Research Article

Demographic and ABO/Rh Blood Group Predictors of SARS-CoV-2 Nucleocapsid IgG Seropositivity in an Unvaccinated Cohort from Northern Egypt

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Abstract | In resource-limited settings, the burden of prior Severe Acute Respiratory Syndrome Coronavirus 2 (ARS-CoV-2) infection and the influence of demographic and immunogenetic factors remain insufficiently characterized. This study aimed to determine the seroprevalence of prior SARS-CoV-2 (COVID-19) infection and identify the demographic and immunohematologic predictors, particularly ABO and Rhesus (Rh) blood groups in an unvaccinated cohort from Northern Egypt. Between June 2023 and June 2024, 200 healthy, non-smoking participants aged 17-40 years were recruited at Alexandria University Hospital. Assays for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and syphilis for all participants were performed, and individuals with chronic comorbidities or current smoking were excluded. Anti-nucleocapsid IgG for SARS-CoV-2, unaffected by spike-based vaccination, was quantified using a high-sensitivity ELISA. Additionally, demographic and immunohematologic data were analyzed by multivariable logistic regression. The obtained seroprevalence was 76.0%, exceeding previous regional estimates. Multivariate analysis identified several independent predictors of SARS-CoV-2 nucleocapsid IgG seropositivity, including male sex (adjusted odds ratio [aOR] = 2.8, $p < 0.001$), blood group A (aOR = 2.1, $p = 0.001$), Rh-positive status (aOR = 2.4, $p = 0.030$), and blood group AB (aOR= 1.7, $p = 0.047$). In contrast, blood groups B and O, and age were not remarkably associated with seropositivity. To our knowledge, this is the first study in Northern Egypt to integrate high-sensitivity N protein serology with ABO/Rh profiling in an unvaccinated cohort. The current findings indicate a substantial reservoir of previously undiagnosed SARS-CoV-2 infections and highlight host-related susceptibility markers with potential public health implications. The obtained results supported the use of ABO and Rh blood typing as low-cost, accessible tools to guide targeted vaccination strategies. They also underscored the need for multi-center, longitudinal studies that integrate genomic surveillance with comprehensive immunological profiling.

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Introduction

The emergence of coronavirus disease 2019 (COVID-19) in late 2019 triggered an unprecedented global public health crisis. By mid-2020, over 5 million cases and 330,000 deaths had been reported worldwide. As of July 2025, the cumulative number of confirmed infections has exceeded 770 million, with 6.9 million fatalities (WHO, 2025). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); an enveloped *Betacoronavirus* measuring 120–160 nm in diameter, possesses one of the largest known RNA viral genomes (27-32 kb). Its spike (S) glycoprotein mediates ACE2-dependent cell entry, while the nucleocapsid (N) protein responsible for packaging viral RNA is abundantly expressed and highly immunogenic. These features render anti-N assays particularly valuable for distinguishing natural infections from vaccine-induced immunity (Burbelo *et al.*, 2020).

Although reverse transcription-polymerase chain reaction (RT-PCR) remains the diagnostic gold standard for acute SARS-CoV-2 infection; however, it can't detect prior, asymptomatic, or resolved cases. Serological assays; therefore, provide critical insight into true population exposure and can inform public health interventions (Cao et al., 2020; Alahmari et al., 2021). However, most published serosurveys have focused on S-based platforms or high-risk cohorts, risking underestimation of silent transmission and conflation of infection- versus vaccine-derived immunity (Ripperger *et al.*, 2020; Demmer *et al.*, 2021; Duarte *et al.*, 2022; Arkell *et al.*, 2022; Vicentini *et al.*, 2022). In low-resource settings such as Egypt, reported seroprevalence estimates (34-63%) have been largely derived from convenience samples, often without consistent adjustment for confounders such as smoking or blood-borne infections, limiting their validity (Younas et al., 2020; Eldesoukey et al., 2022).

Beyond assay design, host immunohematologic factors may influence susceptibility to SARS-CoV-2 (Zhao *et al.*, 2020; Butler *et al.*, 2023). Evidence from SARS-CoV-1 and early SARS-CoV-2 (COVID-19) cohorts suggests increased infection risk among individuals with blood group A and relative protection in group O, while associations with Rhesus (Rh) status remain inconclusive (Cheng et al., 2005; Li et al., 2020; Muñiz-Díaz et al., 2021). To date, no study in Northern Egypt has systematically evaluated these blood group phenotypes alongside highly specific N-protein serology in a rigorously screened, healthy population.

