Original Research Article

ABO, Rhesus Blood Groups and Haemoglobin Variants Distribution among Individuals with *Helicobacter pylori* in Igwuruta-Ali, Rivers State

ABSTRACT

Aims: The aim of the study is to determine the distribution of ABO & Rhesus blood groups, and haemoglobin variants amongst individuals in igwuruta-ali with *Helicobacter pylori* infection.

Study design: This was a cross-sectional, field-based study carried out in Igwuruta-Ali, Ikwerre Local Government Area, in Rivers State.

Place and Duration of Study: All samples were analyzed at the Haematology Laboratory, Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria, between July and September, 2018.

Methodology: Qualitative determination of *Helicobacter pylori* antigens using serological method. Qualitative determination of ABO and Rhesus blood group using tube method. Qualitative determination of hemoglobin genotype using electrophoretic method (cellulose acetate method). Blood samples were collected randomly based on convenient sampling from a total of 120 volunteers (age 10 to 70 years).

Results: A total of 103 subjects were sero-positive for *Helicobacter pylori* (37 males and 66 females; 35.9% and 64.1% respectively), while 17 subjects were sero-negative (8 males and 9 females; 47.1% and 52.9% respectively). ABO blood group distribution amongst sero-positive individuals were A(22.3%), B(13.6%), AB(8.7%), O(55.3%). The Rhesus blood group distributions in sero-positive individuals were Rhesus D positive (30.8%) and Rhesus D negative (6.67%); Sero-positivity for *Helicobacter pylori* was increased in females than in males and subjects with ABO blood group O were more prone to *Helicobacter pylori* than in other groups. The distribution of haemoglobin variants amongst sero-positive subjects were AA(71.84%), AS(22.33%), SS(5.83%).

Conclusion: The study reveals that O blood group individuals are more susceptible to *Helicobacter pylori* infection and they have more cellular and immunological response to it (expressed by sero-positivity) than other ABO blood groups (group B in particular), while no strong relationship exist between Rhesus D positive and Rhesus D negative subjects, and also no clear relationship based on haemoglobin genotype. Based on the fact that 55.3% of the study population that tested positive for *Helicobacter pylori* was O blood group individuals, O blood group is therefore associated with *Helicobacter pylori*. Also, the study revealed that females were more prone to *Helicobacter pylori* infection.

Keywords: Igwuruta-Ali; Helicobacter pylori; ABO and Rhesus Blood Group; Haemoglobin Variants

1. INTRODUCTION

Blood groups are inherited characteristics present on red blood cell surface and can be detected by specific antibodies. Blood groups are associated with proteins and glycoproteins that are part of the integral structure of the red blood cell membrane. Blood groups are detected based on antigens found on red blood cells and thus can be defined by a specific antibody. The importance of blood to humans cannot be over-emphasized, its importance in health and diseases is critical and various blood types have its own unique association with diseases, hence the need to re-evaluate and underpin established findings that *Helicobacter pylori* infection is associated with O blood group, but in Igwuruta-Ali.

Helicobacter pylori can be identified as a spiral shaped gram negative bacteria that usually colonize the human stomach [1]. The ABO and Rhesus blood groups has remain immunologically important to the blacks and ABO and Rhesus blood groups have been associated with some infectious and non-infectious diseases [2]. Most studies [3];[4] have shown that ABO blood groups individuals are susceptible to infection of Helicobacter *pylori*, however, there are paucity of scientific based information to verify if this association exist between Helicobacter *pylori* and Rhesus blood groups system, and also with heamoglobin variants.

Helicobacter pylori have multiple flagella at one pole and are actively motile. *Helicobacter pylori* grow in 3–6 days when incubated at 37°C in a micro-aerophilic environment [5]. The colonies are translucent, 1–2 mm in diameter. *Helicobacter pylori* are oxidase positive and catalase positive, has a characteristic morphology, is motile, and is a strong producer of urease. It is present in 20 to 50% of the population in developed countries and 80% of the population in developing countries. *Helicobacter pylori* represent the most widely reported causative pathogen of most gastrointestinal ulcerations and gastric cancer; this has been found to cause considerable morbidity and mortality worldwide [6].

