**Analysis of Co-infection patterns and their relationship with Blood Types and Hemoglobin Genotypes among Subjects in River states of Nigeria**

**ABSTRACT**

This study investigated the correlation of infections and phenotypic distribution of common blood types and haemoglobin genotypes among subjects in Rivers State, Nigeria. The aim was to assess genetic traits influencing health outcomes and infection susceptibility within this population. Specifically, the objectives were to analyze the distribution of ABO and Rh blood types, investigate haemoglobin genotypes, and explore associations with HIV, HBsAg, and Tuberculosis infections. A cross-sectional study design was employed, enrolling 392 subjects from December 2023 to February 2024 at the University of Port Harcourt Teaching Hospital (UPTH). Blood samples were collected and analyzed for ABO and Rh blood types, haemoglobin genotypes, and infection markers. The data were analyzed using descriptive statistics, Chi-square association tests, and correlation analysis. Results revealed a predominant distribution of blood types O+ (52.0%) and A+ (26.0%) within the study population. Haemoglobin genotype AA was prevalent (75.8%), with no observed SS variants. Significant associations were found between sex and haemoglobin genotypes (χ² = 29.820, p = 0.00), indicating sex as a determinant factor. The lack of significant associations between demographic variables and blood group phenotypes suggests complex genetic determinants. Correlation analysis demonstrated significant positive relationships between HIV and HBsAg (r = 0.352, p = 0.000), as well as between HIV and Tuberculosis (r = 0.282, p = 0.000), underscoring the need for co-infection monitoring. These findings highlight the importance of genetic factors in infection susceptibility and health outcomes. Understanding these relationships has implications for public health interventions and clinical practice. Further research is recommended to elucidate underlying mechanisms and explore additional genetic markers influencing susceptibility to infections in this population. This study contributes to the broader understanding of genetic epidemiology and informs targeted healthcare strategies to improve health outcomes in Rivers State, Nigeria.

**Keywords:** *Blood Types, Genetic Epidemiology, Haemoglobin Genotypes, Infection Susceptibility, Phenotypic Distribution*

**INTRODUCTION**

The study of human genetics, particularly blood types and haemoglobin genotypes, has been a subject of interest for many years due to their significant implications for health outcomes. Blood types, classified under the ABO and Rh systems, are genetic traits that vary significantly among different populations. The ABO system categorizes blood into four types: A, B, AB, and O, based on the presence or absence of certain antigens on the surface of red blood cells [1][2]. The Rh system, on the other hand, classifies blood as either Rh positive or Rh negative, depending on the presence or absence of the Rh antigen [3]. The distribution of these blood types is influenced by various factors, including but not limited to geography, ethnicity, and genetic drift. Blood type O is the most common worldwide, but its prevalence varies from region to region [1]. In contrast, haemoglobin, a protein in red blood cells, binds oxygen in the lungs and releases it to body cells as blood travels throughout the body. Variants of haemoglobin, such as S and C, can be detrimental to health when inherited [4].

The relationship between genotypic and phenotypic traits and susceptibility to infections is a complex area of study. Certain blood types and haemoglobin genotypes have been associated with varying degrees of susceptibility or resistance to specific infections. For instance, individuals with blood type O are speculated to be less susceptible to severe malaria compared to those with other blood types [5][6][7].

In Rivers State, Nigeria, the primary aim of this research is to explore the genetic traits that influence health outcomes. This region presents a unique context due to its specific demographic and health profile, making this research particularly relevant. Specifically, the research will assess and compare the phenotypic distribution of common blood types in the local population. Furthermore, it will investigate and compare the demographic distribution of haemoglobin genotypes [31,32]. These investigations are crucial as they can provide valuable insights into the health status of the populace and inform medical interventions and public health policies. Additionally, this research will explore the relationship between certain infections and genotypic/phenotypic traits. Specifically, the research will focus on HIV, a virus that attacks the immune system; Tuberculosis (TB), a contagious bacterial infection that primarily affects the lungs; and HBsAg (Hepatitis B Surface Antigen), a protein on the surface of the hepatitis B virus indicating an active hepatitis B infection [8][9][10].

