DISTRIBUTION OF ABO AND RHESUS BLOOD GROUPS AMONG COVID-19 POSITIVE SUBJECTS IN RIVERS STATE, NIGERIA

Abstract

Coronavirus-2019 (COVID-19) is a novel disease that is ravaging ~~the~~ society today. It has been reported that individuals with some blood group antigens are more predisposed or susceptible to certain diseases. This study was carried out to determine the distribution of some blood group antigens among~~Coronavirus-2019 (COVID-19)~~COVID -19-infected individuals. The study involved one hundred (100) subjects, comprising 75 males and 25 females~~subjects,~~ within the ages of 20 and 60 years, who were infected with COVID-19, and receiving treatment ~~fat~~at the COVID-19 Isolation Centre. The bio-data of the subjects were obtained using a well-structured questionnaire. Five mililitres (5ml) of blood collected asceptically from each of the subjects who gave informed consentweredispensed into K_3EDTA bottles for the determination of the ABO and ~~Rhesus~~Rhblood groups.The bio-data show that 74% of the subjects were married while 26% were single. Also, 87% of the subjects had university degree or HND, 6% had OND while 7% had only secondary school education, indicating that majority of the subjects were educated. The results from the study showed that the distribution of ABO blood group among the subjects were 75% blood group O, 11% blood group A, 10% blood group B, and 4% blood group AB. Also, 91% of the subjects were ~~Rhesus~~Rhpositive while 9% were ~~Rhesus~~Rhnegative. The results indicate that the blood group antigens found in the subjects were similar to the pattern reported in the population where the research was conducted. It is concluded that the prevalent blood group in this study was blood group O, while the most prevalent ~~rhesus~~ Rhgroup was Rh-D positive. It is recommended that blood groups be considered in the testing menus for the management of COVID-19 infection, since some subjects may have such blood group antigens that can predispose to the infection.

Keywords: Coronavirus, COVID-19, Blood Groups, ABO, Rhesus, Port Harcourt

Introduction

Blood group systems can be defined as one or more antigens that are controlled at a single gene locus, or by two or more very closely linked homologous genes with little or no observable recombination between them (Intharanut and Nathalang, 2018). In other words, a blood group system refers to a group of antigens that are encoded by alleles at a single gene locus or by a number of gene loci that are so closely linked that crossing over between them is very rare or does not occur (Daniel and Reid, 2010). These blood group antigens are either sugars or proteins that are attached to various components in the red blood cell membrane. For example, the

antigens of the ABO blood group are sugars, while those of the Rhesus blood group are proteins (Yelima*et al*., 2019).

The ABO blood group system, which was discovered by Landsteiner in 1900, was the first blood group ever to be discovered and still remains the most clinically significant of all the blood group systems (Mita*et al*., 2014). Landsteiner classified the blood groups based on the antigens, A and B, that he identified on the surface of the blood of his subjects (Groot *et al*., 2020), and the expression of these antigens is determined by the ABO gene located on chromosome 9 (9q34.2) (van Hylckama, 2014).

The ~~Rhesus (Rh)~~Rhblood group system is the most complex, and perhaps the second most studied blood group system (Ewald and Summer, 2016). There are over 50 antigens in the human ~~Rhesus (Rh)~~Rhblood group system but the principal ~~Rhesus~~Rhantigens of medical importance are D, C, E, c and e (Gundrajukuppam*et al*., 2016). A person with ~~Rhesus~~Rhantigen is referred to as ~~Rhesus~~Rhpositive while individuals lacking the antigen are ~~Rhesus~~Rhnegative (Jahanpour*et al*., 2017). The ~~Rhesus~~Rhantigen was so named because a related antigen was first discovered in Rhesus monkeys (Pratima*et al*., 2013).

Inheritance of ~~Rhesus~~Rhantigens is determined by a complex of two closely linked genes, namely RHD and RHCE, which are found on the short arm of chromosome 1(1p36.11) and positioned in opposite directions to each other (Vege and Westhoff, 2019). One of the genes encodes the protein carrying D antigen (~~Rhesus~~RhD); the other encodes the protein carrying C or c and E or e antigen (~~Rhesus~~RhCE). In the ~~Rhesus~~Rhsystem different antigen combinations (or haplotypes) are observed, with the following as the most common: Dce (Ro, Rho), DCe (R1, Rh1), DcE (R2, Rh2), DCE (Rz, Rz), ce (r,rh), cE (r", hr") (Wafi*et al*., 2016).

