ORIGINAL ARTICLE

Association of ABO and RH Blood Group with Dengue Infection: A Cross-Sectional Study at a Tertiary Care Hospital in Rawalpindi

Jawairia Zarrar[1*], Sunila Tashfeen[1], Samra Shahid[2], Saima Bashir[2], Imtenan Sharif[3], Zunera Sajjad[1], Azka Zulfiqar[1]

ABSTRACT

Objective: To determine an association of ABO and Rh blood group with dengue infection susceptibility.

Study Design: Cross-sectional study.

Place and Duration of Study: This study was conducted at Department of Pathology, Army Medical College in alliance with Pak-Emirates Military Hospital (PEMH) Rawalpindi, Pakistan from November 2022 to December 2022.

Methods: The study was conducted on two groups, one group had 102 dengue positive patient and the other group had 928 normal sample population presenting to the hospital. We applied Fisher's Exact/*Chi-square* test. Samples were collected by convenience sampling technique. Patients with any other concomitant infection such as malaria or typhoid the study were excluded from group 1. ABO and Rh blood grouping done by tube method and verified by forward tile method. Serological testing for dengue for presence of IgM and NS1 done by Rapid chromatographic immunoassay test (ICT) method.

Results: The distribution of ABO and Rh blood groups in both the normal population and dengue-positive patient groups was similar. In the normal sample population, the prevalence of O+ blood type was 30.1%, closely aligning with the 38.2% observed in dengue-positive patients. While the 38.2% figure is higher than that of the normal population, it reflects a notable increase. Similarly, other blood group distribution is matched with O- 3.3% in normal sample and 2.0% in dengue positive. Blood group B+ is 28.0% in normal sample and 26.5% in dengue positive patient. Blood group A+ being 22.4% in normal sample population and 24.5% in dengue positive patients.

Conclusion: According to this study, blood group O has a slightly higher percentage of dengue patients compared to its prevalence in the general population. The prevalence of other blood groups in dengue patients closely mirrors their prevalence in the general population. Further research is needed to assess if HLA and ABO are dependent or independent risk factors and if certain blood groups have a higher chance of DENV infection.

Keywords: *ABO Blood Group, Dengue, Rh-Hr Blood-Group System.*

[1]*Department of Hematology/Pathology* [2]
Army Medical College Rawalpindi, Pakistan
[3]*Department of Community Medicine*
Quetta Institute of Medical Sciences (QIMS) Quetta, Pakistan
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Correspondence:
Dr. Jawairia Zarrar
Department of Hematology
Army Medical College Rawalpindi, Pakistan
E-mail: tjawesome@hotmail.com
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Introduction

The dengue virus is an Arbovirus belonging to the family, Flaviviridae. Dengue virus is caused by an Aedes aegypti mosquito's bite, which transmits the Arbo virus infection having 4 serotypes which are as follows DENV-1, DENV-2, DENV3 and DENV4.[1] This virus can infect a person four times owing to 4 different serological types. A secondary infection with a different type of serotype may lead to a very serious form of dengue with more complication and a higher chance of fatality. Dengue virus is present in

higher densities in Southeast Asian and Pacific regions like Latin America making it endemic in more than hundred countries.[1] It is more common in densely populated cities and towns. Dengue disease has wide range of presentations. Patient may have serious flu symptoms, bone-breaking fever, and pain behind eyes or may be asymptomatic. Very severe infection can have complication e.g., Dengue hemorrhagic fever and Dengue shock. Various factors play key role in the disease severity, infection, tendency and its outcome, one of which is the ABO blood group and Human Leukocyte antigen haplotype. Blood group has shown to have influence and susceptibility to develop different infectious and viral diseases.[2] It is being proposed that dengue infection is influenced by various factors like age, nutrition, gender, viral strains and reinfection. Blood group of an individual can be easily tested in hospital and it would be of great clinical significance if found to predict the prevalence and disease susceptibility in dengue infection.[3] As there is no specific treatment for Dengue disease, most of the treatment is supportive. Hence, timely and early detection of the disease helps in well-timed treatment and lowers the mortality associated with dengue disease. This study aims to investigate the association of dengue infection to various major blood groups admitted at tertiary care hospital. This study aims to investigate whether there is an association between ABO and Rh blood groups and dengue infection. By identifying such associations, we can enhance our understanding of risk factors for dengue. If a significant correlation is found, it could facilitate early detection and intervention, allowing healthcare providers to identify individuals at higher risk based on their blood group. This proactive approach may lead to timely diagnosis and treatment, ultimately improving patient outcomes and reducing the disease's impact.[4] Dengue has emerged as one of the leading causes of hospitalization in Pakistan. There have been researches done in Pakistan which show an association or link between human leukocyte antigen haplotype to severe form of dengue, like dengue hemorrhagic fever or dengue shock syndrome.[5] Another recent study observed comparative study of different blood cell parameter