Understanding these relationships can contribute significantly to the development of targeted prevention and treatment strategies for these infections. The findings of this research could have profound implications for public health policies and medical practices in the region. By shedding light on the genetic factors that influence susceptibility to infections, this research could guide the development of more effective public health interventions, potentially reducing the burden of disease in Rivers State and contributing to improved health outcomes for the population.

**MATERIALS AND METHOD**

**Study Area**

This study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), a premier teaching healthcare institution located in the heart of Port Harcourt, the vibrant capital city of Rivers State, Nigeria [11]. UPTH stands out as a beacon of advanced medical care, providing an extensive array of specialized health services to the community. The strategic placement of UPTH within this bustling metropolis enables it to cater to a wide-ranging patient demographic, encompassing individuals from various backgrounds and regions within Rivers State. This diversity is particularly beneficial for the study, as it ensures a comprehensive representation of the population’s phenotypic and genotypic traits. The hospital’s role as a central healthcare hub attracts patients from all corners of the state, offering a unique opportunity to observe and analyze the correlation between blood types, haemoglobin genotypes, and susceptibility to infections such as HIV, HBsAg, and TB within a well-represented demographic cross-section

**Study Design**

This cross-sectional study was designed to evaluate the phenotypic distribution of common blood types and haemoglobin genotypes, as well as their correlation with certain infections among subjects in Rivers State. From December 2023 to February 2024, a total of 392 subjects suspected of having varying susceptibilities to infections such as HIV, HBsAg, and TB were enrolled. Blood samples were systematically collected from each participant to perform comprehensive examinations for ABO and Rh blood types, haemoglobin genotypes, and the aforementioned infections. Additionally, sputum samples were systematically collected for TB examinations,

**Sampling Method**

The sampling method employed in this study was a simple random sampling technique, which is a fundamental approach to ensure unbiased selection. Instead of a binary numbering system, participants were subjected to a coin toss to determine their inclusion in the study. Each potential participant was assigned a coin flip, with the outcome of ‘heads’ signifying inclusion in the study sample, and ‘tails’ indicating exclusion. This method provided a clear and straightforward mechanism for participant selection, maintaining the integrity of the random sampling process.

 **Measures**

**Processing of Sputum Samples for Culture:**

The processing of sputum samples for culture was meticulously carried out using the N-Acetyl-L-Cysteine (NALC)-NaOH method [12]. Initially, 4% NaOH and 2.9% Sodium citrate were prepared and sterilized separately. These reagents were then mixed in equal volumes to create the working solution. To this, 1 gram of NALC was added freshly. An equal volume of the NALC-NaOH solution was then added to the sputum samples, which were gently mixed by inversion and subsequently vortexed for 30 seconds. The mixture was allowed to stand for 15 minutes, with intermittent mixing to ensure proper lysis of the sputum. Following this, the content was neutralized using phosphate-buffered saline (PBS) at a pH of 6.8. The samples were then centrifuged at 3000xg for 15 minutes. After discarding the supernatant, the pellet was resuspended in 2 ml of PBS. Finally, 3 drops of the resuspended sample were inoculated into the Lowenstein-Jensen media in duplicate and incubated at 37°C for 8 weeks to allow for the growth and identification of mycobacteria.

**ABO, Rh Grouping & Hb Genotyping**

The determination of ABO blood groups and Rh factor was conducted using the standard agglutination technique [13]. Blood samples were collected in EDTA tubes and centrifuged at 2500xg for 5 minutes to separate plasma from erythrocytes. The erythrocyte layer was washed three times with saline to remove the plasma completely. Two drops of the test erythrocytes were mixed with one drop of anti-A, anti-B, and anti-D sera, respectively, on a glass slide. The slides were gently rocked to observe agglutination, indicating the presence of corresponding antigens.

For haemoglobin genotype analysis, the alkaline denaturation method was utilized [14]. Hemolysates were prepared by mixing 2 ml of blood with an equal volume of distilled water, followed by centrifugation at 3000xg for 5 minutes. The clear supernatant was treated with an alkaline solution and incubated at room temperature for 20 minutes. The degree of haemoglobin denaturation was measured spectrophotometrically at 540 nm. The absorbance values were used to determine the haemoglobin genotype based on established reference standards.

These procedures were performed under stringent laboratory conditions to ensure the accuracy and reliability of the blood typing and genotyping, which are critical for the subsequent correlation analysis with infection rates among the study subjects.