Helicobacter pylori is a bacteria that is found in the gastric mucous layer or adherent to the epithelial lining of the stomach. *Helicobacter pylori* causes more than 90% of duodenal ulcers, up to 80% of gastric ulcers, gastritis (inflammation of the lining of the stomach) [1]. The mechanisms by which *Helicobacter pylori* cause mucosal inflammation and damage are not well defined but probably involve both bacterial and host factors. The bacteria invade the epithelial cell surface to a limited degree. Toxins and lipo-polysaccharide may damage the mucosal cells, and the ammonia produced by the urease activity may also directly damage the cells [7]. Studies carried out by [8];[9] and others have associated *Helicobacter pylori* with some blood groups. Their different outcomes necessitated our quest to investigate risk factors associated with *Helicobacter pylori* infection in one of our own local community based on blood type.

Igwurutali-ali is an area in Rivers state with poor socio-economic amenities which increases their risk to microbiological infections especially of the bacterial origin. This community is in Ikwerre Local Government Area of Rivers State, in Nigeria.

The aim of the study is to determine the distribution of ABO, Rhesus blood groups, and haemoglobin variants amongst individuals in Igwuruta-Ali with *Helicobacter pylori* infection. The objectives of this study are: (i) to determine the ABO and Rhesus Blood groups distribution among *Helicobacter pylori* infected individuals, (ii) to determine the haemoglobin variants distribution among *Helicobacter pylori* infected individuals, (iii) to determine the distribution of *Helicobacter pylori* infection among male and female individuals in Igwuruta-Ali.

2. MATERIAL AND METHODS

2.1 Research Design

This is a cross sectional study and was conducted in Igwuruta-Ali, a town in Ikwerre Local Government Area of Rivers State, Nigeria, specifically to determine the ABO, Rhesus blood groups and haemoglobin variants distribution pattern among natives of Igwuruta that also reside in the community.

2.2 Study Area

Igwuruta-Ali is located in Nigeria about 458km south of Abuja, the country's capital. The Port Harcourt International Airport is about 7km northwest from the community. The community is along the Bonny River having an estimated population of about 111,000 inhabitants as at 2016. The climate in Igwuruta-Ali is usually rainy and dry season. Igwuruta-Ali is situated at longitude and latitude 4.9682° N, 6.9847° E respectively. The indigenes of Igwuruta-Ali are mainly farmers and as such survive from its proceeds. The analysis was carried out at the Haematology Laboratory of Medical Laboratory Science Department, Rivers State University, Port Harcourt.

2.3 Study Population

A total of 120 subjects [45 males and 75 females] within the age range of 10-70 years were randomly selected based on convenient sampling method for the study and a total of 103 subjects were confirmed to be positive for *Helicobacter pylori* and were part of the study.

2.4 Eligibility of Subjects, Ethical Clearance and Informed Consent

Non-indigenes of Igwuruta-Ali were excluded and indigenes who have not been residing in Igwuruta-Ali for a period of not less than one year were also excluded from the study. Informed consent was obtained from subjects who were apparently healthy prior to enrolment. Ethical clearance was given by the Ethics Committee of the Department of Medical Laboratory Science, Rivers State University. The Community Leaders of Igwuruta-Ali gave us verbal approval to carry out the study on their kinsmen.

2.5 Sample Collection

4 ml of blood was collected by venipuncture into a K_3EDTA anticoagulated (at a concentration of 1.2 mg/ml), with sample container already labelled with patient's name, sex and age. Analysis was carried out within two hours of sample collection.

2.6 Sample Analysis

For the serological detection of *Helicobacter pylori*, the test is based on the principle of double antigen-sandwich technique. When an adequate volume of test specimen antibody of *Helicobacter pylori* present in commercially prepared anti-sera binds to red cell antigen(sensitization), it results in an observable clumping of red cells(agglutination). Presence of agglutinated red cells indicates *Helicobacter pylori* infection. This one step test for the qualitative detection of IgG antibodies of H. pylori in the serum was done using Rapid Diagnostic Test Kit (ACON, USA). A drop of patient plasma is placed in the specimen well of the test cassette and then one drop of buffer is added too on same specimen well. 15 minutes after, the result was read. The burgundy colored T band indicates a positive result; absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immune-complex conjugate regardless of the presence of any antibodies to *Helicobacter pylori*. Otherwise the test is invalid.