in dengue.[6] In summary, this study aims to investigate the association between ABO and Rh blood groups and susceptibility to dengue infection.

Methods

This is a cross-sectional study carried out in November and December 2022 at the Department of Hematology, Army Medical College in alliance with Pak-Emirates Military Hospital Rawalpindi, Pakistan. The Ethical Review Committee granted permission to carry out the research. The ERC number is ERC/ID/238. Two group of patients were considered for comparison. First group consisted of patients who presented to the hospital during the month of November and December who tested positive for dengue by serological method of ICT to detect either IgM or NS1. One hundred and two patients of all ages and gender with dengue admitted to the Military Hospital were included. Patients with concomitant infections such as Malaria and Typhoid were excluded from the study. Second group consisted of patients presented to hospital for blood donation and were dengue negative. Their blood grouping was done to assess the sample population blood group presenting to this single center. Three ml of blood in ethylene diamine tetra acetic acid (EDTA) tubes was collected, of 102 patients with clinical symptoms and serologically positive (IgM/NS1) dengue patients and normal sample population of 928. All patients with dengue (NS1 and IgM positivity) were serologically confirmed using the Rapid Card Method and recorded. The names and detail of the patients were kept confidential. Blood grouping was performed using tile method as well as by tube method. The first technique (forward method) includes blending the patient's red blood cells, cleansed with saline, with commercially generated antiserum containing agents against A, B, and D, all within a test tube. Following this, the mixture is allowed to rest at room temperature and is subsequently subjected to centrifugation. In the second method (reverse method), the patient's serum is combined with commercially procurable reagent red blood cells that belong to recognized A, B, and O groups. After allowing this combination to incubate at room temperature, it is then centrifuged. Agglutination was examined by observing the red blood cell button at the bottom of the test tube. The similar was done

in tile method when 3 drops of blood are taken and every drop is mixed with Anti-A, Anti-B and Anti-D sera separately and the checked for agglutination.[5] We performed Blood grouping for 928 samples admitted in our hospital of random patients and these were used as comparison group for blood group distribution in the particular area. The comparison group will comprise of subjects in which dengue is ruled out, whose blood grouping done. The two groups compared to check the association of dengue to a particular blood group as compared to general sample population presenting to this hospital in month of November and December. Detailed proforma made regarding patient age, gender, CBC, dengue infection status and blood group type. The data entered and analyzed in SPSS 25.

Statistical Analysis

IBM SPSS 25 for windows, Armonk, NY, USA was used to analyze the data. Continuous data was presented in table like age giving a mean and standard deviation. The categorical data was presented as percentages or frequency. The groups were compared using Fisher's Exact/*Chi-square* test considering *P*<0.05 as significant.

Results

Our study included 102 dengue positive patients by convenience sampling in one month's time; their calculated mean value of age with standard deviation was 41.61±13.92 years. As shown in table-1 the packed cell volume (PCV) has a mean and standard deviation of 0.42±0.057 (%) in dengue positive patients. Other parameters like platelets had a mean with standard deviation of 78.60±44.04 ($\times 10^9$/L). The White blood count (WBC) has a mean with standard deviation of 4.04±1.56 ($\times 10^9$/L). The total red blood cell count (TRBC) has mean with standard deviation of 4.99±0.64 ($\times 10^{12}$/L). The hemoglobin values are given in table-1 below.

