Original Research Article

Association *of H. Pylori* with serum iron levels and some risk factors in children aged 1-12 years attending the Buea regional hospital.

ABSTRACT

Background/Objective: *Helicobacter pylori* (*HP*) is a very common human infection worldwide, colonizing the stomach of 50% of the world's population. *H. pylori* play a major role in the development of iron deficiency, chronic gastritis, peptic ulcer and gastric cancer. *H. pylori* infection is more prevalent in developing countries and its acquisition is predominant in childhood. The aim was to determine the prevalence of HP and its association with serum iron levels in children aged 1-12 years attending the Buea Regional Hospital.

Methods: This was a hospital based cross sectional study involving 189 children. About 2ml venous blood was collected and analyzed using immunoassay diaspot one step *H. pylori* Test Device and spectrophotometers to determine *H. pylori* immunoglobulin G and Serum iron level respectively.

Results: A prevalence of 31.7% (60/180) and 47.1% (73/155) for *H. pylori* and low serum iron level was observed respectively. *H. pylori* was significantly associated with normal serum iron level (P=0.004). *H. pylori* was more prevalent among males 38.04% (35/92) to 25.77% (25/97) for females. There was no significant association of *HP* with personal hygiene and living Conditions.

Conclusion: This study shows that some iron deficiency anemia is not caused by *HP* and that other causes such as parasitic infections should be checked. Also, that the mode of transmission of *HP* is still not clear defined, since as it wasn't associated with personal hygiene and living condition. Health education to limit infection should target this gender.

Keywords: *H. pylori*; iron deficiency; parasitic infection; personal hygiene.

Introduction

Helicobacter pylori is a spiral-shaped, flagellated, micro-aerophilic Gram-negative bacillus that colonizes the gastric mucosa of humans. *Helicobacter pylori* is the most common human infection worldwide, colonizing the stomach of 50% of the world's population [1]. *H. pylori* was discovered in 1982 by Barry Mashall and Robin Warren after several attempts made by researchers to describe this organism in the stomach of patients with gastritis and gastric cancer [2]. It is acquired predominantly in early childhood, especially in preschool age. Since *H. pylori* has no environmental reservoir, transmission is likely by fecal-oral route [3].

The prevalence of *H. pylori* infection is lower in developed countries than in developing countries [3]. The increased infection rate in developing countries is likely due to poor sanitation and/or living conditions like living in a crowded area, having infected household members, eating with dirty hands, drinking contaminated water and eating contaminated food [4]. The incidence of *H. pylori* infection in infancy is also high and has also been associated with malnutrition and growth faltering in developing countries [2-4]. The prevalence of *H. pylori* among children below 10years living in developing countries (13% to 60%) is higher compared to those in developed countries (0% to 5%) [5]. In 2004, 52.2% asymptomatic children age 0-10 years in the Buea and Limbe health district were reported with *H. pylori* [6].

Iron deficiency anemia is diminished red blood cell production due to low iron stores in the body. Iron deficiency (ID) and iron deficiency anemia (IDA) are major public health problems, especially in children and women of childbearing age in developing countries [7]. It has been estimated that approximately 1.6 billion people are anemic globally [8]. ID is considered to account for 50% of identified anemia, and 800,000 deaths worldwide can be attributed to IDA. Iron deficiency is estimated to be the most common nutritional disorder worldwide and the most common cause of anemia [9]. Deficiency of this trace element has adverse implications on health at all stages of life [7]. Iron deficiency anemia can result from inadequate iron intake, decreased iron absorption, increased iron demand, and increased iron loss. Identifying the underlying etiology and administering the appropriate therapy are keys to the evaluation and management of this condition. Risk factors include low birth weight, history of prematurity, exposure to lead, exclusive breastfeeding beyond four months of life, and weaning to whole milk and complementary foods without iron-fortified foods [10]. Iron deficiency anemia is diagnosed by laboratory confirmation of anemia, as well as evidence of low iron stores.

