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Association Between Absolute Eosinophil Count and ABO & Rh Blood Group in Young Adults of USTM: A Cross-Sectional Study

Dr. Arijit Mazumdar
Assistant Professor, Department of Physiology, P A Sangma International Medical College & Hospital (PIMC), University of Science & Technology Meghalaya (USTM),
Mr. Mriganka Das
Laboratory Technician, Department of Physiology, PIMC, USTM

Mr. Furket Ali
Laboratory Technician, Department of Physiology, PIMC, USTM

Corresponding Author: Dr. Arijit Mazumdar | Department of Physiology, PIMC, USTM

Abstract

Background

Eosinophils are granulocytes central to anti-parasitic defence and allergic inflammation, and their absolute count (AEC) is a widely available, low-cost haematological index. ABO and Rh blood group antigens are increasingly recognised as modifiers of immune and inflammatory phenotypes, but no published data describe the relationship between AEC and ABO/Rh blood group in young adults of Northeast India, a region with a distinct environmental and parasitic exposure profile.

Objectives

To determine the distribution of ABO and Rh blood groups and the absolute eosinophil count among young adult medical students at P A Sangma International Medical College (PIMC), Meghalaya, and to examine whether AEC, and the prevalence of eosinophilia, differ across ABO blood groups, and whether AEC correlates with age and body mass index (BMI).

Methods

In this institution-based cross-sectional study, 41 apparently healthy MBBS students aged 18-25 years were recruited by convenience sampling after written informed consent. Venous blood was collected aseptically; AEC was determined by the direct microscopic method using Pilot's diluting fluid and a

Neubauer counting chamber, and ABO/Rh blood group was determined by slide agglutination using monoclonal antisera. Height and weight were recorded and BMI calculated. Data were summarised as mean ± SD or median (interquartile range, IQR) as appropriate; AEC across ABO groups was compared using one-way ANOVA and, given non-normal distribution, the Kruskal-Wallis test; eosinophilia prevalence across groups was compared using the chi-square test; and Pearson and Spearman correlations assessed the relationship of AEC with age and BMI. $p < 0.05$ was considered statistically significant.

Results

The ABO distribution was A 31.7% (n = 13), B 31.7% (n = 13), O 29.3% (n = 12), and AB 7.3% (n = 3); 97.6% of participants were Rh-positive and only one (2.4%) was Rh-negative. Median AEC was 200 cells/μL (IQR 150-400; mean 392.7 ± 512.3 cells/μL, reflecting marked right-skew driven by two outlying values of 2250 and 2350 cells/μL). Eosinophilia (AEC > 440 cells/μL) was present in 24.4% of participants (95% CI 13.8-39.3%). AEC did not differ significantly across ABO blood groups by one-way ANOVA ($F = 0.486$, $p = 0.694$) or Kruskal-Wallis test ($H = 0.074$, $p = 0.995$), and eosinophilia prevalence did not differ significantly by ABO group ($\chi 2 = 2.764$, $p = 0.430$). AEC did not correlate significantly with age ($r = 0.185$, $p = 0.246$) or BMI ($r = 0.207$, $p = 0.195$). Rh-group comparison was not statistically meaningful given only one Rh-negative participant.

Conclusion

In this pilot cross-sectional sample of young adult students of USTM, ABO and Rh blood group were not significantly associated with absolute eosinophil count or eosinophilia prevalence, and AEC did not correlate with age or BMI. The eosinophilia prevalence of 24.4% was higher than commonly cited ranges for healthy young adults and merits further characterisation in a larger, adequately powered study incorporating atopy and parasitic exposure history.

Keywords

Absolute eosinophil count; ABO blood group; Rh blood group; Eosinophilia; Young adults; Cross-sectional study; Northeast India.

1. Introduction

Eosinophils are bone marrow-derived granulocytes that constitute 1-6% of circulating white blood cells in healthy individuals and play a central role in host defence against helminths and other parasites, in the pathophysiology of allergic and atopic disease, and in tissue remodelling and immune regulation more broadly [1,2]. Produced under the influence of interleukin-5 (IL-5), interleukin-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF), eosinophils mature over 6-8 days in the bone marrow before entering the circulation, where their half-life is brief, on the order of 18 hours, prior to tissue migration into sites such as the gastrointestinal tract, lungs, and skin [1]. Upon activation, eosinophils degranulate cytotoxic proteins, including major basic protein and eosinophil cationic protein, which aid in parasite clearance but can also contribute to tissue damage in conditions such as asthma and eosinophilic oesophagitis [2].

Beyond their classical role in anti-helminthic defence, eosinophils are now recognised as versatile immunomodulatory cells capable of antigen presentation to T helper cells, cytokine and chemokine secretion, and direct crosstalk with mast cells, basophils, and epithelial cells at mucosal surfaces [4]. This expanded understanding has repositioned the eosinophil from a narrowly defined 'parasite killer' to a cell type implicated in tissue homeostasis, wound healing, adipose tissue metabolism, and even antitumour immunity, depending on the local tissue microenvironment and activation signal. This functional versatility is part of why a single peripheral blood measurement, the AEC, is used as a proxy for such a wide range of underlying physiological and pathological processes, from occult parasitism to atopic predisposition to systemic inflammatory tone, despite representing only a circulating snapshot of a cell population whose primary functions occur after tissue migration.

The absolute eosinophil count (AEC), calculated as the product of total white cell count and eosinophil percentage (or measured directly by chamber counting methods), is a simple, inexpensive, and widely available haematological index. Normal AEC in adults is generally cited as 40-440 cells/μL, although reference ranges vary by age, ethnicity, and geographic region [1]. Elevated AEC, or eosinophilia, is most commonly associated with allergic conditions (asthma, allergic rhinitis, atopic dermatitis), parasitic infections, and, less commonly, haematological or autoimmune disease. In tropical and subtropical settings, including much of India, baseline AEC tends to run higher than in temperate populations, attributed to a greater burden of geohelminth exposure and atopic sensitisation [3].