To address these knowledge gaps, this study aimed to determine the seroprevalence of prior SARS-CoV-2 infection in a rigorously screened, unvaccinated Egyptian cohort and evaluate demographic and blood group predictors, particularly ABO and Rhesus (Rh) blood groups.

By integrating rigorous serological screening with host-factor analysis in a resource-limited context, this study provided the first comprehensive evaluation of blood group-related predictors of SARS-CoV-2 exposure in Egypt, refined estimates of silent transmission, elucidated blood group-related risk profiles, and informed targeted public health strategies where diagnostic and vaccination resources remain constrained.

Materials and Methods

Study design and sample size

This cross-sectional comparative study was conducted between June 2023 and June 2024, enrolling 200 healthy, unvaccinated adult blood donors from Alexandria University Teaching Hospital. Alexandria, Egypt. A priori sample-size calculation using G*Power v3.1 demonstrated that 200 participants would provide 80% power to detect an odds ratio of 1.8 for SARS-CoV-2 seropositivity across ABO blood groups at a significance level of α=0.05 (Faul *et al.*, 2007). This estimation was consistent with the World Health Organization (WHO) formula for cross-sectional studies, which yielded an approximate sample size of 196 when assuming a prevalence (p) of 0.5, a 95% confidence interval, and a 7% margin of error (Lwanga and Lemeshow, 1991). All study procedures complied with the ethical principles outlined in the Declaration of Helsinki (2013) (World Medical Association. 2013).

Participant recruitment and data collection

Participants were recruited from voluntary blood donors under strict eligibility criteria to ensure a healthy and homogeneous study population:

- Inclusion criteria were adults aged ≥ 18 years, in apparent good health; with no history of COVID-19 vaccination, and who were non-smokers.

- Exclusion criteria encompassed history of COVID-19 vaccination, active or past infectious diseases, malignancies, autoimmune disorders, or chronic metabolic conditions (e.g., diabetes, hypertension, and atherosclerosis), and current or former smokers.

After eligibility confirmation, all participants completed a structured questionnaire capturing demographic details (*i.e.*, age, sex, and residence), comorbidities, smoking history, and prior COVID-19 infection history, including severity and complications. Both smoking and comorbidities were specifically documented, as they are known to influence immune responses and COVID-19 outcomes. These measures minimized confounding and enabled precise evaluation of the ABO association with SARS-CoV-2 seropositivity.

Blood sampling and processing

From each participant, two venous blood specimens (total volume = 5 mL) were obtained under aseptic conditions. For ABO and Rh(D) phenotyping, 0.5 mL of whole blood was collected into an EDTA-anticoagulant tube to maintain red cell integrity for agglutination testing (CLSI, 2020a; Cohn *et al.*, 2020). The remaining 4.5 mL were drawn into a sterile plain tube, allowed to clot at room temperature for 30 min, and centrifuged at 3,000 × g for 5 min. The resulting serum was aliquoted for serological screening and SARS-CoV-2 antibody assays. All specimens were processed within 2h of collection and stored at ≤ -20°C until analysis (CLSI, 2020b).

Serological assays

Serological screening for confounding infections: To rule out confounding blood-borne infections, all sera were screened using qualitative ELISA kits with manufacturer's-provided positive and negative controls.

- Anti *Treponema pallidum* IgG was detected using the Aria Syphilis Ab ELISA Kit (CTK Biotech, 2017). Samples with OD ratio < 1.0 were considered negative, those ≥ 1.0 positive, and borderline results were retested. The kit demonstrated 100% sensitivity and 99.8% specificity (Henao-Martínez and Johnson, 2014).
- Anti-HCV IgG was tested using the NANBASE C-96 V4.0 Anti-HCV ELISA Kit (General Biologicals Corporation, 2023a). Samples with OD < cutoff were negative, those with ≥ cutoff positive, and borderline results were retested. The kit demonstrated 100% sensitivity and 99.8% specificity (Tabll *et al.*, 2015).
- HBsAg was detected using the SURASE B-96 TMB ELISA Kit (General Biologicals Corporation, 2023b). Specimens with OD < cutoff were negative; those ≥ cutoff were retested in duplicate, and only repeat-reactive samples were considered positive. The kit demonstrated 100% sensitivity and 99.6% specificity (De Almeida Pondé, 2022).
- HIV IgG/Ag was tested using the GB HIV Ag-Ab COMB Kit (General Biologicals Corporation, 2023c). Samples with OD < cutoff were negative, those with ≥ cutoff positive, and borderline results were retested; only repeat-reactive samples were confirmed positive. The kit demonstrated 100% sensitivity and high specificity (Bangalee *et al.*, 2021).