We demonstrated in table-2 the comparison of blood group ABO and Rh in positive dengue patients and in control group presenting to the hospital who tested negative for dengue. The percentages of A-, A+, B+ and O+ are comparable to each other. The A- is 2.0% in dengue positive group and 2.7% in the normal sample population presenting to the hospital. The table shows that 24.5% of dengue positive patient had A+ blood group, which closely matches the normal sample population presenting to hospital taken in our sample as being 22.4%. The blood group A when compared in positive cases and normal sample population is not significant with a value of 0.448 and a fisher's exact value of 0.592.

Blood group B+ is 26.5% in dengue positive patient and 28% in normal status sample group. The *P*-value 0.365 for blood group B among dengue positive patient is insignificant, with a fisher exact value of 0.471. Blood group O+ has 38.2% presence in dengue positive cases and 30.1% presence in normal sample population. The fisher exact value is 0.291 with a *P*-value for blood group O is 0.228, which is not significant. Similarly, blood group AB also has insignificant *P*-value at 0.423 and a fisher exact value of 0.326.

These evaluations show that the ABO and Rh blood groups in dengue patient included in our study follow the pattern of normal distribution of blood group distribution when compared to our sample population. We can thus say that there is no association between the blood group and dengue infection. Alternately, from these values we can assess that whichever blood group has more frequency in sample population e.g. O+ 30.1%, similar percentage 38.2% of population are dengue positive patient. The blood group AB-, AB+, B- and O- not compared as they have very few samples in dengue positive patients.

The figure.1 also illustrates the distribution of

Table-1: Complete blood picture parameters in Dengue positive patients				
Variables	Mean ± SD	Median	Minimum	Maximum
Age (years)	41.61±13.92	40.50	14	86
PCV (%)	0.42±.057	0.42	0.14	0.53
PLT ($\times 10^9$/L)	78.60±44.04	72.50	8.00	245.00
WBC ($\times 10^9$/L)	4.04±1.56	3.80	2.00	9.80
TRBC ($\times 10^{12}$/L)	4.99±0.64	5.03	3.46	6.72
Hb (g/L)	14.41±1.44	14.35	9.60	17.90

Table-2: Demographic distribution of ABO and Rh blood group in dengue positive patients and Normal sample population

Blood group (ABO &Rh)	Positive for dengue	Normal sample	Total	Fisher's Exact/ Chi-square test	P-value
A -ve	2 (2%)	25 (2.7%)	27 (2.6%)	0.592	0.448
A +ve	25 (24.5%)	208 (22.4%)	233 (22.6%)		
AB -ve	0 (0.0%)	15 (1.6%)	15 (1.5%)	0.326	0.423
AB +ve	5 (4.9%)	77 (8.3%)	82 (8.0%)		
B -ve	2 (2.0%)	33 (3.6%)	35 (3.4%)	0.471	0.365
B +ve	27 (26.5%)	260 (28.0%)	287 (27.9%)		
O -ve	2 (2.0%)	31 (3.3%)	33 (3.2%)	0.291	0.228
O +ve	39 (38.2%)	279 (30.1%)	318 (30.9%)		
Total	102 (100.0%)	928 (100.0%)	1030 (100.0%)		

dengue positive cases in red and normal population sample in green. It shows all the data described in table from in bar chart.
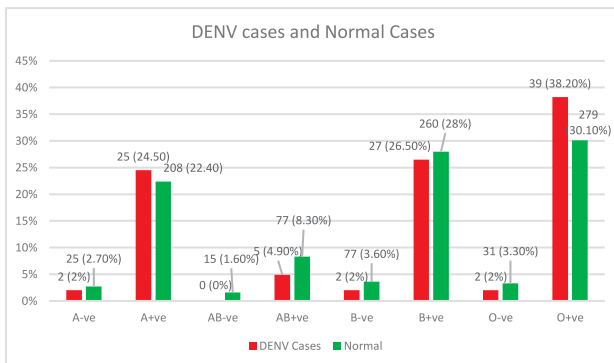

