DISSERTATION

TITLE OF STUDY:

THE PREVALENCE OF HELICOBACTER PYLORI IN TONSILLAR TISSUE OF PATIENTS UNDERGOING TONSILLECTOMY AT KENYATTA NATIONAL HOSPITAL.

Principal researcher:

Name: Dr. Ochung'o. Peter

MBChB - University of Nairobi.

Registration number: H58/71001/09

Supervisors:

1. Dr. Peter Mugwe.

Consultant ENT Surgeon and Senior Lecturer Department of Surgery,

University of Nairobi

2. Dr. Peter Masinde.

Consultant ENT Surgeon, ENT Department,

Kenyatta National Hospital.

3. Dr. Waweru Wairimu

Consultant Pathologist and Lecturer

Department of Human Pathology.

University of Nairobi

A dissertation submitted in partial fulfillment of the requirement of the University of Nairobi, for the Award of the Degree of Master in Medicine in ENT, Head and Neck Surgery.

DECLARATION

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

Signed_____ Date 28/5/2012

Dr. Peter. O. Ochung'o

This thesis was supervised by:

1. Signed_____ Date 29/5/2012

Dr. Mugwe P.

Consultant ENT Surgeon.

Senior Lecturer, Dept. of Surgery,

University of Nairobi.

2. Signed_____ Date 28/5/2012

Dr. Masinde P.

Consultant ENT Surgeon, KNH

3. Signed_____ Date 29/5/12

Dr. Waweru W.

Consultant Pathologist and Lecturer, Dept of Human Pathology

University of Nairobi

TABLE OF CONTENTS

1.0 LIST OF TABLES AND FIGURES 6

1.1 ACRONYMS AND ABBREVIATIONS 7

2.0 ABSTRACT 8

3.0 INTRODUCTION 9

3.1 EMBRYOLOGY.....9

3.2 ANATOMY 9

3.3 FUNCTIONAL MORPHOLOGY -TONSIL.....10

3.4 H. PYLORI – STRUCTURE AND PHYLOGENY 12

3.5.H. PYLORI COLONIZATION AND EXTRAGASTRIC RESERVOIRS..... .12

3.6 ADAPTATION OF H. PYLORI TO THE HOST 13

3.7 COCCOID FORMS OF H. PYLORI 15

3.8 AGE OF ACQUISITION OF H. PYLORI 15

3.9 H. PYLORI AND CHRONICITY OF TONSILS 15

4.0 LITERATURE REVIEW 17

5.0 JUSTIFICATION FOR THE STUDY 23

6.0 RESEARCH QUESTION 23

6.1 HYPOTHESIS 23

6.2 NULL HYPOTHESIS 24

7.0 AIMS AND OBJECTIVES 24

7.1 GENERAL OBJECTIVES 24

7.2 SPECIFIC OBJECTIVES 24

8.0 MATERIALS AND METHODS 24

8.1 STUDY DESIGN 24

8.2 SAMPLE SIZE 25

8.3 SAMPLING METHOD 25

8.4 STUDY DURATION 25

8.5 INCLUSION CRITERIA 26

8.6 EXCLUSION CRITERIA 26

8.7 CONFOUNDING FACTORS 26

8.8 CLINICAL EVALUATION 27

8.9 MATERIALS AND EQUIPMENT.....26

8.10 HISTOPATHOLOGICAL INVESTIGATION 28

9.0 QUALITY CONTROL 29

10.0 DATA MANAGEMENT 30

11.0 ETHICAL CONSIDERATIONS 31

12.0 RESULTS.....32

13.0 DISCUSSION.....47

14.0 CONCLUSIONS.....50

15.0 RECOMMENDATIONS.....50

16.0 LIMITATIONS OF STUDY.....51

17.0 APPENDIX 52

17.1 PATIENT INFORMATION AND CONSENT.....52

17.2 QUESTIONNAIRE 56

17.3 ICSD – DIAGNOSTIC AND CODING MANUAL 57

17.4 PARADISE CRITERIA FOR TONSILLECTOMY.....58

17.5 LABORATORY PROCEDURE FOR RAPID UREASE TEST 59

17.6 REFERENCES 60

TABLES AND FIGURES

FIGURE 1.....10

FIGURE 2.....11

FIGURE 3.....16

FIGURE 4.....33

FIGURE 5.....34

FIGURE 6.....35

FIGURE 7.....36

FIGURE 8.....37

FIGURE 9.....38

FIGURE 10.....39

FIGURE 11.....40

FIGURE 12.....43

FIGURE 13.....45

FIGURE 14 46

TABLE 1 – FINDINGS BY VARIOUS AUTHORS.....22

TABLE 2 – AGE DISTRIBUTION 32

TABLE 3 – BASELINE CHARACTERISTICS 41

TABLE 4 – PRESENCE OF H PYLORI 42

TABLE 5 - DIFFERENCE IN COLONISATION 43

TABLE 6 – LOGISTIC REGRESSION/ TABLE 7 – AGE ADJUSTED RISK 44

TABLE 8 - PRESENCE OF ACTIVE AND COCCOID FORMS OF HPYLORI.....45

1.1 ACRONYMS AND ABBREVIATIONS:

AAO HNS	American Academy of Otolaryngology Head and Neck Surgery
ATH	Adenotonsillar Hypertrophy
CLO	Campylobacter Like Organism
CRT	Chronic Recurrent Tonsillitis
E N T	Ear Nose and Throat
H. pylori	Helicobacter Pylori.
ICSD	International Society for Sleep Disorders
IgA N	Immunoglobulin A Nephropathy
IgG	Immunoglobulin G
KNH	Kenyatta National Hospital.
MALT	Mucosa associated Lymphoid Tissue
OSAS	Obstructive Sleep Apnea Syndrome
PCR	Polyclonal Chain Reaction
RUT	Rapid Urease Test
SPSS	Statistical package for the social sciences.

2.0 Abstract:

Background: Human Palatine Tonsils are lympho epithelial tissues which are part of the Mucosa Associated Lymphoid Tissue which play a vital role in sampling and effector functions for the upper respiratory tract. Palatine tonsils may also serve as a reservoir for pathogens including H. pylori. It has been suggested that this may be responsible for the chronicity and recurrent nature of tonsillitis in some patients and may serve as an extra gastric reservoir for H. pylori.

Objective: To determine if H. pylori colonises tonsillar tissue and to analyse the difference in patterns of H. pylori colonization in patients with Chronic Recurrent Tonsilitis compared to those with adeno tonsillar hypertrophy.

Study Design: Prospective Cross Sectional Comparative Study

Material and Methods: A total of 78 cases were recruited from patients booked for tonsillectomy or adenotonsillectomy at the ENT satellite theatre. History was elicited from each patient recruited using preformatted questionairres. After tonsillectomy, one sample was taken from either tonsil and analyzed using Rapid Urease Test Kit and Histology for detection of H. pylori in the tonsil tissue.

Study setting: Kenyatta National Hospital – A tertiary teaching hospital

Data Analysis: Data was entered into preformatted worksheets and analysed using SPSS 17.0. Categorical variables were presented as percentages while continuous variables as means and standard deviation. Data was presented in the form of tables and graphs. Baseline characteristics was compared and Students T-Test and Pearsons' Chi Square test was used to test associations. Logistic regression was used to analyse statistically significant data.

Results: A total of 78 tonsils were analysed for H. pylori by Rapid Urease Test and by Histology. H. pylori was present in 30.5% (n=24) of tonsillar tissues. Colonisation of palatine tonsils by H. pylori was found in 38.5% (n=15) of patients with Chronic Recurrent Tonsillitis with OSAS and 23% (n=9) of patients with Adenotonsillar Hypertrophy with OSAS. There was a statistically significant difference in risk of colonization by H. pylori when adjusted for age [OR 2.5 (1.6-3.9) P= <0.001]. Colonisation of tonsil tissues by H. pylori using histology was found in 10.3% (n=4) of tonsil tissues. All were found in chronically recurrent tonsil tissues. A total of 12.8% (n=5) of cases had colonization by coccoid forms of H. pylori. There was no statistically significant risk in colonization by coccoid forms of H.pylori between tissues with Chronic Recurrent Tonsillitis with OSAS and those with Adenotonsillar Hypertrophy with OSAS.

Conclusion: H. pylori colonization of the palatine tonsils is a new frontier with early results showing colonization of tonsils by H. pylori. This may lead to change in management protocols for chronically recurrent tonsils and also lead to new methods of treating H. pylori related gastric disease.

3.0 INTRODUCTION:

Overview:

H. pylori is among the most common infections of mankind. Recently the human palatine tonsil has been identified as a possible extra gastric reservoir for H. pylori.[1] [2]

Surgical anatomy of the human palatine tonsil:

3.1 Embryology:

The human palatine tonsil develops from the second pharyngeal pouch from about the sixth week of embryonal development. The epithelial part of the tonsils develop from the foregut endoderm (Pouches I and II), while the surface epithelium and lining of the crypts of the palatine tonsils develop from endoderm of the second pharyngeal pouch. The capsule of the tonsil develops from a condensation of mesenchyme. [3]

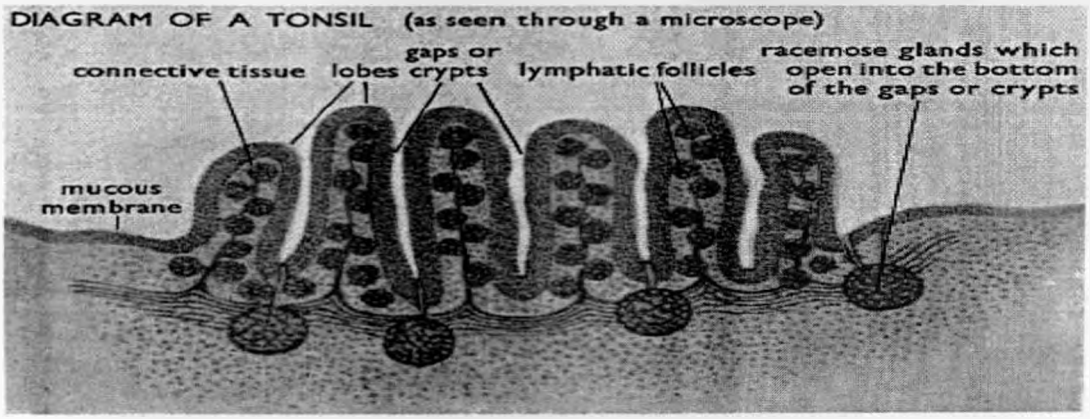
3.2 Anatomy:

The Human Palatine Tonsils are paired lymphoid organs in the tonsillar fossa located between the palatoglossal and palatopharyngeal arches of the oropharynx. They form a component of the well described mucosa associated lymphoid tissue(MALT). They are part of the internal waldeyers ring. They constitute the first line of mucosal defence against invading pathogens. [4]

The tonsillar lymphoid mass doesn't fill the whole area between the two arches. Instead a small area, the supratonsillar fossa, is found at the upper part of the interval. The tonsil then extends under cover of the glosso palatine arch and is covered by a fold of mucous membrane. The superior part of this fold extends across the supra tonsillar fossa, between the two arches. This fold is the plica triangularis. Between the plica triangularis and the surface of the tonsil is a space named the tonsillar sinus. The tonsillar fossa is bordered by the Palatopharyngeal muscle dorsally and the palatoglossal muscle ventrally. Laterally the fossa is adherent to a fibrous

capsule separated from the inner surface of the superior constrictor muscle by loose connective tissue. [5]

Figure 1: Microscopic cross section of the human palatine tonsil: (Adapted from The Encyclopedia of Science.)