**HIV and HBsAg screening**

The screening for Human Immunodeficiency Virus (HIV) was conducted using the recommended serological testing methods [15]. Individuals were screened using a combination of antibody tests, antigen/antibody tests, and nucleic acid tests (NAT). Blood samples were collected either by venipuncture or finger prick, and oral fluid swabs were also utilized for some tests. The antibody tests detect antibodies to HIV in the blood or oral fluid and can take 23 to 90 days post-exposure to identify HIV antibodies. Antigen/antibody tests look for both HIV antibodies and antigens, providing a quicker diagnosis. NATs detect the actual virus in the blood and are used in cases where early infection is suspected or for confirmation of infection status.

HBsAg Screening Measures for Hepatitis B surface antigen (HBsAg) screening, the Centers for Disease Control and Prevention (CDC) recommends the use of the triple panel test, which includes HBsAg, antibody to hepatitis B surface antigen (anti-HBs), and total antibody to hepatitis B core antigen (total anti-HBc) [16]. This comprehensive approach ensures the detection of current infection, past or resolved infection, and immunity due to vaccination. The screening process involves collecting blood samples and testing for these markers to determine the hepatitis B infection status of an individual.

**Data Analysis**

Descriptive statistics were employed to summarize the phenotypic distribution of ABO and Rh blood types, as well as haemoglobin genotypes, within the study population. The results are presented as percentages. The Chi-square test was utilized to determine the associations between these phenotypic distributions and various socio-demographic variables. An alpha (α) level of 0.05 was set as the threshold for significance, where p-values less than 0.05 were considered indicative of statistically significant results. Correlation coefficients were calculated to explore the relationships between infections (HIV, HBsAg, and Tuberculosis) and genetic/phenotypic traits (Hb Genotype and Blood Group).

**RESULTS AND DISCUSSION**

**Phenotypic Distribution of ABO and Rh Blood Types by Demographics**

Table 1: Crosstab showing Distribution of ABO and Rh Blood Types in the Study Population

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Demographics** | **A+** | **O+** | **B+** | **AB+** | **O-** | **A-** | **B-** | **Total** |
| **Age (Years)** |  |  |  |  |  |  |  |  |
| Less than 40 | 76 (25.2%) | 158 (52.5%) | 38 (12.6%) | 14 (4.7%) | 10 (3.3%) | 4 (1.3%) | 1 (0.3%) | 301 (100.0%) |
| 40 and Above | 26 (28.6%) | 46 (50.5%) | 8 (8.8%) | 3 (3.3%) | 2 (2.2%) | 5 (5.5%) | 1 (1.1%) | 91 (100.0%) |
| Total  | 102 (26.0%) | 204 (52.0%) | 46 (11.7%) | 17 (4.3%) | 12 (3.1%) | 9 (2.3%) | 2 (0.5%) | 392 (100.0%) |
| **Education** |  |  |  |  |  |  |  |  |
| Educated | 99 (26.5%) | 193 (51.6%) | 44 (11.8%) | 15 (4.0%) | 12 (3.2%) | 9 (2.4%) | 2 (0.5%) | 374 (100.0%) |
| Uneducated | 3 (16.7%) | 11 (61.1%) | 2 (11.1%) | 2 (11.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 18 (100.0%) |
| Total | 102 (26.0%) | 204 (52.0%) | 46 (11.7%) | 17 (4.3%) | 12 (3.1%) | 9 (2.3%) | 2 (0.5%) | 392 (100.0%) |
| **Sex** |  |  |  |  |  |  |  |  |
| Male | 37 (24.0%) | 82 (53.2%) | 7 (4.5%) | 12 (7.8%) | 7 (4.5%) | 8 (5.2%) | 1 (0.6%) | 154 (100.0%) |
| Female | 65 (27.3% | 122 (51.3%) | 39 (16.4%)  | 5 (2.1%) | 5 (2.1%) | 1 (0.4%) | 1 (0.4%) | 238 (100.0%) |
| Total | 102 (26.0%) | 204 (52.0%) | 46 (11.7%) | 17 (4.3%) | 12 (3.1%) | 9 (2.3%) | 2 (0.5%) | 392 (100.0%) |
| **Marital Status** |  |  |  |  |  |  |  |  |
| Married | 48 (24.2%) | 102 (51.5%) | 27 (13.6%) | 6 (3.0%) | 7 (3.5%) | 6 (3.0%) | 2 (1.0%) | 198 |
| Unmarried | 54 (27.8%)  | 102 (52.6%) | 19 (9.8%) | 11 (5.7%) | 5 (2.6%) | 3 (1.5%) | 0 (0.0%) | 194 (100.0%) |
| Total | 102 (26.0%) | 204 (52.0%) | 46 (11.7%) | 17 (4.3%) | 12 (3.1%) | 9 (2.3%) | 2 (0.5%) | 392 (100.0%) |