The Coronaviridae is a family of enveloped, single-stranded, positive-sense RNA viruses with the largest genome among RNA viruses. The club-shaped spike (S) proteins on their surface make them look like a crown. Other structural proteins include the haemagglutinin esterase (HE) (only found in some of them), small membrane (E), membrane (M), nucleocapsid (N) and internal (I) protein (Weiss and Leibowitz, 2011). The most common symptoms at thestart of COVID-19 are fever, cough, and fatigue. Other symptoms include sputum production, headache, rhinorrhoea, sneezing, sore throat, haemoptysis, diarrhoea, dyspnoea, and lymphopenia, with an incubation period of about 5 days, and a time frame between the onset of COVID-19 symptoms and death ~~being~~isabout 6 to 41 days (Wang *et al*., 2020).

The presence or absence of the red blood cell antigens has been implicated in the susceptibility and pathogenesis of some bacterial, viral and parasitic infections, such as Vibrio cholera, Escherichia coli (Croxen*et al*., 2013), Helicobacter pylori (Jaff, 2011), Norovirus (Barb*éet al*., 2018), Rotavirus and Human Immunodeficiency Virus (HIV) (Cooling, 2015).

This study seeks to determine the distribution of ABO and ~~Rhesus~~Rhblood group antigens among COVID-19 positive subjects, a piece of information that is scanty in our area of study.

Methodology

A. Study Design

A cross-sectional study design was used for this study, comprising subjects who were positive for COVID-19, and were on admission and isolation at the Rivers State COVID-19 Isolation Centre, Eleme Local Government Area, Rivers State, Nigeria.(Please, indicate the duration of the

study)

B. Study Area

This study was carried out in the Rivers State COVID-19 Isolation Centre in Eleme local government area (LGA), Rivers State, Nigeria. The facility was the only facility used in the testing and management of COVID-19 in the State.

Eleme Local Government Area is one of the twenty-three Local Government Areas inRivers State, South-South, Nigeria, witha population of 190,884 as ~~at~~of the 2006 National Census, and is located between longitude 7^0 and 7^0 15" East of the Meridian and latitudes 4^0 60" and 4^0 35" North of the Equator. The total area of Eleme is about 138 square kilometers (km^2), and is bordered on the North by Obio/Akpor Local Government Area and Oyigbo Local Government Area, on the East by Tai Local Government Area, on the South by Ogu/Bolo Local Government Area and Okrika Local Government Area (Moses *et al.*, 2020).

