative	17	17.65	76.47	5.88

From figure 15 it can be seen that the majority of the patients across all HIV, *H. pylori* and anti-*CagA* groups were black.

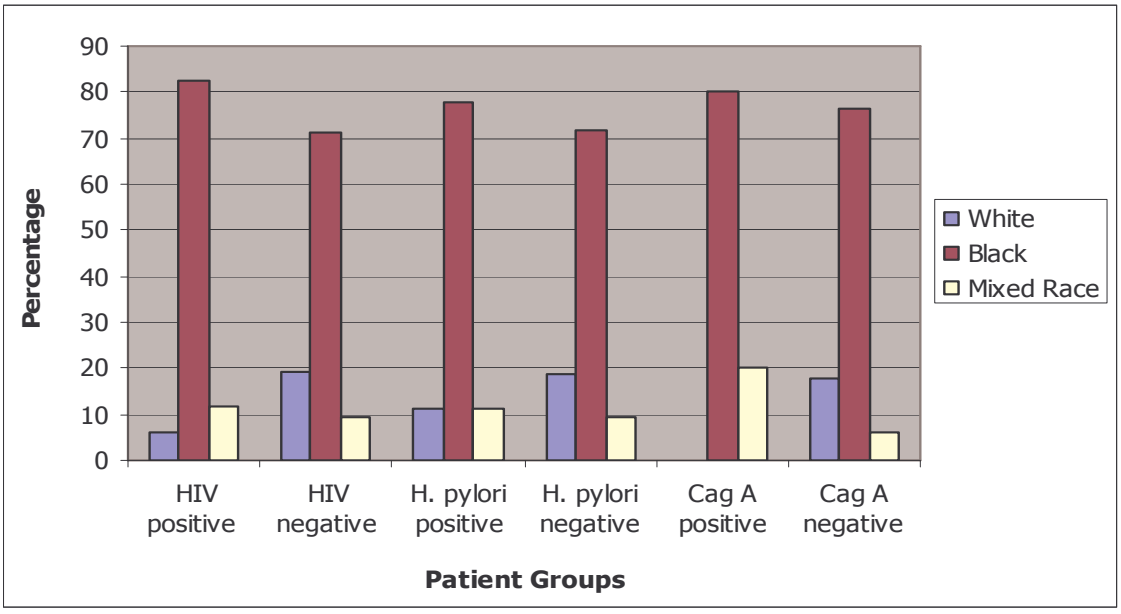


Figure 15: Race in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients

The differences in race distribution seen in the HIV positive and negative patients were not statistically significant (White P = 0.2630, Black P = 0.5164 and Mixed race P = 1.0000) (Figure 15). The differences in race distribution seen in the *H. pylori* positive and negative patients were not statistically significant (White P = 0.4677, Black P = 0.6039 and Mixed race P = 1.0000), (Figure 15).

The anti-*CagA* positive patient group did not include any white patients, whereas the anti-*CagA* negative patient group included black, mixed race and white patients. The differences in race seen in the anti-*CagA* positive and negative patients were not statistically significant (White P = 0.2735, Black P = 1.0000 and Mixed race P = 0.5350),(Figure 15).

Table 11 showed race in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Table 11: Race in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Patient group	N	White (%)	Black (%)	Mixed race (%)
HIV positive *H. pylori* positive	8	0	100	0
HIV positive *H. pylori* negative	9	11.11	66.67	22.22
HIV negative *H. pylori* positive	19	15.79	66.42	15.79
HIV negative *H. pylori* negative	23	21.74	73.91	4.35
HIV negative anti-*CagA* positive	4	0	100	0
HIV negative anti-*CagA* negative	4	0	100	0
HIV positive anti-*CagA* positive	6	0	66.67	33.33
HIV positive anti-*CagA* negative	13	23.08	69.23	7.69

From figure 16 it can be seen that the majority of the patients with known HIV and *H. pylori* status were black.

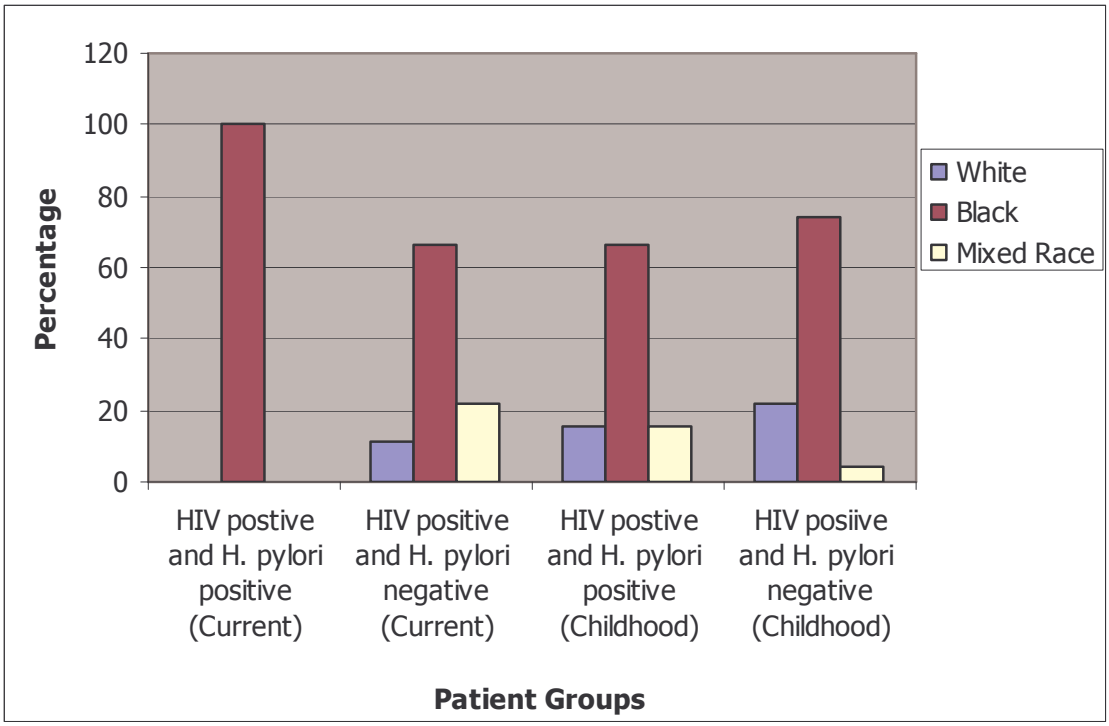


Figure 16: Race in relation to patients with known HIV and *H. pylori*.

The difference in race distribution between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (White P = 1.0000, Black P = 0.2059 and Mixed race P = 0.4706), (Figure 16). The difference in race ratio between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative were not statistically significant (White P = 0.7092, Black P = 0.6950 and mixed race P = 0.3129), (Figure 16).

Figure 17 illustrates race in relation to patients with known HIV and anti-*CagA* status.

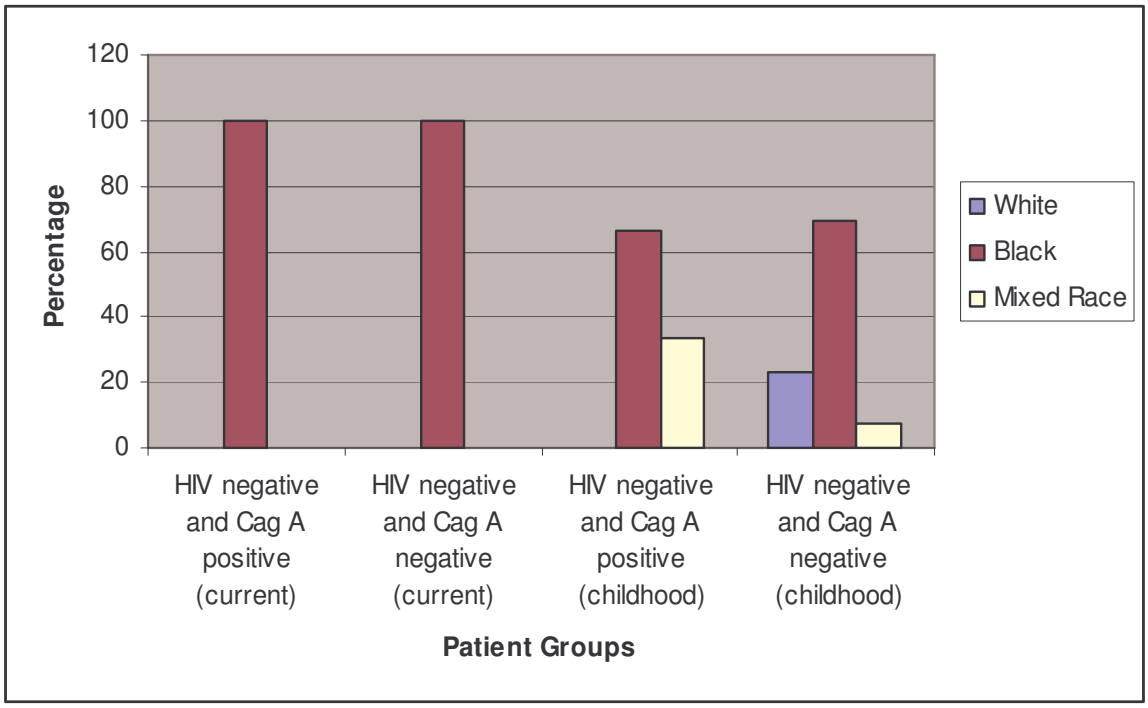


Figure 17: Race in relation to patients with known HIV and anti-*CagA* status.

For the patient groups where patients were HIV negative anti-*CagA* positive and HIV negative and anti-*CagA* negative, all of the patients were black (Figure 17). However, in the subset of patients who were HIV positive anti-*CagA* positive the race was divided into black and mixed race; and the race of the patients who were HIV positive anti-*CagA* negative were divided into black, mixed race and white (Figure 17).

Due to the low patient numbers for patients who were HIV negative anti-*CagA* positive and patients who were HIV negative and anti-*CagA* negative, statistical significance was not calculated for race in these patients (Figure 17).

5.10 Water source

Fourty-eight patients had running water and 11 patients needed to fetch water from external sources. This included 1 patient who collected from a tank, 1 patient who collected from a river and 9 patients who collected from a tap in the street.

During childhood 25 patients had running water and 34 patients needed to collect water. Patients collected water bore holes (9 patients), tanks (1 patient), rivers (4 patients), dams (5 patients), well (1 patient) and tap in the street (16 patients). Eight patients collected water as needed from a tap outside the dwelling and 6 patients used more than one type of source.

Table 12 shows water source in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients. The "other" water source specified was a "well" for all patients.

Table 12: Water Source in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Patient group	N	Tap water on premises (%)	Tap water from communal source (%)	Bore Hole (%)	Tanks (%)	River (%)	Dam (%)	Other (%)
HIV positive (Current Source)	17	70.59	17.65	0	5.88	5.88	0	0
HIV negative (Current Source)	42	85.71	14.29	0	0	0	0	0
HIV positive (Childhood Source)	17	41.18	41.18	5.88	5.88	0	11.76	0
HIV negative (Childhood Source)	42	42.88	21.43	19.05	0	9.52	7.14	2.38
H. pylori positive (Curent Source)	27	66.67	29.63	0	3.70	0	0	0
H. pylori negative (Current Source)	32	93.75	3.13	0	0	3.13	0	0
H. pylori positive (Childhood Source)	27	37.04	18.52	14.81	3.70	11.11	18.52	3.7
H. pylori negative (Childhood Source)	32	46.88	34.3	15.83	0	3.13	0	0
Anti-*CagA* positive (Current Source)	10	70.00	20.00	0	10	0	0	0
Anti-*CagA* negative (Current Source)	17	64.71	35.29	0	0	0	0	0
Anti-*CagA* positive (Childhood Source)	10	40.00	0	30.00	10	10	30	0
Anti-*CagA* negative (Childhood Source)	17	32.29	29.41	5.88	0	11.76	11.76	5.88

Figure 18 illustrates the relationship between HIV positive and negative patients with regards to water source, both currently and from childhood days.

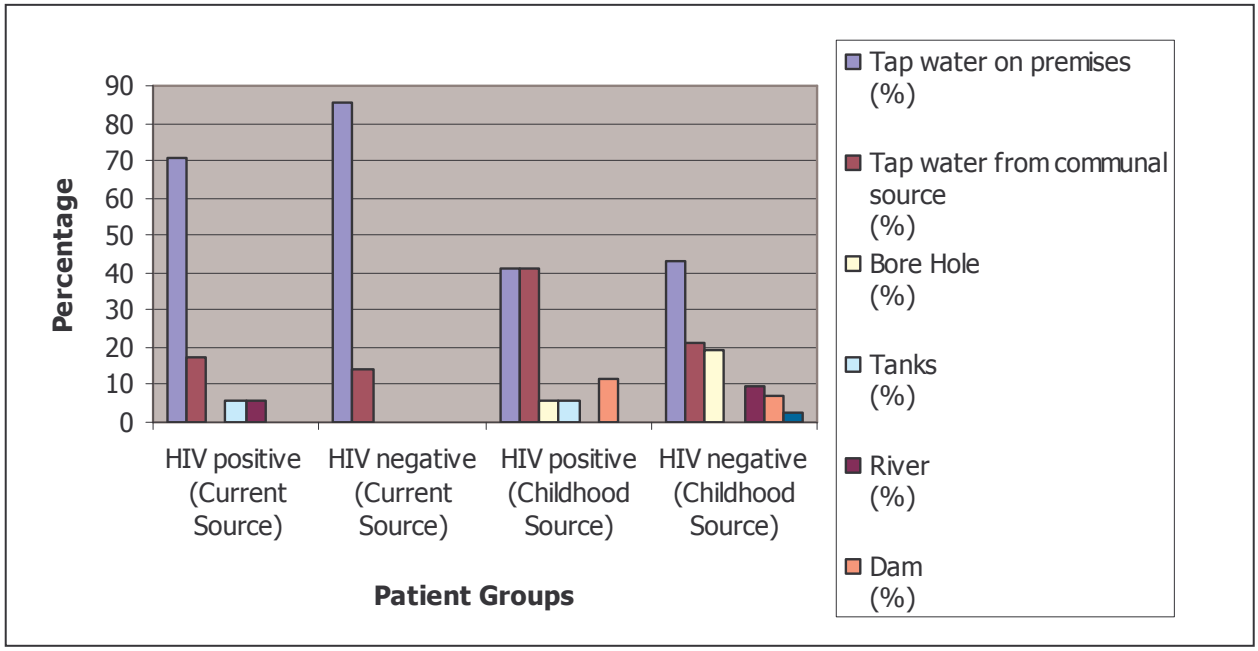


Figure 18: Water source in relation to HIV status both currently and during childhood.

The differences in current water source seen in the HIV positive and negative patients were not statistically significant (Tap water on premises P = 0.2671, Tap water from communal source P = 0.7080, Bore hole P = NA, Tank P = 0.2881, River P = 0.2881, Dam P = NA and Other P = 1.0000), (NA = option not used by patients), (Figure 18).

Similarly, the differences in water source from childhood seen in the HIV positive and negative patients were not statistically significant (Tap water on premises P = 0.9058, tap water from communal source P = 0.1945, Bore hole P = 0.2630, Tank P = 0.2881, River P = 0.3139, Dam P = 0.6199 and Other P = 1.0000), (Figure 18).

Figure 19 illustrates the relationship between *H. pylori* positive and negative patients with regards to water source, both currently and from childhood days.

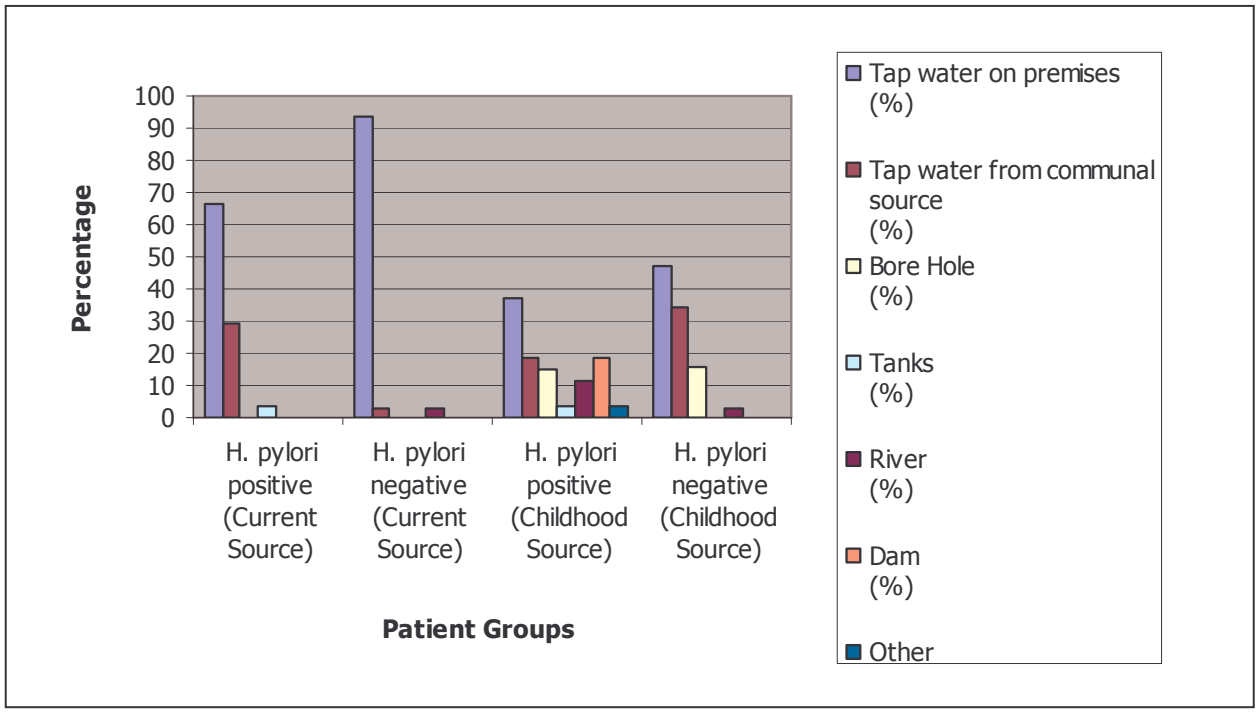


Figure 19: Water source in relation to *H. pylori* status, both currently and during childhood.

The differences in current water source seen between the *H. pylori* positive and negative patients were statistically significant for tap water on premises and tap water from a communal source. With tap water on premises (P = 0.0078) being predominately used in both *H. pylori* positive and negative patient groups, however, a larger percentage of *H. pylori* positive patients used tap water from communal source (P = 0.0063). The use of a bore hole, a tank, a river or a dam showed no statistical significance, (Bore hole P = NA, tank P = 0.4576, river P = 1.0000 and dam P = NA), (NA = option not used by patients), (Figure 19).

The differences in percentage of water source from childhood seen in the *H. pylori* positive and negative patients were statistically significant for dam water as a water source, (Dam P = 0.0161). *H. pylori* positive patients showed 18.82% of the patients collected dam water whereas none of the *H. pylori* negative patients collected dam water. The use of other water sources showed no significant differences (Tap water on

premises P = 0.4461, tap water from communal source P = 0.1723, bore hole P = 1.0000, tank P = 0.4576, river P = 0.3232 and other P = 0.4576), (Figure 19).

Figure 20 illustrates water source in relation to anti-*CagA* status, both currently and during childhood.

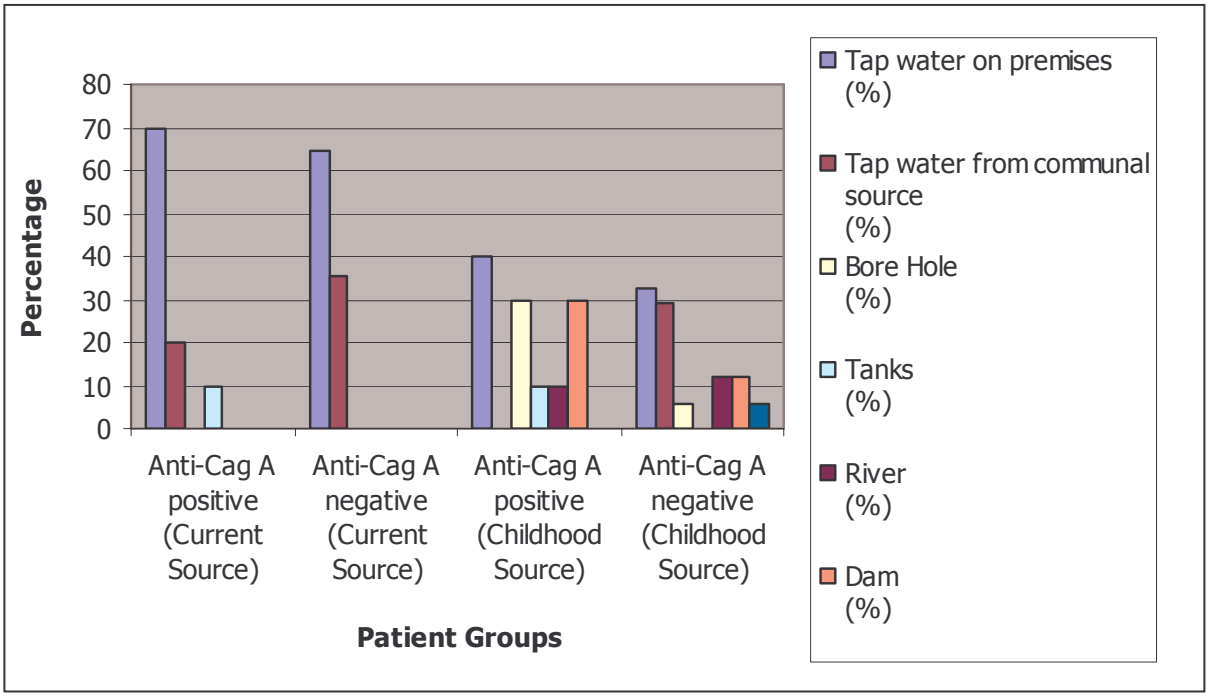


Figure 20: Water source in relation to anti-Cag A status, both currently and during childhood.

The differences in percentage of current water source seen in the anti-*CagA* positive and negative patients were not statistically significant (Tap water on premises P = 1.0000, tap water from communal source P = 0.6655, bore hole P = NA, Tank P =0.3704, river P = NA and dam P = NA), (NA = option not used by patients), (Figure 20).

Similarly, the differences in percentage of water source from childhood seen in the anti-*CagA* positive and negative patients were not statistically significant (Tap water on premises P = 1.0000, tap water from communal source P = 0.1240, bore hole P = 0.1282, tank P = 0.3704, river P = 1.0000, dam P = 0.3261 and other P = 1.0000),(Figure 20).

Table 13 shows water source in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Table 13: Water source in relation to patient groups with combined HIV and *H. pylori* status; and combined HIV and anti-*CagA* status, both currently and during childhood.

Patient group	N	Tap water on premises (%)	Tap water from communal source (%)	Bore Hole (%)	Tanks (%)	River (%)	Dam (%)	Other (%)
HIV positive *H. pylori* positive (Current Source)	8	50	37.5	0	12.5	0	0	0
HIV positive *H. pylori* negative (Current Source)	9	88.89	0	0	0	11.11	0	0
HIV positive *H. pylori* positive (Childhood Source)	8	25	37.5	12.5	12.5	0	25	0
HIV positive *H. pylori* negative (Childhood Source)	9	55.56	44.44	0	0	0	0	0
HIV negative *H. pylori* positive (Current Source)	19	73.68	26.32	0	0	0	0	0
HIV negative *H. pylori* negative (Current Source)	23	95.65	4.35	0	0	0	0	0
HIV negative *H. pylori* positive (Childhood Source)	19	42.11	10.53	15.79	0	15.79	15.79	5.28
HIV negative *H. pylori* negative (Childhood Source)	23	43.48	30.43	21.74	0	4.35	0	0
HIV positive anti-*CagA* positive (Current Source)	4	50	25	0	25	0	0	0
HIV positive anti-*CagA* negative (Current Source)	4	50	50	0	0	0	0	0
HIV positive anti-*CagA* positive (Childhood Source)	4	50	0	25	25	0	25	0
HIV positive anti-*CagA* negative (Childhood Source)	4	0	75	0	0	0	25	0
HIV negative anti-*CagA* positive (Current Source)	6	83.33	16.67	0	0	0	0	0
HIV negative anti-*CagA* negative (Current Source)	13	69.23	30.77	0	0	0	0	0
HIV negative anti-*CagA* positive (Childhood Source)	6	33.33	0	33.33	0	16.67	33.33	0
HIV negative anti-*CagA* negative (Childhood Source)	13	46.15	15.38	7.69	0	15.38	7.69	7.69

Figure 21 illustrates water source in relation to patient groups with combined HIV and *H. pylori*, both currently and during childhood.

The differences in percentage of current water source between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Tap water on premises P = 0.1312, tap water from communal source P = 0.0624, bore hole P = NA, tanks P = 0.4706, river P = 1.0000 and dam P = NA), (NA = option not used by patients), (Figure 21). The differences in percentage of water source from childhood between patients who were HIV positive *H.*

pylori positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Tap water on premises P = 0.3346, tap water from communal source P = 1.0000, bore hole P = 0.4706, tanks P = 0.4706, river P = NA and dam P = 0.2059), (NA = option not used by patients), (Figure 21).

The differences in percentage of current water source between patients who were HIV negative *H. pylori* positive and patients who are HIV negative *H. pylori* negative were not statistically significant (Tap water on premises P = 0.0754, tap water from communal source P = 0.0754, bore hole P = NA, river P = NA, dam P = NA), (NA = option not used by patients), (Figure 21). The differences in percentage of water source from childhood between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative are not statistically significant (Tap water on premises P = 1.0000, tap water from communal source P = 0.1494, bore hole P = 0.7092, tank P = NA, river P = 0.3129, dam P = 0.0844 and other P = 0.4524), (NA = option not used by patients), (Figure 21).