Haemoglobin electrophoresis was carried out with method as described by Brown [10]. A small quantity of haemolysate of venous blood from each of the subjects was placed on the cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris/EDTA/Borate buffer at a pH of 8.9. The electrophoresis was then allowed to run for 15 to 20 minutes at an electromotive force (emf) of 160V. The results were read immediately. Haemolysates from blood samples of known haemoglobin (i.e. AA, AS, AC) were run as controls at the same time.

Red cell phenotyping was carried out with standard tube techniques as described by Judd [11] and Brecher [12]. For ABO blood phenotyping, a drop of anti-A, anti-B, and anti AB (Biotec, Ipswich, UK) each was placed in clean test tubes labelled 1, 2, 3. To each tube was added a drop of 5% red blood cell suspension in saline. The contents were gently mixed together and centrifuged for 30 seconds at 1000 g. The cell buttons were re-suspended and observed for agglutination. Agglutination of tested red cells constituted positive results. A smooth cell suspension after re-suspension followed by a microscopic confirmation constituted negative test results.

2.7 Statistical Analysis

Data were analyzed to obtain percentage distribution using Microsoft excel packages for the various parameters. Data are represented in Tables and Figures.

3. RESULTS

3.1 Demographic Details of Participants

A total of 120 subjects including 45 males and 75 females within the age range of 10-70 years were recruited for this research in Igwuruta-Ali, Rivers State, Nigeria within the period of July and September 2018. The subjects were screened for *Helicobacter pylori* infection and then after their ABO and Rhesus blood group type and haemoglobin variants were screened.

3.2 Distribution of Male and Female Subjects in Relation to *Helicobacter pylori* Infection

Sero-positivity for *Helicobacter pylori* was observed in 103 subjects of which 37 were male and 66 were female (64.1% and 35.9% respectively) and absent in 17 subjects of which 8 were male and 9 were female (47.1% and 52.9% respectively). The graphical representation can be seen in Figure 1.
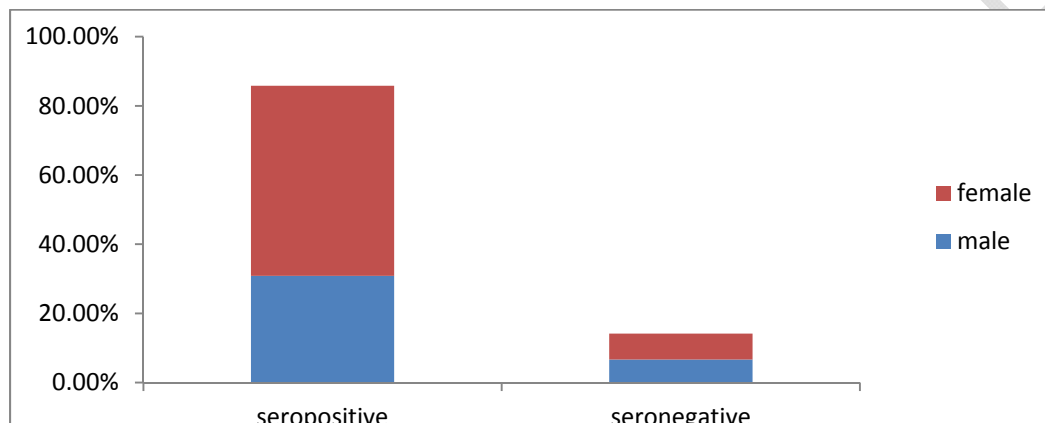

Figure 1. Percentage Distribution of Male and Female Subjects in Relation to *Helicobacter pylori* Infection

3.3. Distribution of ABO and Rhesus Blood Group in Relation to *Helicobacter pylori*

The ABO blood group distribution from the total population of study were 28 for A (23.3%), 17 for blood group B (14.2%), 65 for blood group O (54.1%), 10 for blood group AB (8.3%). The graphical representation can be seen in Figure 2.1.