Several studies have demonstrated a relatively strong association of H. pylori infection with iron deficiency anemia (IDA) [11]. It has been reported that *H. pylori* inhibits secretion of ascorbic acid into the gastric juice which may lead to decreased absorption of iron, since the acid aids in the solubilization and reduction of ferric iron to the more absorbable ferrous iron form [12]. A few case reports indicate that successful eradication of *H. pylori* results in improving iron status and anemia [1]. Epidemiologic studies have shown that persons seropositive for *H. pylori* infection have a significantly lower serum ferritin level [13].

Cameroon as a developing country, risk condition/factors (poor sanitation/ living conditions) associated with *H. pylori* infection are common. Moreover, children are at risk since they

scarcely observe hygiene and as such, are prone to infection. Studies on *H. pylori* done by others in this area have not looked at *H. pylori* and its association with serum iron and its risk factors. This study therefore was aimed at determining the association of H. Pylori with serum iron levels and some risk factors in children aged 1-12 years attending the Buea regional hospital.

Methods

Study Area

This study was conducted in Buea, South West region of Cameroon. Buea is located at the foot of mount Cameroon (an active volcano 4010mm) at an elevation of 1000m above the sea level with a surface area of 870Km^2. The urban areas include: miles 14, 15, 16 and 17, Bomaka Bunduma, Molyko Great Soppo, Muea, Bokwaongo, Clerk's and Federal quarters, Buea Town, Likoko-Membea and Govornment Residential Area. Daily temperature ranges from 20-28^{o}C annually. The municipality is characterised by a hilly topography, a dense network of spring and streams, high humidity and fertile volcanic soil [14]. This region experiences two seasons: the dry (November - March) and the rainy (March - October) seasons with and annual rainfall of 3000-5000mm, although the pattern is now changing. Buea has several clinics and health facilities which include: Buea town health centre, Regional hospital annex Buea, Seventh Day Adventist hospital, Mount Marry hospital and the Muea district hospital. The economy of Buea has been described as moderate with agricultural, administrative, tourism and the financial sector taking the central stage [14]. The study site included Buea Regional Hospital.

Type of Study Design

This study was a hospital based cross sectional study.

Study Population

The population of the study consisted of children aged 1-12years attending the Buea Regional Hospital. Purposive sampling technique was used to recruit participants to the study.

Inclusion Criterion:

All children age 1- 12years brought to the Buea Regional Hospital by their parents were eligible for recruitment into the study and were recruited into the study after their parents have signed the consent form.

Exclusion Criteria

- Children who have tested positive for *H. pylori* within three months before the study.
- Children who were positive for malaria infection.
- Hospitalised patients.

Ethical Considerations

The protocol of the study was approved by the Institutional Review Board of the Faculty of Health Sciences of the University of Buea. Administrative clearance was also obtained from the Regional Delegation of Public Health of the South West Region, Cameroon and Buea Regional hospital. Individuals who fulfill the specific inclusion criterion and volunteer to participate after adequate sensitization on the project objectives, methods and possible benefits/risks by signing written guardian consent and an assent forms were enrolled in to the study. Confidentiality was maintained using codes to identify study participants after their recruitment and only authorized

laboratory personals could the study enrolment registers. Possible risks associated with the study were minimized by use of trained professionals in the conduct of the study.

Data Collection

Parents who signed the consent form for their children wer who wanted their children to take part in the study by signing the consent form were given questionnaires to fill patients' information such as demographic data and other relevant information such as family history of *H. pylori*, symptoms of gastritis, number of household members, number of rooms in the house and if the child ate at school. Parents who could neither read nor write were interviewed and their questionnaires filled. Participants' data and test results were entered a log book.

