Evaluation of ABO and Rh Blood Groups Among *Helicobacter Pylori* Infection Among Patients Attending Thika Level 5 Hospital Kiambu, Kenya

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Abstract

Background: Blood group A and B carbohydrate are thought to impact the risk of getting *H. pylori* through sticking to the gastric mucosa in the crypts. Knowing the risk factors connected with the infection, interventions and guidance on appropriate preventative measures are required.

Methods: The study included 201 patients with dyspepsia symptoms referred from outpatient department for investigation between age 16-75 years. Data on demographic information was gathered before they were given questionnaires. All patients were investigated for blood group phenotype and rhesus dependent through slide hemagglutination test. *H. pylori* stool antigen test was also conducted for its positivity. The data was analyzed using SPSS version 25.

Results: The frequency of ABO and Rhesus blood group among *H. pylori* infected patients was O, A, B, AB (48.2%), (31.8%), (14.1%), (5.9%) respectively. The majority of the patient were female 51 and 34 males.

Conclusion: *H. pylori* infection is an endemic problem that needs to be addressed by improving hygiene, providing purified water and taking additional steps for its eradication. Blood type O are more likely to contract *H. pylori* infections while female and younger adults are more prone to the disease.

Keywords: *H. pylori* infection, ABO blood groups, dyspepsia.
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1. Introduction

Blood group ABO was discovered by Karl Landsteiner in 1901. It is concealed by a gene located on the long arm of chromosome nine. ABO antigens are starch molecules in nature and are produced by the temporal action of ABO glycosyltransferases enzymes (Gasim et al., 2017). A and B antigens are also found in saliva, semen and sweat (Teshome et al., 2019) when this antigen is secreted in different organs and tissues, they play a role in resistance or susceptibility of infectious and non-infectious diseases (Kadhum Baqir *et al.*, 2016).

H. pylori is a public health significant in developing countries owing to poor hygienic environment and resources constrain. Young ones are more susceptible to this bacterium at childhood and harbor it without any sign or symptom of infection till adulthood. Most of the patients complain of abdominal pain, nausea, vomiting weight loss and lack of appetite (Aitila et al., 2019). A lot of methods are used in medical field to diagnose *H. pylori* infection which includes sero immunoglobulin G (IgG) antibody test and stool antigen tests which is more reliable, specific and higher sensitivity.

The recommended treatment for *H pylori* is normally triple therapy whereby the patient is given antibiotic, metronidazole and proton pump inhibitor. (Aitila *et al.*, 2019). In Africa there is no continental way to determine the management, diagnosis and treatment of *H. pylori* which led to rise in bacteria resistance to antibiotics. (Smith *et al.*, 2019) A better understanding of underlying mechanism between *H. pylori* and ABO blood group association provided indication to potential prevention strategies for *H. pylori* infection. The data would be useful to the ministry of health to give guideline in policy making.

2. Materials and methods

2.1 Study design and site

The study involved a cross-sectional design and random sampling of the patient attending Thika level 5 hospital for dyspepsia treatment in Kiambu County, Kenya. It serves a large number of Thika town residence and its affluent neighborhood. The target population composed of patient who attended Thika level 5 hospital, Kiambu county seeking for healthcare services with dyspepsia. The study was ethically approved by Mount Kenya University Ethical committee and permission sought from the hospital while the permit was accorded by National Council for Science Technology and Innovation (NACOSTI). ABO and Rhesus (Rh) blood groups were studied to determine whether there may be a link between H. pylori infection and these blood types in patients who presented with dyspepsia.

2.2 Target population and sampling

The sampled dyspepsia respondent were 201 patients and who met the recruitment criteria for the study. The sampling started at the point when the patients were referred to the laboratory for *H. pylori* test. simple random sampling was used for selection until the sample size was achieved. An informed consent form was given to every participant and taken through consenting. Those who consented were advised to sign and a unique identifying number was assigned to every consented form. A random number was also assigned to the questionnaire and recorded on research book.

2.3 Sample size determination

The sample size was determined by fisher's formula 2014: this was so because in ideal sense the time reversal test and the factor reversal test were satisfactory.