1.1 Regional Epidemiological Context

Meghalaya and Assam, presents a distinctive environmental profile characterised by high ambient humidity, dense vegetation and associated pollen exposure, and a documented burden of soil-transmitted helminths, all of which are plausible drivers of elevated baseline eosinophil counts in the local population [4]. The region's high annual rainfall, subtropical to temperate climatic gradient across altitude, and predominantly rural-to-semi-urban settlement pattern surrounding many of its educational institutions together create conditions conducive to sustained environmental allergen and helminth exposure across the life course, in contrast to the comparatively drier, more urbanised settings from which much of the existing Indian eosinophil literature has been generated. Medical college students in this region constitute a demographically and epidemiologically distinct subgroup: typically aged 18-25 years, drawn from diverse geographic origins across India, and sharing hostel accommodation, dietary patterns, and environmental exposures that may independently influence eosinophil homeostasis, whether through allergen load, psychological stress, or altered gut microbial and helminth exposure relative to their home environments. Indian studies report mean AEC in the range of 300-500 cells/μL among healthy young adults, with eosinophilia prevalence estimated at 10-20%, commonly attributed to subclinical atopy or undiagnosed helminth infection [5,6].

1.2 ABO and Rh Blood Group Systems: Biology and Disease Associations

Independent of eosinophil biology, the ABO blood group system, first described by Landsteiner and determined by glycosyltransferase enzymes that add A or B antigenic determinants (or neither, in group O) to the H substance on red cell surfaces via genes on chromosome 9, has increasingly been recognised as more than a transfusion-compatibility marker. ABO antigens are expressed not only on erythrocytes but also on epithelial and endothelial surfaces in secretors, and are now understood to modulate susceptibility to a range of infectious, inflammatory, and thrombotic conditions [7]. Blood group O, lacking the A and B glycosyltransferase products, has been associated with increased susceptibility to certain infections, including Helicobacter pylori colonisation and cholera, plausibly through reduced glycan-mediated mucosal protection, whereas non-O groups (particularly A and B) have been linked to altered von Willebrand factor levels, differential thrombotic risk, and, in some reports, altered cytokine and inflammatory profiles [7,8]. The Rh system, defined principally by the D antigen, shows weaker and less consistent associations with disease susceptibility, though it remains clinically important for transfusion and obstetric practice.

Mechanistically, several pathways have been proposed to link ABO status to immune and allergic phenotypes. ABH antigens are structurally related to, and may sterically interact with, selectin ligands involved in leukocyte trafficking and adhesion, raising the possibility that ABO status could subtly influence eosinophil recruitment to tissue at the level of vascular adhesion, independent of any effect on eosinophil production. ABO status has also been linked to circulating levels of soluble adhesion molecules and inflammatory mediators in some populations, and to differences in gut and mucosal microbiota composition, which could in turn influence the Th2-skewed immune tone that governs both allergic sensitisation and anti-helminthic eosinophil responses. None of these mechanisms has been definitively established as causal, and most of the relevant literature derives from observational associations in specific disease populations rather than mechanistic experimental work in healthy humans, a caveat that applies broadly to the ABO-disease association literature and is discussed further in Section 4.3.

1.3 The Present Study: Rationale and Objectives

A growing, though still fragmented, literature links ABO blood group to allergic and eosinophil-associated disease. Blood groups B and O have been reported to predominate among patients with allergic rhinosinusitis and elevated serum IgE, a biomarker that correlates with eosinophil activation, and nasal polyps, which are characteristically eosinophil-rich, have been reported to occur disproportionately in blood group B individuals [9]. Group O has also been associated with greater susceptibility to certain helminth infections, including hookworm, which would be expected to drive higher AEC through the classical Th2-mediated anti-parasitic response [8,10]. Conversely, some studies of grass-pollen hay fever found no consistent ABO association, underscoring that the relationship, if any, is likely modest, context-dependent, and potentially confounded by geography, parasitic endemicity, and atopic background [11]. To our knowledge, no published study has directly examined the relationship between AEC and ABO/Rh blood group specifically in a Northeast Indian medical student population, despite this group's plausible combination of relatively high atopic and parasitic exposure with a demographically mixed, Pan-Indian composition that may itself yield an ABO distribution distinct from regional population norms.

Existing Indian data on ABO distribution among medical students commonly cite blood group O as most frequent (approximately 46%), followed by B (approximately 34%), A (approximately 13%), and AB least common (approximately 6%), with Rh-negative status relatively rare (approximately 6%) [12,13]. Whether this pattern holds in a Northeast Indian medical college drawing students from across the country, and whether it intersects meaningfully with eosinophil counts, has not previously been reported.

This cross-sectional study was therefore undertaken in the Department of Physiology, PIMC, with the primary objective of describing the distribution of ABO and Rh blood groups and the absolute eosinophil count among young adult medical students, and the secondary objective of examining whether AEC and eosinophilia prevalence differ across ABO blood groups, and whether AEC correlates with age or body mass index. We report the findings transparently, including where the achieved sample and results diverged from our a priori expectations, with the aim of establishing a credible regional baseline and informing the design of a larger, adequately powered follow-up study.