Figure 22 illustrates water source in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

For patients who were HIV positive anti-*CagA* positive the current water source mostly used was tap water on the premises, with tap water from a communal source and water from a tank being the next mostly used. The patients who were HIV positive anti-*CagA* negative all currently received water from a treated water source which was either tap water on the premises or tap water from a communal source (Figure 22). During childhood, the most used water source for the patients who were HIV positive anti-*CagA* positive was tap water on the premises, where as for the patients who were HIV positive anti-*CagA* negative the most used water source was tap water from a communal source (Figure 22).

For both the patients who were, HIV negative anti-*CagA* positive, and the patients who were HIV negative anti-*CagA* negative the mostly used water source was tap water on premises with the next most used water source being tap water from a communal

source. A range of different water sources were used during childhood for both these two patient groups (Figure 22). However, due to the low patient numbers for patients who were HIV negative anti-*CagA* positive and patients who were HIV negative and anti-*CagA* negative, statistical significance was not calculated for water source in these patients.

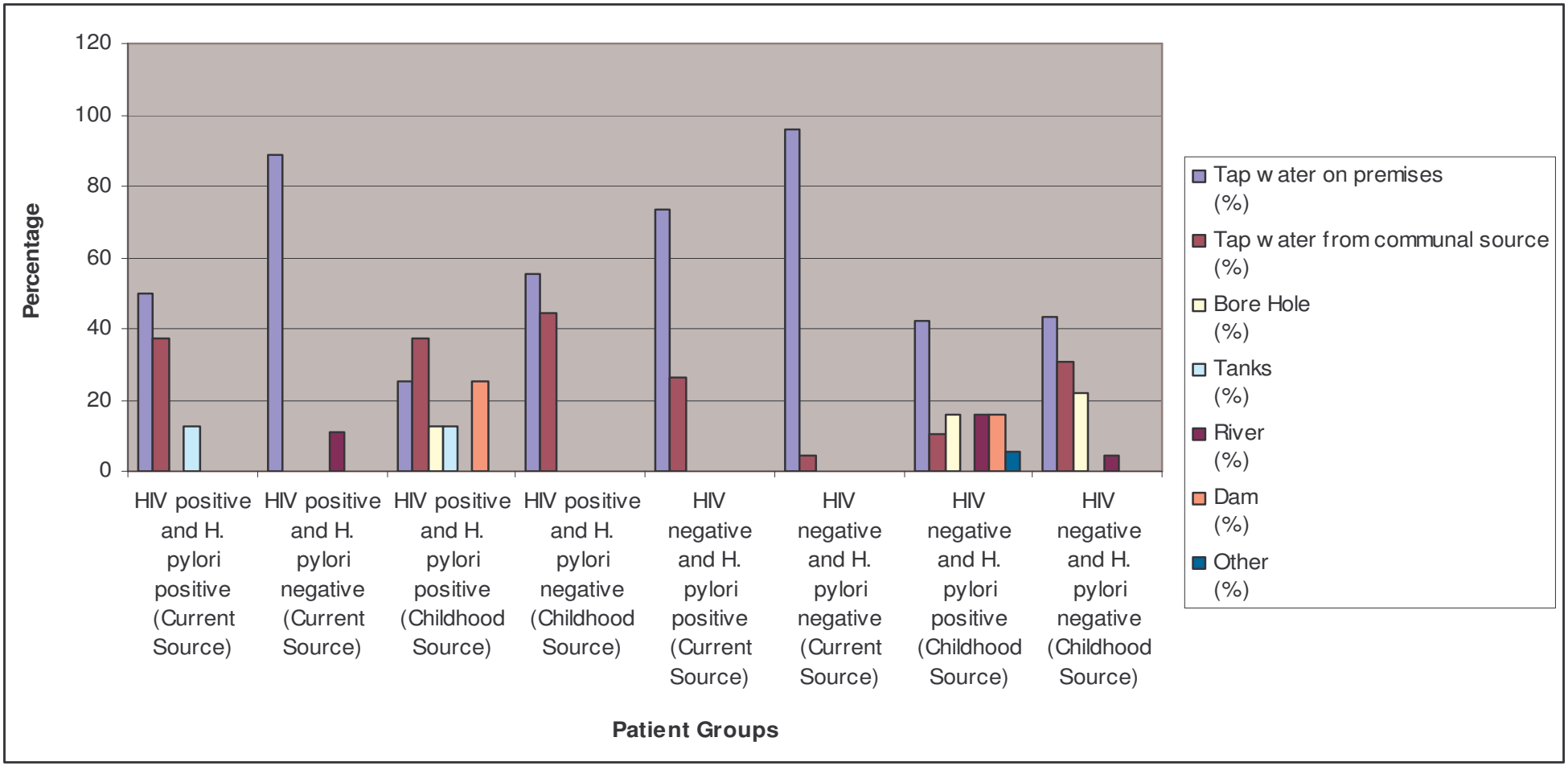


Figure 21: Water source in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

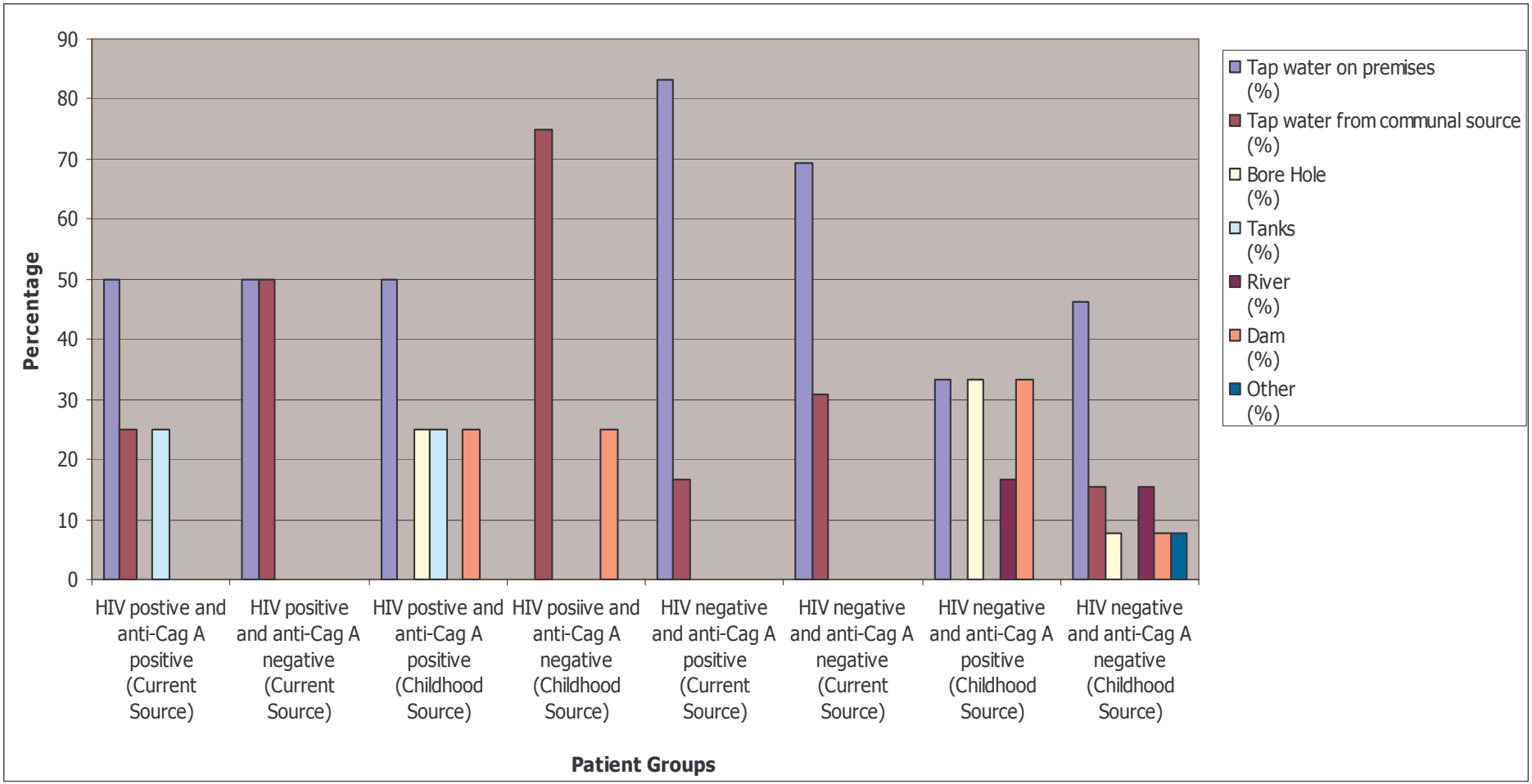


Figure 22: Water source in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

5.11 Transport of water

Only patients who needed to fetch water, transported water. All of the patients who currently needed to collect water did so using buckets. However, during childhood although the majority used buckets (33 patients), some patients used other containers such as glass bottles (1 patient), plastic bottles (1 patient) and drums (2 patients). One patient used more than one source.

Table 14 shows the water transportation method in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Table 14: Water transportation method in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Patient group	N	Bucket (%)	Plastic bottle (%)	Glass bottle (%)	Drum (%)
HIV positive (Current usage)	17	23.53	0	0	0
HIV negative (Current usage)	42	11.9	0	0	0
HIV positive (Childhood usage)	17	58.82	0	5.88	5.88
HIV negative (Childhood usage)	42	54.76	2.38	0	2.38
H. pylori positive (Current usage)	27	29.63	0	0	0
H. pylori negative (Current usage)	32	3.13	0	0	0
H. pylori positive (Childhood usage)	27	62.96	0	0	0
H. pylori negative (Childhood usage)	32	50	3.13	3.13	6.25
Anti-*CagA* positive (Current usage)	10	30	0	0	0
Anti-*CagA* negative (Current usage)	17	29.41	0	0	0
Anti-*CagA* positive (Childhood usage)	10	60	0	0	0
Anti-*CagA* negative (Childhood usage)	17	64.71	0	0	0

Figure 23 illustrates the water transportation method used for patients not having running water on their premises in relation to HIV status, both currently and during childhood.

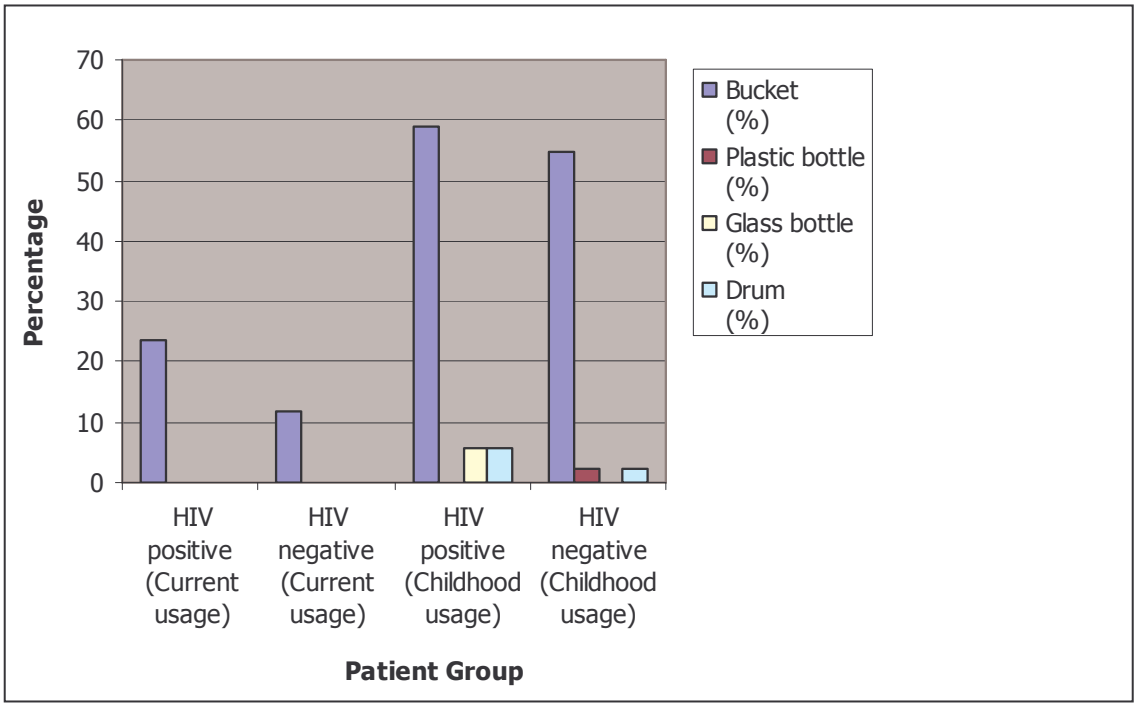


Figure 23: Water transportation method in relation to HIV status, both currently and during childhood.

The differences in current water transportation method seen in the HIV positive and negative patients were not statistically significant (Bucket P = 0.4241, plastic bottle P = NA, glass bottle P = NA and drum P = NA), (NA = option not used by patients), (Figure 23). Similarly, the differences in water transportation method used in childhood seen in the HIV positive and negative patients were not statistically significant (Bucket P = 0.7760, plastic bottle P = 0.288, glass bottle P = 1.0000 and drum P = 0.4968)

Figure 24 illustrates water transportation method in relation to *H. pylori* status, both currently and during childhood.

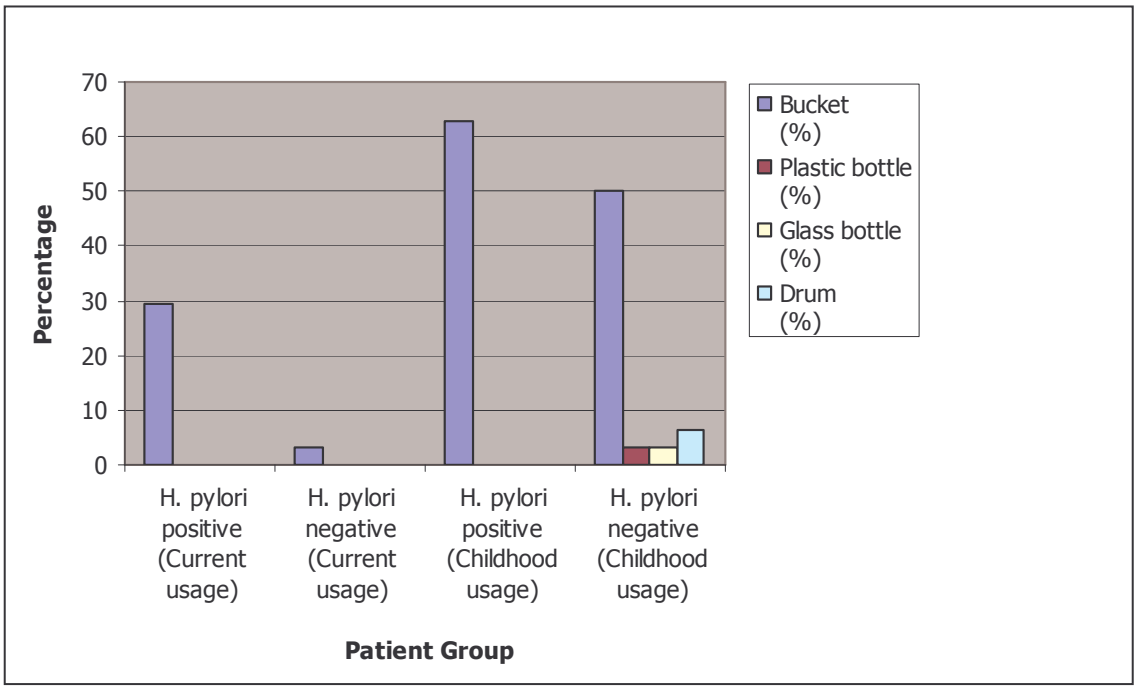


Figure 24: Water transportation method in relation to *H. pylori* status, both currently and during childhood.

The only current water transportation method used for both the *H. pylori* positive and negative patients was buckets. The difference in percentage of patients using buckets between the two groups is statistically significant (Bucket P = 0.0083), (Figure 24).

During childhood the *H. pylori* positive patients only made use of buckets, while the *H. pylori* negative patients also made use of other means of transportation. The differences in percentage of water transportation method used in childhood seen in the *H. pylori* positive and negative patients were not statistically significant (Bucket P = 0.3177, plastic bottle P = 1.0000, glass bottle P = 1.0000 and drum P =0.4950), (Figure 24).

Figure 25 illustrates water transportation method in relation to anti-*CagA* status of patients, both currently and during childhood.

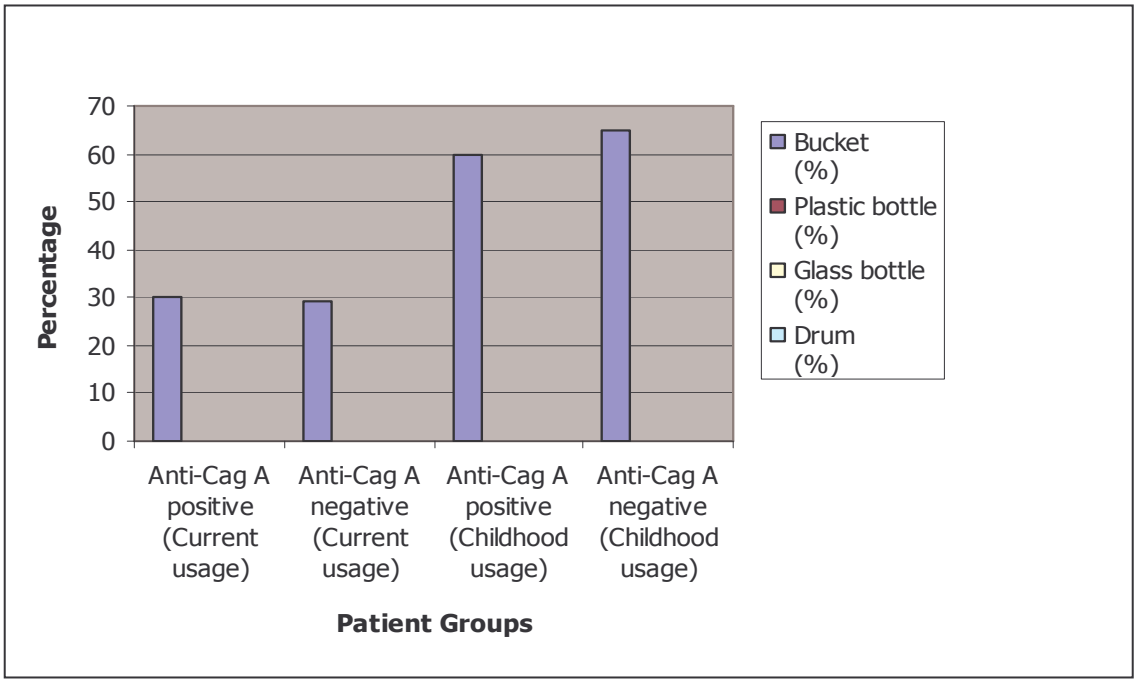


Figure 25: Water transportation method in relation to anti-*CagA* status, both currently and during childhood.

All of the anti-*CagA* positive and negative patients made use of buckets, both in the past and presently as water transportation method. No statistical significance was found for water transportation method between anti-*CagA* positive and negative currently (Bucket P = 1.0000) nor for water transportation between anti-*CagA* positive and negative patients in childhood (bucket P = 1.0000), (Figure 25).

Table 15 shows water transportation method in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Table 15: Water transportation method in relation to patient groups with combined HIV and *H. pylori* status; and combined HIV and anti-*CagA* status, both currently and during childhood.

Patient group	N	Bucket (%)	Plastic bottle (%)	Glass bottle (%)	Drum (%)
HIV positive *H. pylori* positive (Current usage)	8	50	0	0	0
HIV positive *H. pylori* negative (Current usage)	9	0	0	0	0
HIV positive *H. pylori* positive (Childhood usage)	8	75	0	0	0
HIV positive *H. pylori* negative (Childhood usage)	9	44.44	0	11.11	11.11
HIV negative *H. pylori* positive (Current usage)	19	21.05	0	0	0
HIV negative *H. pylori* negative (Current usage)	23	4.35	0	0	0
HIV negative *H. pylori* positive (Childhood usage)	19	57.89	0	0	0
HIV negative *H. pylori* negative (Childhood usage)	23	52.17	4.35	0	4.35
HIV positive anti-*CagA* positive (Current usage)	4	50	0	0	0
HIV positive anti-*CagA* negative (Current usage)	4	50	0	0	0
HIV positive anti-*CagA* positive (Childhood usage)	4	50	0	0	0
HIV positive anti-*CagA* negative (Childhood usage)	4	100	0	0	0
HIV negative anti-*CagA* positive (Current usage)	6	16.67	0	0	0
HIV negative anti-*CagA* negative (Current usage)	13	23.06	0	0	0
HIV negative anti-*CagA* positive (Childhood usage)	6	66.67	0	0	0
HIV negative anti-*CagA* negative (Childhood usage)	13	53.85	0	0	0

Figure 26 illustrates water transportation method in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

Only buckets were used as a means of transportation for water in the HIV positive *H. pylori* positive group. None of the patients who were HIV positive *H. pylori* negative used any of the mentioned transportation methods, indicating that all 9 patients had running water on the premises or collected water from a communal source as needed. This difference was statistically significant (Bucket P = 0.0294), (Figure 26). The patients who were HIV positive *H. pylori* positive only used buckets as means of transportation for their water during childhood. The HIV positive *H. pylori* negative patients, although predominately also using buckets during childhood, also made use of other transportation methods in their childhood. The differences in percentage of water transportation method used in childhood seen between the patients who were HIV

positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Bucket P = 0.3348, plastic bottle P = NA, glass bottle P = 1.0000 and drum P = 1.0000), (NA = option not used by patients), (Figure 26).

Only buckets were used as a current means of transportation for water for both patients who are HIV negative *H. pylori* positive and patients who are HIV negative *H. pylori* negative. The difference in percentage of patients using buckets between these groups was not statistically significant (Bucket P = 0.1580, plastic bottle P = NA, glass bottle P = NA and drum P = NA). The patients who were HIV negative *H. pylori* positive only used buckets as means of transportation for water in their childhood. The HIV negative *H. pylori* negative patients, although predominately also using buckets in their childhood, also made use of other transportation methods in their childhood. The differences in water transportation method between patients who are HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative were not statistically significant (Bucket P = 0.7106, plastic bottle P = 1.0000, glass bottle P = NA and drum P = 1.0000), (NA = option not used by patients), (Figure 26).

Figure 27 illustrates water transportation method in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

All patients included in the HIV vs anti-*CagA* status evaluation used buckets. Figure 27 illustrates water transportation method in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood. Although there were differences in the percentage of patients using buckets in each subgroup, due to the low patient numbers for patients who were HIV negative anti-*CagA* positive and patients who were HIV negative and anti-*CagA* negative, statistical significance was not calculated for water transportation method in these patients.