Sample Collection and Processing

Blood and stool collection

About 2ml of venous blood was collected into EDTA and dry tubes. Stool samples were collected from 189 participants. Samples collected were transported to the Faculty of Health Sciences Laboratory where they were analyzed. The coagulated blood samples were centrifuged for 5minutes to obtain serum which was stored in the freezer at -20^{o}C for later analysis.

Measurement of Packed Cell Volume

Anticoagulated blood samples collected then were mixed for 2minutes with a mixer, the anticoagulated microhaematocrit tube were ¾ filled with mixed whole blood, sealed with clay sealant and centrifuged in a microhaematocrit centrifuge for 5minutes and the packed cell volume (PCV) was read using a microhaematocrit reader. Normal values in children is 34- 45.

Values less than 34 were considered anemic, the anemia was classified in to three classes: mild (30<PCV<34), moderate (21≤PCV≥30) and severe (PCV<21) [15].

Measurement of serum iron

Serum iron was detected with the use of a spectrophotometer and ferrozine reagent following manufacturer's procedure.

The working solution was prepared by emptying the content of reagent 2 (ascorbic acid) into reagent1 (acetate pH 4.9) and was gently mixed. Reagent 1 acts as the buffer while reagent 2 as the reductant. Sterile plastic tubes were labeled blank, standard blank, standard, sample blank and sample respectively. 1ml of the working solution was put in each tube. 200µl of the standard was put in the tubes labeled standard blank and standard. 200µl of the sample was put in the tubes labeled sample blank and sample. One drop of reagent 3 (ferrozine) was put in the tubes labeled sample and standard. The tubes were vortexed for 1munite and kept for 10munites for the reaction to take place, the absorbance was read, and the iron concentration was calculated using the following formula *Conc = Abs of sample – Abs of sample blank/Abs of standard × 100.*

Normal values of serum iron concentration in children are 35-140µg/dl. Concentration below 35µg/dl were considered as low serum iron, while those above 35-140µg/dl was considered as normal and those above 140µg/dl were considered as high.

Detection of *H. pylori* antibody

The *H. pylori* One Step Device was used and it is a qualitative membrane strip based immunoassay for the detection of *H. pylori* antibodies in serum or plasma. In this test procedure, anti-human IgG is immobilized in the test line region of the device. After 75µl of serum was

transferred into the specimen well of the test device, were it reacts with *H. pylori* antigen coated particles in the test and results were read after 15minutes. NO result was read after 20 minutes.

Occult blood test

A rapid diagnostic faecal occult blood test was performed with the stool samples for 64 randomly selected participants. All samples collected per day were given numbers which were written on pieces of papers, put in a ballot box and half the total number was selected.

10drops of the extraction buffer were put in to the extraction cup; 10-50mg of the stool sample was transferred in to the extraction cup and mixed to become homogeneous. The test strip was immersed into the reaction cup and result read after five minutes. The presence of the control line alone indicated a negative result while the presence of both the control and the test lines indicated a positive result and the test was considered invalid if the control line was absent.

Thereafter, wet mount preparations of stool were performed and then observe under the microscope at 40x objective for the presence of cyst or ova of parasites. Since Hook worms cause bleeding of the intestinal epithelium it can also lead to iron deficiency anemia. Thus, these tests were to rule out any other cause of iron deficiency anemia apart of *H. pylori*.

Data Management and Data Analysis

Identification codes were given to participants and were written in their questionnaires. Also, at the end of each day questionnaires were put into a file and patients' data were later being entered in to the computer. Data were analyzed using IBM SPSS version 19 (SPSS Inc., Chicago, IL), and all tests were performed at a 5% level of significance. Chi-square statistics was

performed to determine if there was an association of *H. pylori* infection and serum iron level among children attending the Buea Regional Hospital.

Results

Characteristics of the demographic/risk factors of the study Participants.

A total of 189 children aged 1 – 12 years were enrolled in this study. The prevalence of *H. pylori* in the study population was 31.7% (60/189) (Fig 1)

Ninety-two (48.7%) of these 189 participants were males and ninety-seven (51.3%) were females. The participants were divided into three age groups (< 5, 5 - < 9, and ≥ 9) years with children less than 5 years being the most represented (Table 1).