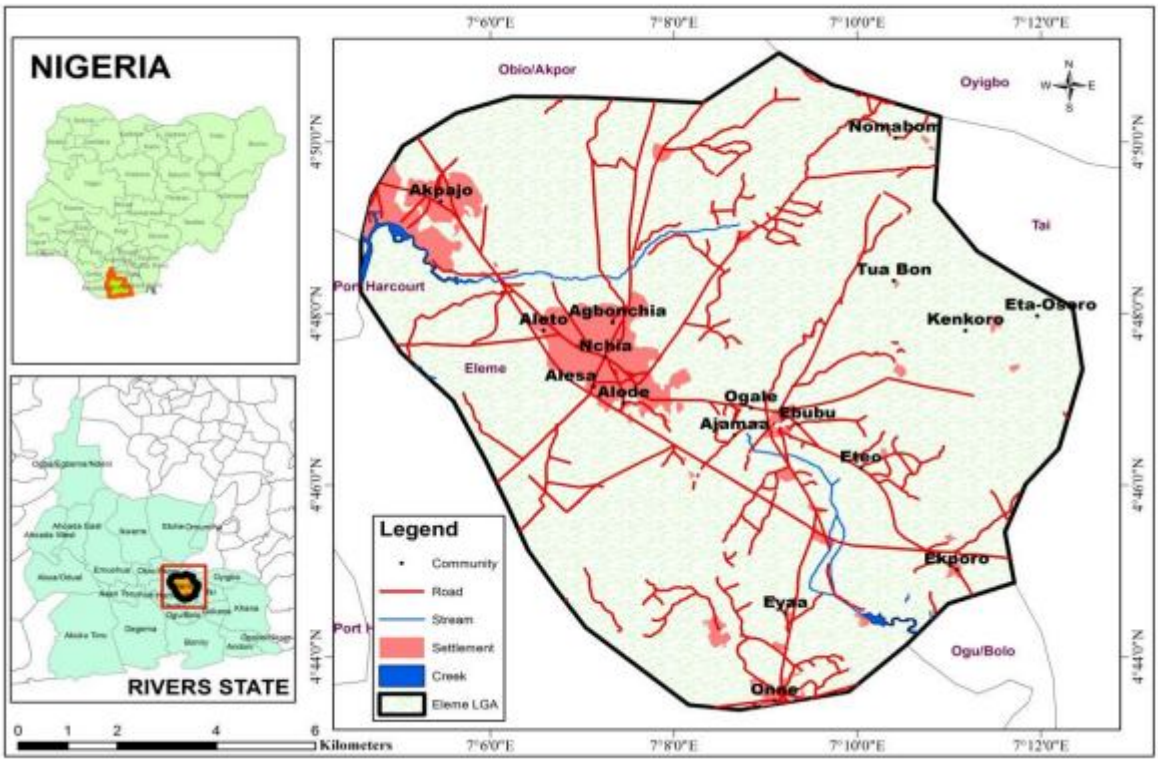

Figure 1: Location of Eleme Local Government Area (Moses *et al*., 2020).

C. Study Population

This study involved a total of 100 subjects, comprising 75 male and 25 female subjects, who were ~~within~~between theages of 20 and 60 years and who were tested and ~~were~~ confirmed positive for COVID 19. A convenient sample size was used to determine the sample size for this study, because COVID-19 is a novel disease that is still infecting several people globally, and the prevalence in the study area was not yet established ~~as~~atthe time of this study.

The subjects gave their informed consent before being recruited for this study. The demographic data of the subjects were obtained using a structured questionnaire.

D. Eligibility Criteria

(a) Inclusion Criteria

Subjects within the age bracket of 20 to 60 years, who tested positive ~~to~~forCOVID-19, confirmed for the infection, and admitted in the isolation center were used for this study.

(b) Exclusion Criteria

Subjects who were unconscious or those who were experiencing severe difficulty in breathing as a result of COVID-19 were excluded from the study (because they were not able to give informed consent), as well as those who did not give their consent to participate in the study.

E. Ethical Consideration

Ethical approval for this study was obtained from the Research Ethics Committee of the Ministry of Health, Port-Harcourt, Rivers State, Nigeria.

F. Sample Collection

Five mililitres (5mls) of whole blood sample were collected from each subject by venipuncture technique and dispensed into ethylene diamine tetraacetic acid-anticoagulant bottles; the blood was properly mixed with the anticoagulant by several gentle inversions, which was followed by the analysis of ABO and~~Rhesus~~Rhblood groups.

G. Sample Analysis

The Determination of ABO and RhD Blood Groups was done using PRO-MED for AB (Lot Number B 191007 and Lorne Laboratories Ltd, Lot Number A200101; Expiry Date: 09/2021)

Method: The slide method was used according to themanufacturer's instruction.

Principle: The ABO and Rh blood grouping system is based on agglutination reaction. When red blood cells carrying one or both antigens are exposed to the corresponding antibodies, they interact with each other to form visible agglutination or clumping.

Procedure:

A clean and dry glass slide was divided into four sections with a glass marking pencil. The sections were labelled as Anti-A, Anti-B and Anti-D to identify the antisera. Then, one drop of anti-A serum, one drop of anti-B serum, and one drop of anti-D serum were placed in the center of the corresponding sections of the slide. One drop of the blood sample to be tested was added to each antisera. Each mixture (antiserum and blood) was mixed together with the aid of separate sticks, and the contents weretilted and examined for agglutination after two minutes.

Result Interpretation:

Positive (+) result: Little clumps of red cells were seen floating in a clear liquid, indicating a positive result.

Negative (-) result: Red cells were floating homogenously in a uniform suspension, indicating a negative result.

Confirmation of ~~Rhesus~~RhD negative results: The blood sample was used to prepare 5% red cell suspension. One drop of theanti-D antiserum was dispensed into a clean test tube, andthen one drop of the cell suspension was added to it. The mixture was incubated at 37^{o}C in a water bath for 30 minutes. Then themixture was centrifuged, and then, using a Pasteur pipette, the mixture was placed on a clean slide and viewed under the microscope using X10objective. There was no

agglutination, confirming the ~~Rhesus~~ RhD negative status.