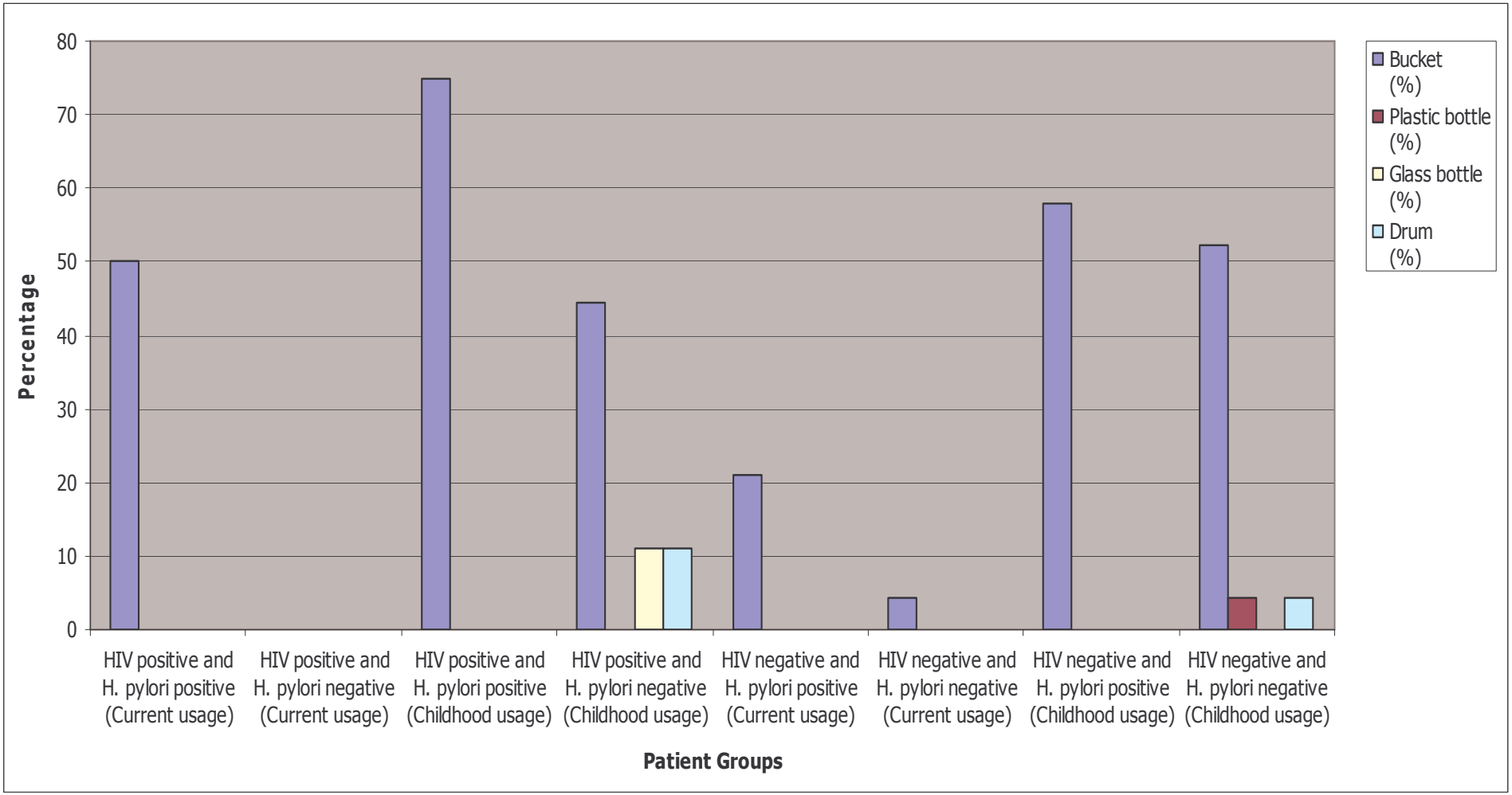


Figure 26: Water transportation method in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

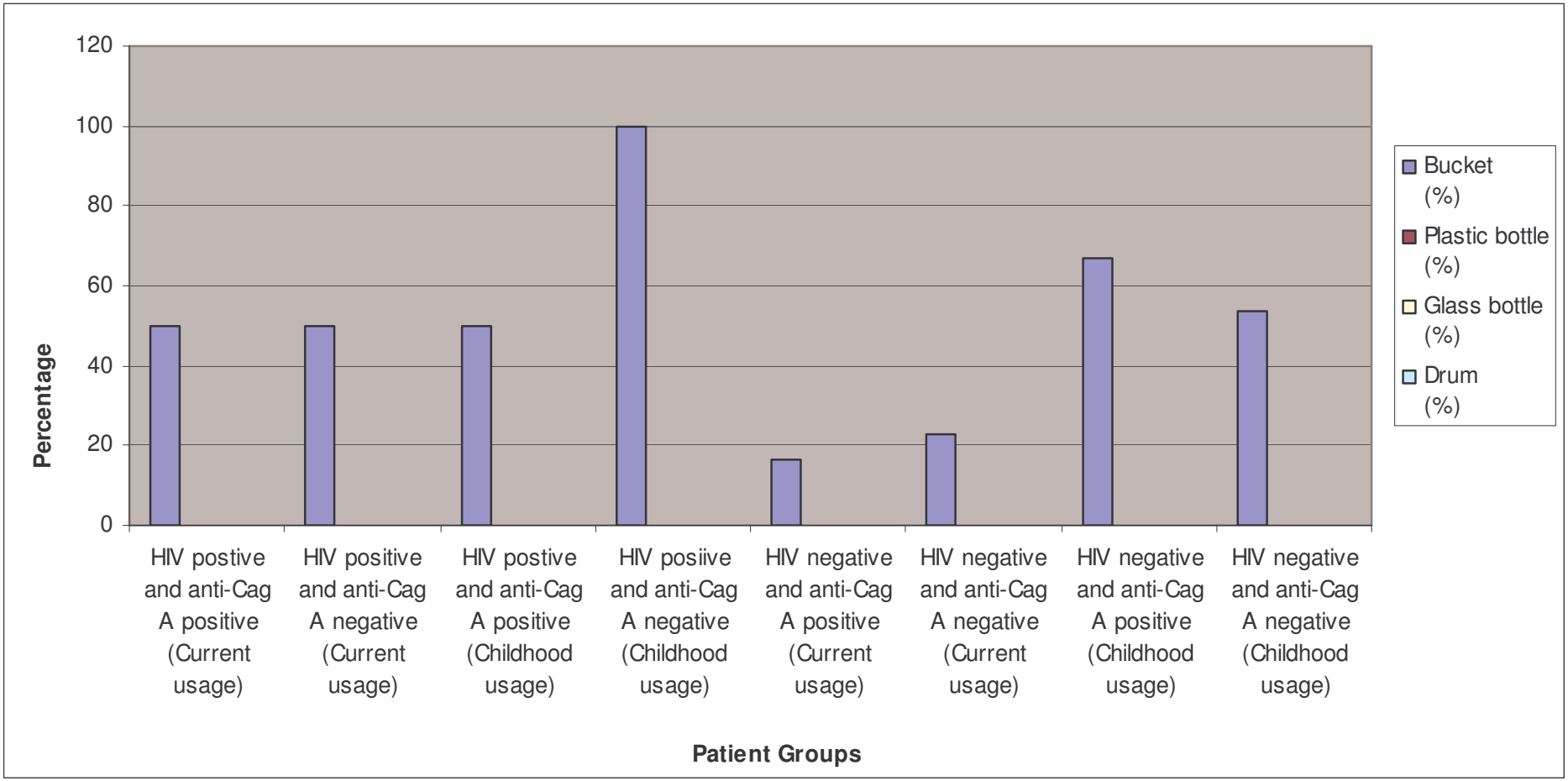


Figure 27: Water transportation method in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

5.12 Water storage

Only the patients who had to fetch water needed to store their water. Eight of the patients who had to collect water stored their water indoors and only 1 patient stored their water outside. In a similar pattern, during childhood 30 patients (who needed to store their water) stored water indoors and only 4 patients stored their water outside.

Table 16 shows water storage location in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Table 16: Water storage location in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Patient group	N	Indoors (%)	Outside (%)
HIV positive (Current storage)	17	17.65	5.88
HIV negative (Current storage)	42	11.9	0
HIV positive (Childhood storage)	17	58.82	0
HIV negative (Childhood storage)	42	47.62	9.52
H. pylori positive (Current storage)	27	25.93	3.7
H. pylori negative (Current storage)	32	3.13	0
H. pylori positive (Childhood storage)	27	51.85	11.11
H. pylori negative (Childhood storage)	32	50	3.13
Anti-*CagA* positive (Current storage)	10	30	0
Anti-*CagA* negative (Current storage)	17	23.53	5.88
Anti-*CagA* positive (Childhood storage)	10	50	10
Anti-*CagA* negative (Childhood storage)	17	52.94	11.76

Figure 28 illustrates the relationship in water Storage location, indoors or outside, in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

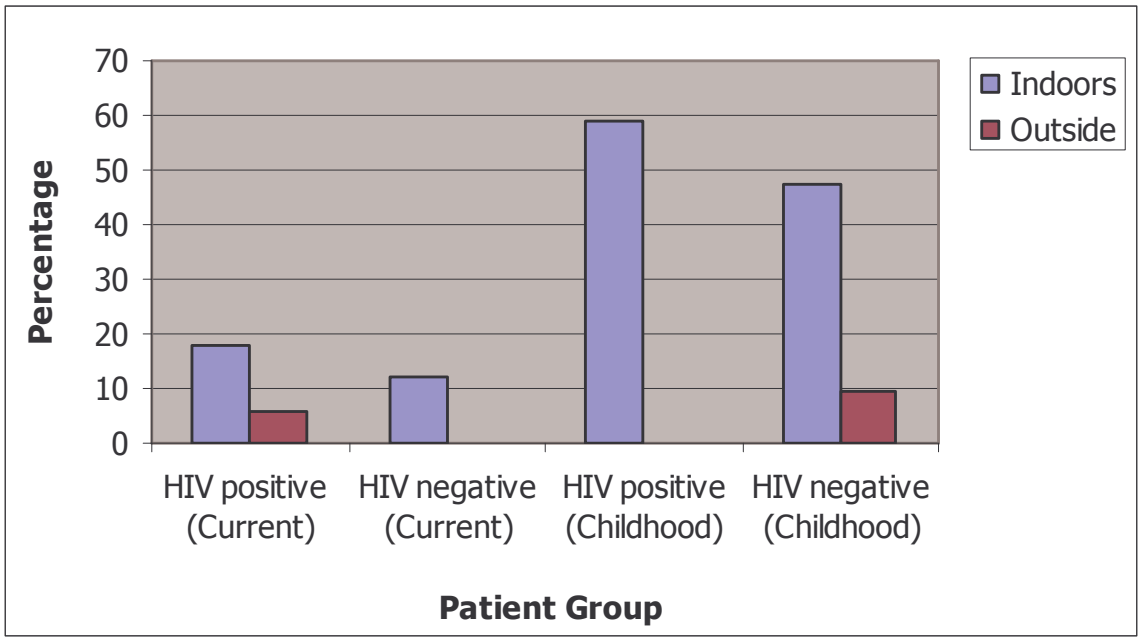


Figure 28: Water storage location in relation to HIV status, both currently and during childhood.

All of the HIV negative patients currently stored their water indoors. Although currently the HIV positive patients also predominantly stored their water indoors, a percentage of the patients did store their water outside. This difference was not statistically significant, (Indoors P = 0.6783 and outside P = 0.2881), (Figure 28).

All of the HIV positive patients stored their water indoors during childhood. Although HIV negative patients also predominately stored their water indoors during childhood, a percentage of the patients did store their water outside. This difference was not statistically significant (Indoors P = 0.4356 and outside P = 0.3139), (Figure 28).

Figure 29 illustrates water storage in relation to *H. pylori* status, both currently and during childhood.

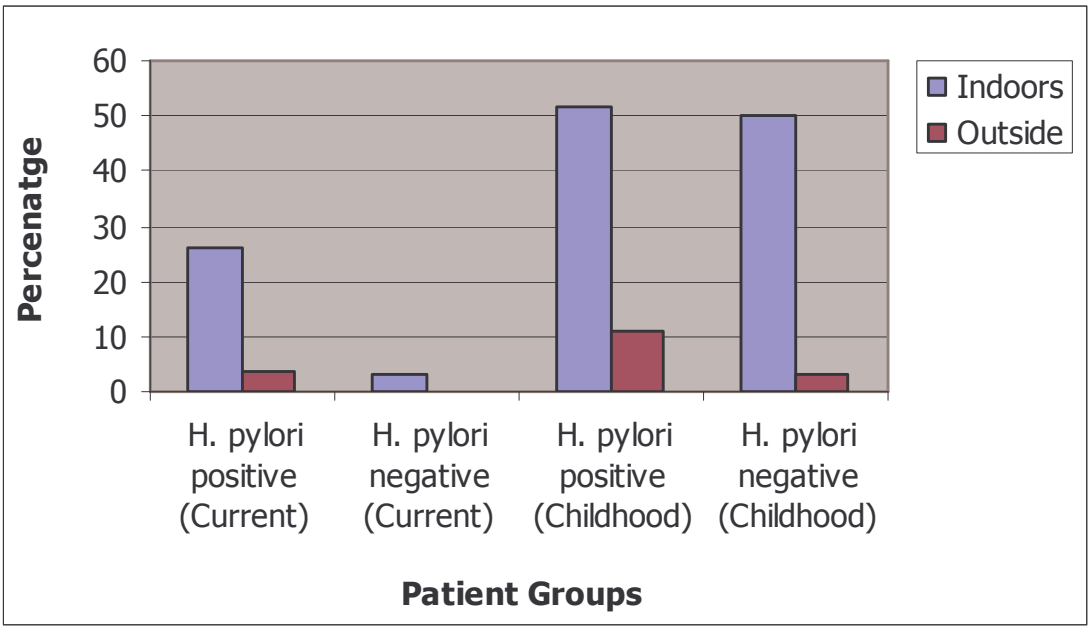


Figure 29: Water storage in relation to *H. pylori* status, both currently and during childhood.

The *H. pylori* negative patients currently only store their water indoors. In the *H. pylori* positive patients, although the majority also store their water indoors a percentage do stored their water outside. The difference in water storage location seen in the *H. pylori* positive and negative patients was statistically significant for indoors storage (Indoors P = 0.0166) however not for outside storage (Outside P = 0.4576), (Figure 29).

The differences in percentages of water storage location from childhood seen in the *H. pylori* positive and negative patients were not statistically significant (Indoors P = 0.6673 and outside P = 0.3232), (Figure 29)

Figure 30 illustrates water storage in relation to anti-*CagA* status, both currently and during childhood.

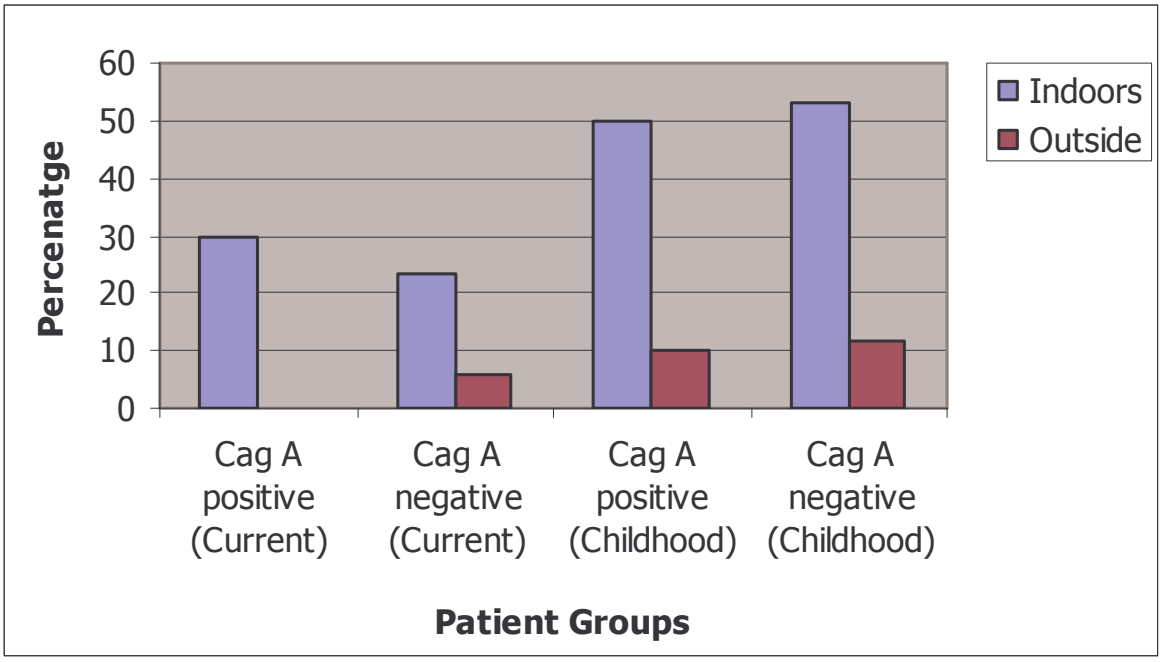


Figure 30: Water storage in relation to anti-*CagA* status, both currently and during childhood.

Although all the anti-*CagA* positive patients currently only stored their water indoors, the anti-*CagA* negative patients had some patients storing water indoors and another percentage storing outside. This difference was not statistically significant (Indoors P = 1.0000 and outside P = 1.0000), (Figure 30).

The differences in percentages of water storage location during childhood seen in the anti-*CagA* positive and negative patients were not statistically significant (Indoors P = 1.0000 and outside P = 1.0000), (Figure 30).

Table 17 shows water storage location in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Table 17: Water storage location in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Patient group	N	Indoors (%)	Outside (%)
HIV positive *H. pylori* positive (Current storage)	8	37.5	12.5
HIV positive *H. pylori* negative (Current storage)	9	0	0
HIV positive *H. pylori* positive (Childhood storage)	8	75	0
HIV positive *H. pylori* negative (Childhood storage)	9	44.44	0
HIV negative *H. pylori* positive (Current storage)	19	21.05	0
HIV negative *H. pylori* negative (Current storage)	23	4.35	0
HIV negative *H. pylori* positive (Childhood storage)	19	42.11	15.79
HIV negative *H. pylori* negative (Childhood storage)	23	52.17	4.35
HIV positive anti-*CagA* positive (Current storage)	4	50	0
HIV positive anti-*CagA* negative (Current storage)	4	25	25
HIV positive anti-*CagA* positive (Childhood storage)	4	50	0
HIV positive anti-*CagA* negative (Childhood storage)	4	100	0
HIV negative anti-*CagA* positive (Current storage)	6	16.67	0
HIV negative anti-*CagA* negative (Current storage)	13	23.08	0
HIV negative anti-*CagA* positive (Childhood storage)	6	50	16.67
HIV negative anti-*CagA* negative (Childhood storage)	13	38.46	15.38

Figure 31 illustrates Water storage in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

No water storage location was shown for patients who were HIV positive *H. pylori* negative indicating that all of these patients have a running water source on their premises. The difference in water storage location between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative is not statistically significant (Indoors P = 0.0824 and outside P = 0.0824), (Figure 31). A percentage of all the patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative stored their water indoors during childhood, with a higher percentage of the HIV positive *H. pylori* positive patients storing their water outside during childhood, figure 31. This difference in water storage location during

childhood between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative was not statistically significant (Indoors P = 0.3348 and Outside P = 0.3348)

A percentage of all of the patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative stored their water indoors, with a higher percentage of the HIV negative *H. pylori* positive storing their water outside, figure 31. The difference in water storage location ratio between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative was not statistically significant (Indoors P = 0.1580 and outside P = NA). The difference in water storage location during childhood between patients who were HIV negative *H. pylori* positive and patients who are HIV negative *H. pylori* negative was not statistically significant (Indoors P = 0.5155 and outside P = 0.3129).

Figure 32 illustrates water storage in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

All HIV patients who were also anti-*CagA* positive currently store their water indoors, where as 25% of the patients who were HIV positive anti-*CagA* negative store their water outside and another 25 % store their water iindoors. The majority of both patients who were HIV negative anti-*CagA* positive and patients who are HIV negative anti-*CagA* negative stored their water indoors, however there are small percentages of patients in both groups which stored their water outside.

However, due to the low patient numbers for patients who are HIV negative anti-*CagA* positive and patients who are HIV negative and anti-*CagA* negative, statistical significance was not calculated for water storage location in these patients.

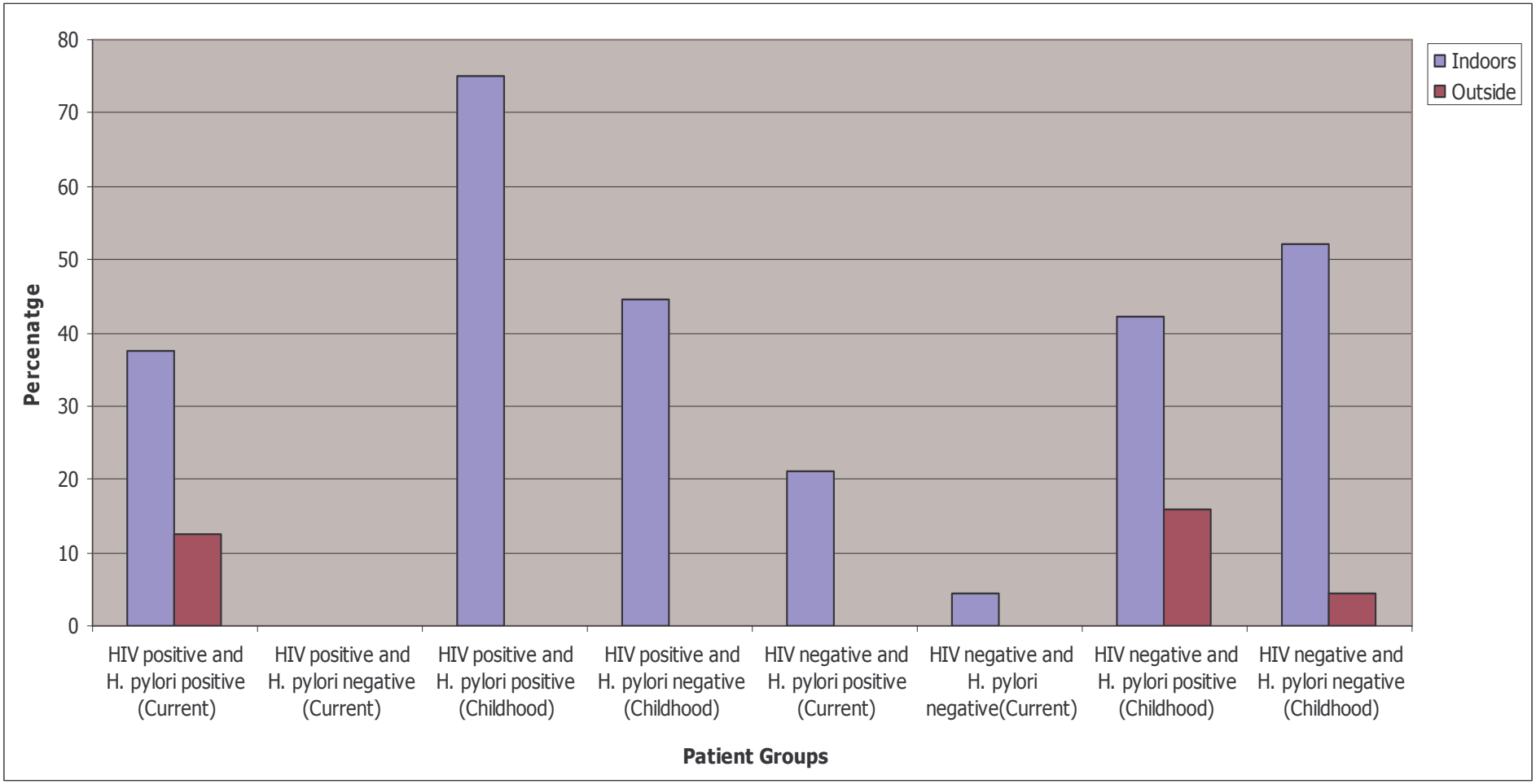


Figure 31: Water storage in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

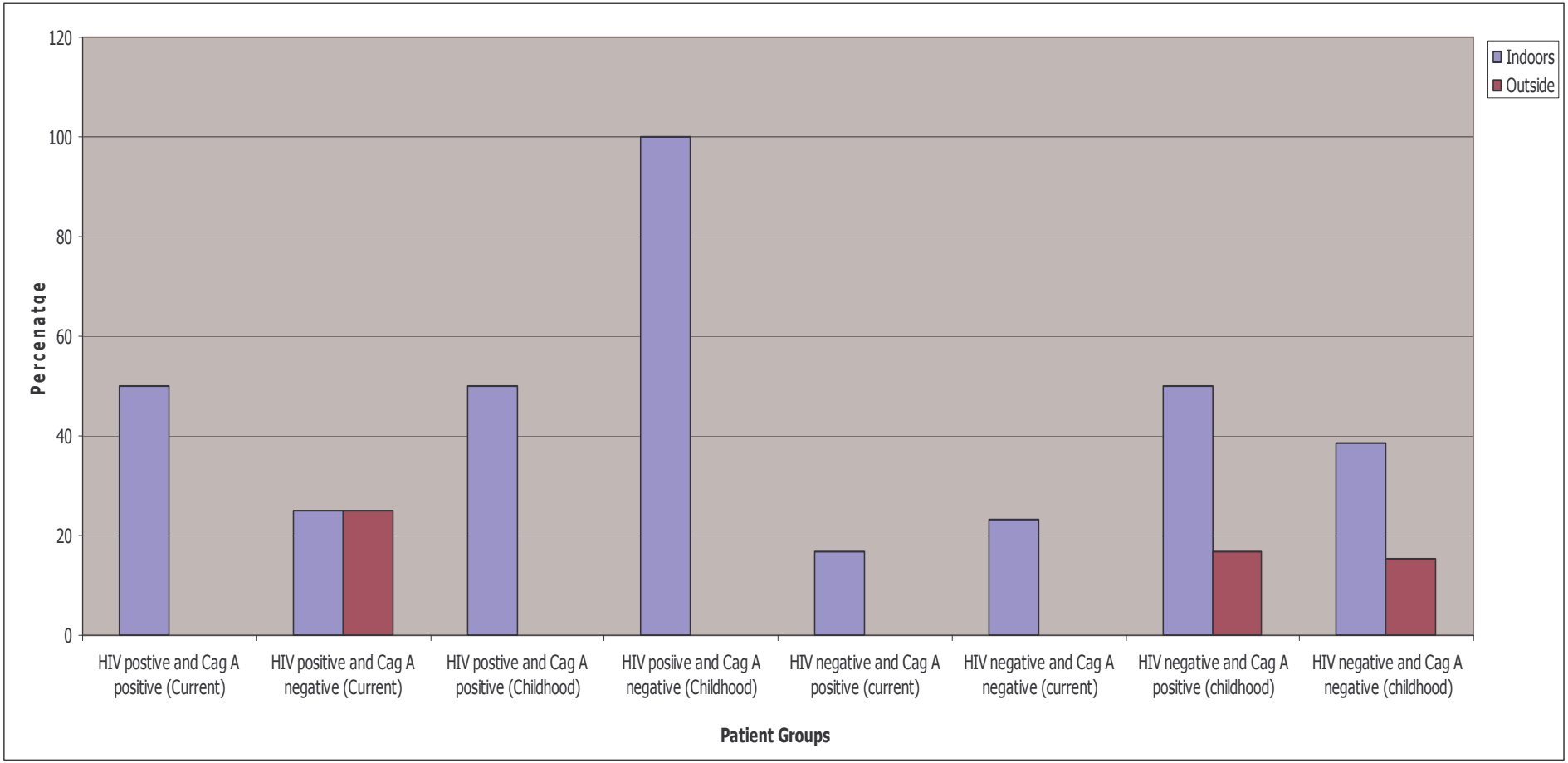


Figure 32: Water storage in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

Only the patients who needed to fetch water needed to store their water. Currently 4 patients stored their water in an open bucket and 5 stored their water in closed bucket. During childhood 8 patients stored their water in an open bucket, 26 stored their water in closed buckets and 2 stored their water in a large plastic container. Two patients used more than one storage facility.

Table 18 shows water Storage container in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Table 18: Water storage container in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Patient group	N	Open Bucket (%)	Closed Bucket (%)	Large open plastic container (%)
HIV positive (Current storage)	17	5.88	17.65	0
HIV negative (Current storage)	42	7.14	4.76	0
HIV positive (Childhood storage)	17	5.88	52.94	5.88
HIV negative (Childhood storage)	42	16.67	40.48	2.38
H. pylori positive (Current storage)	27	11.11	18.52	0
H. pylori negative (Current storage)	32	3.13	0	0
H. pylori positive (Childhood storage)	27	14.81	48.15	0
H. pylori negative (Childhood storage)	32	12.5	40.63	6.25
Anti-*CagA* positive (Current storage)	10	10	20	0
Anti-*CagA* negative (Current storage)	17	11.76	17.65	0
Anti-*CagA* positive (Childhood storage)	10	10	50	0
Anti-*CagA* negative (Childhood storage)	17	17.65	47.06	0

Figure 33 illustrates the relationship between HIV status and water storage containers, both currently and during childhood.