H. pylori was more prevalent among males with 35(38.0%) out of 92 of them having *H. pylori* as opposed to 25 (25.8%) of the 97 females and this difference was significant (P=0.049) (Table 1). Participants within the 5- 9 years, had the highest prevalence of *H. pylori* with 24 (38.7%) of the 62 having *H. pylori*, followed by those <5years with 25 (30.9%) of the 81 having *H. pylori* and then ≥9 years with 11 (23.9%) out of 46 having *H. pylori*, however, this difference was not significant (P=0.257) (Table 1).

Out of 83 participants from crowded homes, 28 (33.7%) were positive for *H. pylori* while 32 out of 106 (30.2%) children from non-crowded homes were positive for *H. pylori* but this difference was not significant (P=0.358). The one participant whose source of drinking water was well had *H. pylori* (100%). We had 33.5% (56/167) for those whose drinking source was tap had *H. pylori*. Out of 8 whose drinking source was stream, 2 (25%) were positive for *H. pylori* and of

the 13 whose drinking source was mineral water, 1 (7.69%) was positive for *H. pylori*. However, this difference was not significant (P=0.11) (Table 1).

Out of 73 participants with dirty nails, 25 (34.24%) were positive for *H. pylori* and 35 out of 116 (30.17%) without dirty nails were positive for *H. pylori.* This difference was not significant (P=0.335). Out of 144 children who reportedly ate out of their homes, 48 (42.1%) were positive for *H. pylori* and 12 out of 45 (26.7%) children who ate only in their homes were positive for *H. pylori*. This difference was also not significant (P=0.259). Eighteen out of 55 (32.72) children who did not practice hand washing regularly were positive for *H. pylori* and 42 of the 134 (31.34) who practiced hand washing regularly were positive for *H. pylori*. However, these differences were not significant (P=0.491) (Table 1)

Table 1: Prevalence of *H. pylori* with respect to demographic/risk factors.

Category	Number enrolled (%)	*H. pylori*		P-value (X^2)
		Negative (%)	Positive (%)	
Gender				0.049(3.281)
Male	92 (48.67%)	57 (30.15%)	35 (38.04%)	
Female	97 (51.32%)	72 (38.09%)	25 (25.77%)	
Age Group				0.257(2.719)
< 5	81(42.85%)	56 (29.62%)	25 (30.9%)	
5 – 9	62 (32.80%)	38 (20.10%)	24 (38.7%)	
9- 12	46 (24.33%)	35 (18.51%)	11 (23.9%)	
Living Condition				0.358(0.270)
Crowded	83 (43.91%)	55 (29.10%)	28 (33.7%)	
Normal	106 (56.08%)	74 (39.15%)	32 (30.2%)	
Source of Water				0.011(6.035)
Tap	167 (88.35%)	111 (58.73%)	56 (33.5%)	
Mineral	13 (6.87%)	12 (6.34%)	1 (7.69%)	
Stream	8 (4.23%)	6 (3.17%)	2 (25%)	
Well	1 (0.53)	0 (0)	1 (100)	
Dirty nails				0.334(0.343)
Yes	73 (38.62%)	48 (25.39%)	25 (34.24%)	
No	116 (61.37%)	81 (42.85%)	35 (30.17%)	
Eat at school				0.259 (0.703)
Yes	144 (76.19%)	96 (50.79%)	48 (33.33%)	
No	45 (23.80%)	33 (17.46%)	12 (26.66%)	
Hand Washing				0.491(0.034)
Yes	134 (70.89%)	92 (48.67%)	42 (31.34%)	
No	55 (29.10%)	18 (9.52%)	18 (32.72%)	

The Prevalence of Serum Iron Level in the Study Population

Of the 155 participants whose serum iron concentration was measured, 73/155 (47.1%) had low serum iron levels and 82/155 (52.9%) had normal serum iron level (figure 2). The whole189 participants were not involve in this test because their samples were not collected for this and contamination of their samples.