**Demographic Distribution of Haemoglobin Genotype**

Table 2: Demographic Distribution of Haemoglobin Genotype in the Study Population

|  |  |  |  |
| --- | --- | --- | --- |
| **Demographics** | **Normal Hb** |  **Haemoglobin Variants** | **Total** |
|  | **AA** |  **AS** | **SS** |  |
| **Age** |  |  |  |  |
| Less than 40years | 228 (75.7%) | 73 (24.3%) | 0 (0.0%) | 301 (100.0%) |
| 40years and Above | 69 (75.8%) | 22 (24.2%) | 0 (0.0%) | 91 (100.0%) |
| Total Examined | 297 (75.8%) | 95 (24.2%)  | 0 (0.0%) | 392 (100.0%) |
| **Sex** |  |  |  |  |
| Male | 113 (73.4%) | 41 (26.6%) | 0 (0.0%) | 154 (100.0%) |
| Female | 184 (77.3%) | 54 (22.7%) | 0 (0.0%) | 238 (100.0%) |
| Total Examined | 297 (75.8%) | 95 (22.7%) | 0 (0.0%) | 392 (100.0%) |
| **Marital Status** |  |  |  |  |
| Married | 149 (75.3%) | 49 (24.7%) | 0 (0.0%) | 198 (100.0%) |
| Unmarried | 148 (76.3%) | 46 (23.7%) | 0 (0.0%) | 194 (100.0%) |
| Total Examined | 297 (75.8%) | 95 (24.2%) | 0 (0.0%) | 392 (100.0%) |
| **Education** |  |  |  |  |
| Educated | 285 (76.2%) | 89 (23.8%) | 0 (0.0%) | 374 (100.0%) |
| Uneducated | 12 (66.7%) | 6 (33.3%) | 0 (0.0%) | 18 (100.0%) |
| Total Examined | 297 (75.8%) | 95 (24.2%) | 0 (0.0%) | 392 (100.0%) |

Table 3: Demographics and Blood Group Phenotypes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Demographics | Chi Square | Df | p-value | Remark |
| Age | .000 | 1 | 0.99 | Not Significant |
| Sex | .788 | 1 | 0.38 | Not Significant |
| Marital Status | .057 | 1 | 0.81 | Not Significant |
| Education | .851 | 1 | 0.36 | Not Significant |

Table 4: Demographics and Haemoglobin Genotype

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Demographics** | **Chi Square** | **Df** | **p-value** | **Remark** |
| Age | 7.892 | 6 | 0.25 | Not Significant |
| Sex | 29.820 | 6 | 0.00 | Significant |
| Marital Status | 6.508 | 6 | 0.37 | Not Significant |
| Education | 4.042 | 6 | 0.67 | Not Significant |

|  |
| --- |
| **Table 5: Correlations showing relationship between Infections and Genetic/Phenotypic Identity (**Hb Genotype and Blood Group) |
| Variables Statistics | HIV | HBsAg | Tuberculosis | Hb Genotype | Blood Group |
|  | **HIV** | Correlation Coefficient | 1.000 | .352\*\* | .282\*\* | -.002 | -.046 |
| p-value | . | .000 | .000 | .971 | .362 |
|  |  |  |  |  |  |
| **HBsAg** | Correlation Coefficient | .352\*\* | 1.000 | .125\* | -.143\*\* | -.047 |
| p-value | .000 | . | .013 | .005 | .353 |
|  |  |  |  |  |  |
| **Tuberculosis** | Correlation Coefficient | .282\*\* | .125\* | 1.000 | .050 | -.059 |
| p-value | .000 | .013 | . | .327 | .244 |
|  |  |  |  |  |  |
| **Hb Genotype** | Correlation Coefficient | -.002 | -.143\*\* | .050 | 1.000 | -.059 |
| p-value | .971 | .005 | .327 | . | .247 |
|  |  |  |  |  |  |
| **Blood Group** | Correlation Coefficient | -.046 | -.047 | -.059 | -.059 | 1.000 |
| p-value | .362 | .353 | .244 | .247 | . |
| N | 392 | 392 | 392 | 392 | 392 |
| \*\*. Correlation is significant at the 0.01 level (2-tailed), \*. Correlation is significant at the 0.05 level (2-tailed).. |