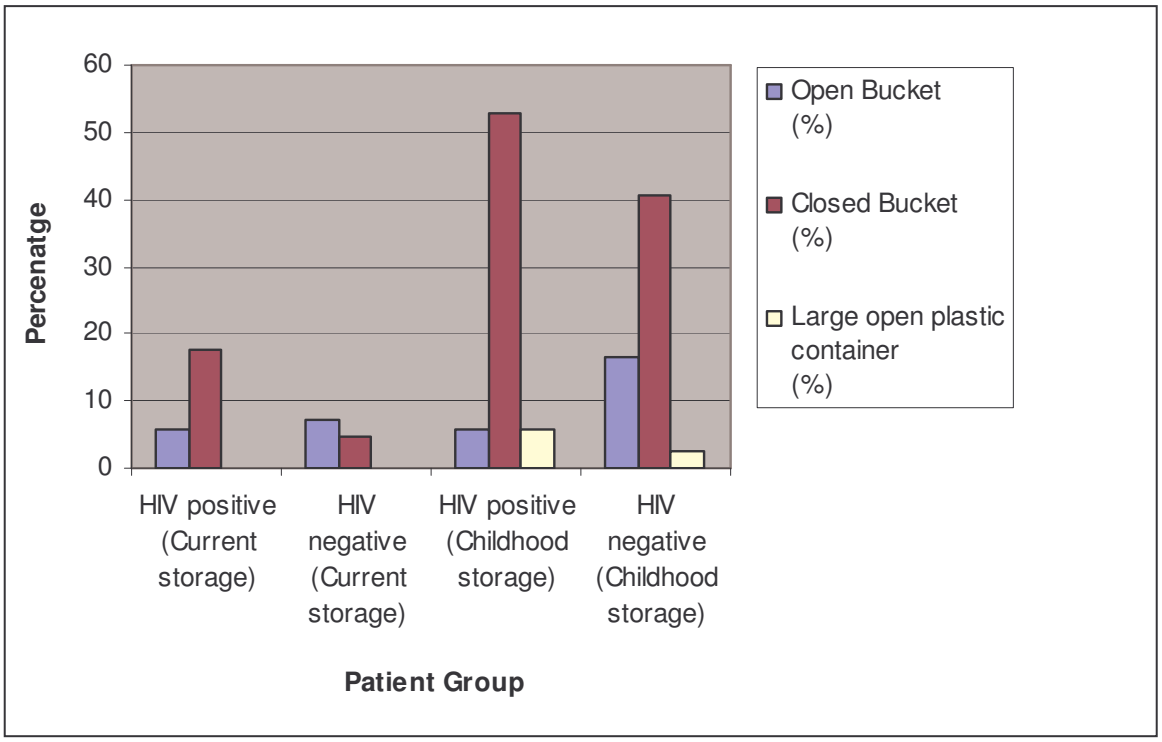


Figure 33: Water storage container in relation to HIV status, both currently and during childhood.

The differences in percentage of current water storage containers seen in the HIV positive and negative patients were not statistically significant (Open bucket P = 1.0000, closed Bucket P = 0.1381 and large open plastic container P = NA), (NA = option not used by patients), (Figure 33). Similarly, the differences in percentage of water storage containers used during childhood seen in the HIV positive and negative patients were not statistically significant (Open Bucket P = 0.4174, closed Bucket P = 0.3624 and large open plastic container P = 0.4968), (Figure 33).

Figure 34 illustrates Water storage container in relation to *H. pylori* status, both currently and during childhood.

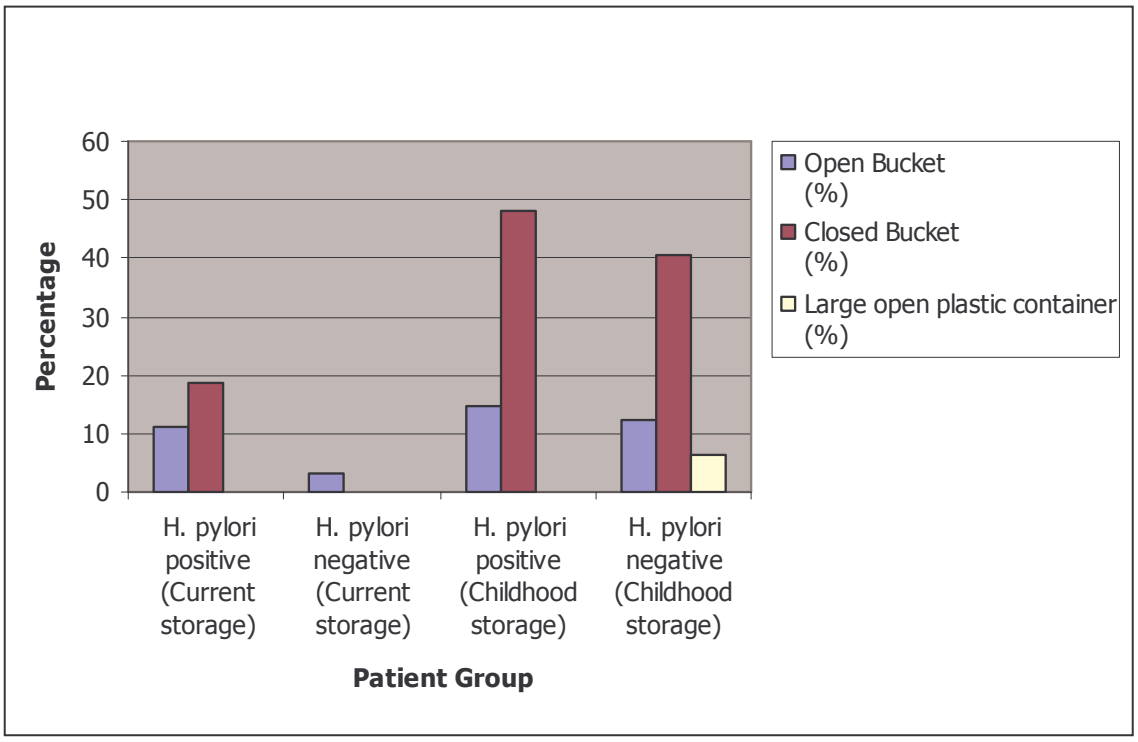


Figure 34: Water storage container in relation to *H. pylori* status, both currently and during childhood.

The *H. pylori* negative patients did not use a closed container to store their water, where as the *H. pylori* negative patients used open or closed buckets to store their water. This difference in percentage of water storage containers seen in the *H. pylori* positive and negative patients was statistically significant for a closed bucket (Closed bucket P = 0.0161) however not for an open bucket (Open Bucket P = 0.3232), (Figure 34).

The differences in ratio of water storage containers used during childhood seen in the *H. pylori* positive and negative patients were not statistically significant (Open Bucket P = 1.0000, closed bucket P = 0.5620 and large open plastic container P = 0.4950), (Figure 34).

Figure 35 illustrates Water storage container in relation to anti-*CagA* status, both currently and during childhood.

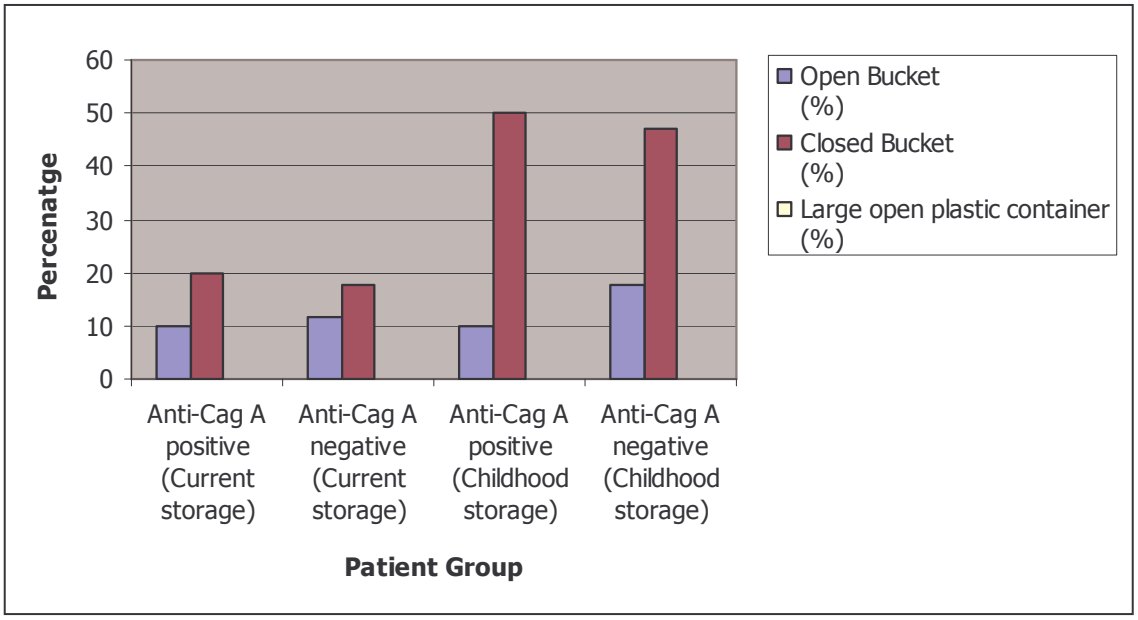


Figure 35: Water storage container in relation to anti-*CagA*, both currently and during childhood.

Both currently and in childhood only buckets were used for water storage containers for all anti-*CagA* positive and negative patients (Figure 35). The differences in percentage of current water storage containers seen in the anti-*CagA* positive and negative patients were not statistically significant (Open Bucket P = 1.0000 and Closed Bucket P = 1.0000), (Figure 35). Similarly, the differences in percentage of water storage containers during childhood seen in the anti-*CagA* positive and negative patients were not statistically significant (Open Bucket P = 1.0000 and closed Bucket P = 1.0000), (Figure 35).

Table 19 shows Water storage container in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Table 19: Water storage container in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Patient group	N	Open Bucket (%)	Closed Bucket (%)	Large open plastic container (%)
HIV positive *H. pylori* positive (Current storage)	8	12.5	37.5	0
HIV positive *H. pylori* negative (Current storage)	9	0	0	0
HIV positive *H. pylori* positive (Childhood storage)	8	12.5	62.5	0
HIV positive *H. pylori* negative (Childhood storage)	9	0	44.44	11.11
HIV negative *H. pylori* positive (Current storage)	19	10.53	10.53	0
HIV negative *H. pylori* negative (Current storage)	23	4.35	0	0
HIV negative *H. pylori* positive (Childhood storage)	19	15.79	42.11	0
HIV negative *H. pylori* negative (Childhood storage)	23	17.39	39.13	4.35
HIV positive anti-*CagA* positive (Current storage)	4	100	0	0
HIV positive anti-*CagA* negative (Current storage)	4	75	25	0
HIV positive anti-*CagA* positive (Childhood storage)	4	0	50	0
HIV positive anti-*CagA* negative (Childhood storage)	4	25	75	0
HIV negative anti-*CagA* positive (Current storage)	6	83.33	16.67	0
HIV negative anti-*CagA* negative (Current storage)	13	92.31	7.69	0
HIV negative anti-*CagA* positive (Childhood storage)	6	16.67	50	0
HIV negative anti-*CagA* negative (Childhood storage)	13	15.38	38.46	0

Figure 36 illustrates water storage container in relation to patient groups with known HIV and *H. pylori* status, both currently and during childhood.

No current water storage container was shown for patients who were HIV positive *H. pylori* negative indicating that all of these patients have a running water source on their premises. The difference in current water storage container ratio between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Open Bucket P = 0.4706, closed Bucket P = 0.0824 and large open plastic container P = NA), (NA = option not used by patients), (Figure 36). The difference in ratio of water storage container use during childhood between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Open Bucket P = 0.4706, closed Bucket P = 0.6372 and large open plastic container P = 1.0000), (Figure 36).

Patients who were HIV negative *H. pylori* negative only stored their water in an open bucket, whereas the patients who were HIV negative *H. pylori* positive used different storage containers. However, these differences in ratio of water storage containers between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative were not statistically significant (Open bucket P = 0.5613, closed Bucket P = 0.1986 and container P = NA), (NA = option not used by patients), (Figure 36). The difference in ratio of water storage containers during childhood between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative were not statistically significant (Open Bucket P = 1.0000, closed Bucket P = 1.0000 and large open plastic container P = 1.0000), (Figure 36).

Figure 37 illustrates water storage container in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

The patients who are HIV positive anti-*CagA* positive only used open buckets for water storage, whereas percentage of the patients who were HIV positive anti-*CagA* negative used open buckets and another percentage used closed buckets. The patients who are HIV positive anti-*CagA* positive only currently used closed buckets for water storage during childhood, where as for the patients who were HIV positive anti-*CagA* negative some of the patients used open buckets and others used closed buckets during childhood.

The majority of the patients who were HIV negative anti-*CagA* positive and the patients who were HIV negative anti-*CagA* negative currently use open buckets for water storage. However for both these patient groups the majority of the patients used closed buckets for water storage. Due to the low patient numbers for patients who are HIV negative anti-*CagA* positive and patients who were HIV negative and anti-*CagA* negative, statistical significance was not calculated for race in these patients.

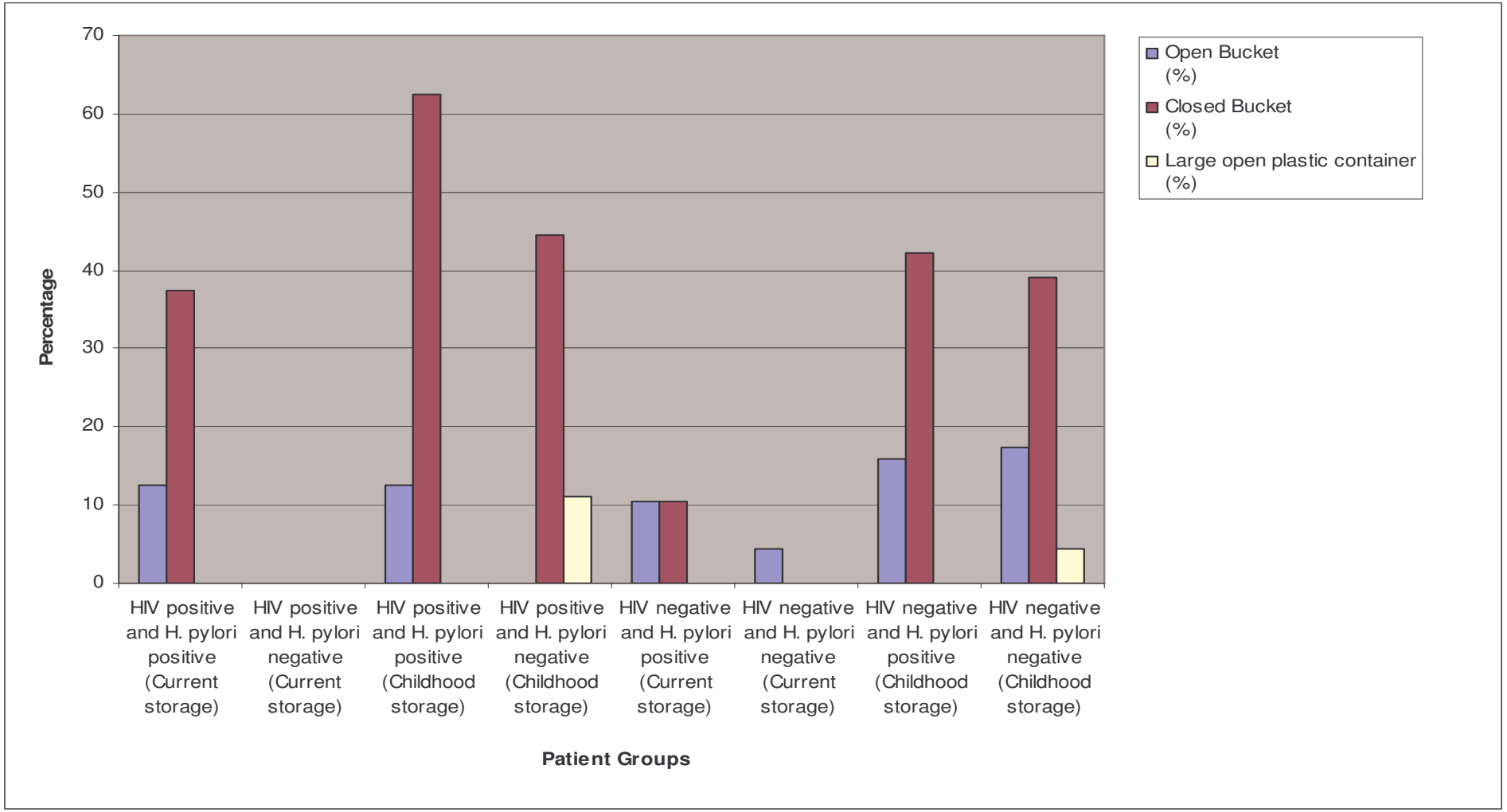


Figure 36: Water storage container in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

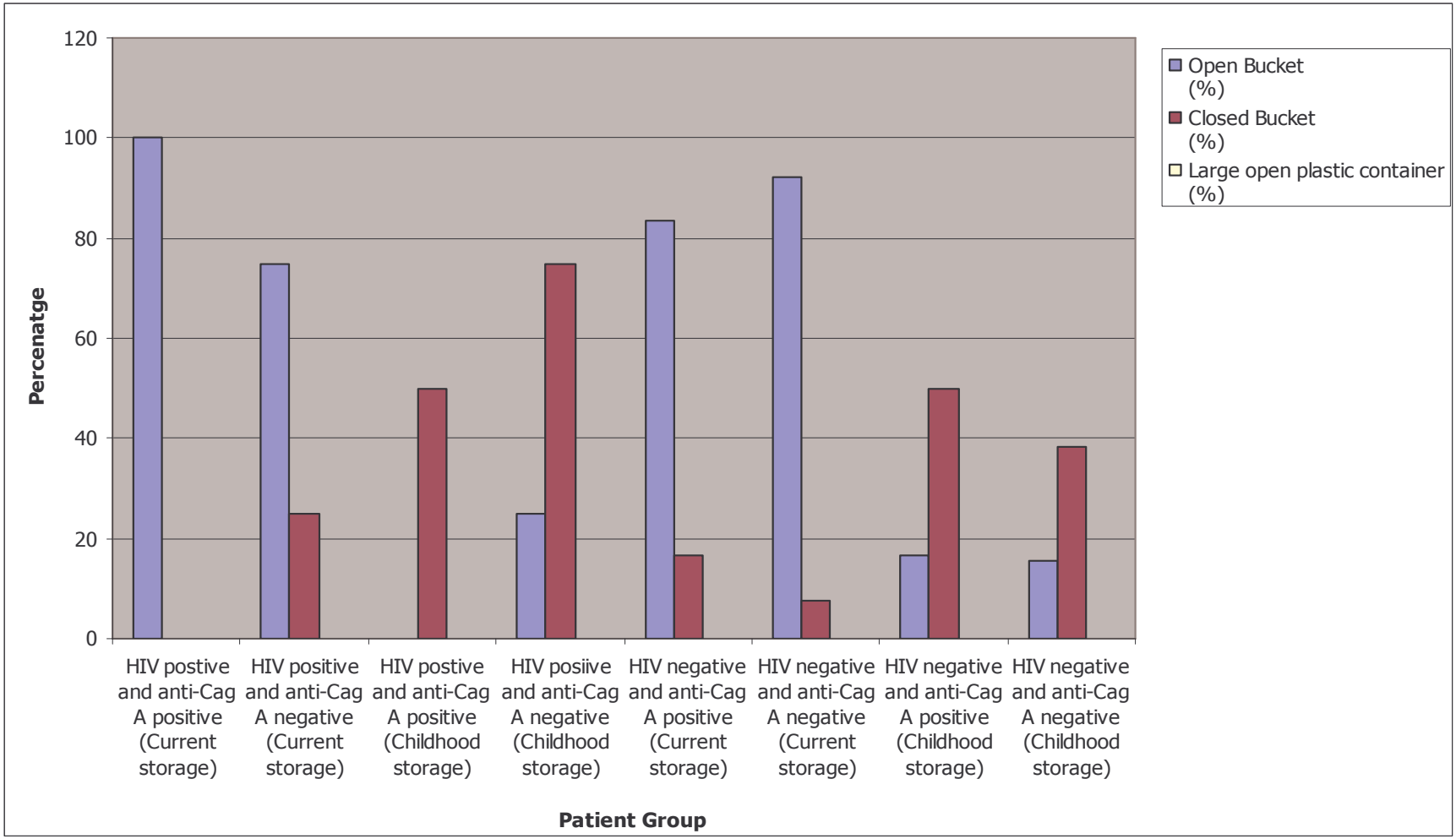


Figure 37: Water storage container in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

5.13 Boiling of Water before consumption

Six patients currently boiled their water and 53 patients did not boil their water. During childhood 3 patients boiled their water, 54 patients did not boil their water and 2 boiled their water only when sick.

Table 20 shows boiling of water before consumption in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Table 20: Boiling of water before consumption in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Patient group	N	Boiled (%)	Not Boiled (%)	Only Boiled when ill (%)
HIV positive (Current usage)	17	23.53	76.47	0
HIV negative (Current usage)	42	4.76	95.24	0
HIV positive (Childhood usage)	17	5.88	88.24	5.88
HIV negative (Childhood usage)	42	4.76	92.86	2.38
H. pylori positive (Current usage)	27	11.11	88.89	0
H. pylori negative (Current usage)	32	9.36	90.63	0
H. pylori positive (Childhood usage)	27	3.7	92.59	3.7
H. pylori negative (Childhood usage)	32	6.25	90.63	3.13
Anti-*CagA* positive (Current usage)	10	20	80	0
Anti-*CagA* negative (Current usage)	17	5.88	94.12	0
Anti-*CagA* positive (Childhood usage)	10	0	100	0
Anti-*CagA* negative (Childhood usage)	17	5.88	88.24	5.88

Figure 38 illustrates the relationship of boiling of water before consumption in relation to HIV status, both currently and during childhood.

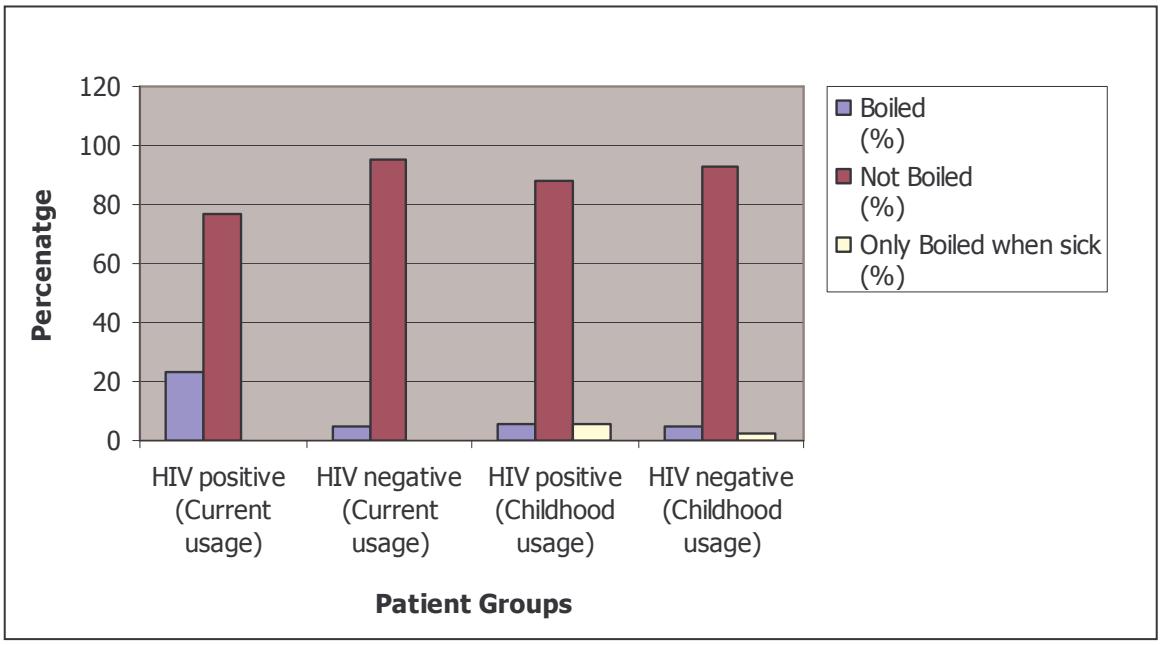


Figure 38: Boiling of water before consumption in relation to HIV status, both currently and during childhood.

The majority of both the HIV positive and HIV negative patients do not currently boil their water before consumption. None of the patients currently boil their water when they are ill. The differences in percentage of boiling water before consumption seen in the HIV positive and negative patients are statistically significant for both boiling and not boiling water before consumption (Boiled water P = 0.05 and not Boiled water P = 0.05). None of the HIV positive or negative patients currently boil their water when they are ill (Figure 38).

The differences ratio of boiling water before consumption during childhood seen in the HIV positive and negative patients were not statistically significant (Boiled water P = 1.0000, Not Boiled water P = 0.6199 and boiled water when ill P = 0.4968), (Figure 38).

Figure 39 illustrates boiling of water before consumption in relation to *H. pylori* status, both currently and during childhood.