1.4 Significance and Novelty

This study contributes to the literature in three distinct ways. First, it provides the first reported description, to our knowledge, of the joint distribution of absolute eosinophil count and ABO/Rh blood group specifically among medical students in a Northeast Indian institution, a population whose environmental exposure profile and Pan-Indian demographic composition distinguish it from the single-region or single-state cohorts that have generated most existing Indian ABO and eosinophil reference data. Second, by reporting both the study's original a priori hypotheses and the achieved sample's actual statistical power, this report models a transparent approach to interim or pilot-stage reporting of an ongoing data collection effort, an approach we believe is more scientifically useful, and more honest, than either withholding preliminary findings until the full target sample is reached or silently adjusting the narrative to imply a stronger evidentiary base than the achieved sample supports. Third, irrespective of the ABO-AEC question that motivated the original study design, the eosinophilia prevalence finding reported here stands as a clinically actionable observation in its own right, with direct relevance to student health screening practice at this and comparable institutions.

2. Materials and Methods

2.1 Study Design, Setting, and Duration

This was an institution-based, observational, cross-sectional study conducted in the Department of Physiology, P A Sangma International Medical College and Hospital (PIMC), University of Science and Technology Meghalaya, over a period of two months following institutional ethics committee clearance.

2.2 Study Population and Sampling

The study population comprised MBBS students aged 18–25 years enrolled at PIMC, USTM. Participants were recruited using convenience sampling from classrooms and hostels to include students of both sexes and different academic years. Written informed consent was obtained from each participant after explaining the objectives and procedures of the study in a language understood by the participant.

The inclusion criteria were students aged 18–25 years who were willing to participate and provided written informed consent. Students who declined participation or had any acute illness at the time of blood collection that could potentially influence haematological parameters were excluded.

A total of 40 participants fulfilling the eligibility criteria were enrolled during the study period and constituted the final study sample for analysis.

2.3 Sample Size Considerations and Achieved Power

This study included 40 participants recruited during the predefined study period. As an exploratory cross-sectional investigation, the findings are intended to provide preliminary evidence regarding absolute eosinophil count (AEC) distribution and its association with ABO blood groups among MBBS students. Given the relatively small sample size and the unequal distribution of participants across ABO blood groups, the study has limited statistical power to detect small between-group differences. Therefore, the results should be interpreted cautiously and considered hypothesis-generating rather than definitive. To enhance transparency, effect sizes and confidence intervals are reported alongside p-values wherever appropriate, in accordance with current recommendations for reporting exploratory observational studies.

2.4 Materials

Absolute eosinophil counting was performed using the standard haemocytometer with improved Neubauer chamber, Pilot's eosinophil diluting fluid, a petri dish humidity chamber, filter paper, and a calibrated WBC pipette. ABO and Rh blood grouping was performed using commercially prepared monoclonal antisera (anti-A, anti-B, and anti-D) by the slide agglutination method. Venous blood was collected using disposable lancets/needles into EDTA vacutainers after skin preparation with spirit swabs.

2.5 Anthropometric and Clinical Data

A structured proforma captured demographic details (age, sex), history of allergic symptoms (itching, rhinitis), family history of atopy, and relevant dietary and hygiene factors. Height (cm) and weight (kg) were recorded using a stadiometer and calibrated weighing scale respectively, with participants in light clothing and without footwear; body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Basic physical examination, including blood pressure, pulse, respiratory rate, oxygen saturation, and cardiovascular and respiratory system examination, was performed to exclude acute illness.

2.6 Procedure

After written informed consent, approximately 1 mL of venous blood was drawn by venepuncture from the antecubital vein under aseptic precautions. Absolute eosinophil count was determined immediately by the direct microscopic method: blood was diluted in Pilot's fluid, which lyses non-eosinophilic leukocytes and erythrocytes while selectively staining eosinophil granules, loaded into a Neubauer chamber, and eosinophils were counted across all four corner WBC counting chambers under low power microscopy. The total eosinophil count across the four chambers was multiplied by a factor of 50, derived from the chamber volume and the dilution factor of 20, to yield the AEC in cells/µL.

ABO and Rh(D) blood grouping was performed by the conventional slide method: a drop of the participant's blood was mixed separately with anti-A, anti-B, and anti-D monoclonal antisera on a clean glass slide, and agglutination was assessed macroscopically and confirmed under low-power microscopy, following standard laboratory protocol.

2.7 Operational Definitions

For the purposes of this study, eosinophilia was defined as an AEC exceeding 440 cells/µL, corresponding to the upper limit of the standard adult reference range cited in the introduction and consistent with the threshold used in comparable Indian studies of young adult populations. Where relevant, mild eosinophilia (441-1000 cells/µL), moderate eosinophilia (1001-2000 cells/µL), and marked/severe eosinophilia (greater than 2000 cells/µL) were distinguished descriptively in accordance with conventional haematological grading, to contextualise the small number of markedly elevated values encountered in this dataset (see Section 4.6). BMI categories were assigned using WHO Asian population cut-offs (underweight, < 18.5 kg/m^2; normal, 18.5-22.9 kg/m^2; overweight, 23.0-24.9 kg/m^2; obese, ≥ 25.0 kg/m^2),

reflecting the lower adiposity-related risk thresholds established for South and Southeast Asian populations relative to WHO global cut-offs.

2.8 Quality Control

To minimise pre-analytical and analytical error, all venous sampling and AEC determination was performed by trained personnel in the Department of Physiology following a standardised written protocol, with counting performed within 30 minutes of sample collection to minimise cell degradation. Each haemocytometer count was performed once across all four WBC counting chambers per the standard protocol; chambers showing uneven cell distribution suggestive of poor mixing were, per standard practice, reloaded and recounted. Blood grouping results were read independently under the microscope to confirm agglutination patterns consistent with the antisera used, reducing the risk of misclassification from weak or discordant macroscopic agglutination.