Samples that were reactive in any of these screening tests were excluded from further analysis.

SARS-CoV-2 serology

Sera that displayed negative results in all pre-screening assays were analyzed for SARS-CoV-2 nucleocapsid (N) protein IgG antibodies using the RayBio® COVID-19/SARS-CoV-2 Nucleocapsid Protein ELISA Kit (Indirect ELISA, Cat. No. ELV COVID19N, RayBiotech Inc., Peachtree Corners, GA, USA) (RayBiotech. 2021). Testing procedures followed the manufacturer's instructions, with OD < cutoff classified as negative, ≥ cutoff as positive, and borderline results retested in duplicate. Equivocal results were tested, and inter- and intra-assay variability was kept below 10%. The kit demonstrated 96.7% sensitivity and 97.5% specificity (Alzabeedi *et al.*, 2022).

ABO and Rh(D) phenotyping

ABO and Rh(D) blood grouping was conducted using the standard agglutination method with monoclonal antisera from Spectrum Diagnostics (Egyptian Company for Biotechnology S.A.E., Obour City, Cairo, Egypt): Anti-A (Cat. No. 810 002), Anti-B (Cat. No. 814 002), Anti-AB monoclonal IgM (Cat. No. 816 002), and Anti-D (Rho) + monoclonal IgM-IgG (Cat. No. 822 002) (Spectrum Diagnostics, 2023a, b). Participants were classified into four groups: A (antigen A, antibody B), B (antigen B, antibody A), AB (antigens A and B,

no antibodies), and O (no antigens, antibodies A and B). Agglutination with the corresponding antisera was considered positive, absence of agglutination was negative. Weak or equivocal reactions were repeated in duplicate. Spectrum diagnostics antisera demonstrated >99% sensitivity and >99% specificity. All typing procedures were carried out according to the manufacturer's recommendations to ensure reproducibility and accuracy.

Multivariate logistic regression modeling

To identify independent predictors of SARS-CoV-2 IgG seropositivity, multivariable logistic regression was performed, including sex, age, ABO group, and Rh status, reporting adjusted odds ratios (ORs) with 95% confidence intervals (CIs). Before model fitting, all predictor variables were assessed for multicollinearity using variance inflation factors (VIF), and no variable exceeded the predefined threshold for exclusion. Blood groups were dichotomized (Group X vs. non-Group X) based on established seroepidemiological methods for risk stratification. All statistical tests were two-tailed, and $p \leq 0.05$ was considered statistically significant.

Statistical analysis

Data were entered, validated, and analyzed using SPSS v22 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were summarized as frequencies and percentages. Group comparisons for categorical variables were performed using Pearson's chi-square test.

Results

Participant characteristics

A total of 200 healthy, unvaccinated adult blood donors (135 males [67.5%] and 65 females [32.5%]) were enrolled. Participant ages ranged from 17 to 40 years, with a mean of 29.34 ± 6.50 years. All participants were asymptomatic, non-smokers, free of chronic comorbidities, and had not received any COVID-19 vaccination prior to testing.

Serological screening for confounding infections

All tested 200 serum samples were negative for HIV antigen/antibody, hepatitis B surface antigen (HBsAg), hepatitis C virus IgG, and syphilis IgG using validated ELISA kits according to the pre-mentioned detailed criteria. Therefore, all donors were deemed eligible for COVID-19 serological testing.

Distribution of ABO and Rh blood groups in the cohort

The ABO blood group distribution was as follows: group A – 83 donors (41.5 %), group B – 57 (28.5 %), group AB – 37 (18.5 %), and group O – 23 (11.5 %). Regarding Rhesus (Rh) factor, 161 donors (80.5 %) were Rh-positive and 39 (19.5 %) were Rh-negative.

SARS-CoV-2 IgG seroprevalence

Anti-nucleocapsid IgG antibodies specific to SARS-CoV-2 were detected in 152 out of the 200 donors, yielding an overall seroprevalence of 76.0 %, indicating high prior exposure to COVID-19 in this unvaccinated cohort. The distribution of seropositivity by sex, age, ABO blood group, and Rh factor is presented in the subsequent analysis.

Univariate analysis of factors associated with SARS-CoV-2 seropositivity

Sex: Among the 152 seropositive individuals, 92 were male (60.5%) and 60 were female (39.5%). In contrast, among the 48 seronegative individuals, 43 (89.6%) were male and only 5 (10.4%) were female. Female donors were considerably more likely to be seropositive than males (92.3% vs. 68.1%; $p < 0.001$) (Table 1, Figure 1a).