The primary objectives of this research were to assess and compare the phenotypic distribution of ABO and Rh Blood Types in the study population by demographics. The most common blood type across all demographics was O+ (52.0%), followed by A+ (26.0%). The least common were B- and AB-, each making up 0.5% of the total. When looking at age demographics, the distribution of blood types was similar for both groups (less than 40, and 40 and above). However, there was a slightly higher percentage of A+ individuals in the 40 and above group (28.6%) compared to the less than 40 group (25.2%). In terms of education, the distribution of blood types was almost identical between the educated and uneducated groups. However, it's important to note that the sample size for the uneducated group was significantly smaller (18 individuals), which may affect the reliability of these results. The distribution of blood types also varied slightly between males and females. Females had a higher percentage of B+ individuals (16.4%) compared to males (4.5%). Conversely, males had a higher percentage of AB+ individuals (7.8%) compared to females (2.1%). These findings contribute and provide valuable insights into the phenotypic distribution of ABO and Rh blood types in Rivers State, Nigeria. Understanding these distributions is crucial for various medical and research purposes, such as blood transfusion services and disease susceptibility studies. The observed distribution of blood types in this study is supported by Akinnuga *et al* [17], Isah *et al* [18], and Andalibi *et al* [19] in their cross-sectional research. Although the specific percentages vary, the overall trend of a higher prevalence of O and A blood types is consistent across these populations. This consistency strengthens the validity of the findings and suggests that similar genetic and environmental factors may be influencing blood type distribution in these populations. However, further research is needed to confirm these findings and explore the factors contributing to these distributions.

Furthermore, a striking uniformity in the distribution of haemoglobin genotypes across different demographic categories was observed. The prevalence of the normal haemoglobin genotype (AA) remains consistently high across all demographics, while the AS variant shows a lower prevalence. Interestingly, the SS variant was not observed in the study population. When examined more closely, subtle differences emerge. The prevalence of AA genoptype is slightly higher in females and educated individuals. These variations, although minor, could point to underlying genetic or environmental factors that warrant further investigation. The high prevalence of the AA genotype and the absence of the SS variant have significant implications for public health planning in Rivers State. These findings suggest that the burden of sickle cell disease, which is associated with the SS variant, may be lower in this population compared to other regions with a higher prevalence of the SS variant. However, the significant presence of the AS variant indicates a considerable proportion of the population are carriers of the sickle cell trait, which could have implications for future generations. While findings of the current study aligns with some previous studies [17][18] in terms of the high prevalence of the AA genotype and the presence of the AS variant, the absence of the SS variant is a divergence. This discrepancy could be due to various factors, including the specific genetic makeup of the Rivers State population, or it could be a result of the study's limitations.

Moving on, none of the demographic variables examined—age, sex, marital status, and education—were significantly associated with blood group phenotypes. These findings suggest that within the scope of this study, demographic factors may not play a significant role in determining blood group phenotypes. These findings necessitate a reevaluation of the conventional assumptions regarding the role of demographic variables in determining blood group phenotypes. While age, sex, marital status, and education are commonly presumed to be influential factors, our study suggests a more nuanced relationship. The lack of significant associations observed in this study prompts a reconsideration of the relative importance of these demographic variables in predicting blood group phenotypes. Healthcare practitioners should exercise caution when relying solely on demographic characteristics for clinical decision-making regarding blood group compatibility or disease risk assessment. Moreover, researchers should explore alternative factors beyond demographic parameters to better understand the underlying determinants of blood group phenotypes. This shift in focus may lead to the identification of novel factors contributing to blood group variability and enhance our understanding of individual differences in blood group expression. These findings interestingly contradicts several previous studies as observed by Andalibi *et al.* [19] and Liu *et al*.[20] that have established significant associations between demographic variables and blood group phenotypes.