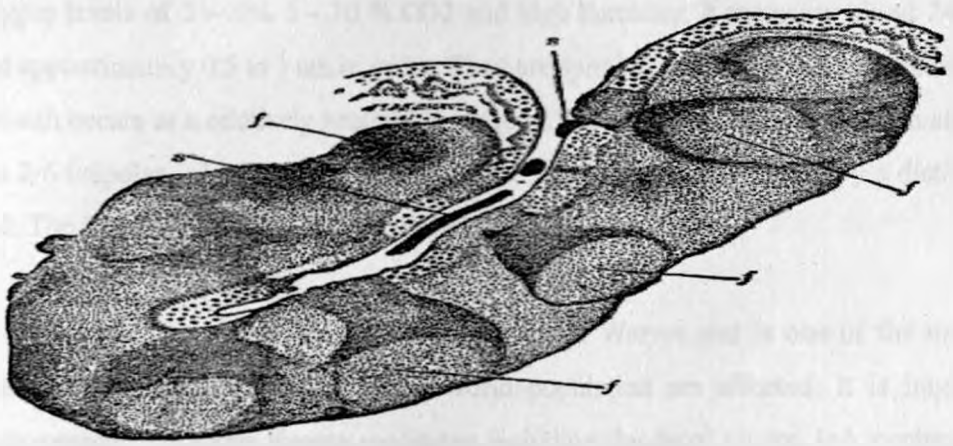


3.3 Functional Morphology of the Human Palatine Tonsil

The human palatine tonsil has 10-30 crypts which are ramified epithelial invaginations. It contains different sub sets of specialized compartments. It forms one of the components of the Mucosa Associated Lymphoid Tissue and the Internal Waldeyers Ring. [4]

Due to easy accessibility, the Human Palatine Tonsils have often been used as the model for human lymphoid organs. Within the upper aerodigestive tract, the Internal Waldeyers Ring acts as a first line of defence against invading pathogens. Immunological processes, both humoral and cellular are initiated in the different specialized compartments of the palatine tonsils including the crypt epithelium, Sub epithelial areas, Lymphoid follicles and Extra follicular regions. Each compartment has a typical composition of lymphocyte and dendritic cell subsets. [4]

Figure 2: Section through one of the crypts of the tonsil. (Stöhr.) Magnified. *e.* Stratified epithelium of general surface, continued into crypt. *f.* Nodules of lymphoid tissue—opposite each nodule numbers of lymph cells are passing into or through the epithelium. *s, s.* Cells which have thus escaped to mix with the saliva as salivary corpuscles.(Adapted From Grays anatomy of the human body fig. 1027)



Recurrent or chronic adenotonsillar infection mainly affect childhood age groups. This may be due to local dysfunction of the epithelial structures. [6]

Chronic recurrent tonsillitis is defined as episodes of tonsillitis occurring more than 7 times per year for one year or more than 5 times per year in the preceding 2 years or more than 3 times per year in the preceding 3 years. Chronic parenchymal tonsillitis affects mainly children and adolescents while chronic membranous tonsillitis affects mainly adults. [32]

The histological configuration of the parenchyma is complex and is vital for the uptake and presentation of antigens to the subepithelial immunocompetent cells. Epithelium that lines the tonsillar crypts is non uniform and contains patches of stratified squamous non keratinized epithelium and patches of reticulated sponge like epithelium. Reticulated patches are associated with disruption in the epithelial basement membrane. [6][7]

3.4 H. pylori – Structure, Genus Description and Phylogeny

H. pylori is a gram negative bacteria that is microaerophilic in nature. Its optimum growth is at oxygen levels of 2 – 5%. 5 – 10 % CO2 and high humidity. It measures about 2-4um in length and approximately 0.5 to 1 um in width. They are spiral shaped but some may be rod shaped. Growth occurs at a relatively neutral PH range of 5.5. to 8 with optimum growth at neutral PH. It has 2-6 unipolar , sheathed flagella which are 3 um in length and may carry a distinct bulb at the end. The flagella gives motility to the organism. [8][9]

H. pylori was discovered in 1983 by Marshall and Warren and is one of the most successful human pathogens. About 50% of the world population are affected. It is implicated in the pathogenesis of various disease conditions including duodenal ulcers, IgA nephropathy, gastric adenocarcinoma and gastric ulcer.[10] For this reason the WHO 's International Agency for Research on Cancer classified H. pylori as a group 1 (definite) carcinogen. [11]

The genus Helicobacter belongs to the subdivision of the Proteobacteria, Order – Campylobacterales, Family – Helicobacteraceae. It consists of over 20 species. [12]

3.5. Etiopathogenesis of H. pylori colonization of extra gastric tissues and Extragastric reservoirs:

Because extra gastric reservoirs of H. pylori is a relatively recent concept, various theories have been put forwards regarding adaptation to the host. Though the gastric tissue is the natural reservoir for H. pylori, other extra gastric tissue have been proposed as potential reservoirs. These include saliva, gall bladder and coronary arteries. [1,12,37]

3.6. Adaptaton of H. pylori to the Host:

Within the human host, the oral cavity is the principal extragastric reservoir. This is because human infection by this pathogen is either oro-fecal or fecal-oral. [2]

H. pylori has a specificity to the human host that has been adapted over many centuries. Transmission requires close contact between individuals and if H. pylori requires to infect another host, it require quick adaptation to the new environment. This ongoing adaptation to the human host is reflected by the very high degree of genetic diversity within H. pylori species. These new population of closely related genetic variants are known as quasi species. [13,14,53]
H. pylori avoids host defences by shedding bacterial proteins and detoxifying reactive free oxygen radicals. It can also survive inside macrophage phagosomes by inhibiting phagosome maturation. Once H. pylori adheres to epithelial cells, it induces a strong immune response. This does not lead to elimination of the bacterium but causes chronic inflammation. [15][16]

Direct injury by H. pylori to the host may occur through production of urease, lipopolyssacharides and the release of various hemolysins and cytotoxins like Vac A. Injury to the host tissue then facilitates direct entry of the H. pylori microbe. [17]

In addition, H. pylori may avoid the host's inflammatory response and remain viable when internalized by epithelial cells. It may also remain viable in macrophage phagosomes by inhibiting phagosome maturation. It may also be involved in regulating the hosts immune response by activating CD25 regulatory T cells and dendritic cells and may also direct immunosuppression of T cells. [18]

H. pylori may then induce activation and maturation of monocyte derived dendritic cells which is coordinated by Toll Like Receptors, which are expressed on antigen presenting cells and which may lead to promotion of Natural Killer and Th1 helper T cell effector responses. These cells have been hypothesized to play a central role in the development of pathologies in the host tissue. [19][20]

Studies have shown that one way in which H. pylori may persist in the human host is through avoiding detection by Toll Like Receptor 5 flagellin receptor by reducing the production of flagellin, whereas other gram negative microbes release it. [21]

Inflammatory response by H. pylori to the host by the use of lipopolyssacharides has been studied. Lipopolyssacharides are a family of toxic gram negative phosphorylated glycolipids on the outer membrane of gram negative bacteria, which include H. pylori. It is composed of a lipid moiety, a core oligosaccharide and a polymeric O specific polysaccharide chain. The reduced levels of lipopolysaccharides and toxic oxygen radicals contribute to the H. pylori inflammatory response.[22]

Histological assay of chronically inflamed tonsils displays crypt epithelium hyperkeratosis. This structural change in the epithelium causes impaired neutrophil chemotactic function and disordered antigen uptake, which leads to recurrence of inflammatory events with higher degrees of severe hyperkeratosis thus triggering a vicious cycle. [22][23] It has been hypothesized that H. pylori could prime the tonsils by inducing macrophage inducible nitric oxide synthase (iNOS) expression and also more marked cytokine responses. This therefore induces pro inflammatory reaction in the palatine tonsils. [23]

H. pylori infection can also cause the same immune changes in the oropharyngeal mucosa as in the gastric mucosa and can contribute to oropharyngeal disease. They may induce production of different cytokines and regulatory molecules which may play a role in oropharyngeal pathology. But more research is needed to clarify how H. pylori persists in tonsillar Tissue. [24]

Another theory put forward for tonsillar colonization by H. pylori is that H. pylori induces a pro inflammatory reaction that is both local and systemic.[26] This is called downstream priming of mucosal immunity by H. pylori and it may account for the various pathologic tissue response observed in infected patients. The finding of H. pylori in the oral cavity suggests that H. pylori does not need an acid environment to survive. [26]

3.7 Coccoid forms of H. pylori and their role in disease transmission

Coccoid forms of H. pylori may represent a structural manifestation of cell death.[25] The possibility that coccoid forms play a role in transmission of H. pylori and in relapse after therapy by antibiotics is still a subject of debate. [25] They have also been found to reduce the sensitivity of test kits to H. pylori.[26] Coccoid forms of H. pylori are also believed to represent a temporary expression of H. pylori to an extra gastric environment.[27] It has been suggested that coccoid forms of H. pylori may represent a viable non culturable state. [27]

3.8 Age of Acquisition of H. pylori

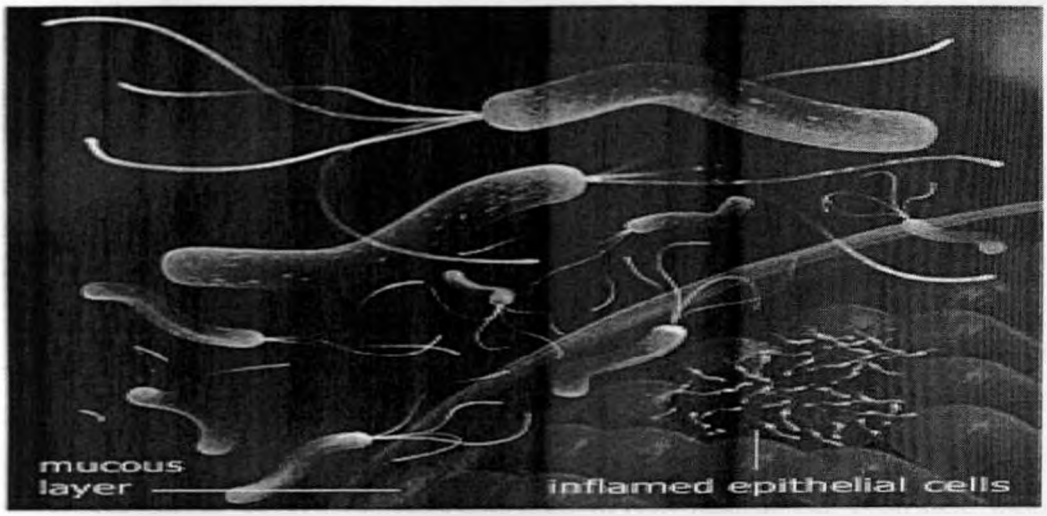
Colonisation by H. pylori shows early infection in developing countries and increases with age. It may be related to socio economic status. Infection is believed to start from as early as one year of age. [28,29]

Overall, H. pylori has a prevalence of 30% in developed and 80% in developing countries. Therefore epidemiological and geographical variations exist across the world. [30,31]

3.9 H. pylori and Chronicity of Tonsils

It has been proposed that H. pylori may induce inflammatory changes that may contribute to persistence of chronicity or recurrence of tonsillitis for which a tonsillectomy either alone or in conjunction with an adenoidectomy may be indicated. [32]

Figure 3: *Helicobacter pylori* invading epithelial cells. (Adapted from Davidson college, U.S.A.)



Chronic recurrent tonsillitis can be diagnosed by both clinical history and histological findings. [31]. This may require tonsillectomy as part of the management. H. pylori may play a role in causing chronicity and recurrence of tonsillitis.[32] This is achieved by its effects on tonsillar epithelial surface and release of inflammatory mediators that may lead to further epithelial tissue damage. [32][33]

4.0 LITERATURE REVIEW:

Although H. pylori has been found to affect approximately half of the worlds' population, its precise modes of transmission have not been fully elucidated.[34] Many confounding factors such as patient and bacterial genetic characteristics, patients socio economic status, use of antibiotic treatment and presence of other infections at the same time, can influence clinical and epidemiological studies of H. pylori colonization and infection. [35]

Moran et al [22] in a study on lipopolysaccharides, postulated that lipopolysaccharides, which are a family of toxic phosphorylated glycolipids in the outer membrane of gram negative bacteria, may induce low immunological activities in test systems. These reduced levels of lipopolysaccharide induced cytokines and toxic oxygen radicals can contribute together with those induced by bacterial proteins to the H. pylori induced inflammatory response. They suggested that the ability of lipopolysaccharides to induce low production plasminogen activator inhibitor type 2 by human mononuclear cell may contribute to the localized inflammatory response and may play a role in extra gastric pathologies.

Although H. pylori has been implicated conclusively as a major pathogen in gastric mucosa of humans, recent studies have also shown that there exists extra gastric reservoirs for the pathogen. [36-41]

Studies, especially from Western Europe have disputed the presence of H. pylori in extra gastric tissues.[34-37]

Vasiyoglu et al [37] evaluated the possible relationship between chronic adenotonsilitis and H. pylori. 91 paediatric patients were recruited. The adenotonsillar tissue was examined using Rapid urease test and Immunohistochemistry. Only 2.2% of adenoidectomy specimens and none of the tonsillar specimens tested positive for H. pylori. Their results suggested that H. pylori could not colonise the tonsil tissue of patients with chronic tonsillitis.

Vilarhino et al [35] investigated 62 children for H. pylori in their adenotonsillar tissues. 3 patients were positive using the Rapid urease test. They concluded that the human palatine

tonsils may not constitute an extra gastric reservoir of H. pylori. They attributed this partly to the currently available methods of detecting H. pylori as not being adequate to evaluate adenotonsillar tissues. This study was also done in Western Europe where H. pylori infection is lower than in developing countries. This reflects the world wide geographic differences in infection by H. pylori.