Table 1: *Univariate analysis of SARS-CoV-2 IgG seropositivity.*

Factor	Seropositive (n = 152)[a]	Seronegative (n = 48)[a]	p Value[b]
Sex			**< 0.001[c]**
Male	92 (60.5%)	43 (89.6%)	
Female	60 (39.5%)	5 (10.4%)	
Age (years)	28.97± 6.53	30.50 ± 6.58	0.155
ABO Group			**< 0.001[c]**
A	70 (46.1%)	13 (27.1%)	
B	32 (21.1%)	25 (52.1%)	
AB	30 (19.7%)	7 (14.6%)	
O	20 (13.2%)	3 (6.3%)	
Rh Status			**0.030[c]**
• Positive	125 (82.2%)	27 (56.3%)	
• Negative	27 (17.8%)	21 (43.7%)	

Where; [a]Data presented as n (%) for categorical variables or mean ± SD for continuous variables. [b]p-Values calculated using Pearson's χ^2 test for categorical variables and an independent t-test for age. [c]Indicates statistically significant p-value ($p \leq 0.05$).

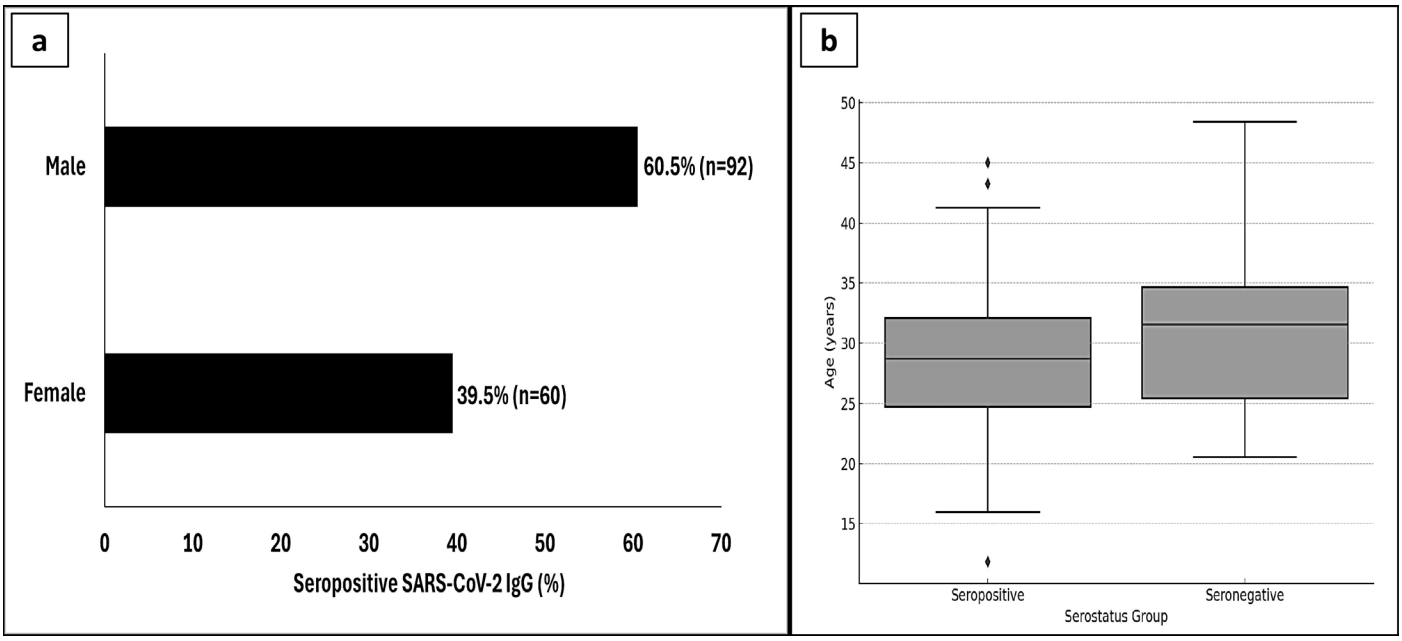

Figure 1: *Distribution of SARS-CoV-2 IgG Seropositivity by Sex and Age. (a) Bar chart showing the proportion of seropositive individuals by sex ($p < 0.001$), (b) Boxplot comparing age distribution (median with interquartile range) between seropositive and seronegative groups ($p = 0.155$). Where; IgG: Immunoglobulin G.*

Age: The mean age of the seropositive donors was 28.97±6.53 years, compared to 30.50 ± 6.58 years for seronegative donors. The difference was not statistically significant ($p = 0.155$) (Table 1, Figure 1b).