From the total population, the ABO blood group distribution amongst sero-positive individuals were 23 for A blood group (22.3%) in which 6 were male and 17 were female; 14 for blood group B (13.6%), 7 were male and 7 female; 9 for AB blood group (8.7%), 6 were male and 3 female; 57 for O blood group (55.3%), 19 were male and 39 female. The ABO distribution amongst sero-negative individuals were 5 for blood group A (29.4%); having 1 male and 4 female, 3 for blood group B (17.6%); having 3 males and no female, 1 for AB blood group (5.9%) having 1 male and no female, 8 for O (47.1%), having 3 males and 5 females. The graphical representation can be seen in Figures 2.2 and 2.3.

The Rhesus D positive blood group individuals in the total population were 103 while Rhesus D negative individuals were 17. Amongst the Rhesus D positive individuals, were 37 (30.8%) *Helicobacter pylori* sero-positive individuals, and 66(55%) *Helicobacter pylori* sero-negative subjects. Among the Rhesus D negative subjects were 8(6.7%) *Helicobacter pylori* sero-positive individuals and, 9(7.5%) sero-negative individuals. Details can be seen in Table 1.
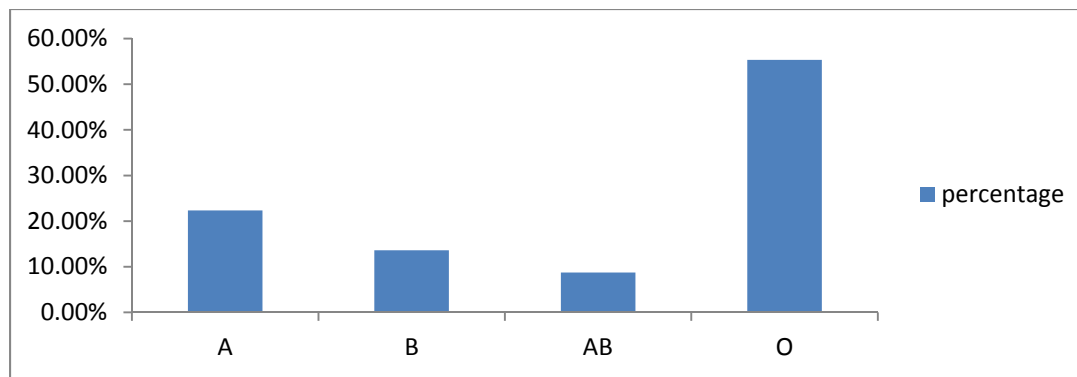
Figure 2.1. Percentage distribution of ABO blood group in sero-positive individuals
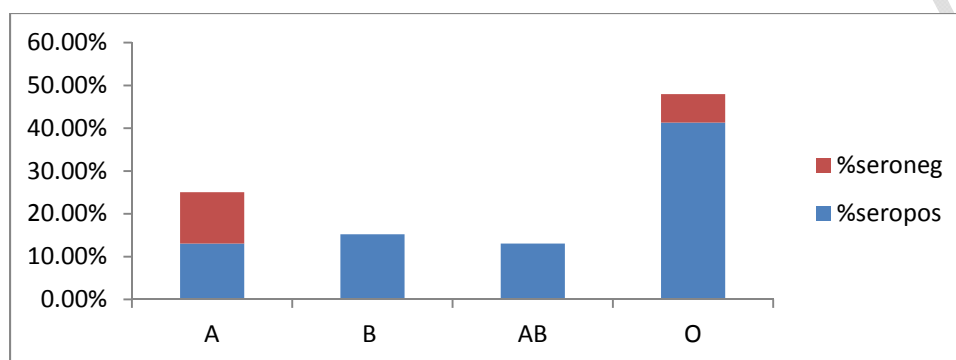