Eyigor et al [38] did a study on the detection of H. pylori in adenotonsillar tissues by Rapid Urease Test and PCR. 47 patients with chronic tonsillitis and adenoid hypertrophy were recruited into the study. They used 35 adenoid and 20 tonsil tissues. 5.5% of patients were positive by Rapid Urease Test. They concluded that further studies needed to be done to clarify the possible role of H. pylori in adenotonsillar tissue hypertrophy and in the pathology of chronic recurrent tonsilitis.

Moghaddam et al [39] did a comparative study to asses H. pylori colonization in tonsillar tissue of children. They recruited 285 children aged between 4 and 14 years. Tests were done using Rapid Urease Test and histopathology. 14% of tonsillar samples were positive by Rapid Urease Test and 39.6% positive on histopathology exam. They concluded that H. pylori was present in tonsillar tissue and has a role as a possible reservoir of H. pylori in children.

Another cross sectional study done by Zahedi et al [33] on 95 adeno tonsillectomy specimens (age range 2 - 35 years), they found 42% we positive for H. pylori on Rapid Urease test and 9.5% positive on histology for H. pylori. They concluded that tonsillar tissue seemed to harbor H. pylori and recommended that further studies be undertaken on the role of H. pylori in reinfection of tonsillitis after treatment.

Lin et al [46], did a retrospective study of 94 patients. The first group had patients with chronic recurrent tonsillitis without Sleep related breathing disorders. The second group had patients with Sleep related breathing disorders without recent tonsillitis. Procedure included taking a 2-3mm diameter piece from each tonsillectomy specimen with ta sterile blade. Each specimen was cut with a different blade and gloves changed after obtaining each specimen. The piece of tissue was placed in a Pronto Dry Test, that detects the urease enzyme of H. pylori. The findings were examined at intervals of 5, 30, and 60 minutes at room temperature. A pink-Magenta color

change was a positive reaction and a negative reaction was yellow color change. They found H. pylori to be positive in 48% of the recurrent tonsillitis group compared with 24% for the group with sleep disordered breathing. They concluded that studies should be done to see whether eradication of H. pylori would decrease the rate of recurrent inflammation in the human palatine tonsils. The advantage of their study is that it was the first to include a control group. This study gave rise to the hypothesis that Colonisation by H. pylori in Chronic recurrent Tonsilitis could be due to structural and morphological changes in the crypt epithelium and immune mechanisms of the tonsil, which may predispose patients to colonization with H. pylori.

A study by Ergur et al [41] investigated the presence and frequency of H. pylori in palatine and pharyngeal tonsils tissues of children. The study had a sample size of 20 patients. They recruited patients based on either a history of chronic recurrent tonsillitis or a history of snoring. Colonisation of tonsillar tissue by H. pylori was found in 55 of specimens by RUT and 20% by immunohistochemistry. The limitation of their study was the relatively small sample size.

In a study by Bulut Y et al [42] with 118 tissue samples of adeno tonsillectomy specimens, they found 24.6 % positive for H. pylori with PCR analysis. In the same study he associated the presence of the Cag A gene with development of adeno tonsillar hypertrophy.

A study by Monem et al [40] on 20 children with chronic tonsillitis, using both PCR and RUT, found that 53.3.% were positive for H. pylori using RUT. They concluded that Human palatine tonsils may constitute an extra gastric reservoir for H. pylori. This was also explored by Nam et al. [43]. They studied 98 patients undergoing tonsillectomy using CLO/RUT test and immuno histochemistry. They found a 62% overall colonization of H. pylori in the tonsil tissues. They did not find any statistically significant difference between the Chronic tonsillitis group and the control group. They concluded that Human palatine tonsil may constitute an extragastric reservoir of H. pylori but not a target tissue. The strength of this study was the inclusion of a control group.

Cho et al [44] recruited 38 patients into their study. They found that 28.8% were positive for H. pylori on Urea Breath Test and 21.1% positive on CLO Test. They concluded that Human

palatine tonsil may be either an extra gastric reservoir for H. pylori or a transmission route. The limitation to the study was the relatively small sample size.

Kusano et al [47] in their study linked the presence of coccoid forms of H. pylori in HPT to the presence of IgA Nephropathy using tonsillar culture and immunohistochemical methods. In their study, all patients with gastric H. pylori also had tonsillar H. pylori colonization. Also all patients with IgA nephropathy had tonsillar H. pylori. Their findings indicated that tonsillar H. pylori in coccoid forms may be one of the causative factors of IgA nephropathy.

Khademi et al [26] in their study on H. pylori in core tonsil tissue in 56 patients undergoing tonsillectomy or adeno tonsillectomy found that 48.2% of patients were positive for H. pylori. They concluded that recurrent nature of H. pylori in the gastric mucosa may be a result of colonization in the tonsillar tissue. They suggested that eradication of H. pylori in the tonsillar tissue may result in reduced levels in the gastric mucosa.

A study by Wibawa et al [48] on 19 patients with chronic tonsillitis demonstrated viable H. pylori in 15.7% of tonsillar tissue. The analysis was both histological and micro biological, using modified Giemsa staining and immunohistochemistry. They concluded that viable H. pylori can be detected in the tonsillar tissues of chronic tonsillitis patients. The limitation of their study was the relatively small sample size and lack of a comparative group.

Another study by Xian et al [51] in China on the prevalence of H. pylori in children presenting with obstructive sleep apnea syndrome reported a high sero prevalence of H. pylori in children with OSAS. The prevalence increased with an increase in severity of OSAS. They concluded that H. pylori may be linked to OSAS. This reflects the variations in results for different studies based on colonisation of palatine tonsils by H. pylori.

Childhood as the age of acquisition of H. pylori has been explored in many studies [30,31,51-54]. Malaty et al [52] concluded that most infections are acquired before the age of 10 years. Siai K [53] found a high prevalence of H. pylori in Tunisian children and attributed this to low socio economic status and crowded conditions.

A study by Rowland M et al [54] concluded that the highest rate of acquisition of H. pylori occurs in childhood and then starts to decline from the age of 5 years. This was especially found in developing countries.

The use of tonsillar core tissue was justified in a study by Di Bonaventura [45] who found H. pylori in tonsillar surface in 53% against 23% in tonsillar core surface. He concluded that surface swab was neither specific nor sensitive in determining colonization of H. pylori at the tonsillar core.

The use of CLO/RUT as a reliable test for H. pylori has been justified by various authors. [26,48,49] Khademi et al [26] chose to use the CLO/RUT test because it was found to be of high specificity and sensitivity for H. pylori . Dye et al [49] reported that the specificity of the CLO/RUT test for H. pylori was 97% and the sensitivity was 98%. Schnell et al [50] reported similar results.

Giemsa stain has been used to stain for H. pylori in tonsil tissue. Giemsa stain is a mixture of Azure B, Methylene Blue and Eosin. The stain is acidophilic and allows for the staining of H Pylori DNA. Giemsa is used in histology because of its high quality staining of chromatin and nuclear membrane, and also the metachromasia of some cellular components leading to a good contrast. It is therefore a popular stain because of its good contrasting ability and its simplicity [55][56]

Table 1. Table of findings by various authors:

Zahedi et al(31)	2009	Cross sectional	95	RUT	42.1% +ve H Pylori
Nam et al (32)	2007	Comparative	98	RUT	61.2% in CRT
Moghadam et al(33)	2009	Prospective	285	RUT	14% +ve H Pylori
Ergur et al(34)	2008	Cross sectional	20	RUT	5% +ve H Pylori
Wibawa et al(35)	2011	Descriptive	19	Mod Giemsa	15.7%
Cho et al(36)	2007	Cross sectional	38	RUT	21.1% +ve H Pylori
Bulut et al(37)	2006	Descriptive	118	PCR	24% +ve
Monem et al(38)	2011	Descriptive	20	RUT	53% +ve
Unver et al(39)	2001	Descriptive	19	RUT/CLO	58% H Pylori +ve
Aslan et al(40)	2007	Cross sectional comparative	94	Pronto dry	42% +ve

5.0 JUSTIFICATION OF STUDY

The association between H. pylori and chronically inflamed tonsillar tissue is a recent finding which requires further study. A finding of positive H. pylori colonization of tonsillar tissue could lead to efforts aimed at eradicating this organism from tonsillar tissue and thus reducing the overall rate of recurrence of chronic tonsillitis and also preventing clinical sequelae of H. pylori colonization in gastric tissue. There has been no similar studies carried out in Kenya and the region. This study provides a foundation for further studies and it will also contribute to the growing knowledge in this new frontier as this is a recent discovery.

This study is set to investigate the prevalence of H. pylori in core tonsillar tissue in patients with chronic recurrent tonsillitis with OSAS compared to patients with adenotonsillar hypertrophy with OSAS.

6.0 RESEARCH QUESTION:

What is the prevalence and pattern of H. pylori colonization among patients with chronic recurrent tonsillitis with clinically diagnosed obstructive sleep apnea syndrome compared to patients with adenotonsillar hypertrophy with clinically diagnosed obstructive sleep apnea syndrome.

6.1 HYPOTHESIS:

H. pylori colonizes tonsillar tissue and there is a difference in the prevalence between patients presenting with chronically recurrent tonsillitis with obstructive sleep apnea syndrome and adeno tonsillar hypertrophy with obstructive sleep apnea syndrome.

6.2 NULL HYPOTHESIS:

There is no difference in prevalence of H pylori in tonsillar tissue between patients with chronic recurrent tonsillitis with obstructive sleep apnea syndrome compared to patients with adenotonsillar hypertrophy with obstructive sleep apnea syndrome.

7.0 AIMS AND OBJECTIVES:

7.1 General Objective

To determine the prevalence of H. pylori in tonsillar tissue, comparing patients presenting with chronic recurrent tonsillitis with OSAS and patients presenting with adeno tonsillar hypertrophy with OSAS.

7.2 Specific Objectives:

a. To determine the presence of H. pylori in core tonsillar tissue in chronic recurrent tonsillitis with OSAS.
b. To determine the presence of H. pylori in core tonsillar tissue in adenotonsillar hypertrophy with OSAS.
c. To determine the difference in colonization by *H. pylori* in tonsillar tissue of patients presenting with chronic recurrent tonsillitis with OSAS and those presenting with adenotonsillar hypertrophy with OSAS.

8.0 MATERIALS AND METHODS:

8.1 Study Design

Prospective cross sectional comparative study

8.2 Sample Size

The sample size was estimated using the following formula for comparing two proportions as outlined below:

n – desired sample size

p_0 – the proportion of exposure (H. pylori) among comparison group (tonsillar hypertrophy) = 24.6% (Bulut et. al, 2006)

q_0 – 1- p_0 = 75.4%

p_1 – the proportion exposure (H. pylori) of among cases (recurrect tonsillitis)

Assuming the expected relative risk (RR) of 2.2 of presence of H. pylori among cases compared to the comparison group

$p_1 = p_0 * RR = 0.246 * 2.2 = 0.541$ (54.1%)

$q_1 = 1 - p_1 = 0.459$

$Z_{1-\alpha/2}$ - Two-sided significance level (1-alpha)-95% = 1.96

$Z_{1-\beta/2}$ – Power (1-beta, % chance of detecting) – 80% = 0.84

By substituting into the formula

n = **39** in each group.

8.3 Sampling Method

Consecutive convenient sampling method was used.

8.4 Study Duration

December 2011 – February 2012 after approval by the KNH/UON ethics and research committee

8.5 Inclusion Criteria

- Patients whom guardians give consent.
- Patients undergoing elective tonsillectomy for chronic recurrent tonsilitis and for adenotonsillar hypertrophy and met the criteria for obstructive sleep apnea syndrome according to the international classification of sleep disorders diagnostic and coding manual(Appendix 3).
- Patients between the ages of 2 years and 14 years.

8.6 Exclusion Criteria

- Patients who decline to participate in the study.
- Patients who have been on anti peptic ulcer drugs(Triple therapy) in the last 2 months
- Patients on any antibiotic during the last two weeks before surgery.
- Patients who have an indication for tonsillectomy other than adenotonsillar hypertrophy or secondary to recurrent chronic tonsillitis
- Patients less than 2 years or more than 14 years.
-

8.7 Confounding Factors:

The main objective of the study was to determine the pattern of colonisation, as such we had to control for confounding factors such as prior antibiotic use. However, to determine if the pattern of colonisation varied between the chronic recurrent tonsillar tissue and adenotonsillar hypertrophy, we had to adjust for known confounding factors. A confounding factor is any factor that is associated with the the outcome (H. pylori) and is also associated with the exposure (Tonsillar enlargement either due to chronic inflammation or simple hyperplasia). A simple questionnaire was used to capture possible confounding factors such as age, and these factors were adjusted in the analysis.