Sample size (n) =(z)2p(1-p)/d2: where

Z=is the standard normal deviate at 95% confidence level= (1.96%)

p =Expected proportion in population based on previous studies

d=absolute error

z=1.96 p=16% d=0.05

Hence 1.96^2x0.16(1-0.16)/0.05^2 =201 subjects

2.4 Sample collection and sampling technique

After consent form was signed, the patient was advised on the procedure for stool sample collection. A well tight fitted lid stool container was labeled with patient unique identifying number which was in the concept form. The patient was given the container and tissue. The patient was advised to collect at least pea size stool sample either in liquid or solid form, close it tightly and return collected sample to the laboratory as soon as possible.

After the patient had brought stool sample, the patient sat comfortably and the arm stretched on phlebotomy area table. The patient was explained on how blood sample was to be collected from the cubital fossa region on either arm. Ethylenediaminetetraacetic acid (EDTA) vacutainer was labelled with unique identifying number corresponding to the patient consent form. A tourniquet was placed on the upper arm and the cubital fossa was examined using index finger. The vein was sterilized with 70 % alcohol swab. With the use of a 5mls and 23gauge needle, the vein was pricked bevel facing down. With the needle inside the vein, 5mls of blood was collected by pulling the syringes bevel until the desired amount was obtained. The tourniquet was removed, the dry swab was placed at the point of prick and the needle was removed. The blood was transferred on to a labelled EDTA vacutainer tube which was inverted several times to ensure proper mixer of blood and EDTA was achieved. The tube was then placed on the collection tray until analysis was done.

2.5 Test procedures

A pea size stool sample was taken from the labeled sample container and placed into a commercially sourced saline buffer 1.5mls, (0.85% saline solution at ph. 7.2) and mixed well to achieve a smooth consistent mixer. A commercially prepared sourced H. pylori antigen test (WONDFO Belgium, Germany) was removed from it foil and placed on the working bench. Three full drops of stool specimen (mixer of stool and buffer) were put into sample well of the test cassette. positive results could be read within 15minute.However negative results were confirmed after 20 minutes. The results were confirmed macroscopically by observation of the colored band that appeared on the test cassette of which double band confirms a positivity and single band confirms negativity of the test.

Blood grouping was done using a commercially sourced Anti sera which included Anti-A, Anti-B and Anti- D. They were taken from cold chain and placed on the working bench to achieve the room temperature which took 4-6minutes.Clean grease free slide were used for the agglutination process. On the slides one drop (45-55 ul) of blood group Anti sera i.e. Anti-A, Anti-B and Anti D were placed on a properly labelled clean slide with a distance separating each slide proportionally. Using a micro pipette,45ul of whole blood was drawn from EDTA vacutainer sample tube and added to each antiserum by the use of applicator stick, the blood was mixed well together with antisera. The slide was rocked for 2 minutes then checked for agglutination macroscopically. The procedure was repeated for all blood sample collected. The results were recorded in the research data collection book.

Questionnaires were used to generate demographic data of patients which included age and gender. Participants completed the questionnaires by providing replies to the questions asked which were used to categorize them in various age groups/clusters in order to determine the occurrence of the infection in different age groups clusters. The validity of the questionnaire was determined prior to the commencement of the study where they were administered to laboratory personnel.

2.6 Data Management, analysis and presentation

Data collected was entered and cleaned using SPSS version 25. Codes were used in place of names to observe confidentiality, integrity and easy retrieval of the data of the study subjects.

Chi-square test was used to assess associations. The descriptive information was presented using graphs and pie charts while tables were used to presents data analyzed using inferential statistics. There was process check sheet (which included books, paper sheets, which were validated), Process standard (rules, regulation and step by step procedure), process documentation and project audit to ensure quality results and proper designing process was followed in all sample product.

2.7 Ethical Considerations

The Mount Kenya University Ethical Research Committee (MKUERC) and the National Council for Science, Technology, and Innovation both granted their approvals for the study (NACOSTI). The Kiambu County Director of Medical Services was also consulted before the study was conducted.

3. Results

The study was able to get 201 samples from patient with dyspepsia attending Thika level 5 hospital. After carrying out *H. pylori* antigen tests,114 of the samples were found negative and 87 were found to be positive. The point prevalence of *H. Pylori* infection in patient attending Thika level five hospital with dyspepsia was 43.3%. The results are as shown in figure 1 below.