H. Data Analysis

The data generated from the study were analysed using Statistical Package for Social Sciences (SPSS),(Please, cite the SPSS correctly.As an example, this is the citation for IBM SPSS version 20: IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) and were expressed in tables. A comparison of the proportion of subjects with the different blood groups was done using Pearson's Chi-Square, and p-values less than 0.05 were considered statistically significant.

Results

Table 1: Demographic Characteristics of Subjects

The number of male subjects in this study was 75, while that of female was 25, giving a total of 100. Seventy-four of these subjects were married while 26 were single. Eighty-seven of these subjects had auniversity degree or HND from a polytechnic, 6 had OND while 7 had O/Level (SSCE).

Variable	Frequency (%)
Gender	
Male	75 (75)
Female	25 (25)
Marital Status	
Married	74 (74)
Single	26 (26)
Educational Qualification	
Degree/HND	87 (87)
OND/NCE	6 (6)
SSCE	7 (7)

Table 2: Percentage Distribution of COVID-19 Positive Subjects Based on ABO Blood Groups

Table 2 describes the distribution of subjects according to ABO blood groups. Eleven (11) subjects had blood group A, 10 had blood group B, 4 had blood group AB while 75 had blood group O.

Blood Group	Number of Subjects	Percentage (%)
A	11	11
B	10	10
AB	4	4
O	75	75
Total	100	100

χ^2-value= 134.480, p-value= <0.001

Table 3: Percentage Distribution of COVID-19 Positive Subjects Based on Rh Blood Group

From the analysis, ninety-one (91) subjects were Rh positive while 9 subjects were Rh negative.(Please, be specific on the type of Rh blood group of your subjects. This should apply to your tables and in the discussion section)

Blood Group	Number of Subjects	Percentage (%)
Rh Positive	91	91
Rh Negative	9	9
Total	100	100

χ^2-value= 67.240, p-value= <0.001

Table 4: Distribution of ABO Blood Groups of COVID-19 Positive Subjects Based on Gender

The number of male subjects with blood group A, blood group B, blood group AB and blood group O were 6, 10, 3 and 56 respectively, while the number of female subjects with the A, B, AB and O blood groups were 5, 0, 1 and 19 respectively.

Blood Group	**Number of Male Subjects (%)**	**Number of Female Subjects (%)**
A	6 (8)	5(20)
B	10 (13)	0(0)
AB	3 (4)	1(4)
O	56 (75)	19(76)
Total	75 (100)	25(100)

Table 5: Distribution of Rh-Blood Group of COVID-19 Positive Subjects Based on Gender

The numbers of male subjects who are Rh-positive were 68, while those who are Rh-negative were 7, with percentages of 91 and 9,respectively. Similarly, the numbers of female subjects who are Rh-positive were 23, while those who are Rh-negative were 2, with percentages of 92 and 8, respectively.

Blood Group	Number of Subjects	Percentage (%)
Male		
Rh-Positive	68	91
Rh-Negative	7	9
Female		

Rh-Positive	23	92
Rh-Negative	2	8

Discussion

This study assessed the distribution of some blood group antigens among COVID-19 infected individuals. The results from this study indicated that there were more male subjects who were positive ~~with~~forCOVID-19 than female subjects. This result is in consonance with a previous report by Wenham *et al.*(2020), which reported that more males than females were prone to COVID-19 infection. It is believed that this happens because women produce more interferon (type 1) than men; interferon is known to be vital in early response during COVID-19 infection (Trovilet-Assant*et al.*, 2020). Furthermore, estrogen, which is known to be produced in women, has also been reported to confer some protection against infections by way of increasing immune responses by T-cells, raising the production of antibodies, increasing neutrophil count as well as producing cytokine via monocytes and macrophages (Rehman*et al.*, 2021).

Data from the study also indicate that 74 subjects were married while 26 subjects were single. It has been reported that married people tend to seek remedies for COVID-19 because of the fear of infecting other family members (Konopinska*et al.*, 2021). This finding agrees with the work of Cvertkovit*et al.*, 2022.

All the subjects recruited for the study had academic achievements (SSCE, University degree or HND from a Polytechnic) and are thus literates. This indicates a high level of literacy among the

subjects. This finding is in agreement with the work of Adefisayo*et al*. (2021), which reported a similar high percentage of educated subjects in their study. This finding is probably because education increases awareness of ~~a~~ disease (Olawuni*et al*., 2020). Oleribe*et al*. (2020) had reported that the high educational attainment in their study may be a reflection of the awareness and understanding of the infection by the subjects.