8.8 Clinical Evaluation

Patient selection was done from the ENT clinic booking diary for elective theatre from among those selected for surgery by the ENT consultants. The relevant clinical history was taken pertaining to the study and this mainly included history of treatment of peptic ulcer disease, history of antibiotic use , history of snoring, morning headaches , obstructed breathing during sleep and other risk or confounding factors. Two groups of patients were be recruited, 39 in each group. The first group were patients undergoing tonsillectomy for chronic recurrent tonsillitis and met the criteria for obstructive sleep apnea syndrome. The second group were patients undergoing adeno tonsillectomy for adeno tonsillar hypertrophy and met the criteria for obstructive sleep apnea syndrome. Obstructive sleep apnea syndrome was diagnosed according to the American academy of sleep medicine, International classification of sleep disorders manual (See Appendix 3) using minimal criteria. The patients were matched for age and sex.

8.9 Materials And Equipment

The materials and equipment that were used for analyzing the tonsillar tissue included:

Rapid Urease Test Kit(Cambridge Life Sciences Ltd. UK, Batch number 311161,exp. 07-2013) – For determining presence of H. pylori in core tonsil tissue
Gloves(Sterile) – For collection of specimen
Blade(Size 15) – For sectioning the tonsil tissue
Modified Giemsa – For staining for H. pylori
Container with formalin – For transport of specimen to the Laboratory
Other Laboratory Equipment

Procedure to Establish Presence of H. pylori in Tonsillar Tissue

Mechanism of action of Rapid Urease Test Kit

Procedure for Detection of H. pylori by Rapid Urease Kit

Rapid Urease Test – A qualitative test based on detection of urease, a hydrolase, produced by H. pylori. The test system – Test well filled with a urea containing gel where the tonsil tissue is inoculated and allowed to incubate.

If H. pylori present, in patients sample, urease will hydrolyse the urea in the gel that will lead to accumulation of ammonium ion. This will cause a rise in PH and this is detected in the PH indicator by a colour change in the test system from yellow to pink or red.

One tonsillar specimen was received from the patient after tonsillectomy and washed with normal saline and then a 3 mm core tonsillar tissue was cut from the specimen. The specimen was placed into a test well of the H. pylori kit. Initial colour was recorded before the use of the test well. Index colour change was recorded at 0 minutes and at 30 minutes by the principle investigator. Test wells were then transported to the laboratory and received by the Laboratory technologist. The laboratory technologist read the test wells at 3hours. 6 hours and at 24 hours. Storage was at room temperature of between 10 degrees and 28 degrees. Any colour change from the initial yellow colour to either pink or red was recorded as positive. Any test well which remained yellow after 24 hours was recorded as negative. No reading was taken after 24 hours

8.10 Histopathological investigation of H Pylori

The gross specimen of tonsillar tissue was placed in formaline and transported to the laboratory. The gross specimen was received in the laboratory. The specimen was described and dimensions taken, the cut into pieces 3 mm thick. The pieceswere put into cassettes and transferred into the automatic processor for dehydration, clearing and wax impregnation (processing) for 14 hours. Tissues were embedded in paraffin wax to produce blocks. Thin slices , 5 micrometer thick were cut from the paraffin wax to produce blocks, mounted on slides and stained with 1% giemsa.

Staining Procedure

The slide was placed in xylene and irrigated using descending grades of alcohol 100%, 90%, 70%. The slide was then washed in tap water and placed in a staining rack. The slide was flooded with 1% giemsa and left to stain for 10 minutes. The slide was rinsed with water, dehydrated, cleared and mounted. Finally the slide was taken to the pathologist for examination.

9.0 QUALITY CONTROL:

The proforma was pre tested prior to commencement of the study and any appropriate changes made.

Only the primary investigator screened the patients and obtained the history and clinical examination data to prevent inter personal bias . Each test kit had a batch number and a serial number. Any test kits whose expiry date lapsed were discarded. One laboratory technician was responsible for tissue handling and analysis once tonsillar tissue was removed.

All specimens were processed in the same laboratory and standard operating procedures for specimen handling, processing and analysis were followed to ensure standardization. Quality control was supervised by the consultant pathologist.

The Batch number was recorded in the laboratory request form and the proforma to ensure internal validity. Guidelines on tissue handling was followed using the University of Nairobi Laboratory Testing Protocol and guided by the UTMB point of care testing procedures policy.[57]

10.0 DATA MANAGEMENT:

Data Entry

The demographic data of the patient, relevant history and findings of the screening tests were recorded in a pro forma and then transferred to a customized MS access database. The data was then separated into pre formatted data sheets under history and clinical findings, age, sex, indication for tonsillectomy, presence of obstructive sleep apnea syndrome. Laboratory Findings included determining the presence of H. pylori in tonsillar specimens by Rapid urease test and determining the presence of H. pylori in tonsillar specimens by Histopathology.

DATA MANAGEMENT AND ANALYSIS

Data recorded in the pre formatted data sheets was analyzed using the statistical package for social sciences (SPSS) 17.0. Binary and categorical variables was presented as percentages while continuous variables are presented as means ±SD or median and inter-quartile range for normally and non-normally distributed data respectively. Baseline clinical characteristics were be compared between those with chronically inflamed tonsillar tissue and those with adenotonsillar hypertrophy

The Pearsons' Chi Square test was used to compare the following observed variables; sex, morning headache, chest retraction , and to compare the results of Rapid Urease Test. Fischers Exact Test was used to test non random associations between categorical variables because of the small numbers. The Unpaired Students T Test was used to determine probabilities of the independent variables being tested with respect to age.

Logistic Regression Table was used to determine the factors independently associated with cases among the factors with the significant associations in the first analysis. This was used to control for confounding variables.

***Data Presentation*:**

Data was presented in form of tables, line and bar graphs.

Risk for H. pylori colonization was estimated by calculating odds ratios.

11.0 ETHICAL CONSIDERATIONS:

The study was carried out only after approval by the Kenyatta Ethics and Research Committee.

Those included in the study were required 'to give an informed consent by their guardians and patients incurred no extra financial costs and their confidentiality was maintained at all times.

Participants reserved the right to withdraw from the study at any time without any penalty and there was no monetary gain by the primary investigator from the study and all costs were incurred by the primary investigator.

Any positive results for H. pylori on both histology and RUT was referred to the gastroenterology clinic.

Results of the research will be published for the benefit of other health practitioners.

12.0 RESULTS

A total of 78 tonsil samples from 78 patients were analysed in the study. All the tonsil tissues were obtained from the ENT satellite theatre.

The Youngest child was 2 years 6 months old while the oldest was 12 years old.

Mean age of the patients was 5.1 years with a standard deviation of 1.8

Median age was 5.0

Table 2: Age Distribution Table

Characteristic	Result
Age	
Mean (SD)	5.1 (1.8)
Median (IQR)	5.0 (4.0-6.0)
Min-Max	2.6-12.0

Figure 4 – Histogram representing age distribution

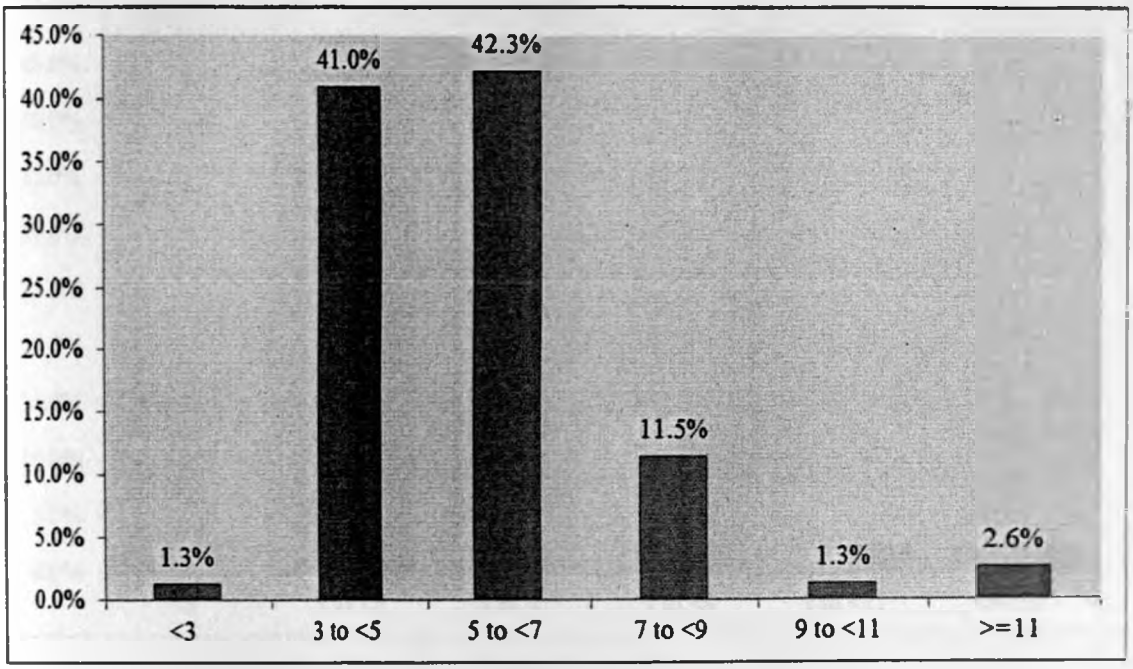
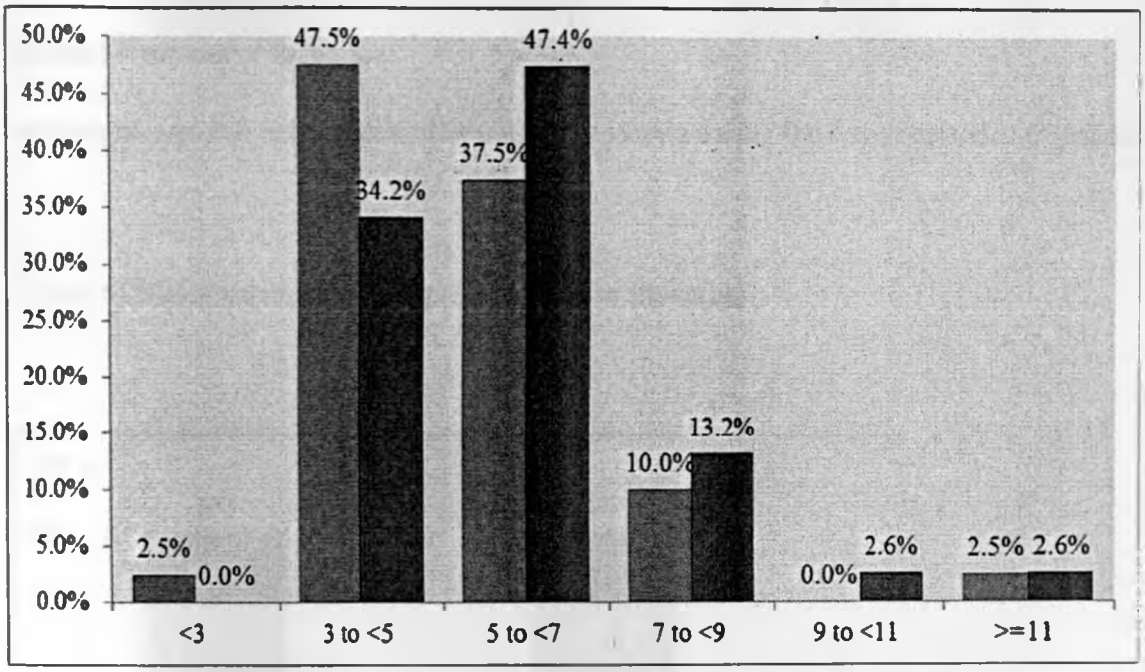


Figure 5 – Graph representing age distribution for ATH and CRT



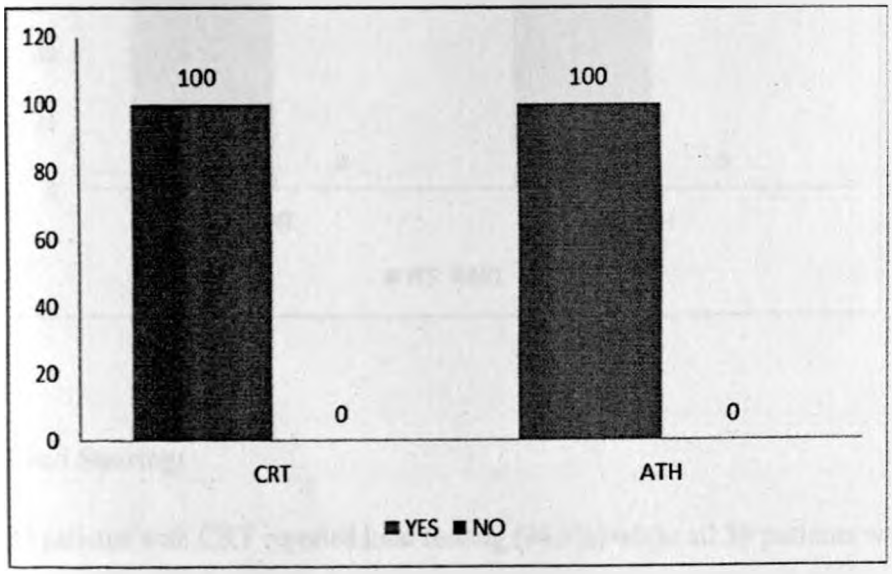
Clinical Characteristics:

The results of the patient characteristics are as shown in the bar graphs below.