ABO blood group: Seropositivity varied appreciably according to ABO blood group (Table 1, Figure 2). Among the 152 seropositive individuals, 70 (46.1%) were group A, 32 (21.1%) group B, 30 (19.7%) group AB, and 20 (13.2%) group O. Statistical analysis revealed that group A donors were considerably more likely to be seropositive compared to those with group B ($p = 0.030$), group AB ($p = 0.290$), or group O ($p = 0.500$). Overall, the association between ABO blood group and COVID-19 IgG seropositivity was highly significant ($p < 0.001$) (Table 1, Figure 2a).

Rh factor: Rh-positive donors represented 125 of 152 seropositive individuals (82.2%), while Rh-negative individuals accounted for 27 (17.8%). In contrast, Rh-negative donors made up 43.7% of the seronegative group. Rh-positive status was significantly associated with seropositivity ($p = 0.030$) (Table 1, Figure 2b).

Multivariate logistic regression analysis

To identify independent predictors, all variables (*i.e.*, sex, age, ABO group, and Rh status) were included in a multivariable logistic regression model. Multivariate logistic regression (Table 2, Figure 3) identified male sex (aOR = 2.8; 95% CI: 1.4–5.6; p < 0.001) and Rh-positive status (aOR = 2.4; 95% CI: 1.1–5.2; p = 0.030) as the strongest independent predictors of SARS-CoV-2 IgG seropositivity. Among ABO blood groups, group A was remarkably associated with increased odds of seropositivity (aOR = 2.1; 95% CI: 1.3–3.5; p = 0.001), followed by group AB (aOR = 1.7; 95% CI: 1.0–3.2; p = 0.047). In contrast, blood group B was not appreciably associated (aOR = 1.2; 95% CI: 0.8–2.0; p = 0.340), and group O showed a trend towards a protective effect, though it did not reach statistical significance (aOR = 0.6; 95% CI: 0.3–1.1; p = 0.098). Age was not a considerable predictor of seropositivity in this cohort (aOR = 0.98 per year; 95% CI: 0.94–1.03; p = 0.412).

Table 2: *Multivariate logistic regression for predictors of SARS-CoV-2 IgG seropositivity.*

Predictor	aOR[a] (95% CI)	p-Value[b]
Male sex (vs. female)	2.8 (1.4-5.6)	< 0.001[c]
Age (per year increase)	0.98 (0.94-1.03)	0.412
Rh-positive (vs. Rh-negative)	2.4 (1.1-5.2)	0.030 [c]
Blood group A (vs. non-A)	2.1 (1.3-3.5)	0.001[c]
Blood group B (vs. non-B)	1.2 (0.8-2.0)	0.340
Blood group AB (vs. non-AB)	1.7 (1.0-3.2)	0.047[c]
Blood group O (vs. non-O)	0.6 (0.3-1.1)	0.098

Where: [a]Adjusted odds ratio (aOR) and 95% confidence interval (CI) from multivariate logistic regression, [b]Model adjusted for all listed predictors; Reference categories: female (sex), per one-year age increase; Rh-negative (Rh status); and non-A, non-B, non-AB, non-O (ABO group). [c]Indicates statistically significant p-value ($p \leq 0.05$).