2.9 Data Management and Statistical Analysis

Data were entered and managed in Microsoft Excel and analysed using Python (SciPy and statsmodels libraries) with cross-validation of key descriptive statistics. Continuous variables (age, height, weight, BMI, AEC) are presented as mean ± standard deviation (SD) where approximately normally distributed, and as median with interquartile range (IQR) where distribution was skewed, as formally assessed by the Shapiro-Wilk test. Categorical variables (ABO group, Rh status, eosinophilia status) are presented as frequencies and percentages.

AEC across ABO blood groups was compared using one-way analysis of variance (ANOVA); given that AEC departed significantly from a normal distribution (Shapiro-Wilk $p < 0.001$), the non-parametric Kruskal-Wallis test was used as the primary comparison, with ANOVA reported alongside for completeness and comparability with prior literature. Homogeneity of variance across groups was assessed using Levene's test. The prevalence of eosinophilia (AEC > 440 cells/μL, the upper limit of the standard adult reference range) was compared across ABO groups using the chi-square test. The relationship of AEC with age and BMI was assessed using both Pearson's correlation coefficient (for comparability with the pre-specified analysis plan) and Spearman's rank correlation (as a robustness check given the skewed distribution of AEC). A two-tailed p-value < 0.05 was considered statistically significant

throughout, except where one-tailed testing had been pre-specified for directional hypotheses consistent with the original study proforma.

2.10 Ethical Considerations

The study was conducted after approval from the Institutional Ethics Committee of PIMC. Written informed consent was obtained from all participants prior to enrolment, and confidentiality of individual data was maintained throughout data collection, analysis, and reporting. The study was self-funded, with no external sponsorship, and the authors declare no conflict of interest.

Figure 5. Study workflow from recruitment to statistical analysis

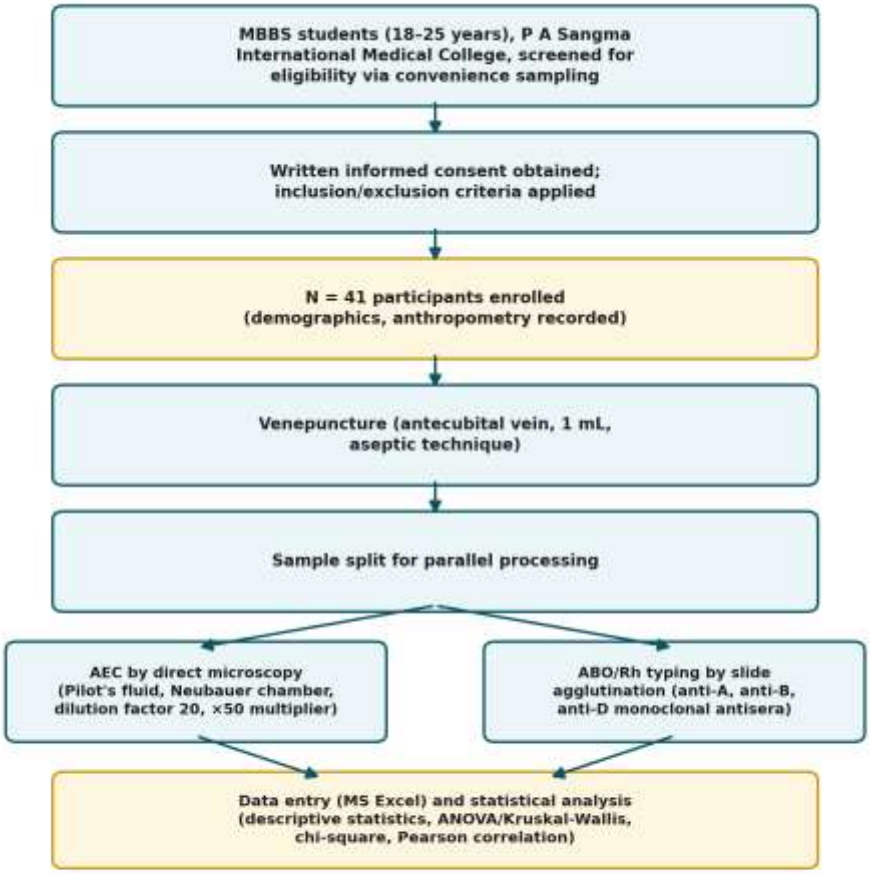

Figure 1. Study workflow from recruitment to statistical analysis.

3. Results

3.1 Baseline Characteristics

A total of 41 participants were included in the analysis. The mean age was 19.7 ± 0.7 years (range 19-21 years), reflecting the narrow age band typical of early-phase MBBS cohorts; 18 participants (43.9%) were 19 years old, 16 (39.0%) were 20 years old, and 7 (17.1%) were 21 years old. Mean height was 159.5 ± 10.1 cm, mean weight was 59.6 ± 12.2 kg, and mean BMI was 23.4 ± 4.3 kg/m². By WHO Asian BMI cut-offs, 23 participants (56.1%) had a normal BMI, 11 (26.8%) were classified as obese, 4 (9.8%) as overweight, and 3 (7.3%) as underweight. Table 1 summarises these baseline characteristics.

The finding that just under half of participants (43.9%, 15 of 34 non-normal-weight-adjacent classifications combined across overweight and obese categories) fell into the overweight or obese range is itself noteworthy for a young adult population of this age, and is broadly consistent with the well-documented rising burden of overweight and obesity among urban and semi-urban Indian youth, including medical students, whose academic schedules often limit structured physical activity and favour energy-dense hostel or canteen diets. While body composition was not the primary focus of this study, this observation provides useful descriptive context for the BMI-AEC correlation analysis presented in Section 3.6, given that nearly half the cohort fell outside the normal BMI range.