Excess Sleepiness or Insomnia:

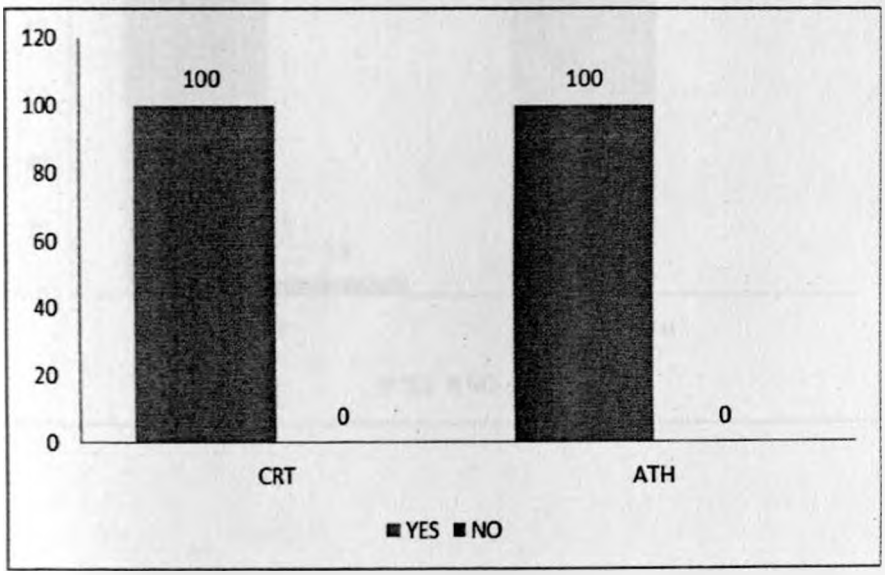
All patients reported either excess sleepiness or insomnia during the day or episodes of apnea .

Figure 6: Histogram representing excess sleep or insomnia.



Frequent Episodes of Obstructed Breathing during sleep:

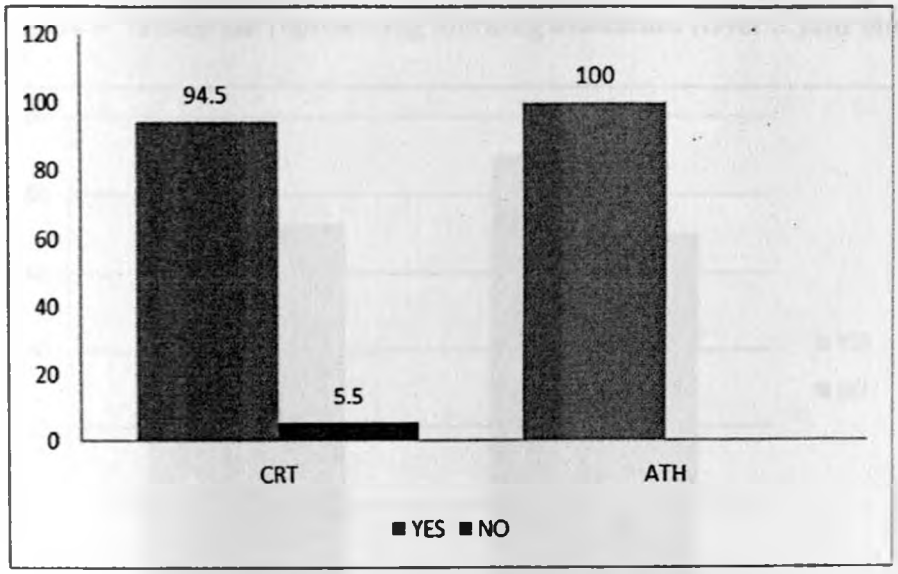
Figure 7: Histogram representing frequent episodes of obstructed breathing during sleep.



Loud Snoring:

37 patients with CRT reported loud snoring (94.9%) while all 39 patients with ATH reported loud snoring(100%). There was no statistically significant difference between the two groups. P=0.494.

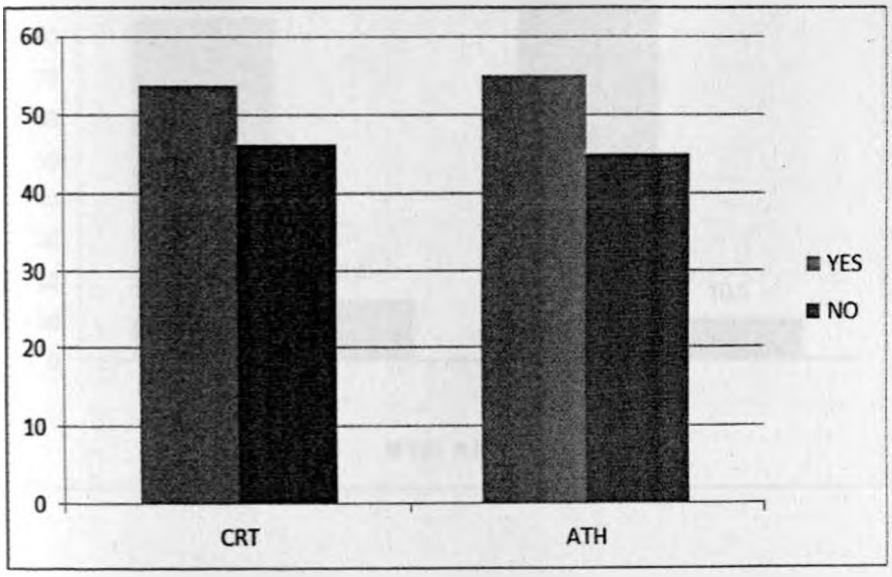
Figure 8: Histogram representing loud snoring



Morning Headaches:

Results were analysed for patients over 5 years old who were able to competently report morning headaches. A total of 20 patients with ATH were over 5 years old and a total of 26 patients with CRT were over 5 years old. Of the patients with CRT, 14 patients reported headaches (53.8%) while 12 patients (46.2%) did not. Of the patients with ATH, 11 patients (55%) reported morning headaches while 9 (45%) did not. (Figure 9). There was no statistically significant difference between the two groups. [OR1.8 (0.7-4.6) P= 0.234].

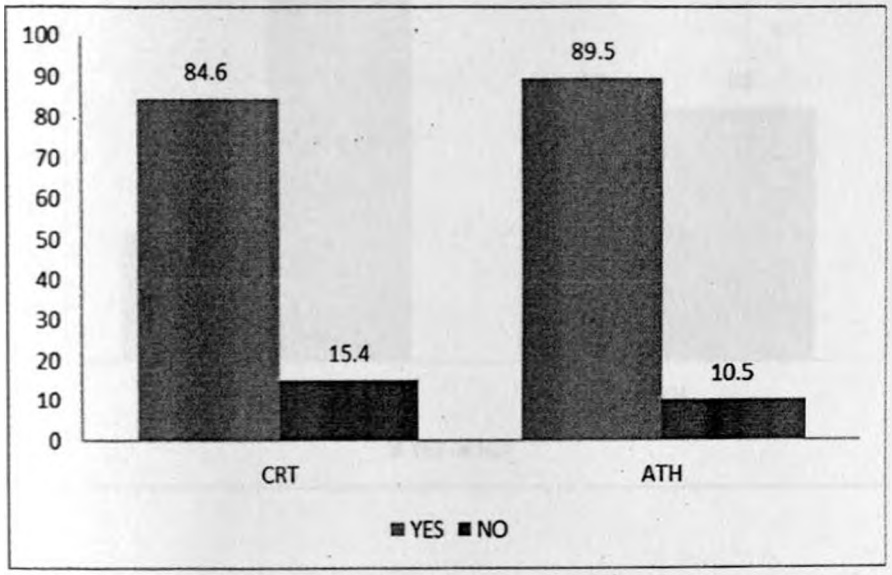
Figure 9: Histogram representing Morning headaches (Over 5 year olds)



Dry Mouth upon awakening:

33 patients with CRT reported dry mouth while awakening(84.6%) while 6 patients did not(15.4%). 34 patients with CRT (89.5%) reported a positive history of dry mouth while 4 (10.5%) did not.(Figure 10) There was no statistically significant difference between patients who reported a dry mouth upon awakening for the CRT group and for the ATH group. OR 0.6 (0.2-2.5) P= 0.737.

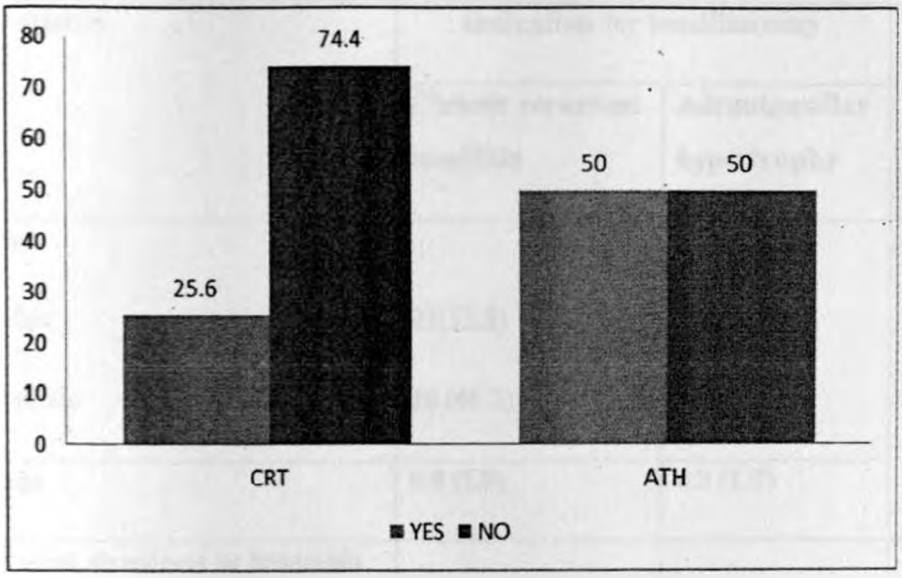
Figure 10: Histogram representing Dry mouth upon awakening.



Chest Retraction During Sleep:

The guardians of 10 patients with CRT reported chest retraction during sleep(25.6%) while 29 (74.4%) did not report retractions. The guardians of 19 patients with ATH reported chest retraction during sleep while 19 patients did not.(Figure 11) There was a statistically significant difference in patients with CRT and in patients with ATH whose quardians reported chest retraction. OR 0.3(0.1-0.9) P=0.027 . But when logistic regression was applied to adjust for independent variables, it was not found to be significant. OR 0.7(0.2-2.2) P=0.553. Thus it was not used to adjust for risk for H. pylori colonization.

Figure 11: Histogram representing Chest retraction during sleep.



Pearsons Chi Square Test was used to compare Age, Sex and Variables that were used to recruit patients according to the International Classification of Sleep Disorders,2001, Coding Manual (780.53-0)

A statistically significant difference was found in relation to age between the 2 groups of patients. CRT - 6.0(1.9 SD) and ATH- 4.3(1.2 SD). This reflects the fact that patients with Chronic Recurrent Tonsilitis tend to be older at presentation than patients with adenotonsillar hypertrophy.

The number of patients whose guardians reported chest retraction when sleeping was found to be higher in the ATH group than in the CRT group. OR 0.3(0.1-0.9) P=0.027

When excess sleepiness, loud snoring and dry mouth on awakening were analysed, no statistically significant difference was found between the two comparative groups.

Table 3: Baseline characteristics

Variable	Indication for tonsillectomy		OR (95% CI)	P value
	Chronic recurrent tonsillitis	**Adenotonsillar hypertrophy**		
Sex				
Male	21(53.8)	19 (48.7)	1.2 (0.5-3.0)	0.651
Female	18 (46.2)	20 (51.3)	1.0	
Age	**6.0 (1.9)**	**4.3 (1.2)**	-	**<0.001**
Excess sleepiness or insomnia				
Yes	39 (100)	39 (100)	2.1 (0.2-23.6)	1.000
No	0	0	1.0	
Obstructed breathing				
Yes	39(100)	39(100.0)	-	1.000
No	0	0		
Loud snoring				
Yes	37 (94.9)	39 (100.0)	-	0.494
No	2 (5.1)	0		
Morning headaches(Over 5 yrs)				
Yes	14 (53.8)	11 (55.0)	1.8 (0.7-4.6)	0.234
No	12 (46.2)	9 (45.0)	1.0	
Not Applicable(Under 5 years)	13	19		

Dry mouth upon awakening				
Yes	33 (84.6)	34 (89.5)	0.6 (0.2-2.5)	0.737
No	6 (15.4)	4 (10.5)	1.0	
Chest retraction during sleep				
Yes	**10 (25.6)**	**19 (50.0)**	**0.3 (0.1-0.9)**	**0.027**
No	**29 (74.4)**	**19 (50.0)**	**1.0**	

Presence of Helicobacter Pylori by Rapid Urease Test:

Presence of H. pylori was analysed among the CRT group and the ATH group. Among the CRT group prevalence was 38.5% (95% CI,23.4-55.4%). Among the ATH group, prevalence was 23.1% (95% CI, 11.1-39.3%).