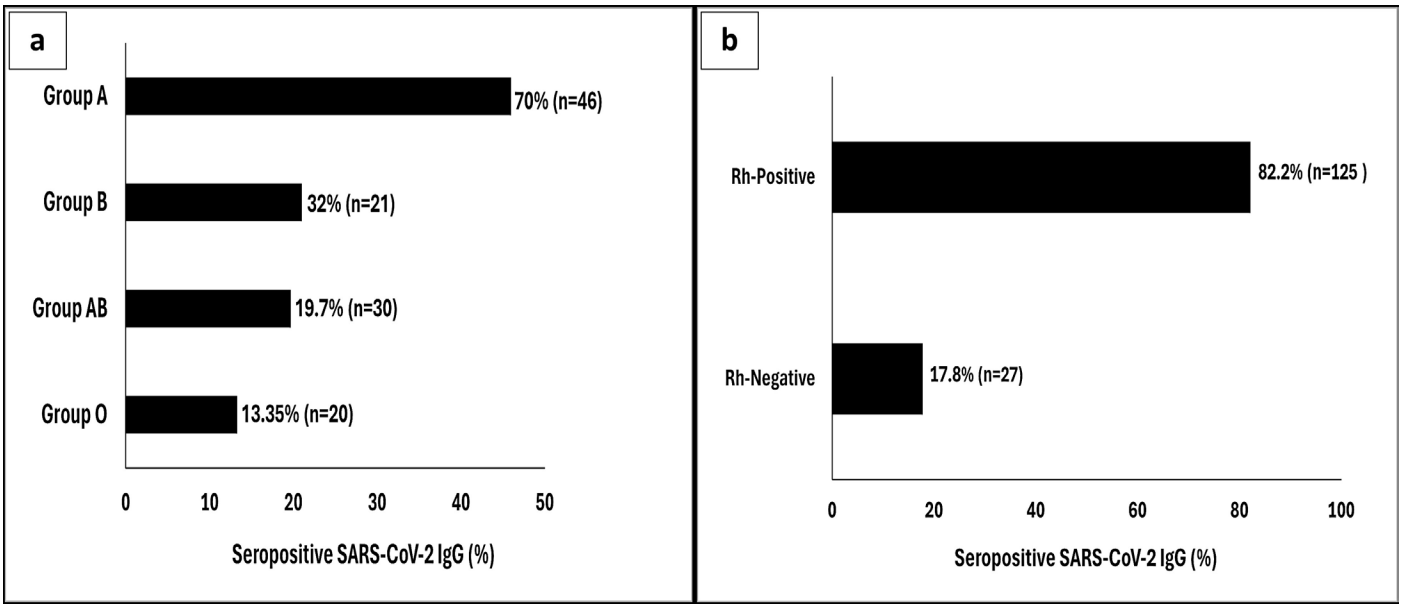

Figure 2: *SARS-CoV-2 nucleocapsid IgG seropositivity by ABO blood group and rhesus factor. (a) Bar chart of seropositivity proportions by ABO blood group (p < 0.001), (b) Bar chart of seropositivity proportions by Rh status (p = 0.030). Where; ABO: ABO blood-group system, Rh: Rhesus, IgG: Immunoglobulin G.*

