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)-infected individuals. The study involved one hundred (100) subjects, comprising 75 male and 25 female subjects, within the ages of 20 and 60 years, who were infected with COVID-19, and receiving treatment fat 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 consent were dispensed into K_3EDTA bottle for the determination of the ABO and Rhesusblood 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 positive while 9% were Rhesus negative. 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 group 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).

Comment [MF1]: The study is not the first globally deal with ABO groups in infected subjects except is new in scanty in your area of study ,so I prefer to add something new to this work ,I suggest to ask patients about :
-what is the chronic infection they had previously ?
-what are the type of vaccines they had received from childhood until now?
-compare CBC in infected subjects
-ask subjects if the y use sterilizer agents usually ,sometimes or often.

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Comment [MF5]: Introduction

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) blood 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) blood group system but the principal Rhesus antigens of medical importance are D, C, E, c and e (Gundrajukuppam*et al*., 2016). A person with Rhesus antigen is referred to as Rhesus positive while individuals lacking the antigen are Rhesus negative (Jahanpour*et al*., 2017). The Rhesus antigen was so named because a related antigen was first discovered in Rhesus monkeys (Pratima*et al*., 2013).

Inheritance of Rhesus antigens 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 D); the other encodes the protein carrying C or c and E or e antigen (Rhesus CE). In the Rhesus system 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

Comment [MF6]: viruses

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 start 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 the time-frame between the onset of COVID-19 symptoms and death being about 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 (Barbé*et al*., 2018), Rotavirus and Human Immunodeficiency Virus (HIV) (Cooling, 2015).

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

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Methodology

A. Study Design

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.

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 Rivers State, South-South, Nigeria, witha population of 190,884 as at 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).

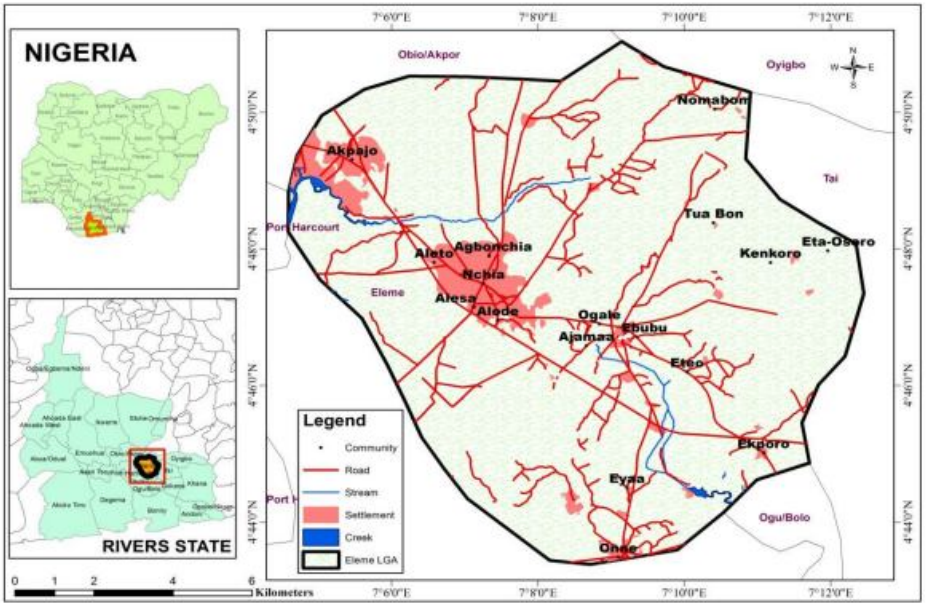

Figure 1: Location of Eleme Local Government Area (Moses *et al.*, 2020).

C. Study Population

This study involved a total of 100 subjects, comprising 75 male and 25 female subjects, who were within ages 20 and 60 years and who were tested and were confirmed positive for COVID 19. 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 the time of this study.

Comment [MF8]: not enough

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 COVID-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 diaminetetraacetic 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 blood 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 manufacturer'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 labeled 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 tilted and examined for agglutination after two minutes.

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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 D negative results: The blood sample was used to prepare 5% cell suspension. One drop of anti-D antiserum was dispensed into a clean test tube, then 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 mixture was centrifuged, and then, using a Pasteur pipette, the mixture was placed on a clean slide and viewed under the microscope using X10. There was no agglutination, confirming the Rhesus D negative status.

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H. Data Analysis

The data generated from the study were analysed using Statistical Package for Social Sciences (SPSS), and were expressed in tables. Comparison of 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.

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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 university degree or HND from a polytechnic, 6 had OND while 7 had O/Level (SSCE).

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Variable	Frequency (%)
Gender	
Male	75 (75)
Female	25 (25)
Marital Status	
Married	74 (74)
Single	26 (26)

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Educational Qualification	
Degree/HND	87 (87)
OND/NCE	6 (6)
SSCE	7 (7)

Comment [MF14]: I has been prefer to ask patients about :
-what is the chronic infection they had previously ?
-what are the type of vaccines they had received from childhood until now?

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

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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 (%)** **female** **male**
A	11	11
B	10	10
AB	4	4
O	75	75
Total	100	100

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χ^2-value= 134.480, p-value= <0.001

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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.

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

$\square^2$-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.

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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)

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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 COVID-19 than female subjects. This result is in consonance with a previous report by Wenham *et al.*(2020), which reported that more male than female 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 production of antibody, 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 singles. It has been reported that married people tend to seek remedy 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 decrease risk of COVID-19 infection (Gerald *et al.*, 2020). SARS-COV-2 which includes the corona virus, has spike proteins which enables it to gain entry into and infect the host cell (Wrapp*et al.*, 2020).

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The findings of this study agrees 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 ABO 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 group 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).

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This study showed that 91% of the subjects were Rhesus positive while 9% were Rhesus negative. In other words, a significant majority of the subjects in this study were Rhesus Positive. This finding is in agreement with the work of Arak *et al*. (2020), who reported that 96.42% of their subjects were Rhesus positive. Rhesus 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 D positive and blood group AB Rhesus D positive are more susceptible to COVID-19 infection than individuals with blood group O- whether Rhesus D positive or negative still had a significant lower risk of COVID-19 infection. The mechanism of Rhesus factor in COVID-19 is not clearly understood, necessitating the need for further research to confirm the role of Rhesus blood group in the susceptibility of individuals to COVID-19 infection (Saify*et al.,* 2021). It has been proposed that the observation in this study may be due to

possible interaction between the Rhesus 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 with Rh-positive constituted the largest fraction; this report is attributed to the distribution pattern of the Rhesus blood group among the subjects. This study has revealed a trend in the Rhesus 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).

Comment [MF30]: Not scientific discussion

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 D positive in the study locality.

It is recommended that more research should be carried out to proffer better 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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Comment [MF31]: The percent of recentness is 66.66 % ,good .

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Comment [MF32]: correct

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