Table 4: Presence of H. pylori

	Prevalence	**95% CI**
Chronic recurrent tonsillitis(CRT)	38.5%	23.4-55.4%
Adenotonsillar hypertrophy(ATH)	23.1%	11.1-39.3%

Figure 12: Histogram representing prevalence of H. pylori

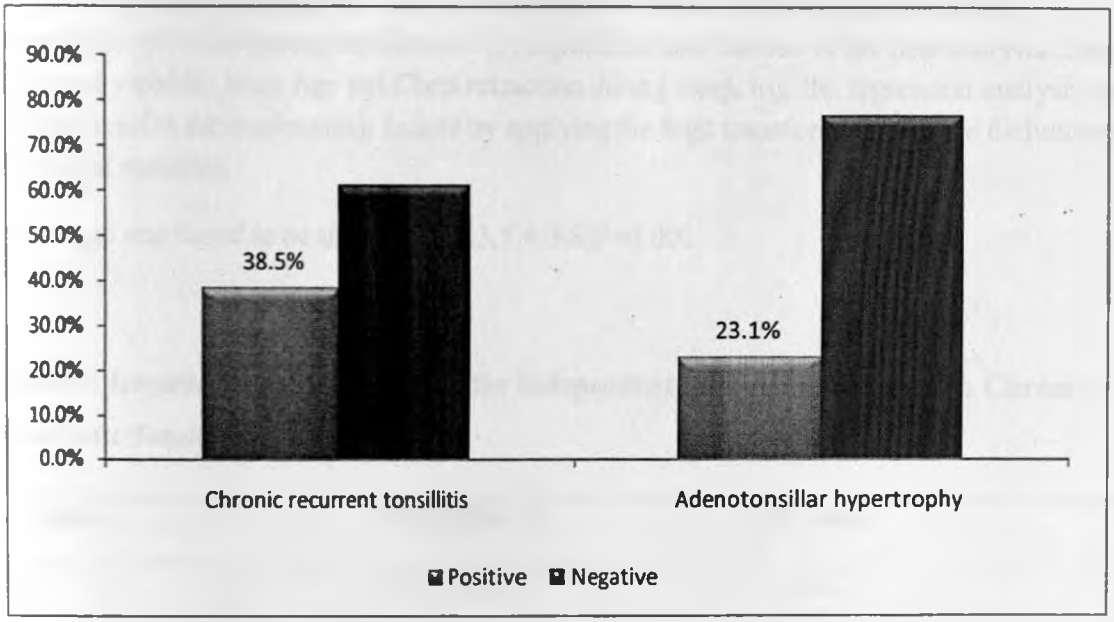


Table 5: Differences in colonization between chronic recurrent tonsillitis and adenotonsillar hypertrophy

Variable	Indication for tonsillectomy		OR (95% CI)	P value
	Chronic recurrent tonsillitis	Adenotonsillar hypertrophy		
Rapid Urease test				
Positive	15 (38.5)	9 (23.1)	2.1 (0.8-5.6)	0.141
Negative	24 (61.5)	30 (76.9)	1.0	
Histopathology				
Positive	4 (10.3)	0	-	0.115
Negative	35 (89.7)	39 (100.0)		

Logistic regression analysis.

Logistic regression analysis was used to determine the factors which are independently associated with cases among the factors with significant associations in the first analysis. Since these two variables were Age and Chest retraction during sleep, logistic regression analysis was applied to solve for confounding factors by applying the logit transformation to the dichotomous dependent variables.

Only Age was found to be significant(.2.3,1.4-3.8)P=0.001

Table 6: Logistic Regression Analysis for independent factors associated with Chronic Recurrent Tonsilitis

Variable	OR (95% CI)	P value
Age	2.3 (1.4-3.8)	0.001
Chest retraction during sleep		
Yes	0.7 (0.2-2.2)	0.553
No	1.0	

Table 7: Age-adjusted risk

Variable	OR (95% CI)	P value
Positive rapid Urease test	2.5 (1.6-3.9)	<0.001

Table 8: Presence of active and coccoid forms of H. pylori in chronic recurrent tonsillitis and adenotonsillar hypertrophy

Variable	Indication for tonsillectomy		OR (95% CI)	P value
	Chronic recurrent tonsillitis	Adenotonsillar hypertrophy		
Active chronic tonsillitis				
Yes	1 (2.6)	3 (7.7)	0.3 (0.0-3.2)	0.308
No	36 (97.4)	36 (92.3)	1.0	
Coccoid forms of Hyplori				
Yes	2 (5.1)	3 (7.7)	0.6 (0.1-4.1)	1.000
No	37 (94.9)	33 (92.3)	1.0	

Figure 13: Presence of active chronic tonsilitis

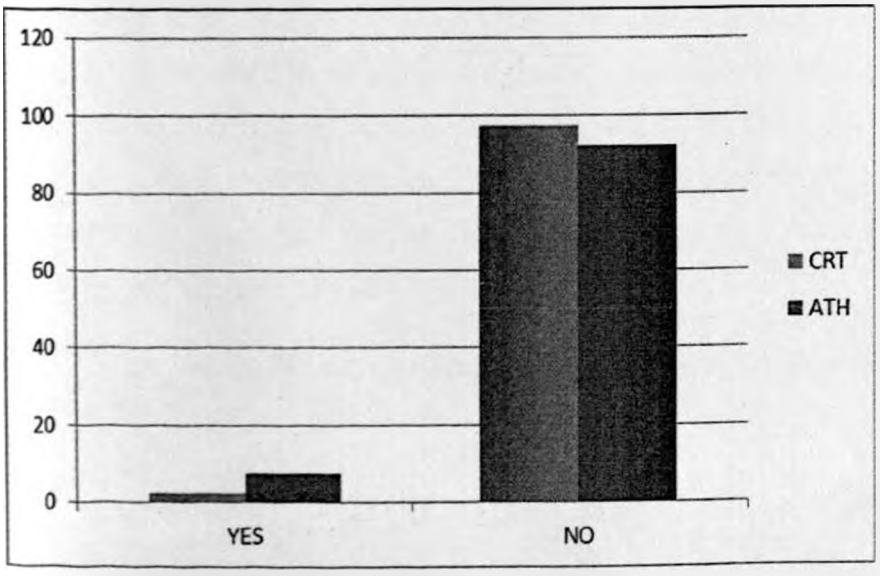
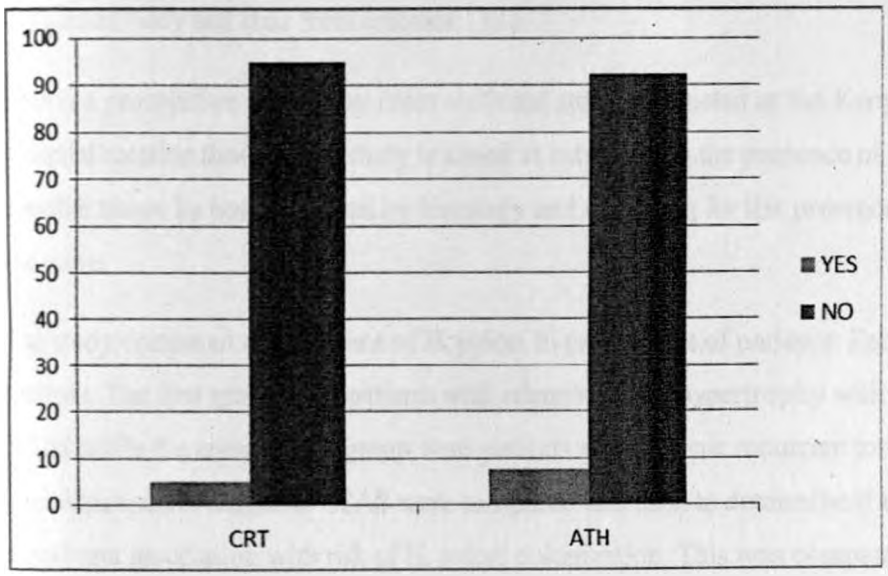


Figure 14: Presence of coccoid forms of H pylori.



13.0 DISCUSSION

H. pylori is a micro aerophilic gram negative bacteria. They are spiral shaped but some may be rod shaped. Described as one of the most successful human pathogens, H. pylori continues to attract a lot of attention due to its ability to change the microenvironment of its various niches in the human body and thus form colonies. [32].

This is a prospective descriptive cross sectional study conducted at the Kenyatta National Hospital satellite theatre. The study is aimed at establishing the presence of H. pylori in core tonsillar tissue by both RUT and by histology and exploring for the presence of coccoid forms of the same.

The study compared the presence of H. pylori in two groups of patients. Each group had 39 patients. The first group were patients with adenotonsillar hypertrophy with clinically diagnosed OSAS while the comparative group were patients with chronic recurrent tonsillitis with OSAS. Variables used to diagnose OSAS were compared and used to determine if there was any significant association with risk of H. pylori colonization. This was comparable to a study by Lin et al [46] which linked the severity of OSAS to colonization of tonsillar tissue by H. pylori. In this present study, OSAS was diagnosed using a clinical tool from the international classification of sleep disorders(Appendix 3). It is worth noting that children with chronic recurrent tonsillitis may have the hypertrophic type which also predisposes them to OSAS.

The age distribution was between the age of two and a half years and twelve years. There was a statistically significant difference in the two groups of patients in terms of age distribution. OR 6.0(1.9) for CRT with OSAS and 4.3(1.2) for ATH with OSAS; P=<0.001. Due to this significance , the age distribution table was analysed in the logistic regression table to eliminate any confounding variables. It was found to be statistically significant. OR 2.3 (1.4-3.8);P=0.001 at 95% Confidence Intervals.

In terms of the sex distribution, both comparative groups had equal number of males and females. The sex ratio was 1:1 and there was no statistically significant difference between the two groups.

Clinical characteristics pertinent to OSAS was analysed. Morning headaches was analysed using a cut off age of five years as children less than five years may not give an accurate description of

their headaches. There was no statistically significant difference between the two groups in terms of morning headaches. Chest retraction was also analysed and a statistically significant difference was found between the two comparative groups. 50.0% ATH with OSAS compared to 25.6% CRT with OSAS. OR 0.3(0.1-0.9)P=0.027. This is comparable to studies that show a higher incidence of chest retraction, use of accessory muscles and paradoxical rib cage motion in children with ATH [60]. In this present study, chest retraction was found to have significant association but when analysed in the logistic regression table for confounding variables, it was not found to be statistically significant OR 0.7 (0.2-2.2) P=0.553 and thus it was not used to analyse for risk of H. pylori colonization.

Histological characteristics of chronic tonsillitis was analysed. 10.3 % of patients in the chronic tonsillitis group were found to have neutrophils in their parenchyma and were thus classified as active tonsillitis. There was also an overall prevalence of H. pylori by histology in 10.3% of the specimens. This finding in the present study compares well with other studies[33,39,44]. Moghaddam et al[39] found a higher positivity by histopathology. This may be due to varying sensitivities of the methods used and also due to the fact that the physical location of the H. pylori may vary depending on the core sample used.

Overall prevalence of H. pylori was 30.5% by RUT. This is comparable to a study by Zahedi et al[33] which found a prevalence of 42% by RUT and 9.5% by histology. When the two comparative groups were separately analysed, the present study found a prevalence of 38.5% for the CRT with OSAS group and a prevalence of 23.1% for the ATH with OSAS group. Analysis of the two gropus after adjustment for age, due to the relatively older age at presentation for patients with CRT with OSAS, showed a higher risk for H. pylori colonization in the CRT with OSAS group compared with ATH with OSAS. OR 2.5(1.6-3.9) P=<0.001. This was the main outcome of the study. This shows that there is a two and a half times risk of colonization in patients with CRT with OSAS compared to ATH with OSAS. The scatter diagram in the logistic regression analysis was a straight line thus rejecting the null hypothesis and accepting the hypothesis that there is a difference in colonization between patients presenting with CRT with OSAS and patients presenting with ATH with OSAS. This is comparable to various studies. [26,40,43,44,46,58]

This finding may help to further expound on the existence of extra gastric reservoirs of H. pylori. It may also explain the higher colonization rate in patients with CRT. This is comparable to a study by Lin et al.[46]

COCCOID FORMS OF H PYLORI

Overall prevalence of coccoid forms of H. pylori was 12.8%. The coccoid forms were found in 2 (5.1%) of patients with CRT and compared to 3 (7.7%) of patients with ATH. There was no statistically significant difference in the two groups when compared. OR 95% CI 0.6 (0.1-4.1) P=1.000. The finding in the present study is comparable to a study by Kusano et al. [47] In that study it was concluded that tonsillar H. pylori may be involved in causation of IgA nephropathy. There is need to further analyse tonsillar tissue to specifically look for coccoid forms of H. pylori.