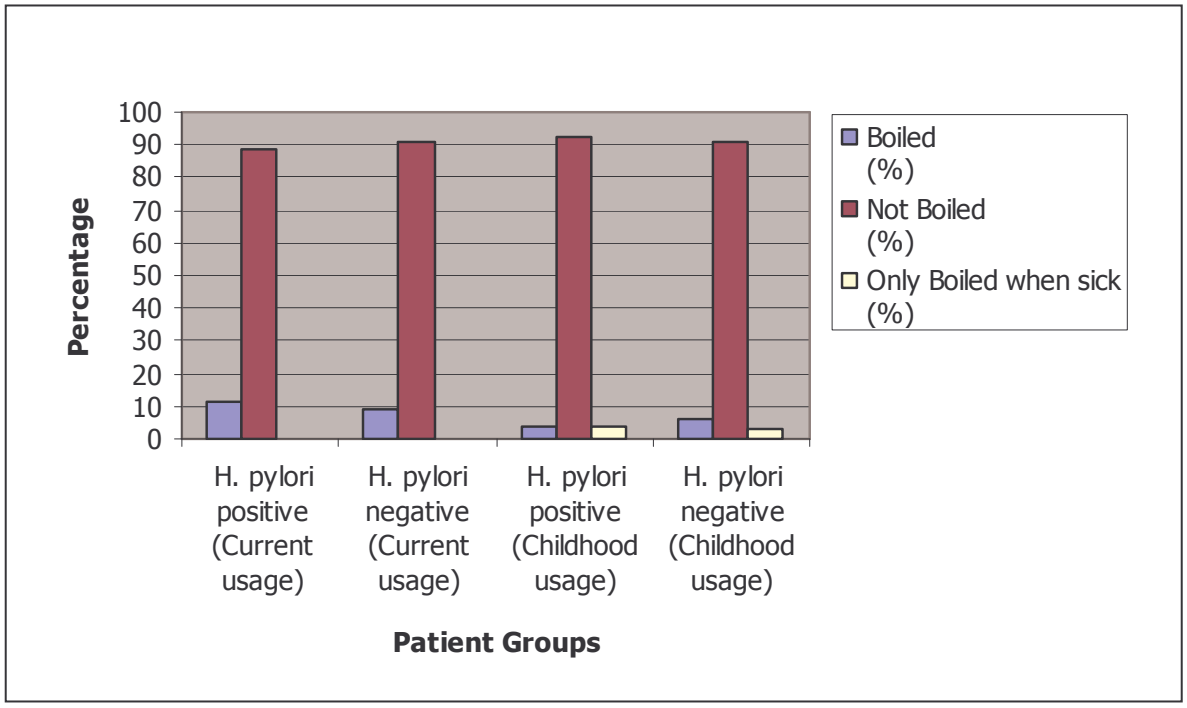


Figure 39: Boiling of water before consumption in relation to *H. pylori* status, both currently and during childhood.

The differences in ratio of currently boiling of water before consumption seen in the *H. pylori* positive and negative patients were not statistically significant (Boiled water P = 1.0000 and not Boiled water P = 1.0000). Neither the *H. pylori* positive nor negative patients currently boil their water when they are ill (Figure 39).

The differences in percentage of boiling of water before consumption during childhood seen in the *H. pylori* positive and negative patients were not statistically significant (Boiled water P = 1.0000, not Boiled water P = 1.0000 and boiled water when ill P = 1.0000), (Figure 39).

Figure 40 illustrates boiling of water before consumption in relation to anti-*CagA* status, both currently and during childhood.

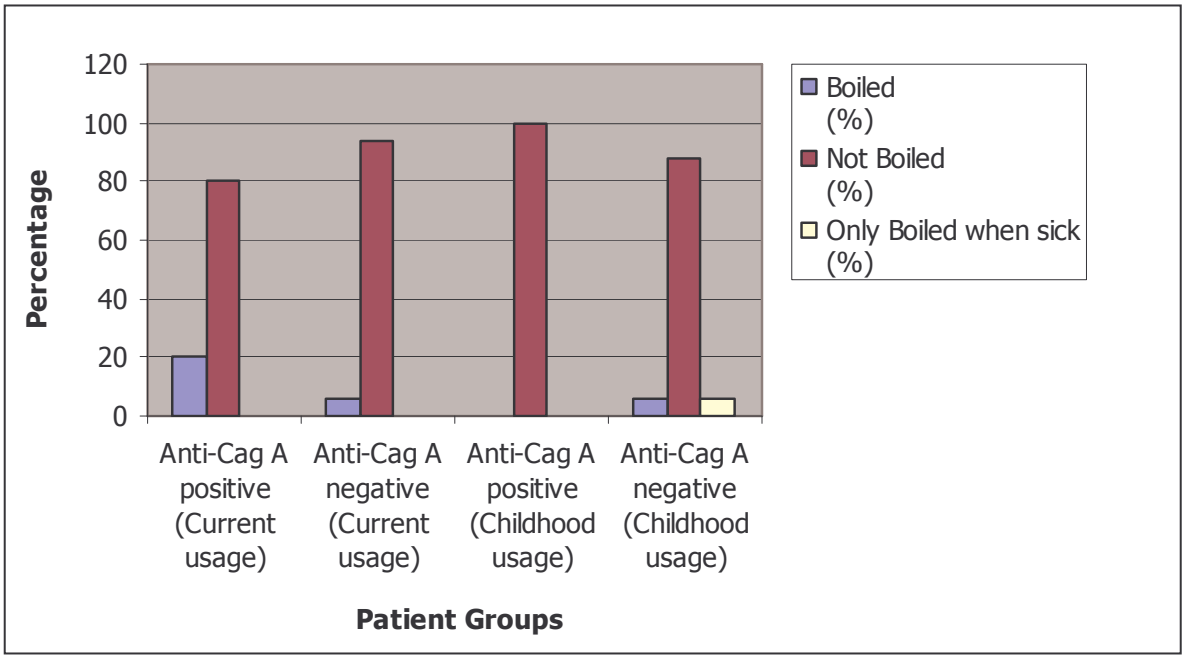


Figure 40: Boiling of water before consumption in relation to anti-*CagA* status, both currently and during childhood.

The differences in percentage of currently boiling of water before consumption seen in the anti-*CagA* positive and negative patients were not statistically significant (Boiled water P = 0.5350, not Boiled water P = 0.5350). Neither the anti-*CagA* positive nor negative patients currently boiled their water when they were ill (figure 40).

None of the anti-*CagA* positive patients boiled their water before consumption during their childhood. Although the majority of the anti-*CagA* negative patients did not boil their water before consumption, a percentage boiled their water before consumption and another percentage boiled their water when ill during their childhood. These differences in percentage of boiling of water before consumption during childhood seen in the anti-*CagA* positive and negative patients were not statistically significant (Boiled water P = 1.0000, not Boiled water P = 0.5157 and boiled water when ill P = 1.0000), (Figure 40).

Table 21 shows boiling of water before consumption in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Table 21: Boiling of water before consumption in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Patient group	N	Boiled (%)	Not Boiled (%)	Only Boiled when ill (%)
HIV positive *H. pylori* positive (Current usage)	8	25	75	0
HIV positive *H. pylori* negative (Current usage)	9	22.22	77.78	0
HIV positive *H. pylori* positive (Childhood usage)	8	0	100	0
HIV positive *H. pylori* negative (Childhood usage)	9	11.11	77.78	11.11
HIV negative *H. pylori* positive (Current usage)	19	5.28	94.74	0
HIV negative *H. pylori* negative (Current usage)	23	4.35	95.65	0
HIV negative *H. pylori* positive (Childhood usage)	19	5.26	89.47	5.28
HIV negative *H. pylori* negative (Childhood usage)	23	4.35	95.65	0
HIV positive anti-*CagA* positive (Current usage)	4	50	50	0
HIV positive anti-*CagA* negative (Current usage)	4	0	100	0
HIV positive anti-*CagA* positive (Childhood usage)	4	0	4	0
HIV positive anti-*CagA* negative (Childhood usage)	4	0	4	0
HIV negative anti-*CagA* positive (Current usage)	6	0	100	0
HIV negative anti-*CagA* negative (Current usage)	13	7.69	92.31	0
HIV negative anti-*CagA* positive (Childhood usage)	6	0	6	0
HIV negative anti-*CagA* negative (Childhood usage)	13	7.69	11	7.69

Figure 41 illustrates boiling of water before consumption in relation to patient groups with known HIV and *H. pylori* status, both currently and during childhood.

The difference in percentage of boiling of water before consumption between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Boiled water P = 1.0000 and not Boiled water P = 1.0000). Neither the HIV positive *H. pylori* positive patients nor the HIV positive *H. pylori* negative patients currently boil their water when they are ill (Figure 41). None of the patients who were HIV positive *H. pylori* positive boiled their water before consumption during childhood, where as the HIV positive *H. pylori* negative patients ranged between boiling their water, not boiling their water and boiling their water when they were ill during childhood. These differences in the ratios of boiling of water before

consumption between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative are however not statistically significant (Boiled water P = 1.0000, not Boiled water P = 0.4706 and boiled water when ill P = 1.0000), (Figure 41).

The difference in percentage of boiling of water before consumption between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative were not statistically significant (Boiled water P = 1.0000 and not Boiled P = 1.0000). Neither of the HIV negative *H. pylori* positive nor patients who are HIV negative *H. pylori* negative currently boil their water when they are ill (Figure 41). None of the patients who were HIV negative *H. pylori* negative boiled their water when they were ill in their childhood. However the patients who were HIV negative *H. pylori* positive ranged between boiling their water, not boiling their water and boiling their water when they were ill during childhood. The differences in ratio of boiling of water before consumption during childhood between patients who are HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative were not statistically significant (Boiled water P = 1.0000, not Boiled water P = 0.5813 and boiled water when ill P = 0.4524), (Figure 41).

Figure 42 illustrates boiling of water before consumption in relation to patient groups with known HIV and anti-*CagA* status, both currently and during childhood.

Fifty percent of the patients who were HIV positive anti-*CagA* positive boiled their water and 50% did not, whereas none of the patients who were HIV positive anti-*CagA* negative boiled their water. Both of these two patients groups did not boil their water during childhood (Figure 42). The majority of the patients who are HIV negative anti-*CagA* positive do not currently boil their water. All of the patients who are HIV negative anti-*CagA* negative do not currently boil their water. All of the patients who were HIV negative anti-*CagA* positive did not boil their water during childhood. Whereas, the HIV negative anti-*CagA* negative had a close to even distribution between patients boiling their water before consumption, patients not boiling their water before consumption and patients who only boiled their water when ill during childhood.

Due to the low patient numbers for patients who are HIV negative anti-*CagA* positive and patients who were HIV negative and anti-*CagA* negative, statistical significance was not calculated for boiling of water before consumption in these patients.

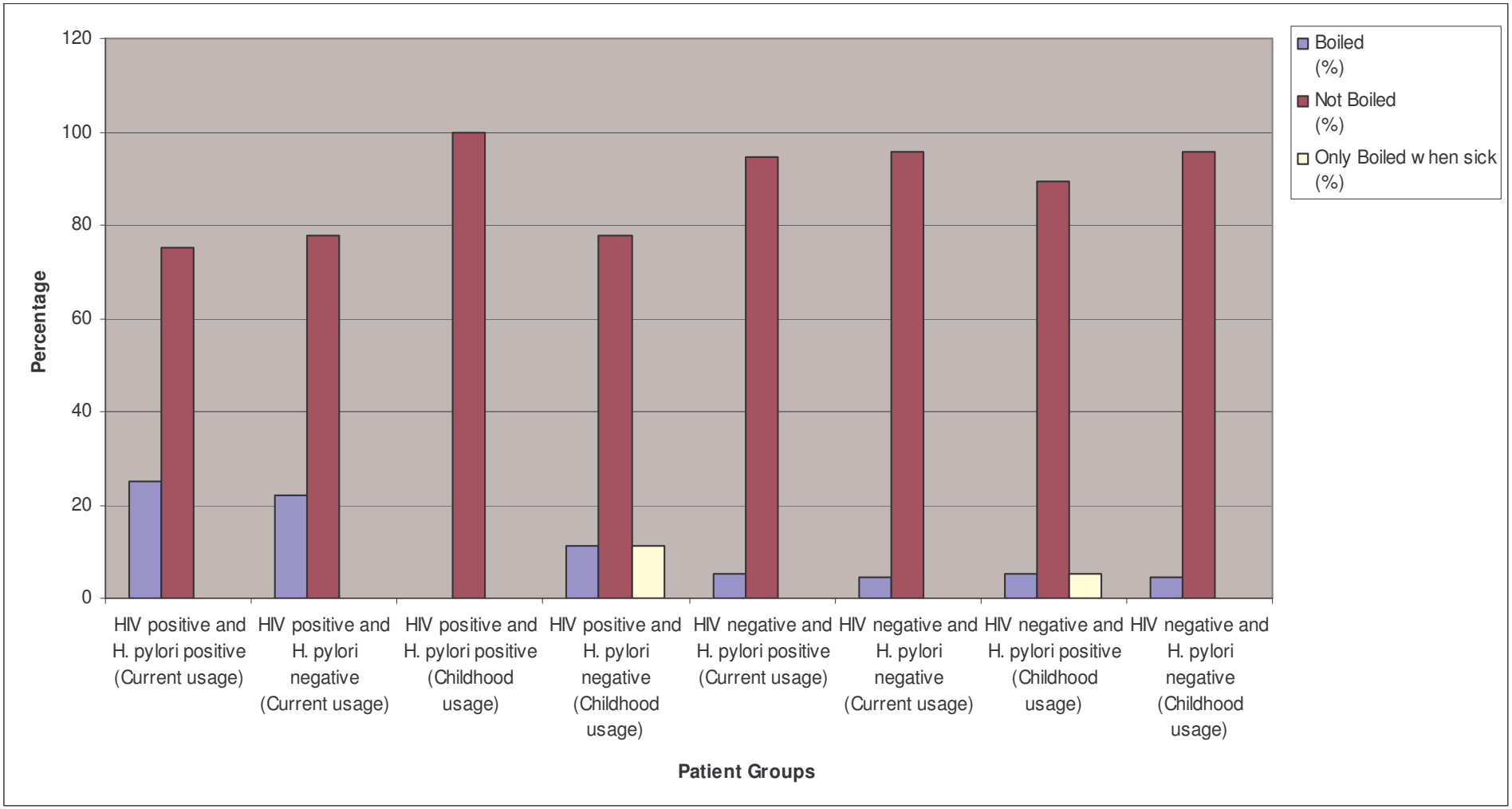


Figure 41: Boiling of water before consumption in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

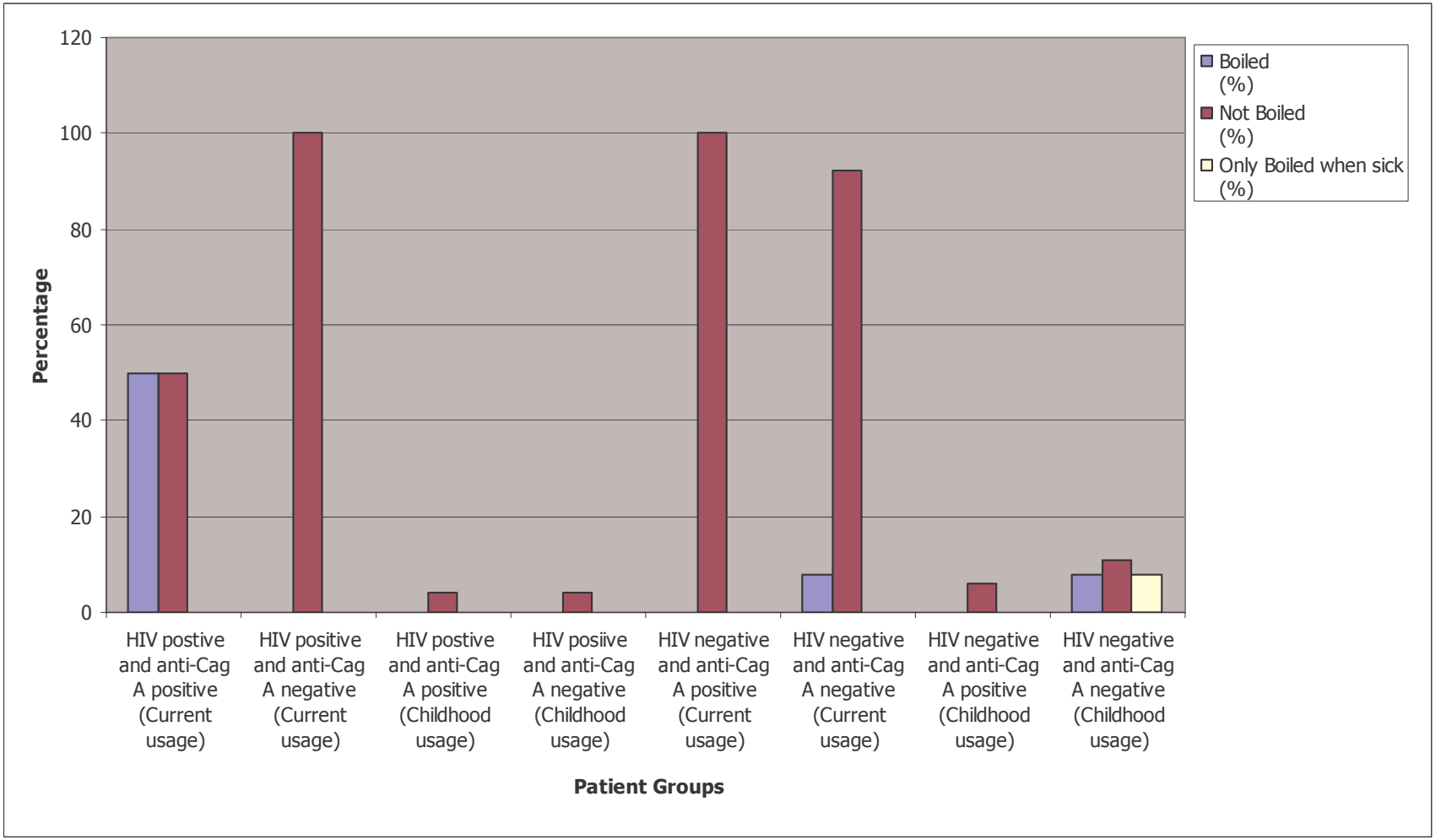


Figure 42: Boiling of water before consumption in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

5.14 Toilet location

All of the toilets which were indoors were flushing toilets and most of the toilets which were outside were pit latrines except for a few patients who had flushing toiles outside. Currently Twenty-eight patients had toilets outside and 31 patients had toilets indoors. During childhood 48 patients had toilets outside and 11 patients had toilets indoors.

Table 22 shows toilet location in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Table 22: Toilet location in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Patient group	N	Indoors (%)	Outside (%)
HIV positive (Current Location)	17	47.06	52.94
HIV negative (Current Location)	42	57.14	42.88
HIV positive (Childhood Location)	17	11.76	88.24
HIV negative (Childhood Location)	42	21.43	78.57
H. pylori positive (Current Location)	27	33.33	62.96
H. pylori negative (Current Location)	32	68.75	31.25
H. pylori positive (Childhood Location)	27	92.59	7.41
H. pylori negative (Childhood Location)	32	71.86	28.13
Anti-*CagA* positive (Current Location)	10	40	60
Anti-*CagA* negative (Current Location)	17	35.29	64.71
Anti-*CagA* positive (Childhood Location)	10	0	100
Anti-*CagA* negative (Childhood Location)	17	11.76	88.24

Figure 43 illustrates toilet location in relation to HIV status, both currently and during childhood.

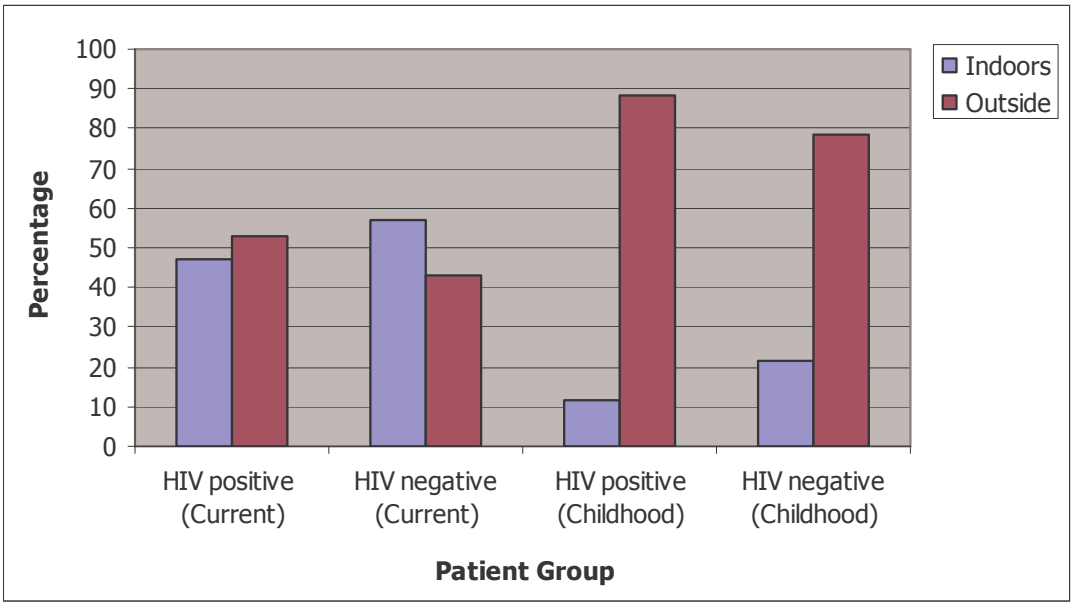


Figure 43: Toilet location in relation to HIV status, both currently and during childhood.

The differences in percentage of current toilet location seen in the HIV positive and negative patients were not statistically significant (Indoors P = 0.4814 and outside P = 0.4814), (Figure 43).

Similarly, the differences in precentage of toilet location during childhood seen in the HIV positive and negative patients were not statistically significant (Indoors P = 0.4638 and outside P = 0.4838), (Figure 43).

Figure 44 illustrates toilet location in relation to *H. pylori* status, both currently and during childhood.

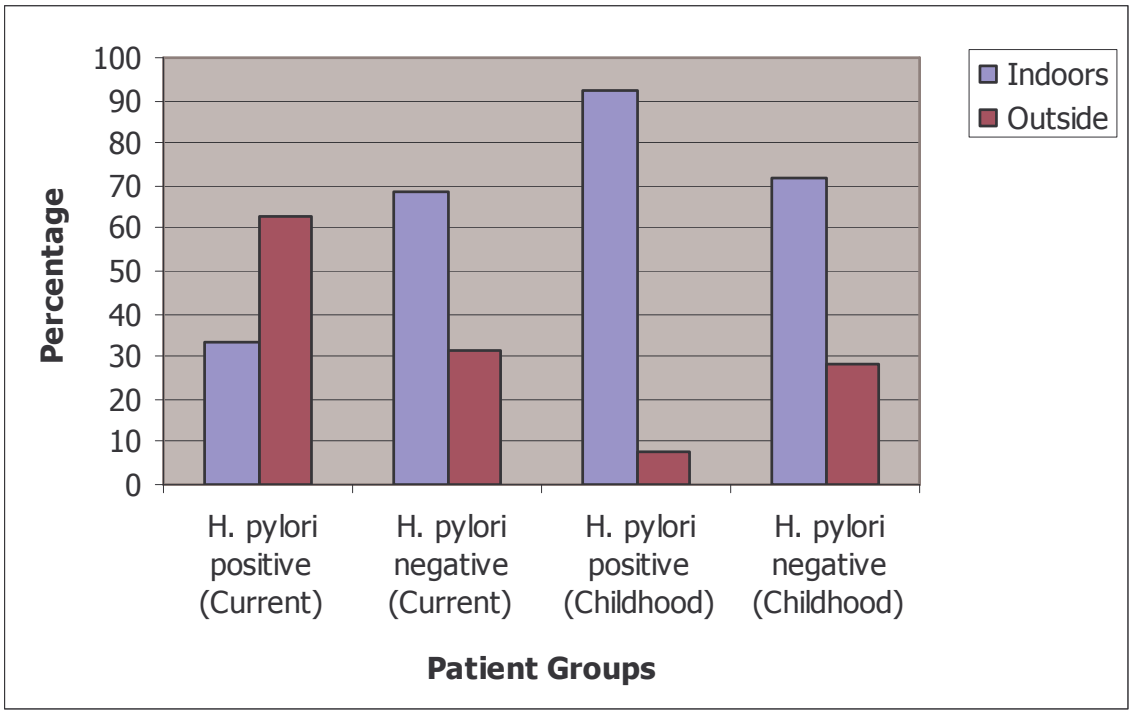


Figure 44: Toilet location in relation to *H. pylori* status, both currently and during childhood.

The differences in precentage of current toilet location seen in the *H. pylori* positive and negative patients are statistically significant (Indoors P = 0.0066 and outside P = 0.0149). The majority of *H. pylori* positive patients currently have toilet facilities outside, where as the majority of *H. pylori* negative patients have toilet facilities indoors (Figure 44).

The differences in precentage of toilet location during childhood seen in the *H. pylori* positive and negative patients were also statistically significant (Indoors P = 0.0418 and outside P = 0.0418). Although both *H. pylori* positive and negative patients predominately had toilet facilities indoors, a higher percentage of the *H. pylori* negative patients had toilet facilities outside during childhood (Figure 44).

Figure 45 illustrates toilet location in relation to anti-*CagA* status, both currently and during childhood.

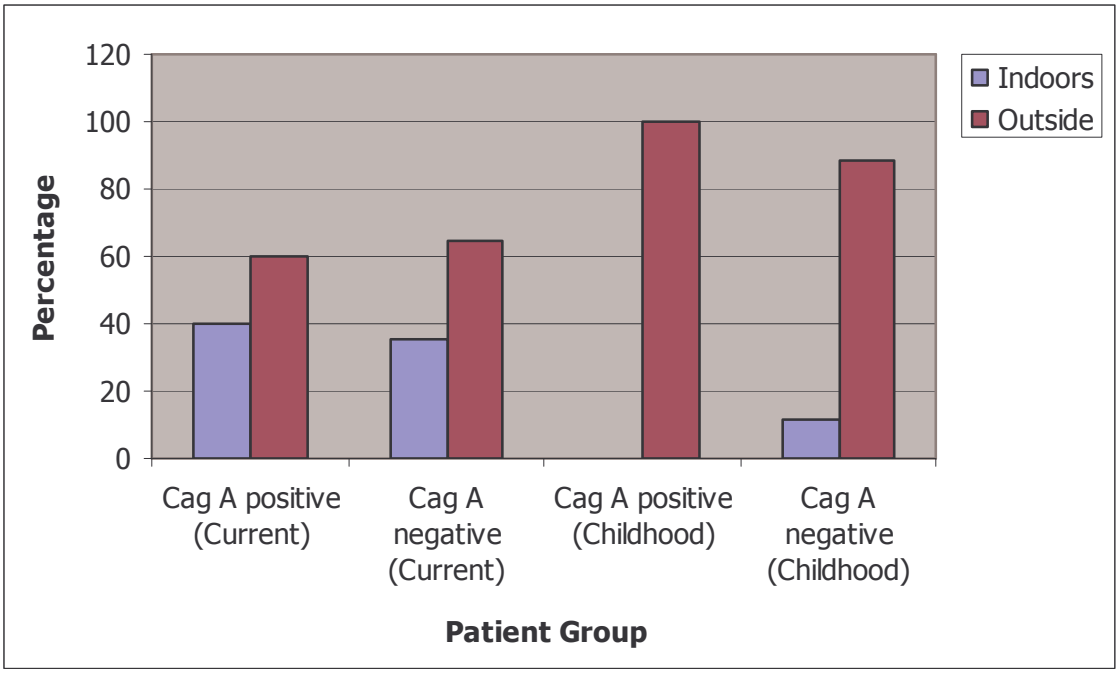


Figure 45: Toilet location in relation to anti-*CagA* status, both currently and during childhood.