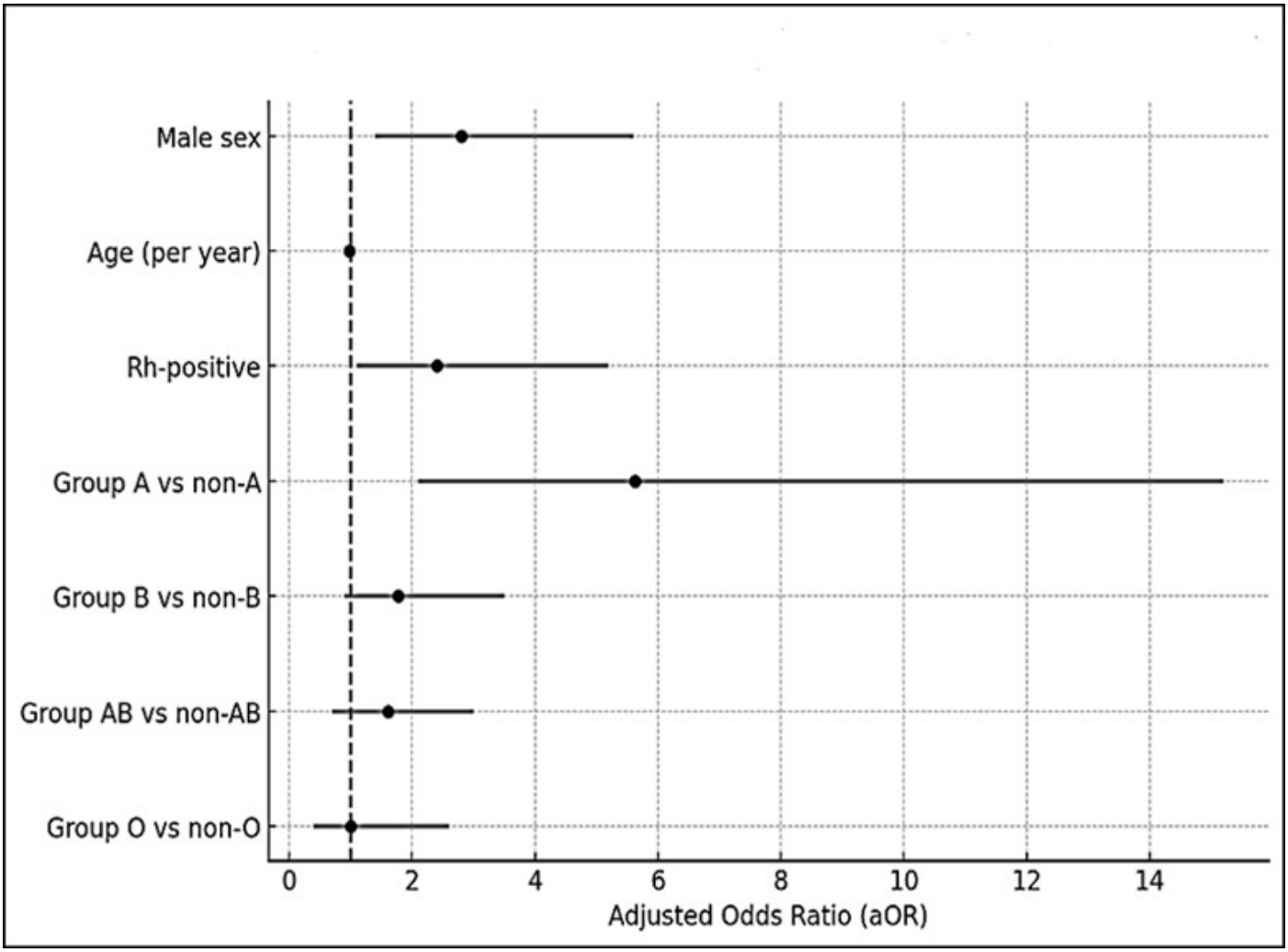

Figure 3: *Forest plot of adjusted odds ratios for predictors of SARS-CoV-2 IgG seropositivity. The plot shows adjusted odds ratios (aORs) with 95% confidence intervals (CIs) for each variable analyzed. Male sex, Rh-positive status, and blood group A were identified as significant predictors (p < 0.05). Where; aOR: adjusted odds ratio, CI: Confidence interval.*

Discussion

Our serosurvey of 200 unvaccinated, current non-smoking blood donors at Alexandria University Hospital, serving both urban and surrounding rural areas, revealed a strikingly elevated 76.0% seroprevalence of anti–nucleocapsid IgG. By excluding current smokers, common bloodborne infections, and vaccine-induced antibodies, and by employing a highly sensitive nucleocapsid-based ELISA, confounders were minimized and low-titer responses were captured often missed by rapid tests (Xia *et al.*, 2020; Eldesoukey *et al.*, 2022). The use of N-protein detection is particularly valuable in settings where spike-only vaccine rollouts can obscure infection-derived immunity (Cucinotta and Vanelli, 2020).

This seroprevalence markedly exceeds regional estimates, 34.4% in Egypt (Eldesoukey *et al.*, 2022), 63.0% in Pakistan (Younas *et al.*, 2020), and 49.4% in India (Chunchu *et al.*, 2022), highlighting the influence of demographic and methodological variables. Our focus on young, socially active donors during periods of escalating community transmission, along with the superior sensitivity of nucleocapsid-targeting ELISA, likely explained this discrepancy (WHO, 2020).

Having established a high baseline seroprevalence, key host factors associated with infection were identified. In multivariate analysis, male sex independently predicted higher odds of seropositivity (aOR = 2.8; p< .001). This obtained result aligns with global reports and may reflect behavioral tendencies such as delayed healthcare-seeking among men, in addition to biological factors such as increased ACE2 expression and lower estrogen-mediated immune protection (Abdollahi *et al.*, 2020; Zhang *et al.*, 2020). Similar trends have been observed in Middle Eastern and Gulf-region cohorts (Assiri *et al.*, 2013; Abdulla *et al.*, 2023).

The current most novel finding relates to the ABO blood group system. Blood group A was considerably associated with seropositivity (aOR = 2.1; p = 0.001), while group O showed a non-significant protective trend (aOR = 0.6; p = 0.098). Group AB had modestly elevated odds (aOR = 1.7; p = 0.047), whereas group B showed no appreciable difference. Proposed mechanisms include enhanced ACE2 binding *via* A-type glycans and potential blocking effect of anti-A isoagglutinins, particularly IgG found in group O individuals (Silva-Filho *et al.*, 2020; Shamikh *et al.*, 2021; Shibeeb and Khan, 2021; Alzabeedi *et al.*, 2022).

Rh-positive status was also associated with higher seropositivity (aOR = 2.4; p = 0.030). While some large-scale studies suggest a protective effect for Rh-negative individuals, the mechanistic basis remains ambiguous. The Rh (D) antigen was not expressed on ACE2-expressing cells, which diminished the likelihood of a direct viral entry role. However, hypotheses involving complement activation, immune modulation, or erythrocyte interactions continue to be explored (Cooling, 2015; Latz *et al.*, 2020).