The data from this study showed that a greater percentage of the subjects (75%) had blood group O. This finding could be attributed to the fact that blood group O is more prevalent (or common) in Nigeria (Anifowoshe*et al*., 2017). However, the observed high frequency of blood group O in the study population does not reflect the susceptibility of the population to COVID 19 infection; the high prevalence of this blood group O could be an evolutionary adoption to confer resistance to diseases that affect our population (Arend, 2018). It has been reported also that blood group O is associated with a decreased risk of COVID-19 infection (Gerald *et al.*, 2020). SARS-COV-2 which includes the corona virus, has spike proteins which enable it to gain entry into and infect the host cell (Wrapp*et al*., 2020).

The findings of this study agree with that of Kwaghe*et al*. (2021) who conducted a study that evaluated the ABO blood group distribution of COVID-19 patients at the University of Abuja Teaching Hospital, stating that no relationship was found between COVID-19 and ABO blood group. On the contrary, a study by Kotila*et al*. (2021) reported an association between theABO blood group and COVID-19, stating that blood group O was less represented among the patients, while blood groups B and AB were significantly more represented. From this study, the COVID-19 positive subjects with blood group O constituted the largest fraction followed by blood groups A, B and then AB; this report is attributed to the distribution pattern of the ABO blood groups

among the subjects. This report follows the trend of normal ABO blood group distribution (blood group O>A>B>AB). This normal ABO trend has been reported by several authors (Udomah*et al*., 2015; Obeagu*et al*., 2019; Christian *et al*., 2020).

This study showed that 91% of the subjects were ~~Rhesus~~Rh positive (which type of Rh?) while 9% were ~~Rhesus~~Rh negative. In other words, a significant majority of the subjects in this study were ~~Rhesus~~Rh Positive. This finding is in agreement with the work of Arak *et al*. (2020), who reported that 96.42% of their subjects were ~~Rhesus~~ Rh positive. ~~Rhesus~~Rh-negative blood group has been reported to be at lower risk of viral infections as well as the severity of illness and death (Zietz*et al.*, 2020). Other studies have indicated similar findings. In their study, Taha*et al*. (2020) reported that individuals with blood group A ~~Rhesus~~Rh D positive and blood group AB ~~Rhesus~~ Rh D positive are more susceptible to COVID-19 infection than individuals with blood group O-whether ~~Rhesus~~Rh D positive or negative still had a significantly lower risk of COVID-19 infection.(The statement in a coloured font is not clear. At one point, you were talking about increased susceptibility and at another, lower risk, implying decreased susceptibility. Please, rewrite the statement).The mechanism of ~~Rhesus~~Rh factor in COVID-19 is not clearly understood, necessitating the need for further research to confirm the role of ~~Rhesus~~Rh blood group in the susceptibility of individuals to COVID-19 infection (Saify*et al.*, 2021). (Please, rephrase this statement. What do you mean by "the mechanism of Rh factor?" You may wish to rephrase it thus: the mechanism by which Rh factor confers increased susceptibility to COVID-19 is not completely understood, therefore, further research is advocated)It has been proposed that the observation in this study may be due to possible interaction between the ~~Rhesus~~Rh factor and SARS-COV-2, but there is need for further research to confirm this (Anderson *et al.*, 2022). In this study, the COVID-19 positive subjects who were~~with~~ Rh D-positive constituted the

largest fraction; this report is attributed to the distribution pattern of the ~~Rhesus~~Rh blood group among the subjects. This study has revealed ~~a~~the trend in ~~the~~~~Rhesus~~Rh distribution in Rivers State to be Rh-positive > Rhesus-negative in consonance with regular Rhesus blood group distribution in Nigeria. (Erhabor*et al.*, 2010; Anifowoshe*et al*., 2017).

Conclusion

The findings from this study revealed that more of the subjects had blood group O in the study locality, followed by blood groups A, B and AB. The results from this study also revealed that more of the subjects had ~~Rhesus~~Rh D positive in the study locality.

It is recommended that more research should be carried out to proffer abetter understanding of the mechanism involved in the interactions of blood group antigens and COVID-19 infections in order to help in the treatment and management of the disease.

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