The differences in percenatge of current toilet location seen in the anti-*CagA* positive and negative patients were not statistically significant (Indoors P = 1.0000 and outside P = 1.0000), (Figure 45). All anti-*CagA* positive patients had toilets outside during childhood, whereas the majority of anti-*CagA* negative patients also had tolites outside during childhood, a percentage did have a toilet indoors during childhood. The differences in percentage of toilet location seen in the anti-*CagA* positive and negative patients were not statistically significant (Indoors P = 0.5157 and outside P = 0.5157), (Figure 45).

Table 23 shows toilet location in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Table 23: Toilet location in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Patient group	N	Indoors (%)	Outside (%)
HIV positive *H. pylori* positive (Current Location)	8	37.5	62.5
HIV positive *H. pylori* negative (Current Location)	9	55.56	44.44
HIV positive *H. pylori* positive (Childhood Location)	8	0	100
HIV positive *H. pylori* negative (Childhood Location)	9	22.22	77.78
HIV negative *H. pylori* positive (Current Location)	19	36.84	63.16
HIV negative *H. pylori* negative (Current Location)	23	73.91	26.09
HIV negative *H. pylori* positive (Childhood Location)	19	10.53	89.47
HIV negative *H. pylori* negative (Childhood Location)	23	30.43	69.57
HIV positive anti-*CagA* positive (Current Location)	4	50	50
HIV positive anti-*CagA* negative (Current Location)	4	25	75
HIV positive anti-*CagA* positive (Childhood Location)	4	0	100
HIV positive anti-*CagA* negative (Childhood Location)	4	0	100
HIV negative anti-*CagA* positive (Current Location)	6	33.33	66.67
HIV negative anti-*CagA* negative (Current Location)	13	38.46	61.54
HIV negative anti-*CagA* positive (Childhood Location)	6	0	100
HIV negative anti-*CagA* negative (Childhood Location)	13	15.38	84.62

Figure 46 illustrates toilet location in relation to patient groups with known HIV and *H. pylori* status, both currently and during childhood.

Although the majority of patients who were HIV positive *H. pylori* positive currently have outside toilet facilities, the majority of patients who were HIV positive *H. pylori* negative currently have indoors toilet facilities. This was of no statistical significance (P = 0.6372), (Figure 46). Although the patients who were HIV positive *H. pylori* positive only had outside toilet facilities in their childhood, the patients who were HIV positive *H. pylori* negative showed a percentage patients making use of toilet facilities indoors in their childhood. These differences were not statistically significant (P = 0.4706), (Figure 46).

The patients who are HIV negative *H. pylori* positive currently predominately use outside toilet facilities, where as the patients who were HIV negative *H. pylori* negative currently predominately use indoor toilet facilities. These differences are statistically significant (P

= 0.0277), (Figure 46). Both patients groups who are HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative show predominately outside toilet facilities in childhood, however the HIV negative *H. pylori* negative group had a higher percentage of toilet facilities indoors than the patients who are HIV negative *H. pylori* positive. These differences were not statistically significant (P = 0.1494), (Figure 46).

Figure 47 illustrates Toilet location in relation to patient groups with known HIV and anti-*CagA* status, both currently and during childhood.

In the patient group where patients were HIV positive anti-*CagA* positive there was an even distribution between the percentage of patients having toilet facilities indoors and having toilet facilities outside. The HIV positive anti-*CagA* negative patient group showed a majority of the patients having toilets outside. Both these two patient groups only had toilets outside during childhood (Figure 47).

In the HIV negative anti-*CagA* positive patient group and HIV negative and anti-*CagA* negative patient group the toilet facilities were outside, both currently and during childhood (Figure 47). However, due to the low patient numbers for patients who are HIV negative anti-*CagA* positive and patients who are HIV negative and anti-*CagA* negative, statistical significance was not calculated for toilet location in these patients (Figure 47).

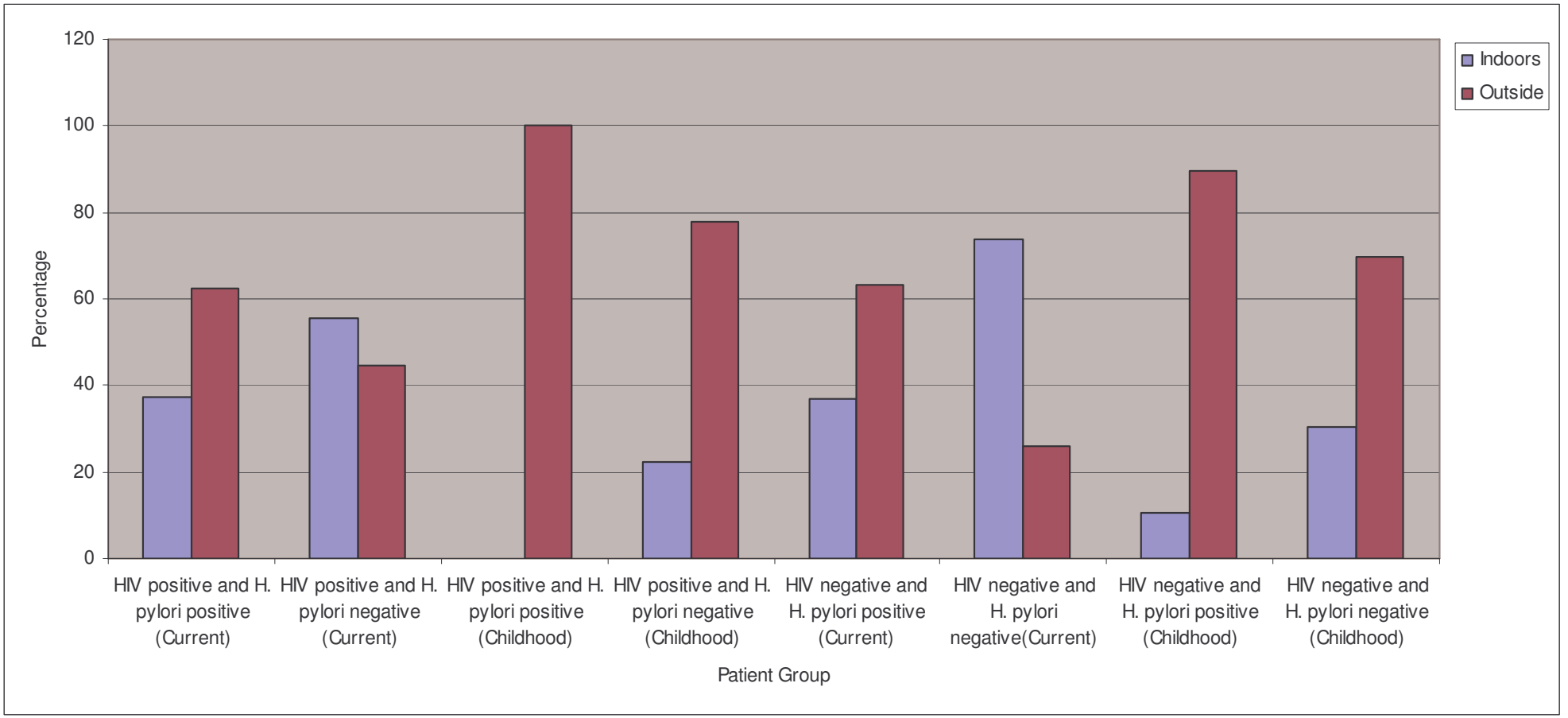


Figure 46: Toilet location in relation to patient groups with combined HIV and *H. pylori* status, both currently and during childhood.

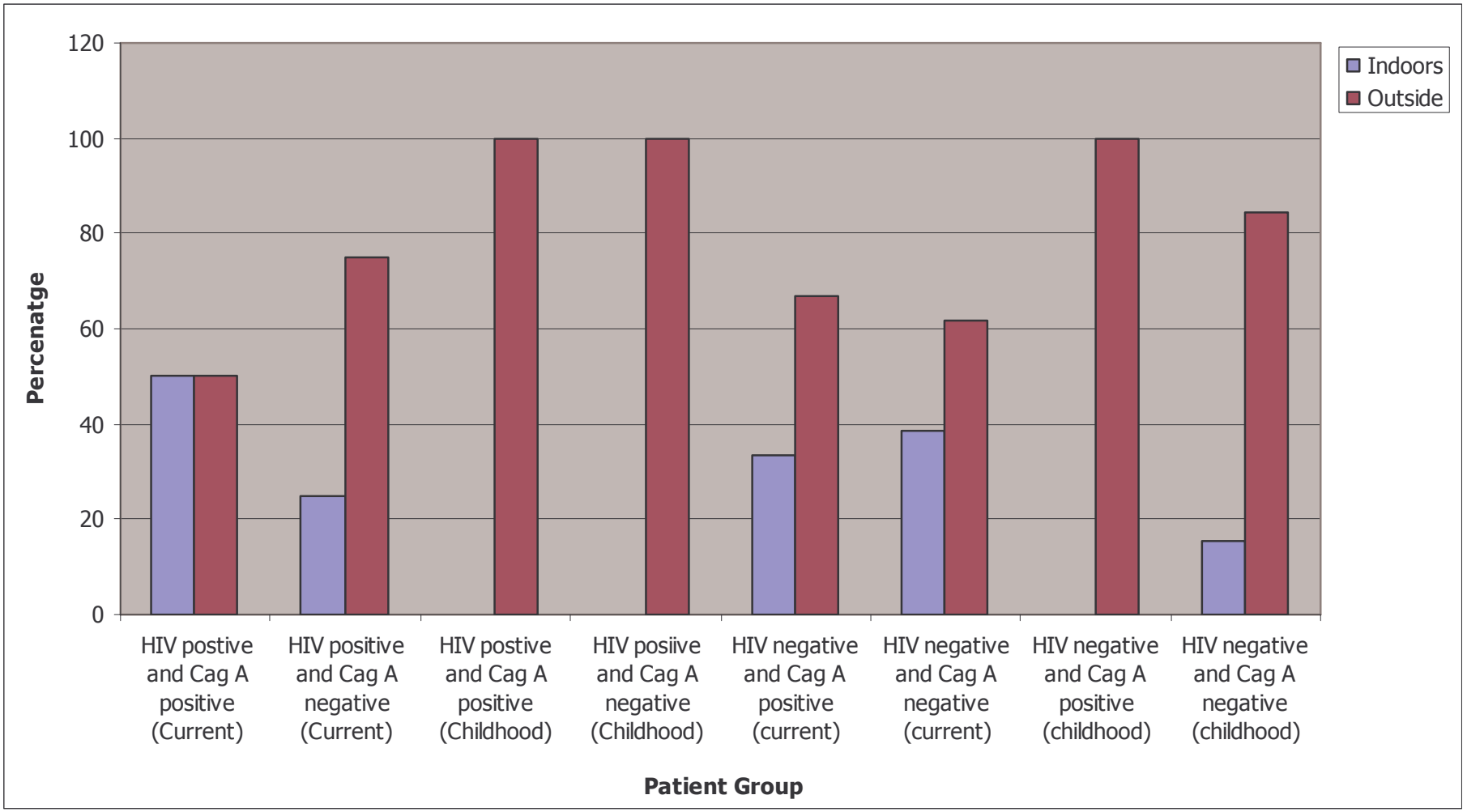


Figure 47: Toilet location in relation to patient groups with combined HIV and anti-*CagA* status, both currently and during childhood.

5.15 Toilet Facilities

Currently 36 patients had flushing toilets and 23 patients had pit latrines. During childhood 13 patients had a flushing toilet, 38 patients had a pit latrine and 2 patients had no toilet facilities.

Table 24 shows toilet facilities in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Table 24: Toilet facilities in relation to HIV status, *H. pylori* status and anti-*CagA* status of patients, both currently and during childhood.

Patient group	N	Flushing (%)	Pit Latrine (%)	No Toilet facilities (%)
HIV positive (Current Facilities)	17	47.06	52.94	0
HIV negative (Current Facilities)	42	66.67	33.33	0
HIV positive (Childhood Facilities)	17	11.76	82.35	5.88
HIV negative (Childhood Facilities)	42	26.19	57.14	16.67
H. pylori positive (Current Facilities)	27	48.15	51.85	0
H. pylori negative (Current Facilities)	32	71.88	28.13	0
H. pylori positive (Childhood Facilities)	27	14.81	59.26	25.93
H. pylori negative (Childhood Facilities)	32	28.13	68.75	3.13
Anti-*CagA* positive (Current Facilities)	10	50	50	0
Anti-*CagA* negative (Current Facilities)	17	47.06	52.94	0
Anti-*CagA* positive (Childhood Facilities)	10	10	50	40
Anti-*CagA* negative (Childhood Facilities)	17	17.65	64.71	17.65

Figure 48 illustrates the relation ship between toilet facilities and HIV status, both currently and during childhood.

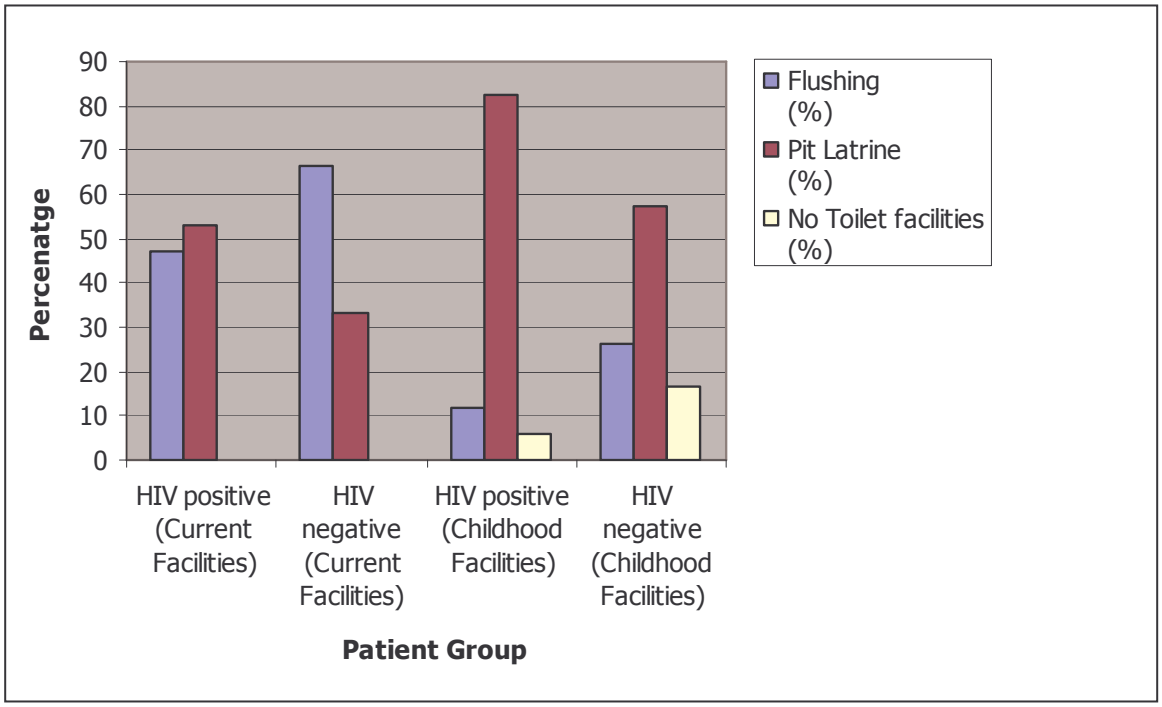


Figure 48: Toilet facilities in relation to HIV status, both currently and during childhood.

Currently, there were more HIV positive patients using a pit latrine than a flushing toilet, while there were more HIV negative patients using a flushing toilet than a pit latrine. These differences in percentage of toilet facilities seen in the HIV positive and negative patients were however not statistically significant (Flushing toilet P = 0.1619 and pit Latrine P = 0.1619), (Figure 48). All HIV positive and HIV negative patients currently have toilets (Figure 48).

Although there is a distribution amongst the HIV positive and negative patients regarding toilet facilities during childhood, both HIV positive and negative patients predominately made use of a pit latrine. The differences in percentage of toilet facilities seen in the HIV positive and negative patients were not statistically significant (Flushing toilet P = 0.3101, pit Latrine P = 0.0670 and no toilet P = 0.4174), (Figure 48).

Figure 49 illustrates toilet facilities in relation *H. pylori* status, both currently and during childhood.

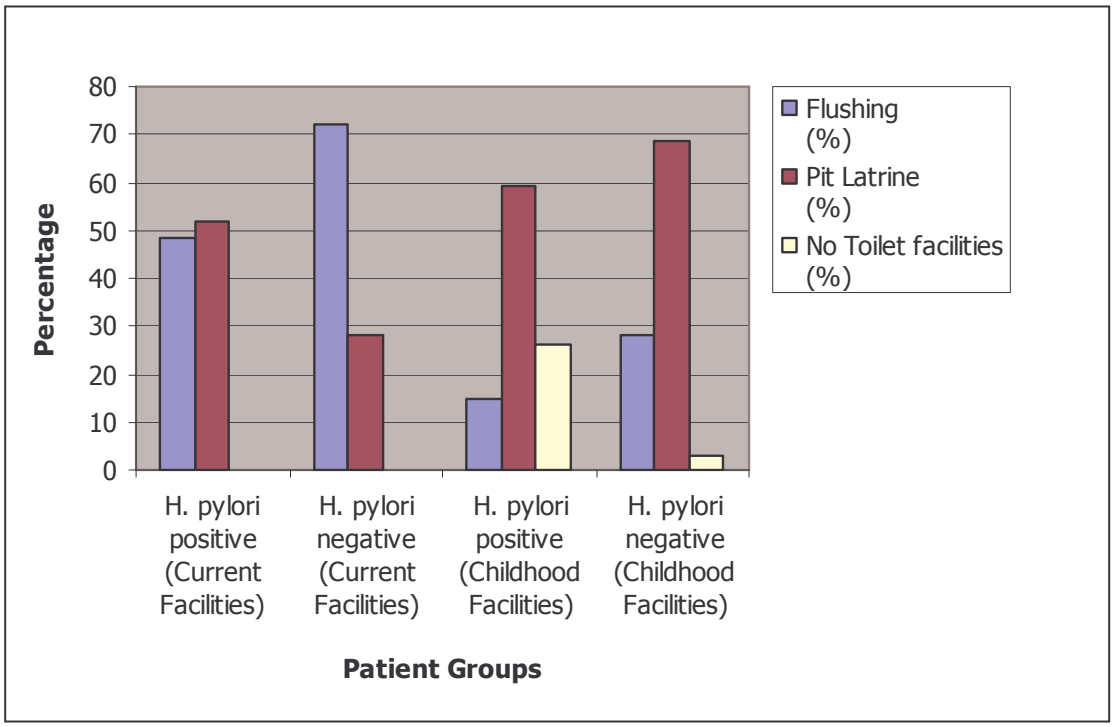


Figure 49: Toilet facilities in relation to *H. pylori* status, both currently and during childhood.

The *H. pylori* negative group showed a higher percentage of flushing toilet users than the *H. pylori* positive group, whereas the *H. pylori* positive group showed a majority of patients used a pit latrine. The differences in percentage of toilet facilities seen in the *H. pylori* positive and negative patients were not statistically significant (Flushing toilet P = 0.0626, pit Latrine P = 0.0626), (Figure 49). Both *H. pylori* positive and negative patients had toilet facilities, (Figure 49).

In both the *H. pylori* positive and negative patient groups a majority of patients used a pit latrine during childhood, with differences in percentage flushing toiler users and patients without toilet facilities. The differences in percentage of toilet facilities seen in the *H. pylori* positive and negative patients were statistically significant for patients without toilet facilities (No Toilet P = 0.0186), where *H. pylori* positive patient groups showed a higher percentage of patients without toilet facilities than the *H. pylori* negative patients (Figure 49). However, the differences in toilet facilities seen in the *H. pylori* positive and negative patients were not statistically significant for patients using a

flushing toilet (Flushing toilet P = 0.2191) nor patients using a pit latrine (Pit latrine P = 0.4481), (Figure 49).

Figure 50 illustrates toilet facilities in relation to anti-*CagA* status, both currently and during childhood.

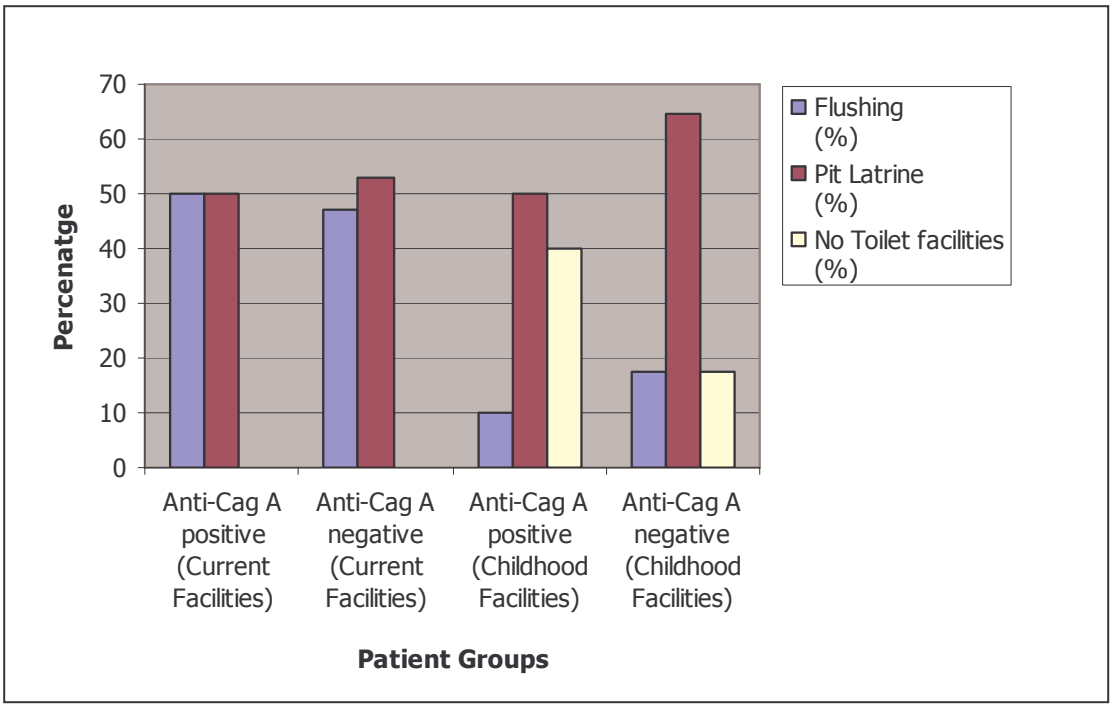


Figure 50: Toilet facilities in relation to anti-*CagA* status, both currently and during childhood.

The differences in percentage of toilet facilities seen in the anti-*CagA* positive and negative patients were not statistically significant (Flushing P = 1.0000 and pit Latrine P = 1.0000), (Figure 50). All anti-*CagA* positive patients and negative patients had toilet facilities (Figure 50).

In both the anti-*CagA* positive and negative patient groups a majority of patients used a pit latrine during childhood, with differences in percentage flushing toiler users and patients without toilet facilities. The differences in percentage of race seen in the anti-*CagA* positive and negative patients were not statistically significant (Flushing toilet P = 1.0000, pit Latrine P = 0.6868 and no toilet P = 0.3648), (Figure 50).

Table 25 shows toilet facilities in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status, both currently and during childhood.

Table 25: Toilet facilities in relation to patients with known HIV and *H. pylori* status and known HIV and anti-*CagA* status.

Patient group	N	Flushing (%)	Pit Latrine (%)	No Toilet facilities (%)
HIV positive *H. pylori* positive (Current Facilities)	8	25	75	0
HIV positive *H. pylori* negative (Current Facilities)	9	66.67	33.33	0
HIV positive *H. pylori* positive (Childhood Facilities)	8	0	87.5	12.5
HIV positive *H. pylori* negative (Childhood Facilities)	9	22.22	77.78	0
HIV negative *H. pylori* positive (Current Facilities)	19	57.89	42.11	0
HIV negative *H. pylori* negative (Current Facilities)	23	73.91	26.09	0
HIV negative *H. pylori* positive (Childhood Facilities)	19	21.05	47.37	31.58
HIV negative *H. pylori* negative (Childhood Facilities)	23	30.43	65.22	4.35
HIV positive anti-*CagA* positive (Current Facilities)	4	25	75	0
HIV positive anti-*CagA* negative (Current Facilities)	4	25	75	0
HIV positive anti-*CagA* positive (Childhood Facilities)	4	0	75	25
HIV positive anti-*CagA* negative (Childhood Facilities)	4	0	100	0
HIV negative anti-*CagA* positive (Current Facilities)	6	66.67	33.33	0
HIV negative anti-*CagA* negative (Current Facilities)	13	53.85	46.15	0
HIV negative anti-*CagA* positive (Childhood Facilities)	6	16.67	33.33	50
HIV negative anti-*CagA* negative (Childhood Facilities)	13	23.08	53.65	23.08

Figure 51 illustrates toilet facilities in relation to patient groups with known HIV and *H. pylori* status, both currently and during childhood.