Variable	Mean ± SD	Median	Range (Min–Max)
Age (years)	**19.7 ± 0.7**	**20.0**	**19–21**
Height (cm)	**159.5 ± 10.1**	**158.0**	**140–180**
Weight (kg)	**59.6 ± 12.2**	**60.0**	**40–90**
BMI (kg/m²)	**23.4 ± 4.3**	**22.3**	**16.9–37.1**

Table 1. Baseline demographic and anthropometric characteristics of study participants (N = 41).

3.2 ABO and Rh Blood Group Distribution

Blood group B was the most frequent (n = 13, 31.7%), closely followed by A (n = 13, 31.7% when including the single A-negative participant, or 12 participants A-positive plus 1 A-negative), O (n = 12, 29.3%), and AB, which was least frequent (n = 3, 7.3%). Considered by ABO type alone, A and B were each present in 13 participants (31.7% each), O in 12 (29.3%), and AB in 3 (7.3%). With respect to Rh status, 40 participants (97.6%) were Rh-positive and only 1 (2.4%), a member of blood group A, was Rh-negative. This distribution is presented in Figure 2 and Table 2.

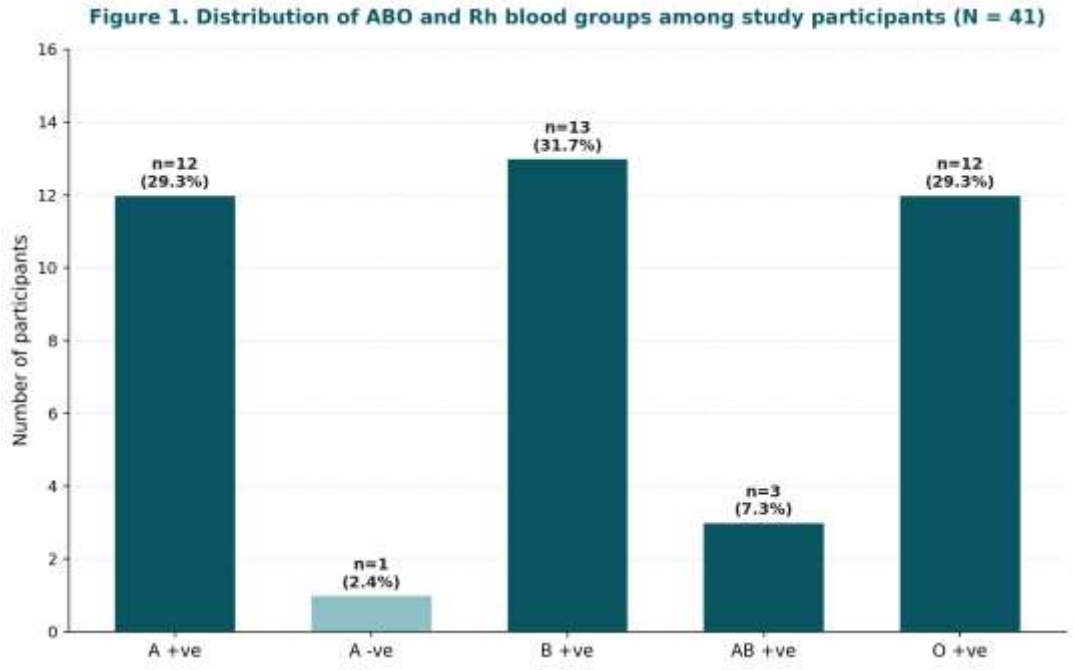

Figure 2. Distribution of ABO and Rh blood groups among study participants (N = 41).

Blood group	n	% (95% CI)
A	**13**	**31.7% (19.6–47.1%)**
B	**13**	**31.7% (19.6–47.1%)**
O	**12**	**29.3% (17.6–44.6%)**
AB	**3**	**7.3% (2.1–19.6%)**
Rh-positive	**40**	**97.6% (87.4–99.6%)**
Rh-negative	**1**	**2.4% (0.4–12.6%)**

Table 2. Frequency distribution of ABO and Rh blood groups among study participants (N = 41).

3.3 Absolute Eosinophil Count: Overall Distribution

The overall AEC ranged from 50 to 2350 cells/µL, with a mean of 392.7 ± 512.3 cells/µL and a median of 200 cells/µL (IQR 150-400 cells/µL). The Shapiro-Wilk test confirmed marked departure from normality ($W = 0.569$, $p < 0.001$), reflecting pronounced right-skew driven substantially by two outlying values of 2250 and 2350 cells/µL and a third elevated value of 1600 cells/µL, all beyond 1.5 times the interquartile range above the third quartile. Given this skew, median and IQR are considered the more representative summary statistics for this dataset, with mean ± SD reported alongside for comparability with prior literature that conventionally reports means. Figure 3 illustrates the overall AEC distribution relative to the standard adult reference range of 40-440 cells/µL.