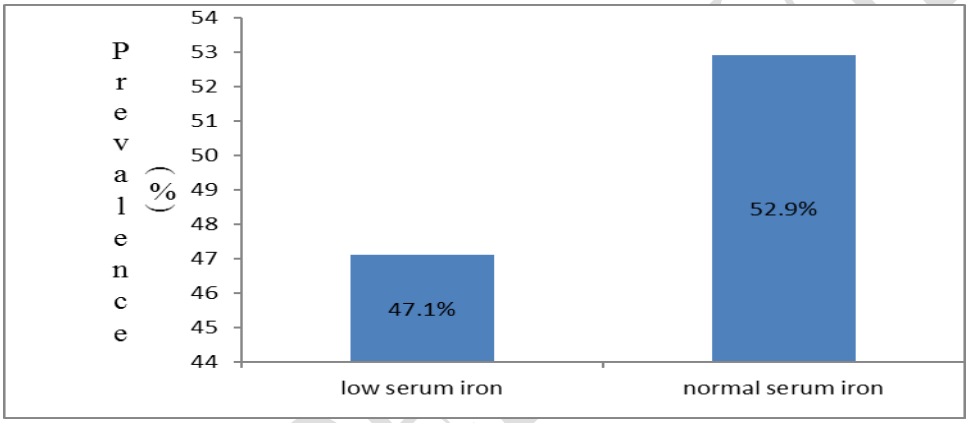

Figure 2: Prevalence of Serum Iron Level in the Study Population

Relationship between Anemia and Low Serum Iron Levels in the study population

Fifty five out of 120 (45.8%) who were anemic had low serum iron levels and 18 out of the 35 (51.4%) who were non-anemic had low serum iron level. This difference was not statistically significant. (P = 0.560) (Figure3).

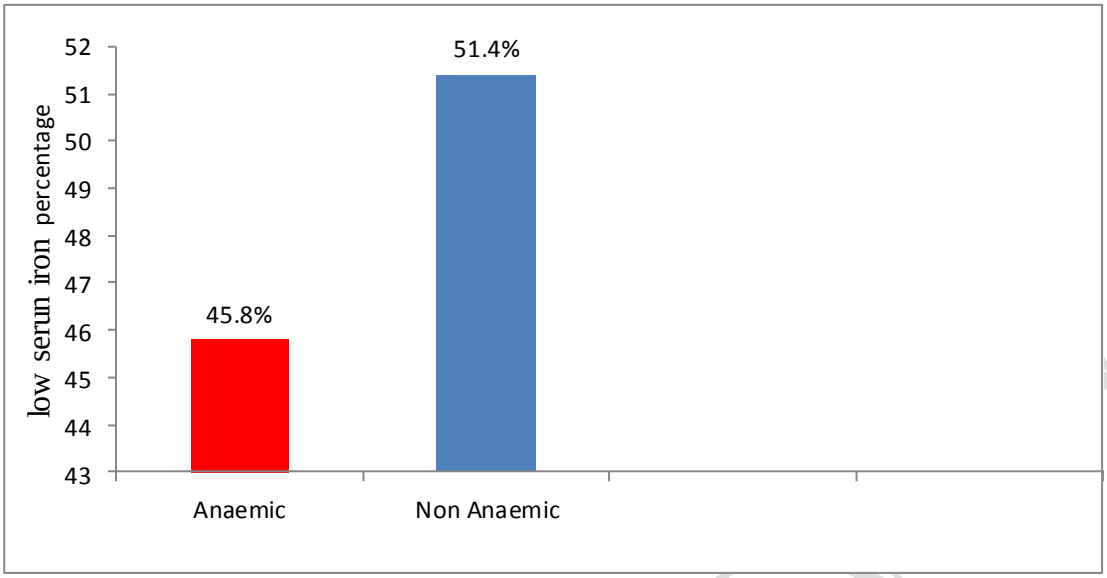

Figure 3: Relationship between Anemia and Low Serum Iron Levels in the Study Population