Fig.1: Blood group distribution among DENV and normal sample population blood group percentages depicted in bar chart. Frequencies of cases in brackets not depicted by bar chart

Discussion

High mortality rate and morbidity rates were linked to Dengue infection, especially in patients with secondary complications of the dengue infection like the hemorrhagic dengue fever and dengue shock syndrome, the increased chances of morbidity and mortality were present. ABO and Rh blood group antigen aid in assessing the chance of disease being present and also assess the possibility of certain blood groups being more disease prone as host whereas in our study we did not follow the patients to observe the clinical outcome and no association was seen with higher rates of infection in any particular blood group.[7] The dengue infection percentages corresponded to the number of blood group percentages. Kaipainen and Vuorinen first proposed the hypothesis in 1960 suggesting a correlation of disease susceptibility with different blood groups.[8] In our study we did not observe any association between the blood group and the dengue infection percentages. Although, in earlier studies different serotypes have differing association with various blood group.[8] Severe forms of serotype 2,3 and 4 of dengue have more association with AB blood group as compared to serotype 1 in a study carried out by Kalayanarooj et al.[9] Additional studies conducted in India found a higher prevalence of individuals with blood group O+ compared to the control group.[9,10] Human cells have carbohydrate markers on their surface according to ABO type, as described by Landsteiner. The A and B blood antigens are made up of tri-saccharide Gal NAcα 1-3 (Fucα-1,2) Gal-β and Galα1-3 (Fucα-1,2) Gal-β, while the O blood group antigens are Fucα-1,2 Gal-β. ABO blood groups are genetic, but environmental factors can affect their prevalence. There have been number of previous studies linking the ABO blood groups with various diseases and infections, including infectious diseases, tumor immunology, and membrane chemistry.

The evaluation of this study reveals that the ABO and Rh blood group occurrence in normal sample population and dengue positive patients are closely related and are comparable. Similar results were shown by other studies where dengue population closely matched the population sample blood group presence.[11] On the other hand another study proposed by Khode et al. blood group O is more susceptible and poses an influence in dengue infection chance in patient.[12] Additionally, studies like Kalayanarooj et al. also proposed that blood group AB is prone to a much severe form of dengue fever ,either dengue hemorrhagic fever or dengue

shock syndrome.[13] Furthermore, it is seen that association with secondary infection of dengue and severity of disease to blood group exists. The role immune system against viral infections due to host genetic factors like blood group affect the response to disease.[14] However, in our study we did not check the patients for secondary infection.

In a national study conducted in Karachi, Pakistan, a statistically significant association was found between blood group B negative (B-) and DENV IgM seropositivity (P = 0.04).[15] Similarly, research from Peshawar indicated that patients with blood group B exhibited a higher prevalence of dengue virus infections, whereas individuals with blood group AB were less likely to contract the virus. However, no significant correlation was observed between specific blood types and the severity of the illness. Additionally, a higher proportion of younger males were identified as being infected with dengue.[16]

Furthermore, a two-year study conducted in Islamabad, Pakistan, reported the distribution of blood groups among recorded cases as follows: A+ (10.4%), B+ (15.0%), O+ (12.7%), AB+ (3.4%), A- (1.0%), B- (1.2%), AB- (0.8%), and O- (3.9%). Notably, this study found that patients with blood group O demonstrated a significantly lower likelihood of developing clinically evident DENV infections.[17] This finding aligns with our own research, which also indicated a higher prevalence of blood group O+ among dengue-positive patients in our sample population. These observations highlight the complexity of the relationships involved and underscore the necessity for further research across diverse populations.