Figure 2.2. ABO blood group distribution among females in relation to *Helicobacter pylori* infection
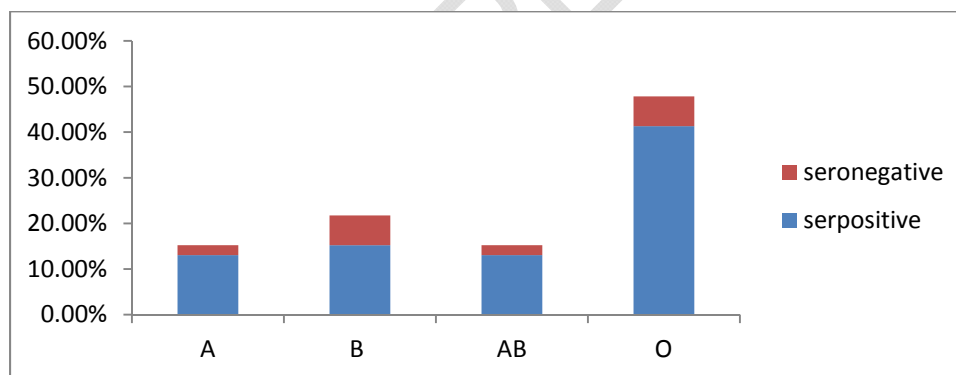

Figure 2.3. ABO blood distribution among males in relation to *Helicobacter pylori* infection

Table 1 Distribution of Rhesus Blood Group in Relation to *Helicobacter pylori* Infection

Parameters	Percentage %
Rhesus positive with *H. pylori*	30.8
Rhesus positive without *H. pylori*	55.0
Rhesus negative with *H. pylori*	6.7
Rhesus without without *H. pylori*	7.5

3.4 The Distribution of Haemoglobin Variants amongst the Total Population

The distribution of haemoglobin variants in the total population were 81 for genotype AA (67.5%), 29 for genotype AS (24.2%), and 10 for genotype SS (8.3%).The graphical representation can be seen in FIgures 3.1

The distribution of haemoglobin variants in sero-positive subjects were 74 for genotype AA (71.84%), having 25 male and 49 female; 23 individuals were of the genotype AS (22.33%), 12 male and 11 female; 6 were of the genotype SS (5.83%), 3 male and 3 female. Haemoglobin variant distribution in sero-negative subjects were 7 (41.2%) of genotype AA, 4 males and 3 females; 6 individuals of genotype AS (35.3%), 2 males and 4 females; 4 of genotype SS (23.5%), 2 males and 2 females. Details are shown in graphical representation (Figures 3.2) and Table 3
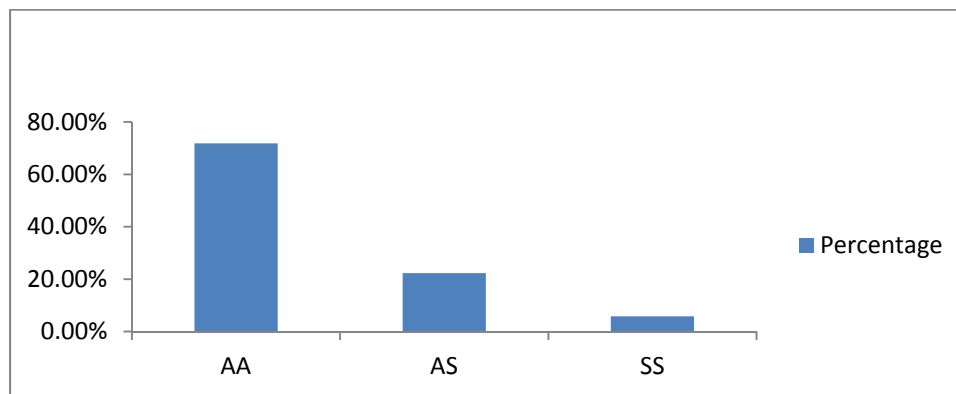

Figure 3.1 Distribution of heamoglobin variants among *helicobacter pylori* seropositive subjects

Table 3. Distribution of haemoglobin variants among females in relation to *Helicobacter pylori* infection