***H. pylori* with respect Iron Level and Anemic conditions of the participants**

Of the 73 (47.1%) participants had low serum iron levels, 16 (21.9%) had *H. pylori* while 36 (43.9%) out of 82 with normal serum iron levels were positive for *H. pylori*. This difference was significant (P=0.004) (Table 3). Of the 54 with mild anemia, 25 (46.3%) were positive for H. pylori, 19 (22.4%) of the 85 with moderate anemia had *H. pylori*, 5 (55.6%) of the 9 with severe anemia had H. pylori and 11 (26.8) out of 41 who were non-anemic had *H. pylori*. This difference was significant (P=0.009) (Table 2)

Table 2: Prevalence of *H. pylori* with respect Iron Level and Anemic conditions of the participants

Category	Total (%)	H. pylori		P-values (X^2)
		Negative (%)	Positive (%)	

Iron level				0.004(8.37) *
Normal	82 (43.38%)	46 (24.33%)	36 (43.9%)	
Low	73 (38.62%)	57 (30.15%)	16 (21.9%)	
Anemia				0.009(11.55) *
Normal	41 (21.69%)	30 (15.87%)	11(26.8%)	
Moderate	85 (44.97%)	66 (34.92%)	19(22.4%)	
Severe	9 (4.76%)	4 (2.11%)	5(55.6%)	

Discussion

Helicobacter pylori is one of the most common human infection worldwide, colonizing the stomach of 50% of the world's population and it's a major risk factor to the development of Iron deficiency, chronic gastritis, peptic ulcer and gastric cancer [1]. H. pylori infection is more widely spread in developing countries than in developed countries and acquisition of bacteria is predominant in childhood [3]. Iron plays an important role in biological systems particularly in oxidation-reduction processes, which are essential in life. H. pylori inhibit secretion of ascorbic acid into the gastric juice which may lead to decreased absorption of iron since the acid aids in the solubilization and reduction of ferric iron to the more absorbable ferrous iron form [12].

This study showed that 31.7% of the children were positive for H. pylori infection (positive IgG test), which is lower compared to the 52.2% previously reported by Ndip and colleagues [6] in a similar population in the same area. However, this result is also lower compared to the 74% prevalence of H. pylori reported by Aquemon [16] and colleagues in a similar population in Benin in 2005. These results however corroborate with the relatively low prevalence of H. pylori reported in a similar population in Iran [17]. This result is however higher compared to the 27.7% of Queiroz and colleagues in a multinational study conducted from 2007 – 2011 [18]. However, studies in symptomatic children in Tombel, Cameroon have reported much higher prevalence of H. pylori (86.6%) [19]. In fact, symptomatic children in this study showed higher

prevalence of H. pylori compared to their asymptomatic counterparts although the difference was not significant.

Likewise, males had a significantly higher prevalence of *H. pylori* compared to females. The higher prevalence of *H. pylori* observed in males could be because they are more active and so tend to indulge in more outdoor activities. This was in conformity with the findings of Zamani and colleagues [17] in Iran, and in contrary to those reported by Queiroz and collaborators [18] in which no difference in prevalence was observed.

Generally, there was an increasing trend of *H. pylori* infection with decrease in age, which could be because of the weak immunity or defense system in general in children below 5years of age. This is partly in conformity with studies by Zamani and colleagues [17] in which prevalence of *H. pylori* decreased with increase in age in the study by Zamani and colleagues.