An international study conducted in Sri Lanka reported that blood group O− exhibited the greatest preferences for landing and feeding.[18] In a study in India, the majority of participants had completed elementary school, with 30 individuals (61% of the total) in the control group and 27 individuals (54% of the total) in the case group. The predominant blood type was O, represented by 21 participants in the case group and 13 in the control group. In the case group, blood type B accounted for 13%, while it constituted 30% in the control group, with 13 and 15 B-type responders in each group, respectively. Blood type AB was present in 19% of the case group and

11% of the control group, while seven participants (14% of the total) in the case group had blood type A, compared to fourteen participants (28% of the total) in the control group.[19]

Consistent with our findings, a study conducted in Bangladesh reported that participants with blood type O exhibited the highest infection rates, at approximately 50%, while blood group AB demonstrated the lowest rates, around 9%. However, when considering the distribution of blood types in the general population, the combined odds of infection for groups B, O, and AB were 2.0, 3.5, and 1.4 times higher, respectively, compared to group A.[20] Similarly, another international study in Indonesia noted that blood group AB exhibited the lowest infection rates, approximately 9%, while blood type O had the highest rates, around 50%. Again, when accounting for the general population's blood type distribution, the cumulative probabilities of infection for groups B, O, and AB were found to be 2.0, 3.5, and 1.4 times greater, respectively, compared to group A. Additionally, individuals who were Rh-negative experienced higher infection rates. Based on adjusted odds ratios, blood groups B, O, and AB exhibited greater susceptibility to infection than blood group A.[21]

In summary, future research on the association between blood groups and Dengue infection should address the limitations of lack of clinical outcome, limited sample size and scope by incorporating longitudinal studies with larger and more diverse samples, considering serotype-specific associations, controlling for confounding factors, employing robust statistical analyses, investigating environmental influences, and providing a comparative analysis with other infectious diseases. These efforts would enhance our understanding of the role of blood groups in Dengue susceptibility and inform more effective prevention and management strategies.

Conclusions

This study found that blood group O is more likely to get dengue, as the percentage of dengue patient is slightly higher than the normal blood group O distribution in normal sample population. Additionally, the prevalence of other blood groups in dengue patients closely mirrors the prevalence of

those blood groups in the normal population. More research is required to determine if HLA and ABO are independent risk factors and if certain blood subgroups have a higher risk of dengue virus (DENV) infection

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REFERENCES

1. Young JW, Gibbons RV, Rothman AL, Tannitisupawong D, Srikiatkhachorn A, Jarman RG, et al. Dengue severity and blood group. Dengue. 2013; 37: 220-2.

2. Hoiland RL, Fergusson NA, Mitra AR, Griesdale DE, Devine DV, Stukas S, et al. The association of ABO blood group with indices of disease severity and multiorgan dysfunction in COVID-19. Blood advances. 2020; 4: 4981-9. doi: 10.1182/bloodadvances.2020002623

3. Hashan MR, Ghozy S, El-Qushayri AE, Pial RH, Hossain MA, Al Kibria GM. Association of dengue disease severity and blood group: a systematic review and meta-analysis. Reviews in Medical Virology. 2021; 31: 1-9. doi: 10.1002/rmv.2147

4. Ravichandran S, Ramya SR, Kanungo R. Association of ABO blood groups with dengue fever and its complications in a tertiary care hospital. Journal of laboratory physicians. 2019; 11: 265-9. doi: 10.4103/JLP.JLP_95_19

5. Noor A, Tashfeen S, Akhtar F, Anwar N, Din HU, Akhtar F. Association of Covid-19 With Abo Blood Groups in Tertiary Care Center of Pakistan. Pakistan Armed Forces Medical Journal. 2020; 70: S251-55.

6. Tashfeen S, Shahid S, Zarrar J, Bashir S, Ellahi A, Mirza IA. A Comparative Study of Red Blood Cell Parameters in Patients of Dengue Fever with Thrombocytopenia in a Tertiary Care Hospital. Pakistan Armed Forces Medical Journal. 2024; 74: 942-5. doi: 10.51253/pafmj.v74i4.10299

7. Stephenson JR. The problem with dengue. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2005; 99: 643-6. doi: 10.1016/j.trstmh.2005.05.003

8. Kaipainen WJ, Vuorinen YV. ABO blood groups in pernicious anaemia and pernicious tapeworm anaemia. Annales Medicinae Experimentalis et Biologiae Fenniae. 1960; 38: 212-3.