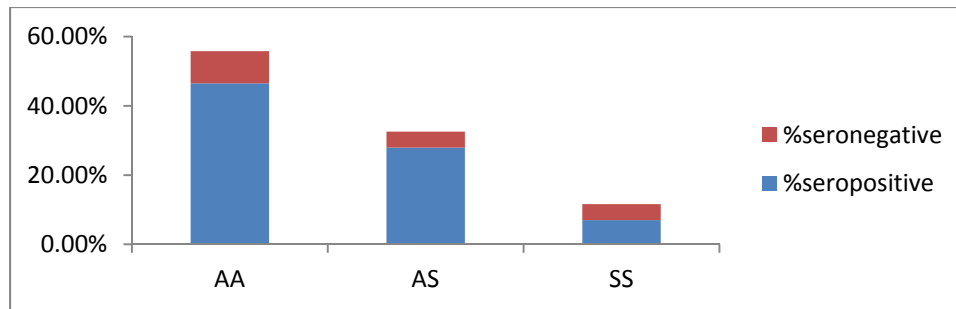| Genotype	Reaction to *H. pylori*	No. (% Distribution)
AA	Sero-positive	74(71.84)
	Sero-negative	7(41.2)
AS	Sero-positive	23(22.33)
	Sero-negative	6(35.3)
SS	Sero-positive	6(5.83)
	Sero-negative	4(23.5)

Figure 3.2: Haemoglobin variants distribution among male subjects in respect to *Helicobacter pylori* **infection**

4. DISCUSSION

Helicobacter pylori is a bacteria that is found in gastric mucous layer or adherent to the epithelia lining of the stomach. It causes more than 90% of duodenal ulcer, up to 80% of gastric ulcer, and gastritis [1]. This research carried out on 120 individuals of both sexes, shows a higher frequency of *Helicobacter pylori* in females than in males (64.1% and 35.9% respectively). This study disagrees with a research survey conducted by [11], that showed percentage distribution of *Helicobacter pylori* among males and females (84.5% and 58.9% respectively).

The ABO blood group contains antigens on red blood cellular surfaces that confer advantage of resistance against some infectious disease. That an individual have a particular surface antigen present on the surface of his/her red cells can help in associating a particular blood group with a disease /infection based on the prevalence of such disease/infection that is dominant in that blood group of the population under study.

The results of this study showed an association between ABO blood groups and *Helicobacter* pylori infection, in which blood group O has a greater tendency towards infection and blood group AB to non-infection. These findings are in agreement with that of other researchers [13], and disagree with some previous studies which demonstrated that the O blood group did not represent a risk factor for *Helicobacter pylori* infection [14].

The Rhesus blood group is an immunological important blood group system second to the ABO blood group. Although its association with helicobacter pylori infection have been widely overlooked, however a study carried out by [4] shows that over 95% of the individuals in the population, who tested positive to *Helicobacter pylori* were Rhesus D positive, and 5% were Rhesus D negative. This study shows that 30.8% Rhesus D positive patients and 6.67% Rhesus D negative patients were positive for *Helicobacter pylori*, and therefore showed no strong differences between those that were positive and negative, indicating that the presence of *Helicobacter pylori* is not associated with Rhesus factor, which is in agreement with a previous study by [15].

Amino acid sequences of the various haemoglobin variants are not the same, these differences forms the basis for the different haemoglobin variants. There is paucity of information on the association of *Helicobacter pylori* infection with haemoglobin variants, however this study shows that there was a higher frequency of haemoglobin genotype AA(71.84%) followed by haemoglobin genotype AS(22.33%) and haemoglobin genotype SS(5.83) among sero-positive individuals.

5. CONCLUSION

Conclusively, O blood group individuals are more susceptible to *Helicobacter pylori* infection and they have more cellular and immunological response to it (expressed by sero-positivity) than other ABO blood groups (group B in particular), while no significant differences between Rhesus D positive and Rhesus D negative patients were seen. Also, haemoglobin AA individuals were mostly affected in Igwuruta-Ali and females were more prone to *helicobacter pylori* infection, probably due to their abstinence from food while trying to "watch their weight", as excessive gastric acid in the stomach mucosa contributes to the ulceration.

CONSENT AND ETHICAL CLEARANCE
Informed consent was obtained from apparently healthy subjects prior to enrolment upon ethical clearance by the Ethics Committee of the Department of Medical Laboratory Science, Rivers State University.

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