Considering hygienic conditions of the children, it was founded that *H. pylori* was more common in children who did not practice hand washing and in those who kept dirty nails although the differences were not significant. No difference was also noticed with the source of drinking water and infectivity with *H. pylori* among the children. Mynepalli and colleagues [20] in a study conducted in Lagos, Nigeria also reported no significant association between drinking water source, hand washing practice after defecation and *H. pylori* positivity.

Also *H. pylori* was common among children who ate in school than in those who ate only in their houses. This may be because food prepared at home for family consumption is done with care and in a more hygienic condition than that prepared for commercial purpose. Likewise, the fact that *H. pylori* is transmitted through fecal-oral means, explains why it was more prevalent in children who did not practice regular hand washing and kept dirty nails compared to those who did the contrary.

H. pylori was more prevalent in children from crowded homes than in those from non- crowded homes although the difference was not significant. This could be due to the low socio-economic status of children from crowded homes. In fact, in a review by Khalifa and collaborators [21], they point out that socioeconomic status is not restricted to income and social class but takes in consideration other factors, including living standards, sanitation, urbanization, and educational level and combined, these factors are likely to increase the risk for infectious diseases in general. They also lay emphasis on the fact that socioeconomic status is reportedly one of the most important factors affecting the spreading of *H. pylori* infection.

Also, 47.1% of the children had low serum iron in this study, while 45.8% of the study population had anaemia and low serum iron level (iron deficiency anaemia). These values are higher than that of Bagget and colleagues [11] in a similar population in Alaska. This difference or these high values could be because, individuals in the developing country such as ours (Cameroon) are prone to malaria and other factors that could cause low iron and anemia compared to developed countries who have more iron fortified food.

Furthermore, results of this study showed that children positive for *H. pylori* had a significant higher serum iron than those negative for *H. pylori*. The difference seen in this study although unexpected is in conformity with the results of Saler and collaborators [22] who concluded that *H. pylori* infection was not associated with iron deficiency. According to literature, a co-morbidity of H. pylori and iron deficiency anemia may be a coincidences, because both of the diseases are highly prevalent and there are many risk factors that could let to the iron deficiency anemia, such as vitamin deficiency, malnutrition, chronic diseases, infections and conditions associated with chronic blood loss [22-24].

Conclusion

This study showed that the prevalence of H. pylori in the studied population was 31.7%. 47.1% of the studied population had low serum iron level. Male gender was significantly associated with *H. pylori* infection and children of age group 5≤ age<9 years had the highest infection. More *H. pylori* positive individuals had high serum iron level and having H. pylori was not associated with low serum iron.

List of Abbreviations

- HP - *Helicobacter pylori*
- 95% C.I - 95% Confidence Interval
- *p*-value - Significance value
- SD - Standard Deviation
- χ^2 - Chi square
- HP –

DECLARATIONS

Ethics approval and consent to participate

Ethical clearances were obtained from the IRB-FHS of the University of Buea. Participation of the study was voluntary, and Consent was also gotten from all the participants. The participants were free to withdraw from the study at any time.

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Nil.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Chey WD, Wong BCY. Management of *Helicobacter pylori* Infection. *Am J Gastroenterol.* 2007; 102:1808–1825

2. Ahuja V, Dhar A, Bal C, Sharma MP. Lansoprazole and secnidazole with clarithromycin, amoxicilin or pefloxicin in the eradication of *Helicobacter pylori* in a developing country. *Aliment Pharmacol Ther.* 1998;12:1-5.

3. Queiroz DMM, Rocha AMC, Crabtree JE.Unintended consequences of *Helicobacter pylori* infection in children in developing countries. *Caspian J Intern Med* .2013; 4(6): 494–50

4. G. Hematologic manifestations of *Helicobacter pylori* infection. *World J Gastroenterol*. 2014; 20 (36):11-16

5. McColl K.E, Omar E, Gillen D, Interactions between *H. pylori* infection, gastric acid secretion and anti-secretory therapy. *Am J Gastroenterol.*1998; 54(1): 21-38.