14.0 CONCLUSIONS:

Colonisation of human palatine tonsils by H. pylori is an exciting new frontier which could change the approach to management of various H. pylori associated disease conditions in future.

This study has demonstrated the presence of H. pylori colonization in human palatine tonsils both by Rapid Urease Tests and histopathological methods. A statistically significant difference in colonization by H. pylori in chronic recurrent tonsil tissue and adenotonsillar hypertrophy was demonstrated after adjustment for age. OR 2.5(95%CI) (1.6-3.9) P=0.001. This may go a long way in demonstrating that H. pylori has a role in sustaining a chronic recurrent state of palatine tonsils. In this study the the null hypothesis was rejected and thus the hypothesis stating that there is a difference in colonization between patients presenting with CRT with OSAS and patients presenting with ATH with OSAS was accepted.

This study concludes that the human palatine tonsil may be an extra gastric reservoir for H. pylori . The study also confirms the presence of coccoid forms of H. pylori within tonsillar tissue which may be responsible for various aetiologies of systemic disease.

15.0 RECOMMENDATIONS

1. Protocols for the management of chronic recurrent tonsillitis should be adjusted to include eradication of extra gastric reservoirs of H. pylori.
2. A protocol for eradication of H. pylori from extra gastric reservoirs which may include adenotonsillectomy may be considered as part of management of H. pylori related gastric disease and sequealae.
3. Future studies to elucidate role of H. pylori in worsening of symptoms of obstructive sleep apnea should be carried out.
4. Further studies are required on the role of coccoid forms of H. pylori in the subclinical activity of human palatine tonsils.
5. More sensitive methods of detecting H. pylori such as real time PCR should be adopted to quantify H. pylori in palatine tonsils by detecting its DNA. Immunofluorescence may also be used to further delineate the structural morphology of H. pylori.

6. Further studies may be needed to elucidate the role of H. pylori in the aetiology of cancer within the oro pharyngeal region.
7. Further local case controlled epidemiological studies may be needed to compare colonization of tonsillar tissue between populations that are symptomatic for recurrent tonsillitis or adenotonsillar hypertrophy and normal populations.

16.0 LIMITATIONS OF THE STUDY

The most sensitive test for evaluation of H. pylori is the PCR. Because of expense, it could not be used in this study.

The study only represents the cohort of patients attending KNH and doesn't represent the whole population of Kenya. Therefore it is a hospital based study and not a population based study which may be more representative of the general population.

The diagnosis of obstructive sleep apnea syndrome in this study used a clinical tool which is subjective and may be subject to bias.

17.0 APPENDIX

17.1 APPENDIX 1 : *GENERAL PATIENT INFORMATION AND CONSENT FORM*

My name is Dr. Peter Ochungo. I would like to seek your consent to participate in a study aimed at understanding the behavior of a bacterium called H. pylori in tonsillar tissue. This will include patterns of infection of this bacterium. The results will go a long way in understanding the way H. pylori colonises tonsillar tissue.

How to participate

1. We will ask you questions seeking to know how the condition started, investigations done and any treatment given.
2. We shall obtain a specimen from the removed tonsil tissue and analyze it.
3. Similar findings from all participants will be used to analyze the behavior of H. pylori in human palatine tissue.

How does your participation affect you?

It does not adversely affect you in any way because:

1. You will receive the same treatment without participating in the study.
2. No treatment will be given to you in addition to what you require and you would ordinarily get were you not participating in the study.
3. All information given by you will be accorded confidential treatment.
4. You are free to withdraw from the study whenever you wish

Are there any hidden dangers?

1. There are no hidden dangers
2. Refusing to consent will not affect the management you receive.

How does your participation help us?

1. The findings in the study will help us to understand the behaviour of H. pylori in tonsillar tissue, .
2. We shall share the findings of the study with other professional colleagues elsewhere. Thus the findings can be published in scientific journals or be presented at scientific conferences without divulging any specific patient information.
3. You are free to discuss this with family members and we shall be ready to answer any questions raised. If you understand everything said and have accepted it then you can sign the consent form provided.

CONSENT FORM (Patients less than 18 years – English)

Study number.....

I Mr/Mrs./Ms.....the parent/guardian of master/miss..... agree to enroll him/her into the study as explained to me by Dr. .....My signature is confirmation that I have understood the nature of the study and that whatever information that I give will remain confidential.

I also confirm that no monetary or material gains have been promised or given to me for participating in the study.

Signed.....(patient/guardian) Relationship.....

Date:.....

Signature of principal investigator..... Date:.....

MAELEZO YA UTAFITI KWA MGONJWA NA KIBALI CHA UTAFITI

Mimi naitwa Dr. Peter Ochungo. Ningependa kukuomba ruhusa (kwa hiari yako) kukuhusisha kwenye utafiti huu. Utafiti huu ni juu maradhi inayoweza kutokea kutokana na bacteria aina ya H. pylori. Itatuwezesha kufanya utafiti maalum jinsi yahiim madhara na itasaidia kuelewa namna hii bacteria inavyoweza kuenea kwa mwili wa binadamu.

Jinsi ya Kushiriki

1. Tutakuuliza maswali kutaka kujua ni lini ugonjwa ulianza na ni matibabu gani uliyopewa
2. Tutachukua kipande cha nyama aina ya tonsil na kupeleka kwa chumba cha utafiti.
3. Habari hii itakusanywa kutoka kwa wagonjwa wengine walio na shida kama yako.

Kushiriki kunakudhuru vipi?

Hakukudhuru kwa njia yoyote ile kwasababu:

1. Utapata matibabu sambamba na wale wasioshiriki.
2. Hakuna chohcote utakachopewa kukusawishi kushiriki kwenye utafiti huu.
3. Habari yoyote utakayotoa itawekwa siri.

Kuna madhara yoyote iliyofichwa yanayoweza kutokana na utafiti huu?

1. Hakuna madhara yoyote.
2. Hata kukataa kushiriki hakutabadili matibabu utakayopewa.

Kushiriki kwako kutatufaidi vipi?

1. Kushiriki kwako ni muhimu kwa sababu matokea ya utafiti huu utasaidia kujua jinsi badhara ya H. pylori inavyo jimudu mwilini mwa binadamu.
2. Matokeo haya yatatumika hata na madaktari wenzetu walioko kwengine.

3. Uko huru kujadiliana na watu wa familia yako kabla ya kukubali kushiriki na maswali yoyote mutakayouliza yatajibiwa.

Iwapo umeridhika na maelezo haya yote, na umekubali kushiriki, basi utatia sahihi kwenye kibali cha utafiti kudhibitisha kwamba umekubali.

KIBALI CHA UTAFITI

Nambari ya utafiti.....

Watoto chini ya miaka 18/wasiojifahamu:

Mimi, Bi/Bwana ..... mzazi wa.....Nimekubali kushiriki katika utafiti huu baada ya kuelezwa na daktari ..... Sahihi yangu ni thibitisho ya kwamba nimeelewa umuhimu wa utafiti huu na kwamba habari yoyote nitakayotoa itawekwa siri.

Pia nathibitisha ya kwamba sijapewa au kuahadiwa pesa au chochote kile, kukubali kushiriki kwenye utafiti huu.

Sahihi ..... Uhusiano .....tarehe .....

Sahihi ya mtafiti ..... Tarehe .....

17.2 APPENDIX 2: QUESTIONNAIRE

SERIAL NO:..... Date .....

File I.P. No.....

Laboratory No. .....

SECTION A: PERSONAL DETAILS

1. Initials:.....
2. Sex: Male ☐ Female ☐

   Age ☐

SECTION B: HISTORY

3. Indication for Tonsillectomy: (Tick one)

   Chronic Recurrent Tonsillitis: ☐

   Adenotonsillar Hypertrophy : ☐

4. Obstructive Sleep Apnea: (Tick One)

   Excess sleepiness or insomnia: Yes ☐ No ☐

   Frequent episodes of obstructed breathing: Yes ☐ No ☐

   Loud snoring: Yes ☐ No ☐

   Morning Headaches(>5yrs):Yes ☐ No ☐ If <5yrs Tick ☐

   Dry Mouth upon Awakening: Yes ☐ No ☐

   Chest Retraction during sleep in young children: Yes ☐ No ☐

   Obstructive Sleep Apnea: Yes ☐ No ☐

SECTION C: LABORATORY RESULTS:

Rapid Urease Test : Positive ☐ Negative ☐

Histopathology: Positive ☐ Negative ☐ Coccoid Yes ☐ No ☐

17.3: APPENDIX 3:

THE INTERNATIONAL CLASSIFICATION OF SLEEP DISORDERS, REVISED, 2001

Diagnostic and Coding Manual

Diagnostic Criteria: Obstructive Sleep Apnea Syndrome (780.53-0)

A. The patient has a complaint of excessive sleepiness or insomnia. Occasionally, the patient may be unaware of clinical features that are observed by others.

B. Frequent episodes of obstructed breathing occur during sleep.

C. Associated features include:

1. Loud snoring
2. Morning headaches
3. A dry mouth upon awakening
4. Chest retraction during sleep in young children

D. Polysomnographic monitoring demonstrates:

1. More than five obstructive apneas, greater than 10 seconds in duration, per hour of sleep and one or more of the following:

a. Frequent arousals from sleep associated with the apneas

b. Bradytachycardia

c. Arterial oxygen desaturation in association with the apneic episodes

2. MSLT may or may not demonstrate a mean sleep latency of less than 10 minutes.

E. The symptoms can be associated with other medical disorders (e.g., tonsillar enlargement).

F. Other sleep disorders can be present (e.g., periodic limb movement disorder

Note: State and code obstructive sleep apnea syndrome on axis A and causative disorders on axis C (e.g., tonsillar enlargement).

Minimal Criteria: A plus B plus C.

Produced by the American Academy of Sleep Medicine in association with the European sleep research society and the Japanese sleep research society.

17.4 APPENDIX 4:

Paradise Criteria for Tonsillectomy

Criterion	Definition
Minimum frequency of sore throat episodes	7 or more episodes in the preceding year, OR 5 or more episodes in each of the preceding 2 y, OR 3 or more episodes in each of the preceding 3 y
Clinical features (sore throat plus the presence of one or more qualifies as a counting episode)	Temperature > 38.3°C, OR Cervical lymphadenopathy (tender lymph nodes or >2 cm), OR Tonsillar exudate, OR Positive culture for group A β-hemolytic streptococcus
Treatment	Antibiotics had been administered in conventional dosage for proved or suspected streptococcal episodes
Documentation	Each episode and its qualifying features had been substantiated by contemporaneous notation in a clinical record, OR *If not fully documented*, subsequent observance by the clinician of 2 episodes of throat infection with patterns of frequency and clinical features consistent with the initial history

This last statement allows children who meet all other criteria for tonsillectomy except documentation to nonetheless qualify for surgery if the same pattern of reported illness is observed and documented by the clinician in 2 subsequent episodes.

17.5 APPENDIX 5: LABORATORY PROCEDURE FOR RAPID UREASE TEST – UTMB POINT OF CARE PROTOCOL

Patient Testing

Immediately before endoscopy, the CLOtest should be placed in a warming plate at 30-40 °C. **This pre-warming stage will help speed the chemical reaction.**

Step	Action
1.	Biopsy an area of normal looking tissue rather than an area affected by erosions or ulceration.
2.	Peel back the label of the CLOtest and with a 19G needle, remove the specimen from the biopsy forceps and **insert it into the gel so it will have maximum contact with the urea and the bacteriostatic agent in the gel.**
3.	Re-seal the CLOtest, and record the time and pertaining patient information on label. Maintain CLOtest card at 30-40 °C.
4.	**Important !!** If a slight pink tinge is noted in the gel, particularly if blood or alkaline bile is present, **observe tinge for color change at 2 minutes**, then re-examine at intervals after that time. Only if the pink area is **deepening in color and expanding in size** may test be called positive.
5.	If a test turns positives at room temperature for the remainder 24 hours.