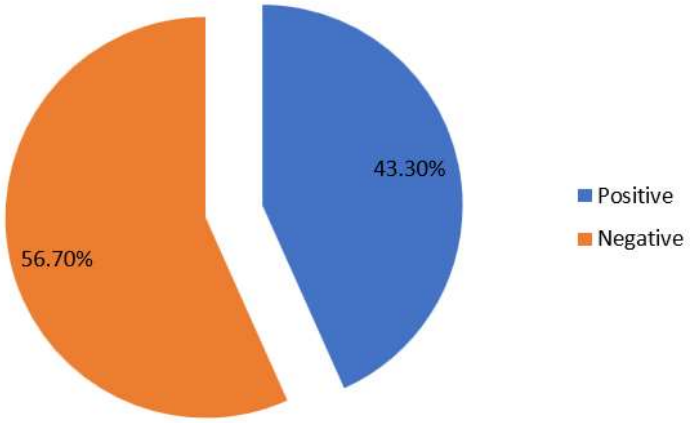

Figure 1: Prevalence of Helicobacter Pylori infection among patient attending Thika Level 5 Hospital. Source field data (2022)

A, B, AB and O were 28(32.18%), 13(14.94%), 5(5.75%) and 41(47.13%) respectively. The study found no statistically significant difference between ABO and H pylori infection (table 1) of the respondents since the p value obtained was 0.967. The study shows that O blood group is more susceptible to H Pylori infection at 47.13%. However, there was no statistical significance among blood group O and H pylori infection since is p value 0.903.

Table 1: Relationship of ABO blood groups in Helicobacter pylori infected patients

Variable		*H. pylori* positivity	Percentage	P – value
Blood group	A	28	32.18%	0.872
	B	13	14.94%	0.881
	AB	5	5.75%	1.212
	O	41	47.13%	0.903
Total		**87**	**87**	

The majority of the patient who were *H. pylori* positive were female 51(58.6%) while male was 36 (41.4%). Gender was found to have insignificant association with the prevalence of *H. pylori* and ABO blood group since p value was 0 .771 (Table 2).

Table 2: Association of gender and ABO and Rhesus Blood Group in H. pylori infection

			Gender		
Variables		**N**	**Male**	**Female**	**P Value**
Blood Group	O	(41)	18	23	
	A	(5)	2	3	
	B	(28)	10	18	0.771
	AB	(13)	6	7	
Rhesus	Rhesus positive	(85)	34	51	0.507
	Rhesus negative	(2)	0	2	

The mean age of the *H. Pylori* positive patient sampled was 32.7±8.2 years with a median age of 25.5 years (range16-75years).

The results showed that those in age group 16-25 years were 27 (31.0%) which were more affected. The relationship between ABO blood group and Age group in *H pylori* infection was found to be statistically significance since p value is 0. 000 (figure 2).

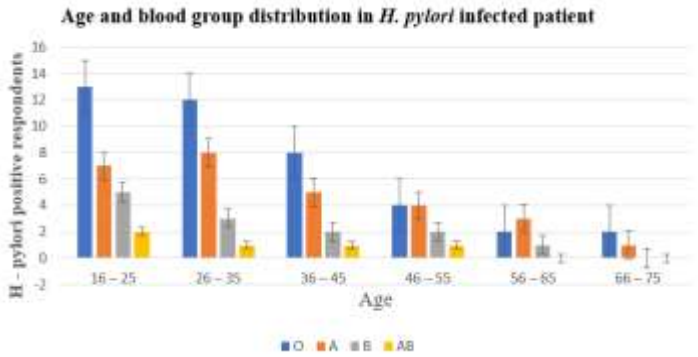

Figure 2: Relationship of age and blood group in H. pylori infected patients

4. Discussion

The result findings showed that *the* point prevalence of *H. pylori* among patient attending TL5H seeking health services with dyspepsia was 43.3%. Globally *H pylori* prevalence as in study done by Yan Zhou et al., 2022 was 50% and in China the prevalence of *H pylori* was 43.6% which is almost in line with this study. According to a study done by Reka et al., 2022 in America and Europe he found out that *H. pylori* prevalence was 50% in America ,66.2% in Portugal, Europe which is higher than the finding of this study. A study done by Smith et al., 2022 in Africa, showed that the *H. pylori* prevalence was 87.8%, 75%,63.5%,88% in Nigeria, Rwanda, Congo, and morocco respectively, which is higher than the findings of a study, Machogu E.M (2020) did a study in Githogoro, Kenya where the prevalence of *H. pylori* was 45% which is almost in line with this study. Another study done in Aga khan hospital by Mwangi et al., 2020 showed that the prevalence of was 54.6% which is higher than the finding of the current study which doesn't agree with this study.