6. Ndip RN, Malange AE, Akoachere JF, MacKay WG, Titanji VP, Weaver LT. *Helicobacter pylori* antigens in the faeces of asymptomatic children in the Buea and Limbe health districts of Cameroon: a pilot study. *Trop Med Int Health*. 2004; 9(9):36-40.

7. World Health Organization. Iron Deficiency Anaemia: Assessment, Prevention, and Control: A Guide for Programme Managers. 2001. Geneva, Switzerland:

8. Wimbley JTD, Graham DY. Diagnosis and management of iron deficiency anemia in the 21st century. *Therap Adv Gastroenterol.* 2011; 4 (3):177-184.

9. Mathew W. Iron deficiency anemia. Madigan Healthcare System, Tacoma, Washington: Jason E; 2013.P. 110-145

10. Hutton EK, Hassan ES. Late versus early clamping of the umbilical cord in full-term neonates: systematic review and meta-analysis of controlled trials. *JAMA.* 2007;297(11):1241-1252.

11. Baggett H C, Parkinson AJ, Muth PT, Gold BD. Gessner BD. Endemic Iron Deficiency Associated with *Helicobacter pylori* Infection Among School-Aged Children in Alaska. *Pediatrics.* 2006; 117:396-404.

12. Mehmood A, Akram M, Shahab U, Ahmed A, Usmanghani K, Abdul H, Mohiuddin E, Asif M. *Helicobacter pylori*: An Introduction. *Inter J App Biol and Pharmacol Technol*.

13. Seo JK, Choi KD. Serum ferritin and *Helicobacter pylori* infection in children: a sero-epidemiologic study in Korea. *J Gastroenterol Hepatol*. 2002; 17:4-7

14. Folefac F, Lifongo L, Nkeng G, Gaskin S. Municipal drinking water source protection in low income Countries: case of Buea municipality Cameroon. *J Ecol and Natural Enviro*. 2009; 1 (4): 73-84

15. Chesbrough M. District laboratory practice in tropical countries. 2nd. New York: Tropical health technology; 2005.

16. Aguemon BD, Struelens MJ, Massougbodji A, Ouendo EM. Prevalence and risk-factors for *Helicobacter pylori* infection in urban and rural Beninese populations. *Clin Microbiol Infect*. 2005; 11(8):611-704

17. Zamani A, Shariat M, Yazdi ZO, Bahremand S, Asbagh PA, Dejakam A. Relationship between *Helicobacter pylori* infection and Serum Ferritin level in primary school children of Tehran-Iran. *J Pak Med Assoc.* 2011; 61(7):658 - 661.

18. Queiroz DMM, Harris PR, Sanderson IR, Windle HJ, Walker MM, Rocha AMC, Rocha GA, Carvalho SD, PFS, Fonseca de Castro LP, Villagrán A, Serrano C, Kelleher D, Crabtree JE. Iron Status and *Helicobacter pylori* Infection in Symptomatic Children: An International Multi-Centered Study. *Plos One.*2013; 8(7): 688.

19. Ebule IA, Longdoh AN, Paloheimo IL. *Helicobacter pylori* infection and atrophic gastritis. *Afr Health Sci*. 2013; 13(1): 112–117.

20. Mynepalli SKC, Osamor M, Mumuni A. Prevalence of *Helicobacter pylori* and hygiene practices among public secondary school students in Ikeja local government area, Lagos, Nigeria. *Health*. 2014;6 (4): 250-258

21. Khalifa1 MM, Sharaf RR, Aziz RK. *Helicobacter pylori*: a poor man's gut pathogen? *Gut Pathog*. 2010; 2:2

22. Saler T, Keşkek SQ, Kırk S, Ahbab S, Ortoğlu G. *H. pylori* May Not Be Associated with Iron Deficiency Anemia in Patients with Normal Gastrointestinal Tract Endoscopy Results. *Adv Haematol*.2014; 2014 (375915):1-4.