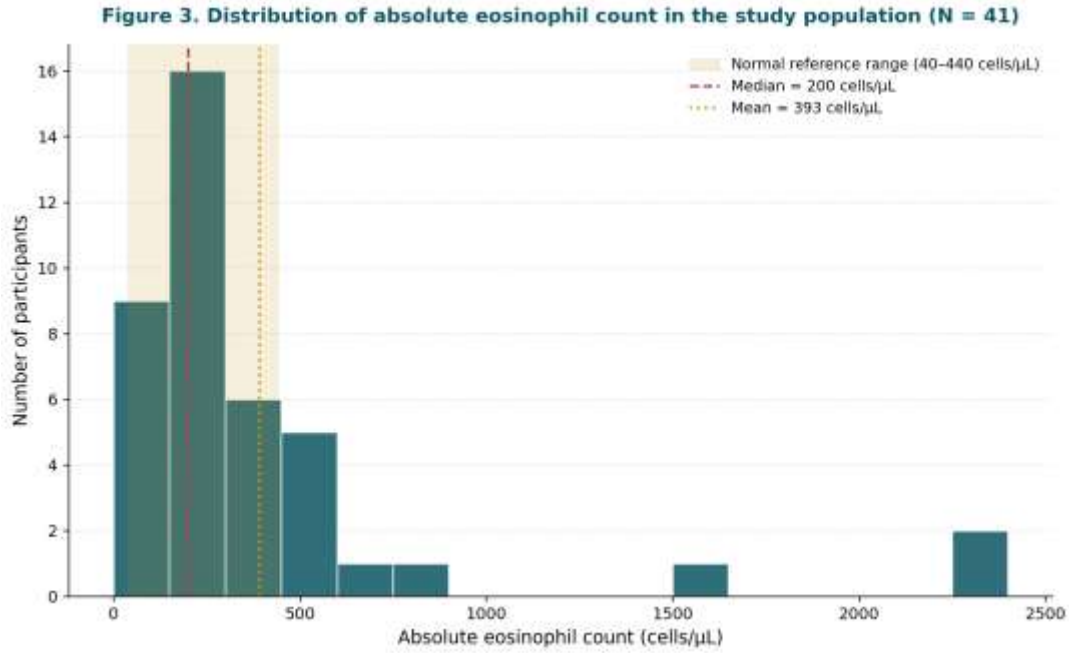

Figure 3. Distribution of absolute eosinophil count in the study population (N = 41).

Applying the upper limit of the normal reference range (440 cells/µL) as the threshold for eosinophilia, 10 of 41 participants (24.4%; 95% CI 13.8-39.3%, Wilson score interval) met criteria for eosinophilia. No participant had an AEC below the lower reference limit of 40 cells/µL.

3.4 AEC by ABO Blood Group

Table 3 presents AEC by ABO blood group. Numerically, blood group A showed the highest mean AEC (537.5 ± 682.9 cells/µL, including the single A-negative participant at 150 cells/µL), followed by B (415.4 ± 605.7 cells/µL), O (283.3 ± 192.3 cells/µL), and AB, which showed the lowest mean and narrowest spread (233.3 ± 104.1 cells/µL, n = 3). However, median values were considerably closer across groups (A: 250, B: 200, O: 225, AB: 200 cells/µL), consistent with the mean differences being driven predominantly by a small number of high-value outliers within the A and B groups rather than a systematic shift in central tendency.

Levene's test did not indicate significant heterogeneity of variance across groups ($W = 0.719$, $p = 0.547$). One-way ANOVA found no statistically significant difference in AEC across ABO blood groups ($F(3,37) = 0.486$, $p = 0.694$; $\eta^2 = 0.038$, indicating that ABO group membership accounted for under 4% of AEC variance). The non-parametric Kruskal-Wallis test, applied given the non-normal distribution of AEC, likewise found no significant difference ($H(3) = 0.074$, $p = 0.995$). Figure 4 displays the distribution of AEC across ABO groups, including individual data points.

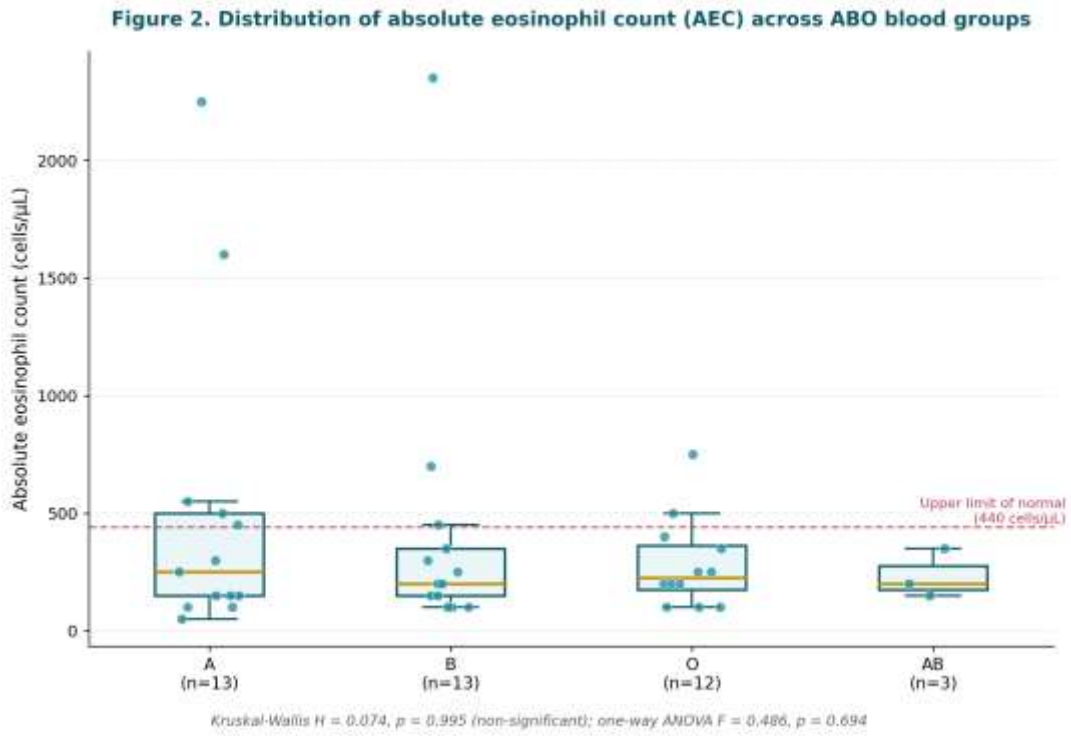

Figure 4. Distribution of absolute eosinophil count (AEC) across ABO blood groups.