The patients who were HIV positive *H. pylori* positive currently predominately use a pit latrine, where as the patients who were HIV positive *H. pylori* negative predominately use a flushing toilet. The difference in race ratio between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Flushing toilet P = 0.1534 and pit latrine P = 0.1534), (Figure 51). All of the patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative had toilet facilities (Figure 51). The patients who were

HIV positive *H. pylori* positive predominately made use of pit latrines during childhood, a smaller percentage of patients did not have any toilet facilities during childhood. The patients who were HIV positive *H. pylori* negative also predominately made use of pit latrines during childhood however a smaller percentage used flushing toilets, with all patients having toilet facilities during childhood. The difference in toilet facilities between patients who were HIV positive *H. pylori* positive and patients who were HIV positive *H. pylori* negative were not statistically significant (Flushing toilet P = 0.4706, pit Latrine P = 1.0000 and no toilet P = 0.4706), (Figure 51).

The difference in toilet facilities between patients who are HIV negative *H. pylori* positive and patients who are HIV negative *H. pylori* negative were not statistically significant (Flushing toilet P = 0.3349 and pit Latrine P = 0.3349), (Figure 51). All patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative had toilet facilities (Figure 51). Both patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative predominately used a pit latrine during childhood, with a smaller percentage of patients using a flushing toilet. A higher percentage of HIV negative *H. pylori* positive patients however, did not have toilet facilities during childhood. This difference in presence of toilet facilities is statistically significant (No toilet P = 0.0341), (Figure 51). However the difference in toilet facilities between patients who were HIV negative *H. pylori* positive and patients who were HIV negative *H. pylori* negative with regards to flushing toilets and pit latrines use at childhood was not statistically significant (Flushing toilet P = 0.7258 and pit latrine P = 0.2447), (Figure 51).

Figure 52 illustrates toilet facilities in relation to patient groups with known HIV and anti-*CagA* status, both currently and during childhood.

For the patients who were HIV positive anti-*CagA* positive and for the patient who were HIV positive anti-*CagA* negative the majority of them have a pit latrine as toilet facilities, both currently and during childhood. However, the HIV positive anti-*CagA* negative patient group showed a substantial percentage of patients not having toilet facilities.

For the patients who were HIV negative anti-*CagA* positive and for the patient who were HIV negative anti-*CagA* negative the majority currently have flushing toilets. However, the majority of the patients who are HIV negative anti-*CagA* positive did not have toilet facilities during childhood, and the patients who are HIV negative anti-*CagA* negative made used of a pit latrine during childhood.

Due to the low patient numbers for patients who were HIV negative anti-*CagA* positive and patients who were HIV negative and anti-*CagA* negative, statistical significance was not calculated for toilet facilities in these patients.

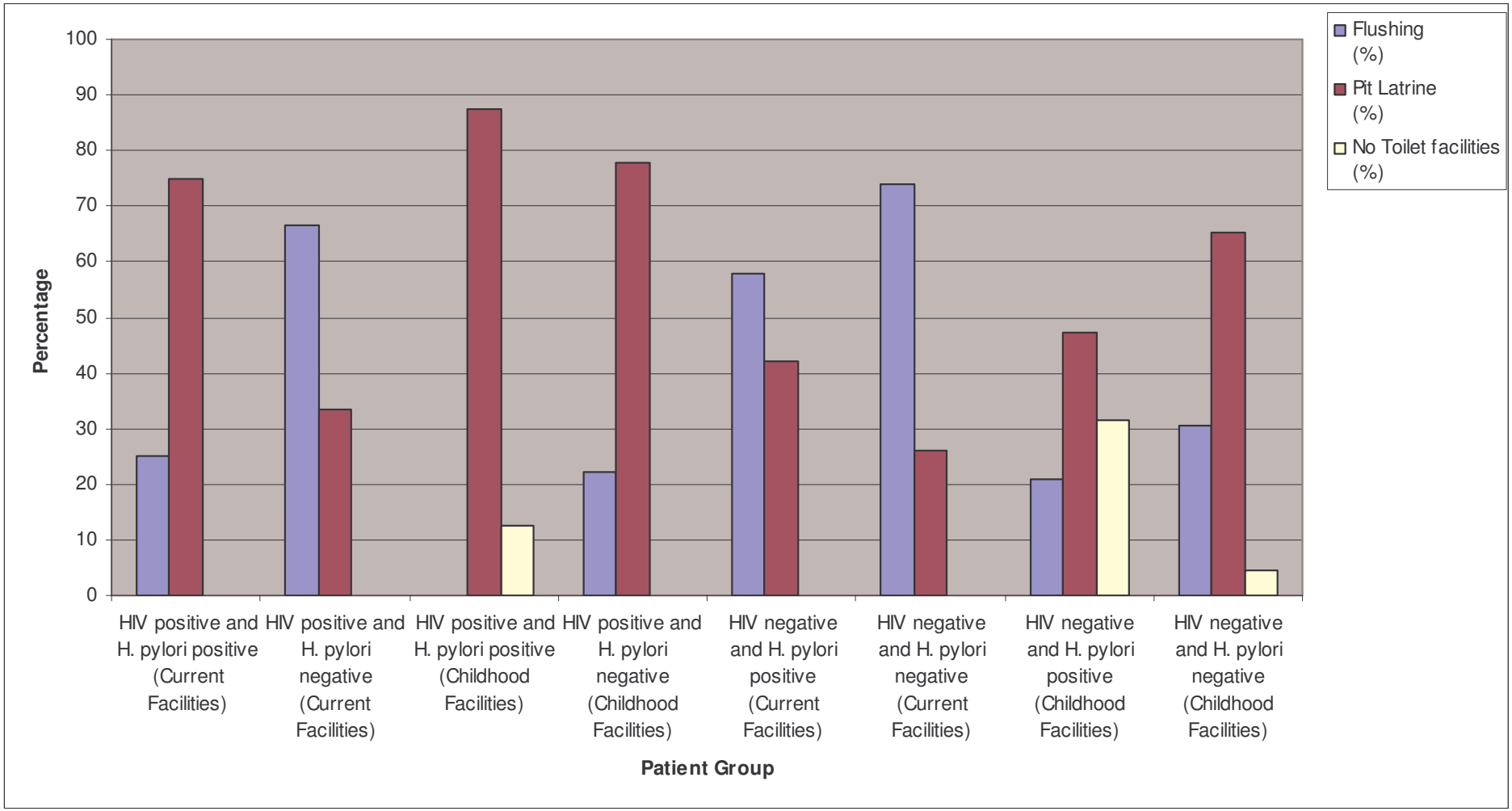


Figure 51: Toilet facilities in relation to patient groups with combined HIV and *H. pylori* status

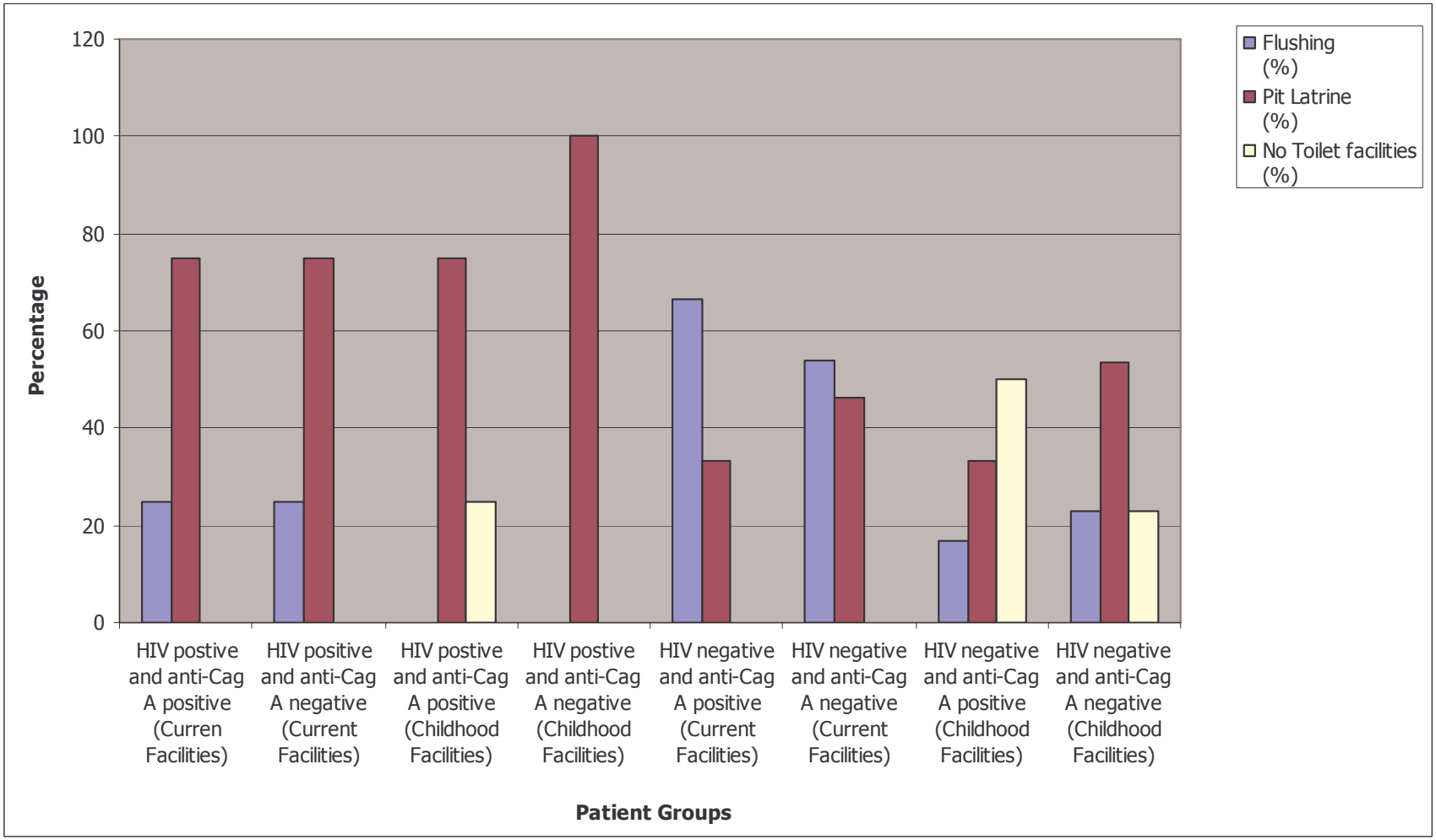


Figure 52: Toilet facilities in relation to patient groups with combined HIV and anti-*CagA* status

5.16 Diagnosis in relation to *H. pylori* and HIV status

The diseases/disorders of particular interest were ITP and iron deficiency as these are currently under investigation with relation to *H. pylori* infection. Table 25 shows the patient diagnosis in relation to HIV, *H. pylori* and anti-*CagA* status.

Table 26: Patient diagnosis in relation to HIV, *H. pylori* and anti-*CagA* status.

Diagnosis	n	HIV Negative	HIV Positive	*H. pylori* Negative	*H. pylori* Positive	Cag Negative	Cag Positive
Acute Lymphoblastic Leukaemia	1	1			1	1	
Acute Myeloid Leukaemia	7	6	1	5	2	2	
Aplastic Anaemia	2	1	1	2			
Auto Immune Haemolytic Anaemia	4	3	1	2	2	1	1
Chronic lymphocytic leukaemia	1	1			1	1	
Chronic Myeloid leukaemia	6	4	2	2	4	2	2
Cold Haemagglutinin disease	1	1		1			
Essential Thrombocytosis	1	1		1			
Idiopathic thrombocytopaenic purpura	9	3	6	6	3	2	1
Iron deficiency anaemia	3	3		2	1		1
Large granular lymphocyte leukaemia	1		1	1			
Lymphoprolifative disorder	1	1			1	1	
Megaloblastic anaemia	2	1	1	2			
Myelodysplastic syndrome	4	3	1	1	3	2	1
Myeloma	8	8		2	6	3	3
Pernicious Anaemia	2	2		2			
Protein C deficiency	2	1	1	1	1	1	
Pure Red cell aplasia	1	1		1			
Thrombotic thrombocytopaenic purpura	2		2	1	1		1
Waldenström /macroglobulinemia	1	1			1	1	

Table 25 shows the number of Auto-immune vs number of Malignant disorders expresses by patients.

Table 27: Malignant versus auto-immune diagnosis in relation to HIV, *H. pylori* and anti-*CagA* status.

Malignant/ Auto immune diagnosis	n	HIV Negative	HIV Positive	*H. pylori* Negative	*H. pylori* Positive	Cag Negative	Cag Positive
Auto-Immune	16	9	7	11	5	3	2
Malignant	30	25	5	12	18	12	6

Due to the amount of diverse disorders none of the above diseases/disorders were proved statistically significant.

6 Discussion

Many studies have been done worldwide looking at different aspects of *H. pylori* infection yet very few of these studies have been performed in South Africa. *Helicobacter pylori* has been linked to many disorders and is of medical interest when treating a patient for a specific disorder. The only study performed on *H. pylori* in the Free State province, South Africa, is a paediatric study performed by Pelser *et al* in 1994.

Furthermore, HIV infection is very prevalent in the community in the Free State Province, South Africa, and by determining the relationship between *H. pylori* and HIV infection, follow-up studies determining the burden of disease within HIV patients will be made possible. This information will be of great importance in contributing to a better understanding of the relationship between these two infections in this community.

Haematology patients are of particular interest as a number of the disorders linked to *H. pylori* infection are of a haematological nature. Therefore the purpose of this study was to investigate the HIV and *H. pylori* prevalence, with relation to anti-*CagA* status in *H. pylori* positive patients, amongst the haematology patients both ambulant and hospitalized living in the Free State province, South Africa.

Different variables were identified within the literature as playing a role in *H. pylori* infection. Therefore, to investigate which variables play a role in *H. pylori* infection in the Free State province, a questionnaire was included in the study asking patients questions relating to: demographics (age, sex, race and socio-economic status), water source (both currently and during childhood), water transportation method if running water was not available at the patients premises (both currently and during childhood), water storage if running water was not available at the patient's premises (both currently and during childhood), water storage container if running water was not available at the patient's premises (both currently and during childhood), boiling of water (both currently and during childhood), toilet location (both currently and during childhood) and toilet facilities (both currently and during childhood).

In the analysis it was seen that the sample group was mainly female, with the race being predominately black with a very low socio-economic status (Table 4), many patients not having any income.

The environment in which a patient lives could encourage or discourage probable *H. pylori* infection. Water source has been determined as an important source for *H. pylori* infection in previous studies (Parsonnet *et al*, 1999; Rolle-Kampezyk *et al*, 2004) and was thus investigated. In this study, current water source for the majority of the patients was tap water on their premises, with very few patients having to collect water from external sources. However, during childhood the majority of the patients did have to make use of external water sources such as bore holes, tanks, rivers, dams or wells. The fact that tap water is more accessable now is most likely due to the improvement in infrastructure over time, making tap water more available to underprivileged communities (Figure 18, 19 and 20).

Water source can also be classified according to treated and non-treated water sources. Treated water sources include a tap on the premises and a tap in the street. Although a tap in the street is an external water source, it is a treated water source and can be categorised with tap water on the premises when looking at water quality.

The transportation and/or the storage of water can decrease the water quality as certain conditions may encourage *H. pylori* proliferation. For this reason it was decided to look at the water transportation method and water storage method used for those patients who needed to fetch water (not having a tap on their premises). Currently, all of these patients use buckets and the majority of these patients then store their water indoors their premises in a bucket (Figures 23, 24, 25, 28, 29, 30, 33, 34 and 35.

It must be taken into consideration that even if water is stored in a potentially pathogen-rich environment, it is possible to eradicate most pathogens through the boiling of water. All patients were asked whether they boiled their water or not regardless of the water source. Interestingly, the majority of the patients do not boil

their water and none of the patients boil their water when sick, although boiling of water when sick seemed to be a common practice during childhood (Figure 38, 39 and 40).

Besides water source, water transportation, and water storage; toilet location and toilet facilities may also play a major role in pathogen infection. In this study, all toilet facilities indoors were flushing toilets while most toilets outside were pit latrines, with the exception of a few patients having flushing toilets outside. Irespective of the toilect location, all patients currently have toilet facilities (Figure 43, 44, 45, 48, 49 and 50).

The majority of the toilets outside were pit latrines where sewerage is left in a deep hole, without any disinfectant, as opposed to being flushed away as in a flushing toilet. Such an environment, with the sewerage build-up and with the toilet outside possibly not being cleaned as regularly as the rest of the house, it could create teh conditions for *H. pylori* proliferation and subsequent infection.

All of the above discussed variables were analysed in each group of patients.

6.1 HIV prevalence

The prevalence of HIV was 29% amongst the haematology patients in this study. This prevalence is higher than that published in 2006 for the Frees state province (12.6%) and that of the national prevalence in 2006 (18.34% for 15 – 49 year olds), (http://www.avert.org/safricastats.htm).

Age difference was found to be statistically significant. The HIV positive patient group had a much lower median age than the HIV negative patient group consistent with local HIV statistics showing that HIV infection is especially prevalent in the younger age groups. Sex, race and socio-economic status were found to have no statistically significant difference between HIV positive and negative patients (Figure 10, 12 and 15).

Similarly, no significant differences in water source, water transport, water storage location and water storage container (neither currently nor during childhood) could be

found for HIV infection (Figure 18), showing that these variables do not play a role in HIV prevalence in this study.

The HIV positive patient group revealed that roughly a third of the patients currently needed to collect water, using buckets for water and the majority storing their water indoors in closed buckets. Whereas the HIV negative patient group had less patients needing to collect water. All of these patients also collected their water in buckets the majority of which stored their water indoors in open buckets (Figure 18, 23, 28 and 33).

During childhood, diverse water sources including taps on premises, taps in the street, bore holes, tanks, rivers, dams and wells, were used by both HIV positive and HIV negative patients groups. The majority of patients transported their water in buckets . A a small difference between the groups was a small number of HIV positive patients who also used glass bottles or drums while the HIV negative patients used plastic bottles or drums. Similarly, the majority of both the HIV positive and negative patient groups, stored their water indoors in a closed bucket during childhood (Figure 18, 23, 28 and 33).

In this study the HIV negative patients currently all used treated water (i.e. water from a source on the premises or water from a communal tap), whereas the HIV positive patients did have a small percentage using tanks and dams as water sources. In contrast, during childhood the majority of the HIV positive patients used treated water, whereas the majority of the HIV negative patients made use of more diverse sources, including bore holes, rivers, dams and wells (Figure 18).

When looking at the boiling of water before consumption, although no statistically significant difference can be seen between HIV positive and negative patients during childhood, there is a statistically significant higher number of patients in the HIV positive patient group which currently do boil their water before consumption (Figure 40). As boiling water would eradicate any pathogens, rather than having a direct link to HIV infection process, the tendency to boil water is probably due to the HIV positive patient trying to prevent infections by boiling water.

Toilet location and toilet facilities may also play a major role in pathogen infection. However in the HIV positive and negative patient groups, toilet location and toilet facilities, both currently and during childhood, was not seen to have an influence on HIV status, showing that toilet location does not play a role in HIV infection in this study. The differences between HIV positive and HIV negative patients was minimal, with the HIV negative patients showing a slightly higher percentage of patients with flushing toilet facilities indoors and the HIV positive patients showing a slightly higher percentage of patients having a pit latrine outside. Both patient groups had a majority of patients with pit latrine facilities outside during childhood (figure 43 and 48). No previous studies could be found which have done such an analysis on HIV infection and the effects which these variables may have.

6.2 *H. pylori* prevalence

Research done in South Africa, and particularly in Bloemfontein, showed the prevalence of *H. pylori* in children 10 – 15 years of age to be 84.2%, with a 95% confidence interval of 77 - 91.3% (Pelser *et al*, 1997). This is consistent with worldwide research where 80 % of adults in developing countries are predicted to be *H. pylori* positive (Suerbaun and Michetti, 2002). Therefore, the *H. pylori* prevalence is lower than expected amongst haematology patients at 46%. This can be due to a number of reasons:

Firstly, the population group for this study is adult patients. Adult patients have generally received a number of antibiotic treatment cycles during their life for various reasons and this may have unintentionally eradicated *H. pylori*.

Secondly, the population group for this study is only haematology patients. For many of the haematology disorders chemotherapy is used to control the disorder and this may also have unintentionally eradicated the *H. pylori* present. As haematology patients are a group at high risk for infections generally and especially in those who have previously

received chemotherapy, it is likely that they may have had more courses of antibiotics than the general population.

Thirdly, chemically treated tap water is more available to the public in the lower socio-economic classes today than a decade ago when the paediatric study was conducted by Pelser *et al*. Socio-economic circumstances have also improved greatly in the Free State and the country as a whole since 1994. This may have led to a generally lower prevalence of *H. pylori* infection in the population.

In contrast to a study done by Zaterka *et al* (2007) in Brazil where it was found that *H. pylori* is related to lower social classes, this study showed no significant differences amongst *H. pylori* positive and negative patients with regards to socio-economic status, (Figure 10 and figure 11). However, as mentioned previously, the majority of the sampling group had a very low socio-economic standard (Table 4) making comparisons difficult. A larger study using more patients from a wider variety of socio-economic backgrounds, both from the private sector and government supported institutions, is suggested to accurately determine the role of socio-economic status in the prevalence of *H. pylori* infection in this region.

Furthermore, it was also concluded that demographic variables including age, sex (Figures 12) and race (Figure 15) do not play a role in *H. pylori* infection. The findings for age and gender are supported by Henriksen (2001) and Zaterka *et al* (2007). However the results for race contradict a study performed by Zaterka *et al* (2007) where race was found to be linked to *H. pylori* prevalence in Brazil. This may be due to the fact that Zaterka *et al* (2007) sampled a larger group of patients, with more variation in socio-economic status which can often be linked to race.

The differences in current water source seen in the *H. pylori* positive and negative patients were statistically significant, where a larger percentage of *H. pylori* positive patients collect tap water from a communal source (Figure 19). Even though a tap in the street is an external water source (water needing to be transported and stored), this water comes from a treated source. Thus, the water quality should be the same as the

water quality of that being received through taps on the premises. Therefore, it is more likely that the patients were infected with *H. pylori* during their childhood or by other means currently, like person to person transmission. The socio-economic status, although not statistically significant, of the *H. pylori* positive patients is slightly lower than the *H. pylori* negative patients.

This is further supported by the finding in this study where the difference in water source during childhood was found to be statistically significant between *H. pylori* positive and negative patients. Dam water, as a water source, was seen to be used amongst the *H. pylori* positive patients whereas none of the *H. pylori* negative patients collected dam water during childhood (Figure 19). With dam water being an ideal environment for *H. pylori* proliferation, with the water being stagnant and relatively warm, if we sepculate this may be the origin of *H. pylori* infection in some patients. These results are consistent with the literature, (Parsonnet *et al* , 1999; Kamezyk *et al*, 2004; Zaterka *et al*, 2007) showing that water source, both currently and during childhood, is related to *H. pylori* prevalence.

Similarly, the differences in the percentage of *H. pylori* positive and negative patients for water transportation method, water storage location and water storage containers were statistically significant. A higher percentage of *H. pylori* positive patients are currently using buckets as a means of water transport and storage, storing their water indoors in closed buckets (figure 24 and 29).

The fact that the *H. pylori* positive patients need to collect and store water more often may be a reflection of the socio-economic status of these patients. Patients with a lower socio-economic status will have less access, if any, to running water on their premises and will therefore need to collect and store water from an external source. The water was most likely stored in the same container in which it was transported and then stored indoors for practical reasons to prevent dirt/dust from spoiling the water. Therefore as previously discussed, patients are probably infected during childhood or are infected currently by person to person transmission in overcrowded circumstances that are typically associated with lower socio-economic status. Alternatively, if the buckets being

used for water transportation or storage are used for other household tasks and are not sterilized before being used for water collection or storage this may be the origin of *H. pylori* contamination.

Differences in the water transportation method, water storage location and water storage containers used during childhood were not found to be statistically significant. *Helicobacter pylori* positive patients only made use of buckets, storing their water indoors using closed buckets to store their water. *Helicobacter pylori* negative patients made use of buckets (predominately) as well as plastic bottles, glass bottles and drums for water transport. Water was stored indoors and, although a majority of patients used closed buckets, uniquely the *H. pylori* negative patients also used large open plastic containers for water storage.

Following the same trend, the majority of the *H. pylori* positive patients do not currently boil their water before consumption, nor did they boil their water before consumption during childhood (Figure 39). Therefore the boiling of water before consumption, both currently and during childhood does not seem to have an effect on the *H. pylori* prevalence in this study.

There is a distinct difference between the *H. pylori* positive patients and the *H. pylori* negative patients when current toilet facilities are analysed. The majority of *H. pylori* positive patients currently have a pit latrine outside their dwelling, whereas the majority of *H. pylori* negative patients have a flushing toilet indoors (Figure 44). The higher number of *H. pylori* positive patient's currently having a toilet outside is statistically significant. Similarly, the differences in toilet facilities (although not statistically significant) shows a trend that suggests a difference, however a larger sampling group is needed to confirm whether this is actually significant or not (Figure 49). This concludes that the current location of toilet facilities does play a role in this study where the outside toilet may be the source of infection. This is consistent with the faecal-oral transmission described as possible by Kelly *et al* (1994).

The toilet location during childhood was also found to be significant where the *H. pylori* positive patients showed a higher percentage of patients with toilet facilities indoors than compared to the *H. pylori* negative patients (Figure 44). This may be due to limited knowledge about bacterial infections in previous years, where the best methods were not used when cleaning toilet facilities, thus unintentionally creating an environment which may have stimulated bacterial proliferation.

A flushing toilet was not common during childhood, where the majority of subjects used a pit latrine. The *H. pylori* positive patients showed a much higher percentage of patients without toilet facilities than the *H. pylori* negative patients (Figure 49). The differences in toilet facilities seen between the *H. pylori* positive and negative patients are statistically significant concluding that not having any toilet facilities during childhood does play a role in *H. pylori* prevalence in this study.

This is supported by Zaterka *et al* (2007) who described toilet facilities to be associated with *H. pylori* prevalence in Brazil, and that the lack of toilet facilities encouraged person to person transmission (Zaterka *et al*, 2007).