9. Kalayanarooj S, Gibbons RV, Vaughn D, Green S, Nisalak A, Jarman RG, et al. Blood group AB is associated with increased risk for severe dengue disease in secondary infections. The Journal of infectious diseases. 2007; 195: 1014-7. doi: 10.1086/512244

10. Periyavan S, Sangeetha SK, Marimuthu P, Manjunath BK, Seema DM. Distribution of ABO and Rhesus-D blood groups in and around Bangalore. Asian journal of transfusion science. 2010; 4: 41. doi: 10.4103/0973-6247.59391

11. Ravichandran S, Ramya SR, Kanungo R. Association of ABO blood groups with dengue fever and its complications in a tertiary care hospital. Journal of laboratory physicians. 2019; 11: 265-9. doi: 10.4103/JLP.JLP_95_19

12. Khode V, Kabbin G, Ruikar K. Association of ABO Rh blood group with dengue fever and dengue hemorrhagic fever: A case-control study. Journal of Applied Hematology. 2013; 4: 145-8. doi: 10.4103/1658-5127.127899

13. Kalayanarooj S, Gibbons RV, Vaughn D, Green S, Nisalak A, Jarman RG, et al. Blood group AB is associated with increased risk for severe dengue disease in secondary infections. The Journal of infectious diseases. 2007; 195: 1014-7. doi: 10.1086/512244

14. Breidung D, Megas IF, Freytag DL, Bernhagen J, Grieb G. The Role of Macrophage Migration Inhibitory Factor (MIF) and D-Dopachrome Tautomerase (D-DT/MIF-2) in Infections: A Clinical Perspective. Biomedicines. 2023; 12: 2. doi: 10.3390/biomedicines12010002

15. Khan MA, Imtiaz K, Shafaq H, Farooqi J, Hassan M, Zafar A, et al. Screening for arboviruses in healthy blood donors: Experience from Karachi, Pakistan. Virologica Sinica. 2022; 37: 774-7. doi: 10.1016/j.virs.2022.07.008

16. Iqbal N, Afridi MAR, Ali Z, Rafiq A. Association of different blood groups in patients with Dengue fever and their relationship with the severity of the illness. Pakistan Journal of Medical Sciences 2023; 39: 1301-6. doi: 10.12669/pjms.39.5.7275

17. Syed F, Arif MA, Mansoor VB, Usman M, Arif SA. Evolving Spectrum of Dengue: A Two-Year Experience from a Tertiary Care Hospital in Pakistan. Cureus. 2024; 16: e53817. doi: 10.7759/cureus.53817

18. Galhena GK, Ganehiarachchi GA, Gunathilaka RA, Jayatunga DP. Effect of ABO and Rh blood groups on host preference, oviposition success, and development of laboratory-reared Aedes aegypti. International Journal of Tropical Insect Science. 2023; 43: 51-60. doi: 10.1007/s42690-022-00923-2

19. Mydeen Sadik M, Merlin RH, Raj AS. To investigate the relationship between the ABO blood group description and the cases of dengue hemorrhagic fever. International

Journal of Mosquito Research 2024; 11: 157-60. doi: 10.22271/23487941.2024.v11.i1c.756

20. Akther T, Karim MN, Akther T, Munshi SU. Investigating the Association Between ABO Blood Groups and Rhesus Factors with Dengue and Chikungunya Virus Infections During the 2017 Outbreak in Bangladesh. Vector-Borne and Zoonotic Diseases. 2024; 24: 321-4. doi: 10.1089/vbz. 2023.0100

21. Rianti P, Mardliyah ZR, Solihin DD. Diversity of ABO blood groups, ethnic groups, and medical histories of university students in Indonesia. Jurnal Natural. 2023; 23: 174-84. doi: 10.24815/jn.v23i3.30596

Authors Contribution

JZ: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading.

ST: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading

SS: Study designing, manuscript writing and proofreading

SB: Study designing, manuscript writing and proofreading

IS: Data analysis, results and interpretation, proofreading

ZS: Manuscript writing and proofreading

AZ: Manuscript writing and proofreading

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