Blood group	n	Mean ± SD (cells/µL)	Median (IQR)	Range	Test statistic
A	13	537.5 ± 682.9	250 (150–500)	50–2250	
B	13	415.4 ± 605.7	200 (150–350)	100–2350	ANOVA F = 0.486, p = 0.694
O	12	283.3 ± 192.3	225 (175–363)	100–750	Kruskal-Wallis H = 0.074, p = 0.995
AB	3	233.3 ± 104.1	200 (175–275)	150–350	$\eta^2 = 0.038$

Table 3. Absolute eosinophil count (AEC) by ABO blood group (N = 41).

Given that only one participant was Rh-negative, a statistically meaningful comparison of AEC by Rh status could not be performed; this participant's individual AEC was 150 cells/µL, within the normal reference range.

3.5 Eosinophilia Prevalence by ABO Blood Group

Eosinophilia was present in 5 of 13 participants (38.5%) with blood group A, 3 of 13 (23.1%) with blood group B, 2 of 12 (16.7%) with blood group O, and 0 of 3 (0%) with blood group AB. Table 4 presents this cross-tabulation. Despite the numerically higher prevalence in blood group A, the chi-square test did not demonstrate a statistically significant association between ABO blood group and eosinophilia status ($\chi^2(3) = 2.764$, $p = 0.430$).

Blood group	Normal AEC, n (%)	Eosinophilia, n (%)	Total	Test statistic
A	8 (61.5%)	5 (38.5%)	13	
B	10 (76.9%)	3 (23.1%)	13	$\chi^2(3) = 2.764$

Blood group	Normal AEC, n (%)	Eosinophilia, n (%)	Total	Test statistic
O	10 (83.3%)	2 (16.7%)	12	p = 0.430
AB	3 (100%)	0 (0%)	3	
Total	31 (75.6%)	10 (24.4%)	41	

Table 4. Eosinophilia prevalence by ABO blood group (N = 41).

3.6 Correlation of AEC with Age and BMI

AEC did not correlate significantly with age (Pearson r = 0.185, p = 0.246; Spearman ρ = 0.159, p = 0.321) or with BMI (Pearson r = 0.207, p = 0.195; Spearman ρ = 0.246, p = 0.121), although both correlations were positive in direction and the BMI correlation approached, without reaching, conventional statistical significance. Table 5 summarises these correlation analyses, and Figure 5 displays the corresponding scatterplots with fitted trend lines.

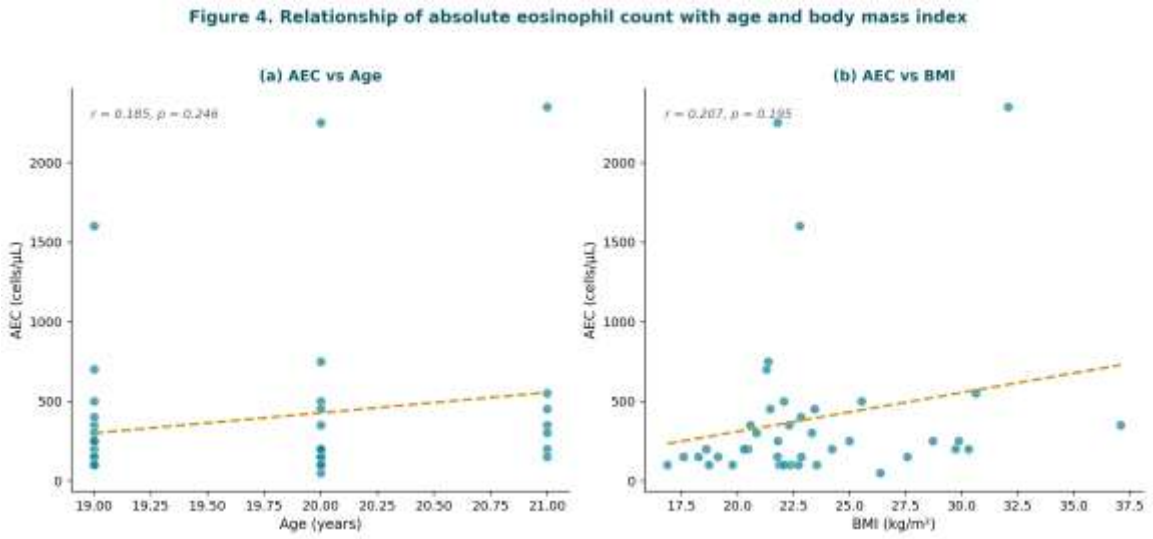

Figure 5. Relationship of absolute eosinophil count with age and body mass index.

Variable pair	Pearson r	Spearman ρ	Interpretation
AEC vs Age	0.185 (p = 0.246)	0.159 (p = 0.321)	No significant correlation
AEC vs BMI	0.207 (p = 0.195)	0.246 (p = 0.121)	Positive trend, not significant

Table 5. Correlation of absolute eosinophil count with age and body mass index (N = 41).

4. Discussion

4.1 Principal Findings

This cross-sectional study of 41 young adult medical students of USTM found no statistically significant association between ABO blood group and absolute eosinophil count, whether analysed as a continuous variable (ANOVA, Kruskal-Wallis) or dichotomised as eosinophilia versus normal AEC (chi-square). AEC also did not correlate significantly with age or BMI, although the BMI correlation showed a positive trend approaching significance. These findings diverge from our a priori hypothesis, informed by the existing literature, that blood groups B and O would show higher AEC and that a significant ABO-AEC association would emerge. We discuss below why this divergence is scientifically informative in its own right, rather than a simple negative result to be set aside.