Blood group antigen which are starch molecules in nature are produced elsewhere in the body which make some individuals resistance or susceptible to infection not excluding *H. pylori*. The finding of this study revealed a high number of patients infected by *H. pylori* were in blood group O were 47.13%. than in others blood groups. This is in agreement with a study done in Nepal Mahaseth et al., 2021, which showed that O blood group were more in H. Pylori infection as it was in Iraq and Iran in the recent study the author findings agreed with the same finding done by Yonas et al., 2019 in Ethiopia. Blood group B and AB had 14.94% and 5.74%. decrease in likely hood of *H. pylori* infection in comparison with A was 32.18%. *Other* researchers found out that Lewis and secretor histological blood system shows the primary mechanism to the connection involving *H. pylori* and ABO blood group. There was a similar association to this study where it indicates gastric pain as the main observed clinical sign in *H. pylori* infection. (Teshome et al. .2019). The author of this study found out that there was no relationship between *H. pylori* and blood group where O were the highest and AB had the lowest.

However, it was found out that blood group with Rhesus positive (Rh +ve) were highly affected compared to Rhesus negative (Rh-ve) blood group as was indicated by the participant who turned Rhesus negative were two and 199 Rhesus positive, which could be attributed by the fact that most population belong to Rhesus positive blood group. (Giada et al., 2016) Individuals with Blood Group O represent an important secretor phenotype which transforms oligosaccharides type 1 into H antigen type 1 but in the presence of GTA and GTB glycosyltransferase enzyme, but if these enzymes lack it did not appear to produced O antigen and instead transforms H type one antigen in to Leb antigen, which represent an important receptor expressed in gastroduodenal mucosal cells to which *Helicobacter pylori* adhere and boost the increase towards *H. pylori* infection. H. pylori is the owner of five of the most significant outer membrane protein (OMP) families (Smith et al., 2019). *H. pylori's* response to IFN showed that IFN-γ can bind to *H. pylori* proteins, including the virulence factor cytotoxin-associated gene A (CagA), whose expression was downregulated, had their expression altered as a result of the binding. In response to a variety of stimuli, including endotoxin and Gram-negative bacteria, effector T-helper cells (CD4+CD25) release IFN-γ, a proinflammatory cytokine. another study found high levels of IFN-γ associated with increased infiltration of mononuclear cells in human gastric mucosa infected with *H. pylori* (Melese et al., 2019), In collaboration with social economic status, environmental factor and life style could actually be significant. (Kayali et al., 2019) there is a detectable level of continued curiosity about the possible function of blood group in contagious disorders and in diseases susceptibility and resistance. However, blood group antigen are receptors for toxic compound, harmful substances which has a capability of interfering with immune surveillance, hence colonization and clearing mechanism is interfered with (Mahaseth et al., 2021).

Conclusion and recommendations

According to the study, it can be concluded that *H. pylori* infection is an endemic problem that needed to be addressed by improving hygiene, providing filtered water, and taking additional steps for its eradication. Blood type O are more likely to contract *H. pylori* infections while female and younger adults are more prone to *H. pylori* infection. To establish a connection between blood type and *H. pylori* contamination in various age groups, more research is required. All patients found to be *H. pylori* positive to be advised to take treatment. Healthcare stakeholders to encourage the public to practice good hygiene to control spreading of *H. pylori*. Patients presenting dyspepsia should be subjected to *H. Pylori* testing to enable more effective and timely therapy of those who test positive, mitigating its spread. More research should be done on ABO blood group antigen to ascertain their susceptibility and resistance to the infection.

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Conflicts of interest

The authors declared no conflicts of interest.

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