6.2.1 *H. pylori* infection related to HIV infection

This study also looked at patients with known HIV and *H. pylori* status to see if any correlations could be found taking into account demographic and environmental factors as listed in the study aims. No statistically significant difference could be found between HIV positive and HIV negative patients in relation to *H. pylori* prevalence, thus showing that HIV infection and *H. pylori* infection did not have a correlation in this study, and each infection is probably independent of the other.

The differences between the HIV positive and HIV negative groups with relation to *H. pylori* were analysed. Patients were further grouped into HIV positive and HIV negative patients and then further into HIV positive *H. pylori* positive patients; HIV positive *H. pylori* negative patients; HIV negative *H. pylori* positive patients; and HIV negative *H. pylori* negative patients.

Socio-economic status was not seen to be statistically significant between the *H. pylori* positive and negative patients in the HIV positive patient group. However, the HIV negative *H. pylori* positive group showed a statistically significant higher number of low socio-economic grading than the HIV negative *H. pylori* negative group, which included more private patients (with medical aid) and therefore a higher socio-economic status (figure 11). This may be due to the fact that HIV infection is currently more prevalent amongst lower socio-economic populations because of a generally lower level of education and lack of HIV infection awareness.

When looking at age in patients with HIV and *H. pylori* status, a significant difference could be seen. The trends looked much like that of the HIV status where HIV positive groups showed a much lower median age (Table 7). The HIV negative patient group showed no statistically significant differences for age, whereas the HIV positive group showed a statistically significant difference for age with *H. pylori* infection being more prevalent in younger HIV positive patients. The HIV negative group was generally a group of older patents whereas the HIV positive group were mainly younger patients. This may have played a role in the difference in statistical outcome as the older patients may have had more antibiotic treatments in their lifetime than the younger patients, and therefore *H. pylori* may have been eradicated unintentionally. Similarly to the results from both the HIV patient analysis and the *H. pylori* patient analysis, sex and race were eliminated as possible factors influencing *H. pylori* prevalence amongst HIV negative and positive patients.

Water source, both currently and during childhood, was proven not to be statistically significant for the HIV negative nor positive patient groups. All of the HIV negative patients currently use treated water with a higher percentage of HIV negative *H. pylori* positive patients needing to collect water from a communal tap (Figure 21). Similarly, most of the HIV positive patients also currently have access to tap water with a higher percentage of HIV positive *H. pylori* positive patients needing to collect water from a communal tap (Figure 21). However, during childhood, various water sources were used between the patient groups, except for the HIV positive *H. pylori* negative patients who

only made use of treated water, either on their premises or from a communal tap in the street, during childhood (Figure 21).

The analysis also showed that neither water transportation method (Figure 26), water storage location (Figure 31) nor water storage containers (Figure 36) play a role in *H. pylori* prevalence, currently or during childhood, amongst HIV positive and negative patients in this study. Consistent with the results from the *H. pylori* analysis, regardless of the HIV status, the *H. pylori* positive patients currently make more use of water transportation using buckets than the *H. pylori* negative patients. The HIV positive patient group generally made use of buckets as means of transportation for their water during childhood with the HIV positive *H. pylori* negative patients included other transportation methods as well (Figure 26). Similarly, the HIV negative patient groups also generally made use of buckets as means of transportation for water in their childhood with the HIV negative *H. pylori* negative patients also including other transportation methods (Figure 26). The water storage location was seen to be predominantly indoors for all patient groups, with a very small, non-significant, percentage of patients storing their water outside. During childhood a similar trend was seen, where all of the patients generally stored their water indoors, with only small differences in percentages seen between patient groups. Lastly, the majority of patients stored their water in closed containers both currently and during childhood, with the exception of HIV positive *H. pylori* negative patients who currently did not store their water as they had taps on their premises (Figure 36). During childhood the HIV positive *H. pylori* negative patient group and the HIV negative *H. pylori* negative patient group also made use of large plastic containers (Figure 36).

The results found when analysing "boiling of water before consumption" were similar to those of the *H. pylori* analysis. It was seen that neither current boiling, nor boiling of water during childhood, play a role in *H. pylori* prevalence amongst HIV positive and negative patients (Figure 41).

Similarly, the current toilet location and toilet facilities were also analysed and showed results very similar to those of the *H. pylori* analyses, where the *H. pylori* positive

patients, no matter their HIV status, showed a higher percentage of patients with pit latrines outside. No statistically significant differences were found between the *H. pylori* positive and negative patients in the HIV positive patient group. However in the HIV negative patient group, although not statistically significant, differences could be seen for toilet facilities (Figure 51). The patients who were HIV negative *H. pylori* positive currently predominately use outside toilet facilities, whereas the patients who are HIV negative *H. pylori* negative currently predominately used toilet facilities indoors. These differences in toilet location between patients who are HIV negative *H. pylori* positive and patients who are HIV negative *H. pylori* negative were statistically significant, concluding that *H. pylori* status is related to current toilet location in HIV negative patients.

None of the differences found between patient groups and toilet location during childhood were identified as statistically significant, concluding that toilet location used during childhood did not play a role in *H. pylori* prevalence amongst HIV positive and negative patients in this study. Similarly, no significant differences were found for toilet facilities during childhood in the HIV positive patient group, where patients were seen to predominately make use of pit latrines, with a smaller percentage of patients not having any toilet facilities during their childhood, regardless of the *H. pylori* status. However, in the HIV negative patient group significant differences were found, where HIV negative *H. pylori* positive patients however showed a higher percentage of patients not having toilet facilities during childhood (Figure 51). This difference in presence of toilet facilities is statistically significant for the presence of toilet facilities confirming that *H. pylori* status was related to toilet facilities in HIV negative patients.

As it was previously proven in this study that *H. pylori* infection was linked to toilet facilities and toilet location, perhaps a larger sampling group in the HIV patient group would have shown significant differences between *H. pylori* positive and negative patients within this group.

No previous studies could be found which have done such an analysis between HIV and *H. pylori* infections and the effects which different variables may have.

6.3 Anti-*CagA* status

The prevalence of IgG antibodies to *CagA* amongst the sampling group was found to be 17% in the total study population and 37% amongst the *H. pylori* positive patients. The anti-*CagA* serology testing method is currently being used in day to day practice to diagnose patients for anti-*CagA* positive *H. pylori* infection accurately with a sensitivity of 85% and specificity of 80% (Yamaoka *et al*, 1998a), and therefore only the *H. pylori* positive patients were tested for anti-*CagA*. The demographic data, sex, race and age was found not to have any statistical significance to anti-*CagA* prevalence (Figures 14 and 17).

Currently the anti-*CagA* negative patients only collect water from a treated source (tap water on the premises or tap water from a communal source) whereas the anti-*CagA* positive patients collect water from tanks as well (Figure 22). However, during childhood, anti-*CagA* positive patients had a higher percentage of patients using river and dam water. When looking at water transportation methods, for those patients who needed to fetch water, it can be seen that all anti-*CagA* tested patients only used buckets, both currently and during childhood.

The anti-*CagA* positive patients currently all stored their water indoors (Figure 32), with anti-*CagA* negative patients being divided between outside and indoor storage. Similarly, anti-*CagA* positive and anti-*CagA* negative patients showed the same trend during childhood, where the majority of patients stored their water indoors with a small minority storing their water outside.

For all of the anti-*CagA* tested patients the current most commonly used container for water storage is a closed bucket, whereas during childhood it was an open bucket (Figure 37). There was very little difference, in water storage containers between anti-*CagA* positive and negative patients both currently and during childhood.

The majority of the anti-*CagA* tested patients do not currently boil their water and none of the patients currently boil their water when ill. Only a small difference can be seen

between anti-*CagA* positive patients and anti-*CagA* negative patients, with the anti-*CagA* positive patients having a higher percentage of patients boiling their water. None of the anti-*CagA* positive patients boiled their water before consumption during their childhood, although the majority of the anti-*CagA* negative patients did not boil their water before consumption (Figure 42).

The number of anti-*CagA* positive and anti-*CagA* negative patients currently having toilet facilities indoors and outside were very similar. All of the anti-*CagA* positive patients had toilet facilities outside during childhood while a percentage of the anti-*CagA* negative patients also had toilets indoors during childhood (Figure 47). This correlates with the *H. pylori* analyses, where the *H. pylori* positive patients had significantly more patients with toilet facilities outside.

All anti-*CagA* positive and negative patients had toilet facilities (Figure 52) with half of the patients using a pit latrine and the other half using a flushing toilet (Figure 52). Both the anti-*CagA* positive and negative patients showed a majority of patients using a pit latrine during childhood with small differences in percentage of patients using a flushing toilet and patients without toilet facilities.

None of the investigated environmental variables (water source, water transportation method, water storage location, water storage containers, boiling of water, toilet facilities and toilet location) were found to have a statistically significant association with anti-*CagA* status. However, this may be related to the small number of patients who tested positive for anti-*CagA*.

No previous studies could be found which have done such an analysis between HIV and *H. pylori* anti-*CagA* infections and the effects which different variables may have.

6.3.1 Anti-*CagA* status in relation to HIV status

Similarly to the *H. pylori* versus HIV infection, anti-*CagA* versus HIV infection was also analyzed to see if any correlations could be identified. Although the percentage of anti-*CagA* positive patients was lower in HIV negative patients, the difference between HIV positive and HIV negative patients was not statistically significant, showing that HIV infection did not have any effect on anti-*CagA* prevalence in this study.

The patients were divided into HIV positive and HIV negative patient groups and these were further divided into HIV positive anti-*CagA* positive; HIV positive anti-*CagA* negative; HIV negative anti-*CagA* positive; and HIV negative anti-*CagA* negative. When dividing the patient groups into HIV status with relation anti-*CagA* status, due to the low number of patients testing positive for anti-*CagA*, the patient numbers were too small to determine statistical significance. Thus none of the relationships between the demographic data or the environmental data (water source, water transportation method, water storage location, water storage containers, boiling of water, toilet facilities and toilet location) could be evaluated for statistical significance. To prove these correlations a larger study would be needed focusing on anti-*CagA* positive patients only, with HIV status available.

Interestingly, although socio-economic status is very low overall, when looking at both HIV infection and anti-*CagA* status in patients, different trends showed in each group. The patients who were HIV positive and anti-*CagA* positive only showed H1 statuses, the patients who were HIV positive and anti-*CagA* negative showed mainly H0 statuses. Similarly, the patients who were HIV negative and anti-*CagA* positive showed a majority of H0 statuses, and the patients who were HIV negative and anti-*CagA* negative had a very close numbers between H0 and H1 statuses. With a larger patient group, these trends may even out and a more definite pattern could arise (Figure 13).

In correlation with the HIV data where HIV patients were found to have a lower median age, the median age was seen to be lower in the HIV positive patient groups compared to the HIV negative patient groups regardless of the anti-*CagA* status (Table 7). These

results also revealed a similar trend as those from the other analyses showing that gender probably does not have an effect on these anti-*CagA* positive *H. pylori* bacteria strains amongst HIV positive and negative patients (Figure 16).

All of the HIV negative patients tested for anti-*CagA* antibodies were black. The majority of the HIV positive patients were black as well, with smaller numbers of mixed race and white patients between anti-*CagA* positive and negative groups. The HIV positive anti-*CagA* positive group consisted of only black and mixed race patients with no white patients and the HIV positive anti-*CagA* negative patients had a smaller percentage of mixed race patients and included white patients. These results correlate with those from the *H. pylori* analyses, concluding that for the HIV anti-*CagA* patient groups race probably does not play a role or have an influence on anti-*CagA* positive *H. pylori* strain prevalence amongst HIV positive and negative patients.

When comparing water sources between patient groups it can be seen that the majority of the patients use treated water, either from a tap on the premises or from a communal tap, with only small differences in other water sources between groups. However, during childhood, the majority of the HIV positive patients had access to tap water, regardless of the anti-*CagA* status, and the HIV negative patients used a number of different water sources including taps on premises, communal tap, bore hole, river, dam and wells. If a larger sampling group would be used, water source may prove to be significant.

All patients included in the HIV versus anti-*CagA* status evaluation used buckets for water transport. Differences can be seen in the percentages of patients using buckets currently compared to percentages of patients using buckets during childhood for all HIV anti-*CagA* patient groups, thus showing that fewer patients need to collect water now days than in the past.

Both now, and during childhood, all patients who were HIV positive and anti-*CagA* positive currently store their water indoors, whereas half of the patients who were HIV positive and anti-*CagA* negative store their water outside and another half store their

water indoors. All the HIV negative patients, both anti-*CagA* positive and anti-*CagA* negative currently store their water indoors. During childhood, all the patients who were HIV negative only stored water indoors whereas a majority of the HIV positive patients stored their water outside and a small number of patients stored their water indoors.

Currently, the majority of the patients who need to store water do so in an open bucket, with little differences between patient groups. It was the opposite during childhood where the majority of the patients stored their water in closed buckets, with the HIV positive anti-*CagA* positive patients only storing their water outside. The majority of the patients currently do not boil their water , nor did they do so during childhood. Very few differences can be seen between the patient groups (Figure 44).

When looking at toilet location, both currently and during childhood, the majority of the patients have toilets outside. Only the HIV positive and anti-*CagA* positive patients have an even distribution in percentage of patients having toilet facilities indoors and those having toilet facilities outside the dwelling. No distinct differences can be seen between the groups.

The toilet facilities available in the HIV positive patients showed a majority making use of a pit latrine. In comparison, the majority of the HIV negative patients, regardless of the anti-*CagA* status, currently have flushing toilets. However, during childhood the majority of the HIV positive and negative patients made use of a pit latrine, with the exception of the HIV negative and anti-*CagA* positive patients a majority of who did not havei toilet facilities during childhood.

No studies could be found where an analysis was done between HIV and *H. pylori* and the effects of all the above mentioned variables..

7 Reliability, validity and trustworthiness

7.1 Reliability

Reliability is the extent to which a procedure produces consistent results using the same methodology. (Joppe, 2000).

The ^{14}C-Urea breath test (UBT) has been validated at the department of medical physics, University of the Free State (Jansen, 2001). This validated methodology was used for this study.

The anti-*CagA* IgG ELISA kit **(Product code: GD33; Genesis, Cambridge, U.K.)** was used as per manufacturers instructions to determine anti-*CagA* status. The serum used for Anti-*CagA* analysis was stored at - 80°C as per manufacturer's instruction.

All patients answered questions from the same questionnaire and translation of the questionnaire was performed when needed. However, when data was collected from patient files two limitations were identified:

1) Socio-economic status was determined by the annual income, lack of annual income, or medical aid coverage. However, as socio-economic standards cannot be determined by income alone these results could be biased. Similarly, patients could be covered by medical aid due to benefits received from an employer, yet still live in a low socio-economic area.

2) The socio-economic standards allocation for each patient, was done by an administrator at the government hospitals. This was done by simply asking the patient whether or not he/she had an income or not. It was also explained to patients, that if they earned an annual income they should give an amount as the higher the income, the more the administration fees would be that they

would have to pay. Thus it is possible that patients may have indicated that they did not have any income in order to avoid having to pay these admission fees.

3) Although all of the patients were either admitted to the haematology ward, or were currently attending a haematology clinic, for treatment of a haematological disorder, the diagnoses presented on the reports in the patient files were not always complete. Thus, it is possible that the diagnoses presented in this study are not complete. Patient files were reviewed with a doctor.

7.2 Validity

Research validity has been defined as the extent to which the research measures what it set out to measure, in other words did the research meet its goals. (Joppe, 2000).

The UBT results gave definite results. None of these results were undeterminable proving the accuracy of the method used.

A standard curve was produced for the Anti-*CagA* analysis using fixed concentrations provided in the kit. Figure 8 clearly shows the accuracy of the results in relation to the standard control solutions, as a clear curve formed as the "positivity" of the standard solutions increased. Therefore the Anti-*CagA* results can be assured as accurate.

The questionnaire was presented to each patient by the researcher herself who explained each question to the patient to ensure it was understood. Further more, an interpreter was used for all patients who did not understand English or Afrikaans. By doing this misinterpretation of questions was avoided.

7.3 Trustworthiness

Trustworthiness is defined as the trait of deserving trust and confidence (http://www.thefreedictionary.com/trustworthiness). Trustworthy data is Valid and Reliable data.

The UBT was tested and validated by Jansen *et al* (2001) for the academic hospitals in Bloemfontein, South Africa, and are currently available to practitioners to use as a diagnostic tool. Mr Le Roux Rabie is an expert in ^{14}C UBT testing, (Department of nuclear physics) all results from the breath tests were validated by him. Further more, the standard curve (Figure 8) produced for the controls of the Anti-*CagA* results, proved these results to be valid. And the questionnaire data was collected by the researcher herself, using translation when needed, avoiding all misinterpretations which could be made by a patient.

Thus, by proving all procedures reliable and valid, these results can be deemed as trustworthy.

7.4 Weaknesses

1) The researcher was not directly involved at the site of patient recruitment therefore it was often difficult to collect information

2) Although all of the patients were either admitted to the haematology ward, or were currently attending a haematology clinic, for treatment of a haematological disorder, the diagnoses presented on the reports in the patient files were not always complete. Thus, it is possible that the diagnoses presented in this study are not complete.

3) Patient numbers were too few to do certain analyses accurately. Eg: The anti-*CagA* HIV analysis could not be determined statistically due to the small numbers.

4) The study populations consisted mainly of black patients therefore race differences could not be accurately calculated.

5) Socio-economic status was determined by the annual income, lack of annual income, or medical aid coverage. However, as socio-economic standards cannot be determined by income alone these results could be biased. Similarly, patients could be covered by medical aid due to benefits received from an employer, yet still live in a low socio-economic area. This was not looked at in this study.

6) The socio-economic standard allocation for each patient, was done so by an administrator at the government hospitals. This was done by simply asking the patient whether or not he/she had an income or not. It was also explained to patients, that if they earned an annual income they should give an amount as the higher the income, the more the administration fees would be that they would have to pay. Thus it is possible that patients may have indicated that they did not have any income in order to avoid having to pay these admission fees.

8 Conclusions

- The prevalence of HIV infection was seen to be higher amongst the haematology patients than the general public (Both in the Free State province and nationaly) for 2006.

- The *H. pylori* prevalence (46%) was lower than expected amongst haematology patients.

- No statistically significant difference was found between HIV positive and HIV negative patients in relation to *H. pylori* prevalence.

- No statistically significant difference was found between HIV positive and HIV negative patients in relation to anti-*CagA* status.

- Due to small numbers no statistically significanct associations could be made between diagnoses and HIV, *H. pylori* or anti-*CagA* status.

8.1 HIV Prevalence

- Only age, no other demographic factor, was identified as a role player in HIV prevalence.

- No significant differences were found with relation to water source, water transport, water storage, neither currently nor during childhood for HIV prevalence.

- Boiling of water currently by HIV positive patients was statistically significant.

- Toilet location and toilet facilities, both currently and during childhood, were seen not to have an influence on HIV infection.

8.2 *H. Pylori* Prevalence

- Demographic variables isuch as age, sex and race did not appear to play a role in *H. pylori* infection.

- Water source, both currently and during childhood, was seen to be related to *H. pylori* prevalence.

- Although not significant during childhood, differences in the percentage of *H. pylori* positive and negative patients for current water transportation methods, water storage location and water storage containers were statistically significant.

- Boiling of water before consumption did had no effect on the *H. pylori* prevalence, neither currently nor during childhood.

- Patients are either most likely being infected with *H. pylori* during their childhood, or by other means currently, such as person to person transmission or by containers being used for other household tasks and not being sterilized before being used for water collection or storage.

- Toilet location was statistically significant to *H. pylori* infection both currently and during childhood.

- The current toilet facilities showed a trend that suggested a difference (although not statistically significant). Childhood toilet facilities were statistically significantly related to *H. pylori* infection.

8.3 *H. Pylori* infection related to HIV infection

- Sex and race did not influence *H. pylori* prevalence amongst HIV negative and positive patients, however age did show a significant difference where the trends looked much like that of the HIV analysis.

- The HIV negative *H. pylori* positive group showed a statistically significant higher number of low socio-economic gradings than the HIV negative *H. pylori* negative group.

- Neither water source, water transportation method, water storage location nor water storage containers played a role in *H. pylori* prevalence, neither currently nor during childhood, amongst HIV positive and negative patients in this study.

- Neither current boiling, nor boiling of water during childhood, played a role in *H. pylori* prevalence amongst HIV positive and negative patients.

- Statistically significant differences were found for toilet location in the HIV negative patient groups.

- Current toilet facilities did not show any significance, however childhood toilet facilities proved to be significant in the HIV negative group.

8.4 Anti-*CagA* Status

- The demographic data, sex, race and age was found not to have any statistical significance to anti-*CagA* prevalence.

- None of the investigated environmental variables (water source, water transportation method, water storage location, water storage containers, boiling

of water, toilet facilities and toilet location) were found to have a statistically significant association with anti-*CagA* status.

8.5 Anti-*CagA* Status in relation to HIV status

- Due to the low number of patients testing positive for anti-*CagA*, the patient numbers were too small to determine statistical significance for relationships with the demographic data or the environmental data (water source, water transportation method, water storage location, water storage containers, boiling of water, toilet facilities and toilet location).

9 Recommendations

1) Researchers in future studies must be involved at the patient recruitment site on a full time basis so that they have a more hands on accessibility to the patient files.

2) A larger study, running for a longer period of time, focusing on anti-*CagA* prevalence, (with HIV status available) will more accurately describe relationship between different variables.

3) A future study focusing on race, where large numbers representing each race are included in the study to determine the prevalence of *H. pylori* between different races.

4) A future study including more patients from a wider variety of socio-economic backgrounds, both from the private sector and government supported institutions, is suggested to accurately determine the role of socio-economic status in the prevalence of *H. pylori i*nfection in this region.

5) Future studies focusing on patients with particular haematological diagnoses will provide great insight into the role of HIV and *H. pylori* within these diseases/disorders.

6) A future study including analysis of the water which is consumed by the patients will be helpfull in determining more accurately the source of infection.

7) A future study including isolation of *H. pylori* from the water which is consumed by the patient, and the DNA finger printing of these bacteria,will help identify the exact strains of *H. pylori* which exist in the Free State area.

8) A future study including isolation of *H. pylori* from the patient, and the DNA finger printing of these bacteria, will help identify the exact strains of *H. pylori* responsible for infection in the Free State community.

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11 Appendix

11.1 Appendix A

Informed consent form for information from patient's file, breath samples for ^{14}C-Urea breath test and blood to be drawn for Anti-*CagA* ELISA.

11.2 Appendix B

Patient questionnaire.

Informed consent form

Department Haematology and Cell biology

University of the Free State

You have been selected to take part in a very important study to see how many people in your community carry a bacterium called *Helicobacter pylori*. The study is being undertaken at the Department of haematology and cell biology, University of the Free State. Details from your file will used to establish that you fulfil the requirements of the study. You will be required to blow into a solution through a straw, drink a capsule and blow another two times into different solutions also through straws. Once after 10 minutes and again after 20 minutes. One tube of blood will be drawn from you to perform tests on and information will be necessary from your patient file. These samples will be taken at your normal appointments with your clinician. No extra visits will be needed. There are **no dangers or risks** involved.

There is no guarantee that this study will be of advantage to yourself, but it will help doctors in your community treat people better in the future. Each patient's files are kept strictly confidential. Each patient is coded; no names or I.D. numbers will be presented on the result sheets. And you are welcome to with draw from the study at any time. In such case, your breath samples will be destroyed and you will be erased from the data bases. If you choose not to take part in this study your consultations and treatment will not be effected. This study is purely on a voluntary basis. For any queries please contact **Tanya Abbott. Cell phone number: (Number provided)**.

Participant: I hereby give my consent to take part in the above-mentioned study.

_____ _____

Name Printed Signature

_____

Date

Researcher:

_____ _____

Name Printed Signature

_____

Date

Questionnaire:

Patient study Number: _____

Personal details:

Race:

White	
Black	
Indian	
Mixed race	
Other	

Age: ☐

Number of children: ☐

Number of people living in same house as yourself: ☐

Financial details:

Are you on pension: YES ☐ NO ☐

Occupation: ☐

Are you Employed: YES ☐ NO ☐

For how many years: ☐

Did you attend school: YES ☐ NO ☐

To what grade: 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐
10 ☐ 11 ☐ 12 ☐ 0 ☐

Do you have any other qualifications: Degree ☐
Diploma ☐
Certificate in ☐

Residence:

City /Town where you live:

Bloemfontein	
Kimberley	
Welkom	

Suburb where you live:

Taba Nchu	
Botshabelo	
Bloemfontein (Township)	

How many bedrooms in house: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Do you have running water: YES ☐ Unknown ☐

NO ☐

If no…..

Where do you get your water:

Bore Hole ☐
Tanks ☐
River ☐
Dam ☐
Tap in street ☐
Other ☐

How do you transport your water:

Bucket ☐
Glass bottles ☐
Plastic bottles ☐
Other ☐

How do you store your water:

Buckets (open) ☐
Bucket (with lid) ☐
Glass bottles ☐
Plastic bottles ☐
Large plastic container ☐
Other ☐

Where do you store your water:

outside ☐
Inside the house ☐
Other ☐

Do you boil the water before you drink it: YES ☐ NO ☐

Is your toilet: Inside ☐ Outside: ☐

What toilet facilities do you have:

Toilet installed by a plumber ☐
Long drop ☐
Other ☐

When you were younger (± 10 years old):

How many bedrooms in house: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

How many people lived in house: ☐

Did you have running water: YES ☐ Unknown ☐

NO ☐

Where did you get your water:

Bore Hole ☐
Tanks ☐
River ☐
Dam ☐
Tap in street ☐
Other ☐

How did you transport your water:

Bucket ☐
Glass bottles ☐
Plastic bottles ☐
Other ☐

How did you store your water:

Buckets (open) ☐
Bucket (with lid) ☐
Glass bottles ☐
Plastic bottles ☐
Large plastic container ☐
Other ☐

Where did you store your water:

☐
outside ☐
Inside the house ☐
Other

Did you boil the water before you drank it: YES ☐ NO ☐

Was your toilet: Inside ☐ Outside: ☐

What toilet facilities did you have:	Toilet installed by a plumber	☐
	Long drop	☐
	Other	☐