Interpretation of Results

Step	Action
1.	Examine the CLOtest at intervals over the next 24 hours. A CLOtest that turns positive prior to the 24 hour period may be reported. Negative CLOtests, however, must not be reported until the end of the 24 hour period. In the event a 24 hour reading is not possible, CLOtest may be read at 72 hours, but not beyond this time limit.
2.	**A color change from yellow to magenta is considered a Positive test; other shades of red such as pink or orange, are also considered positive. Yellow is considered a negative screen.**
3.	TO COMPLY WITH REGULATIONS, ALL NEGATIVE PATIENT CLOtest CARDS **MUST BE INNOCULATED WITH A UREASE TABLET. RESULTS MUST BE DOCUMENTED IN THE APROPRIATE QUALITY CONTROL FORM.**

16.0 REFERENCES

1. Nilsson HO, Taneera J, Castedal M, Glatz E, Olsson R, Wadström T. J Clin Microbiol. 2000 Mar;38(3):1072-6.
2. Nabwera HM, Logan RP. Epidemiology of Helicobacter pylori: Transmission, translocation and extragastric reservoirs. J Physiol Pharmacol 1999;50(5):711-22.
3. Neas JF. Human Development, Embryology Atlas. Chap 23 . Pearson Education Inc; 2003.
4. *Nave H, Gebert A, Pabst R.* Morphology and immunology of the human palatine tonsil .Anat Embryol (Berl). 2001 Nov; 204(5):367-73.
5. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 40th edition. Churchill-Livingstone, Elsevier;2008.
6. Desiderio Passàli, Valerio Damiani Giulio Cesare Passàli,et al Structural and Immunological Characteristics of Chronically Inflamed Adenotonsillar Tissue in Childhood , Clinical and Diagnostic Laboratory Immunology, November 2004, p. 1154-1157
7. M E Perry, The specialised structure of crypt epithelium in the human palatine tonsil and its functional significance. J Anat. 1994 August; 185(Pt 1): 111–127.
8. Sidebotham RL, Worku ML, Karim QN et al. How Helicobacter pylori urease may affect external pH and influence growth and motility in the mucus environment: evidence from in-vitro studies. Eur J Gastroenterol Hepatol. 2003 Apr;15(4):395-401.
9. OToole, P. W., Lane MC, Porwollik. S. Helicobacter pylori motility. Microbes Infect. 2000. 2:1207-1214
10. Kusters J, Arnoud H, Kuipers E et al Pathogenesis of *Helicobacter pylori* Infection . Clinical Microbiology Reviews, July 2006, 449-490, Vol. 19, No. 3
11. International Agency for Research on Cancer (IARC). Agents Classified by the *IARC Monographs*, Volumes 1 – 100. 2011.
12. Fox, J. G. The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. Gut, 2002; 50:273-283
13. Nabwera HM, Logan RP. Epidemiology of Helicobacter pylori: Transmission, translocation and extragastric reservoirs. J Physiol Pharmacol 1999;50(5):711-22.

14. Covacci A., Rappuoli R. *Helicobacter pylori*: molecular evolution of a bacterial quasi-species. Curr. Opin. Microbiol. 1998;1:96–108

15. Ramarao N, Meyer T. *Helicobacter pylori* Resists Phagocytosis by Macrophages: Quantitative Assessment by Confocal Microscopy and Fluorescence-Activated Cell Sorting Infection and Immunity, April 2001, 2604-2611.

16. Crabtree, J. E. Immune and inflammatory responses to Helicobacter pylori infection. *Scand. J. Gastroenterol. Suppl.1996;* 215, 3-10.

17. Lacy B, Rosemore J. Helicobacter pylori: Ulcers and More: The Beginning of an Era J. Nutr. 2001; 131: 2789S–2793S.

18. Blanchard, T, Drakes, M, Czinn S. Helicobacter infection: pathogenesis. *Curr. Opin. Gastroenterol.2004;* 20, 10-15.

19. Hafsi, N., Voland, P., Schwendy, S et al. Human dendritic cells respond to Helicobacter pylori, promoting NK cell and Th1-effector responses in vitro. *J. Immunol.* 2004, 173, 1249-1257.

20. Portal-Celhay, C., Perez-Perez, G. I. Immune responses to Helicobacter pylori colonization: mechanisms and clinical outcomes. *Clin. Sci. (Lond.)2006.* 110, 305-314.

21. Gewirtz AT, Yu Y, Krishna US. *Helicobacter pylori* Flagellin Evades Toll-Like Receptor 5-Mediated Innate Immunity. Journal of Infectious Diseases.2004 Vol 189;10. Pp. 1914-1920

22. Moran A P. Helicobacter pylori lipopolysaccharide-mediated gastric and extragastric pathology. J Physiol Pharmacol. 1999 Dec;50(5):787-805.

23. Passàli D, Damiani V, Passàli G ,et al. Structural and immunological characteristics of chronically inflamed adenotonsillar tissue in childhood. *Clin Diagn Lab Immunol.* 2004;11(6):1154-1157

24. Lukes P, Astl J, Pavik E. Helicobacter Pylori in tonsillar and adenoid tissue and its possible role in oropharyngeal carcinogenesis. Folia Biol(praha).2008;54(2):33-9

25. Ying Liu, Hui Yue, Aimin Li An Epidemiologic Study on the Correlation Between Oral *Helicobacter pylori* and Gastric *H. pylori* Current Microbiology Volume 58, Number 5, 449-453,

26. Khademi B, Imanieh M H, Gandomi B et al, Investigation of *H. pylori* Colonization in Adenotonsillectomy Specimens by Means of Rapid Urease (CLO) Test *Iran J Med* 138 *Sci* September 2005; Vol 30 No 3

27. Steer HW. Ultrastructure of Campylobacter pylori in vivo. In: Rathbone BJ, Heatley RV, eds. Campylobacter Pylori and Gastroduodenal Disease. 2nd ed. Oxford, U.K.: Blackwell Scientific Publishers; 1992:233-45.

28. Elin Hestvik, Thorkild Tylleskar, Kaddu-Mulindwa et al *Helicobacter pylori* in apparently healthy children aged 0-12 years in urban Kampala, Uganda: a community-based cross sectional survey *BMC Gastroenterology* 2010, 10:62

29. Langat AC, Ogutu E, Kamenwa R, et al. Prevalence of Helicobacter pylori in children less than three years of age in health facilities in Nairobi Province. East Afr Med J 2006; 83: 471-477.

30. Baldwin, D.N., B. Shepherd, P. Kraemer, *et al.*, Identification of *Helicobacter pylori* genes that contribute to stomach colonization. Infect. Immunol., 75: 1005-1016,2007

31. Magalhaes Queiroz, D.M. , F. Luzza,. Epidemiology of *Helicobacter pylori* infection. Helicobacter, 2006 11: 1-5.

32. Paradise JL, Bluestone C D, Colborn B K, Tonsillectomy and Adenotonsillectomy for Recurrent Throat Infection in Moderately Affected Children *Pediatrics Vol. 110 No. 1 July 1, 2002 pp. 7 -15*

33. Zahedi M, Darvish Moghadam S, Ahmadi Mosavi M, et al *Helicobacter pylori* Colonization in biopsies of the Adenotonsillectomy Specimens American Journal of Applied Sciences 6 (12): 2050-2053, 2009

34. Uygur-Bayramic¸li O, Yavuzer D, Dabak R, Aydin S, Kurt N. *Helicobacter pylori* colonization on tonsil tissue. *Am J Gastroenterol.* 2002;97(9):2470-2471.

35. Vilarinho S, Guimarães NM, Ferreira RM,. Helicobacter pylori colonization of the adenotonsillar tissue: fact or fiction? Int J Pediatr Otorhinolaryngol. 2010 Jul;74(7):807-11. Epub 2010.

36. Skinner LJ, Winter DC, Curran AJ, et al. Helicobacter pylori and tonsillectomy. Clin Otolaryngol Allied Sci. 2001 Dec;26(6):505-9.

37. Vayisoglu Y, Ozcan C, Polat A, et al Does Helicobacter pylori play a role in the development of chronic adenotonsillitis? Int J Pediatr Otorhinolaryngol. 2008 Oct;72(10):1497-501.

38. Eyigor M, Eyigor H, Gultekin B et al. Detection of Helicobacter pylori in adenotonsiller tissue specimens by rapid urease test and polymerase chain reaction. Eur Arch Otorhinolaryngol. 2009 Oct;266(10):1611-3. Epub 2009 Jan 8.

39. Moghaddam Y, Rafeey M, Radfar R. et al. Comparative assessment of Helicobacter pylori colonization in children tonsillar tissues. Int J Pediatr Otorhinolaryngol. 2009 Sep;73(9):1199-201. Epub 2009 Jun 12.

40. Abdel-Monem MH, Magdy EA, Nour YA, et al. Detection of Helicobacter pylori in adenotonsillar tissue of children with chronic adenotonsillitis using rapid urease test, PCR and blood serology: a prospective study. Int J Pediatr Otorhinolaryngol. 2011 Apr;75(4):568-72. Epub 2011 Feb 15.

41. Dağtekin-Ergür EN, Eren F, Ustün MB et al; [Investigation of Helicobacter pylori colonization in pharyngeal and palatine tonsils with rapid urease test and immunohistochemical analysis]. Kulak Burun Bogaz Ihtis Derg. 2008 Mar-Apr;18(2):85-9.

42.. Bulut Y, Agacayak A, Karlidag T, et al. Association of cagA+ Helicobacter pylori with adenotonsillar hypertrophy. Tohoku J Exp Med. 2006

43. Nam JK, Park KC, Kwon JK, Is Helicobacter Pylori the Pathogen of Chronic Tonsillitis? *Korean J Otorhinolaryngol-Head Neck Surg*. 2007

44. Cho KK, Lee SS, Shim KN. Effect of Palatine Tonsil and Adenoid Tissue on Gastric Infection of Helicobacter Pylori Korean J Otorhinolaryngol-Head Neck Surg, 50(10):907- 912 Oct 2007

45. Di Bonaventura G. Neff M, Neri G, et al. Do tonsils represent an extragastric reservoir for Helicobacter pylori infection? J Infect 2001;42(3):221-2.

46. Hsin-Ching Lin, Pei-Yin Wu,; Michael Friedman et al Difference of *Helicobacter pylori* Colonization in Recurrent Inflammatory and Simple Hyperplastic Tonsil Tissues *Arch Otolaryngol Head Neck Surg.* 2010;136(5):468-470.

47. Kusano K, Inokuchi A, Fujimoto K, et al, Coccoid Helicobacter pylori exists in the palatine tonsils of patients with IgA nephropathy. *IJMS* Vol 30, No 3, September 2005

48. Wibawa T ,Surono A, Widodo I. Isolation of viable *Helicobacter pylori* in the tonsillar tissues of chronic tonsillitis patients. *J Infect Dev Ctries* 2011; 5(7):561-564.

49. Dye KD, Marshall BJ, Frierson HF, et al. Is CLO test alone adequate to diagnose Campylobacter pylori? [abstract] Am J Gastroenterol 1988;83:1032.

50. Schnell GA, Schubert TT, Barnes WG, et al. Comparison of urease, H&E and culture tests for Campylobacter pylori, Gastroenterology 1988;94:A410.

51. Xian Wei, Jun Xiao, Tin Qin. H. pylori seroprevalence in patients in obstructive sleep apnea syndrome among a chinese population Saudi Med J 2009 ; Vol 30(9)

52. Malaty HM, El-Kasabany A, Graham DY, et al. Age at acquisition of Helicobacter pylori infection: a follow-up study from infancy to adulthood. Lancet 2002; 359: 931-935.

53. Siai K, Ghozzi M, Ezzine H, et al Prevalence and risk factors of *Helicobacter pylori* infection in Tunisian children Gastroentérologie Clinique et Biologique , Volume 32, numéro 11 pages 881-886 ; 2008

54. Rowland M, Daly L, Vaughan M, et al. Age-specific incidence of Helicobacter pylori. Gastroenterology 2006; 130: 65-72.

55. Fallon C A.,Loo V.G.,Lough J et al.1997. Hematoxylin and Eosin staining of gastric tissue for the detection of Helicobacter Pylori. Helicobacter 2:32-35

56. Fich A.,Talley N.J.,Shorter R.G. et al 1989. Histological evaluation of Campylobacter pylori from tissue specimens stored in formaldehyde can be misleading. J Clin. Gastroenterol. 11:585

57. UTMB Point of care testing procedures policy - Policy 1.3.25 , University of Texas Medical Branch policy no. 1.3.25; 1999

58. Fahrivar NT ,Pahlevar AA, Johari P. Assessment of H. pylori by real time PCR in Chronic tonsillitis patients JGID Vol4, 2012;1;38-42

59. Minocha A, Raczkowski CA,Richard RJ. Is a history of tonsillectomy associated with a decreased risk of H. pylori infection J. Clinical Gastroenterology 1997;25;580-2

60. Tatlipinar A. The effects of obstructive sleep apnea syndrome; Turkish journal of paediatrics ; 2001. 15(1-2)