The statistical analysis of the relationship between demographic variables and haemoglobin genotypes provides a compelling narrative on the influence of these variables on genetic traits. The most notable result is the significant association between sex and haemoglobin genotypes. This finding suggests that sex is a determinant factor in the distribution of haemoglobin genotypes. The implications of this are multifaceted, affecting clinical approaches to diseases related to haemoglobin, such as sickle cell disease and thalassemia [21]. It also has repercussions for reproductive health, as it could influence genetic counseling for couples and inform public health strategies for screening programs [22][23]. In contrast, age, marital status, and education did not show a significant correlation with haemoglobin genotypes. This suggests that other unexplored factors may be at play, and these demographic variables alone are not strong predictors of haemoglobin genotype distribution. When compared with similar studies, these findings align with research that underscores sex differences in genetic traits. Studies have shown variations in hemoglobin levels and related pathologies between males and females, reinforcing the significance of sex as a variable in genetic and epidemiological research [24][25][26][27]. However, this study contrasts with research that points to geographical and ethnic factors as influential, suggesting that the interplay between genetics and demographics is complex and warrants further investigation [28].

Finally, the correlation analysis between infectious diseases and genetic/phenotypic identity offers a detailed examination of the relationship between various infections and inherent biological traits within a population. The analysis reveals a significant positive correlation between HIV and HBsAg, as well as between HIV and Tuberculosis, suggesting a higher likelihood of co-infections among individuals with HIV. Conversely, the lack of significant correlation between HIV and Hb genotype or blood group suggests that these genetic factors may not influence the susceptibility to HIV within the study population. Similarly, a negative correlation between HBsAg and Hb genotype indicates a potential genetic resistance to HBsAg in certain Hb genotypes.

 These findings have important implications for public health and clinical practice. The significant correlations suggest that individuals with HIV should be closely monitored for co-infections with HBsAg and Tuberculosis. The negative correlation between HBsAg and Hb genotype could inform targeted screening strategies and personalized treatment plans. Moreover, the absence of correlation between certain infections and genetic traits like blood group could redirect research focus towards other potential genetic markers of susceptibility. Research on the correlation between infections and genetic traits has been extensive. Studies have identified genetic variations that influence the immune response to viral infections, highlighting the role of host genetics in disease susceptibility [29]. Other research has focused on the genetic landscape of immune response to common viral infections, emphasizing the importance of the HLA region in host-virus interactions [30]. These studies support the notion that genetic factors can significantly influence the risk and progression of infectious diseases.

**CONCLUSION**

This study provides valuable insights into the phenotypic distribution of common blood types, haemoglobin genotypes, and their correlation with infections among subjects in Rivers State, Nigeria. The findings brings to light the importance of understanding genetic traits in influencing health outcomes and susceptibility to infections within a population. The prevalence of blood types O+ and A+ aligns with global trends, while the absence of the SS haemoglobin genotype suggests a lower burden of sickle cell disease in the study population. Despite minor variations across demographic categories, demographic variables were not significantly associated with blood group phenotypes, highlighting the complexity of genetic determinants. However, sex emerged as a significant determinant of haemoglobin genotype distribution, emphasizing the need for tailored healthcare interventions. The correlation analysis revealed significant associations between HIV and HBsAg, as well as between HIV and Tuberculosis, emphasizing the importance of co-infection monitoring. Conversely, the lack of correlation between certain infections and genetic traits suggests the presence of other factors influencing susceptibility. These findings contribute to the growing body of research on the interplay between genetics, demographics, and infectious diseases, informing targeted public health strategies and personalized clinical interventions. Moving forward, further research is needed to elucidate the underlying mechanisms driving these associations and explore novel genetic markers of susceptibility to infections, ultimately advancing our understanding of human health and disease.

**Ethical Approval and consent**

Ethical approval for this study was obtained from the Ethics Committee of the University of Port Harcourt Teaching Hospital (UPTH) prior to its initiation. Informed written consent was also procured from each participant, ensuring they were fully apprised of the study’s objectives, potential risks, and benefits. This consent process affirmed the participants’ voluntary agreement to partake in the study, thereby respecting and upholding the ethical principle of autonomy.

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