This study offered several notable strengths. It is the first in Northern Egypt to combine high-sensitivity anti-nucleocapsid IgG serology with ABO and Rh blood group profiling in a rigorously pre-screened, unvaccinated, non-smoking donor cohort. The exclusion of individuals with chronic comorbidities or bloodborne infections minimized confounding, while the use of a validated, high-performance ELISA targeting the nucleocapsid antigen enabled detection of prior natural infection independent of vaccination status (Liu *et al.*, 2021). Detailed demographic and immunohematological data allowed robust multivariable modelling, yielding independent host-related susceptibility markers that refine regional seroepidemiological baselines (Eldesoukey *et al.*, 2022; Butler *et al.*, 2023; Carreño *et al.*, 2024).

However, certain limitations in this study should be considered. Thus, cross-sectional design precluded causal inference, and the single-center, age-restricted sample may limit generalizability to the other populations. Antibody waning over time could have led to underestimation of prior infections, particularly for early exposures y in the pandemic. Absence of viral genomic sequencing prevented assessment of how specific SARS-CoV-2 variants or mutants may have influenced seroprevalence patterns or host-factor associations. Additionally, unmeasured variables such as occupational exposure or socioeconomic status could not be fully accounted for. Future multi-centre longitudinal studies incorporating genomic surveillance and comprehensive immunological profiling are warranted to validate and extend these observations.

Together, the present findings offer the first comprehensive sero-epidemiological and immunohematological profile of SARS-CoV-2 exposure in a carefully pre-screened, unvaccinated population from Northern Egypt. Through the integration of high-sensitivity anti-nucleocapsid IgG testing with ABO and Rh blood group analysis, this study enhances regional estimates of silent transmission and highlights host-related susceptibility factors with potential public health relevance (Girgis *et al.*, 2021; Sah *et al.*, 2021; Hafez *et al.*, 2022; Abuawwad *et al.*, 2023; Phan *et al.*, 2023). The obtained results address a critical knowledge gap in resource-limited settings and generate testable hypotheses for future research, particularly regarding the interplay between host phenotypes, viral evolution, and immune response. These insights lay the groundwork for targeted surveillance strategies and population-specific prevention measures that can adapt as the pandemic landscape evolves.

Conclusions and Recommendations

This study reveals a notably high anti-nucleocapsid IgG seroprevalence of 76.0% among unvaccinated, non-smoking blood donors in Northern Egypt, reflecting widespread asymptomatic SARS-CoV-2 transmission. Male sex, blood group A, and Rh-positive status were identified as independent host-related susceptibility markers, offering a valuable foundation for targeted surveillance and risk stratification efforts. In resource-limited settings, ABO and Rh blood typing may serve as cost-effective tools to inform booster vaccination strategies, while group O individuals with high antibody titers could be prioritized for convalescent plasma donation during future variant-driven surges. These findings underscore the need for multi-center longitudinal studies incorporating detailed immunological assessments, including IgM levels, neutralizing antibody activity, T-cell responses, and genomic surveillance to better understand host-virus dynamics and tailor prevention strategies to local populations.

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Novelty Statement

We report the first application of high-sensitivity nucleocapsid-based serology in unvaccinated, non-smoking Egyptian blood donors, revealing 76.0% anti-nucleocapsid IgG seroprevalence. This approach detected low-titer antibodies that were typically missed by spike-only assays. By excluding smokers, vaccine recipients, and individuals with blood-borne infections (*i.e.*, HIV, HBV, HCV, and syphilis), we identified male sex, blood groups A/AB, and Rh-positive status as independent predictors of seropositivity. These findings support the use of phenotype-guided strategies to prioritize booster vaccination and convalescent plasma donation in resource-limited settings.

Author's Contribution

FMA and HHAG: Conceptualization and supervision.
AAMD: Data curation, formal analysis, investigation, methodology, resources, visualization, and writing-original draft.
NH: Data curation, validation, visualization, writing-original draft, and writing-review and editing.
All authors read and approved the final manuscript.

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Ethical approval
The study protocol was reviewed and approved by the Suez Canal University Ethics Committee (REC141/2022). Written informed consents were obtained from all participants before samples collection.

Generative AI or AI-assisted technology statement
The authors declare that no generative artificial intelligence (AI) was used during preparation of this manuscript.

Conflict of interests
The authors have declared no conflicts of interest.

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