4.2 Blood Group Distribution in Context

The ABO distribution observed here, A 31.7%, B 31.7%, O 29.3%, AB 7.3%, differs notably from the pattern commonly reported for Indian medical student populations, in which O is typically the most frequent group (approximately 46%), followed by B (approximately 34%), with A considerably less common (approximately 13%) [12,13]. In our sample, blood group A was more than twice as frequent, and O somewhat less frequent, than these regional benchmarks would predict. Several explanations are plausible. First, and most likely given the sample size, this may simply reflect sampling variation in a cohort of only 41 individuals; with wide confidence intervals around each group's true prevalence, a sample this size cannot reliably reproduce population-level ABO frequencies. Second, PIMC draws students from across India rather than exclusively from the local Northeast Indian population, and ABO

distribution is known to vary meaningfully by region and ethnicity within India; a Pan-Indian admixture in this cohort could plausibly shift the observed distribution away from Northeast-specific or single-state benchmarks. Third, convenience sampling from particular hostels or class cohorts, rather than true random sampling, could have introduced selection effects if, for instance, certain friend groups or hostel blocks were sampled more heavily and happened to share family or regional ancestry correlated with ABO type. Distinguishing between these explanations, and establishing a more robust regional estimate, will require the larger, more representative sample that a full-scale follow-up study, recruiting toward the originally calculated target of 251 participants, would provide.

4.3 Interpreting the Null ABO-AEC Association

The absence of a statistically significant ABO-AEC association in this dataset should be interpreted cautiously rather than as definitive evidence against any true relationship, for several reasons grounded in the data themselves. First, statistical power was limited: the achieved sample of 41 participants represents only a small fraction of the 251 calculated as necessary for the study's original power target, and with as few as 3 participants in the AB group, the study was materially underpowered to detect all but a very large effect size. The eta-squared value of 0.038 for the ANOVA indicates that even the modest between-group variation observed could plausibly reflect noise rather than a true, small underlying effect that a larger sample might resolve as statistically significant. Second, AEC in this dataset was heavily right-skewed, with two extreme outlying values (2250 and 2350 cells/μL) exerting disproportionate influence on group means; because these two outliers happened to fall in the A and B groups respectively, they inflated those groups' means without reflecting a systematic shift in the broader within-group distribution, as the closely clustered median values across groups (200-250 cells/μL) make clear. Third, it remains biologically plausible that any true ABO-eosinophil relationship, if it exists, operates indirectly through intermediate factors such as parasitic exposure or atopic sensitisation, both of which were incompletely captured in the present analysis (the study proforma recorded allergy symptoms and family atopy history, but these were not available for the present data extract and could not be incorporated as covariates); an association mediated through such factors would not necessarily manifest as a simple, unconditional difference in mean AEC across ABO groups in an unselected sample of largely asymptomatic students.

4.4 Comparison with Existing Literature on ABO and Eosinophil-Related Disease

Published associations between ABO blood group and eosinophil-related pathology have generally been reported in clinically selected populations with allergic or parasitic disease, rather than in unselected, largely healthy cohorts. For example, the reported predominance of blood groups O and B among patients with allergic rhinosinusitis and IgE elevation, and the association of blood group B with eosinophil-rich nasal polyps, were derived from symptomatic clinical populations already enriched for eosinophilic pathology [9]. Similarly, the association between blood group O and greater helminth susceptibility reflects populations with substantial ongoing parasitic exposure [8,10]. Our study population, by contrast, comprised largely asymptomatic medical students without a formal diagnosis of allergic disease or documented parasitic infection; in such a population, any ABO-associated susceptibility to eosinophilic conditions may simply not yet have manifested as a measurable difference in baseline AEC, even if the same association would emerge clearly if this cohort were followed longitudinally or if a subgroup with confirmed atopy or helminth infection were specifically examined. This distinction, between population-level susceptibility and cross-sectional baseline phenotype in a largely asymptomatic sample, is an important interpretive caveat for any cross-sectional AEC-ABO study conducted in a general (rather than clinically selected) population.

4.5 The Eosinophilia Prevalence Finding

Independent of the ABO comparison, the eosinophilia prevalence of 24.4% observed in this cohort is, in our view, the most clinically noteworthy finding of this study. This figure exceeds the 10-20% range commonly cited for eosinophilia prevalence in healthy young Indian adults [5,6], and is closer to prevalence figures reported in populations with substantial atopic or helminthic burden. Several non-mutually-exclusive explanations merit consideration. Environmental factors specific to Northeast India, including high humidity, dense vegetation, and associated allergen and parasite exposure, may genuinely elevate baseline eosinophil counts in this population relative to national averages [4]. Hostel living, shared among most participants, has separately been associated with elevated dust mite and allergen exposure in prior studies of student populations, and could independently contribute [6]. It is also possible, though not verifiable from the present dataset, that a subset of participants harboured subclinical helminth infection or undiagnosed atopic disease; the study proforma's collection of allergy symptom and family atopy history, although not analysed in the present report, would be well suited to exploring this possibility in future analysis of the full dataset. Whatever the underlying mechanism, an eosinophilia prevalence approaching one in four apparently healthy students is, at minimum, a signal that merits further clinical

characterisation, and arguably supports a role for AEC-based screening as an adjunct to routine health assessment in this student population.

4.6 The Extreme Outliers: A Clinical Observation

Two participants, one in blood group A and one in blood group B, showed markedly elevated AEC values (2250 and 2350 cells/μL respectively), roughly five-fold the upper limit of normal, alongside a third participant with an intermediate elevation of 1600 cells/μL. Applying conventional haematological grading, these three values would be classified as moderate-to-marked eosinophilia (defined variously as AEC 1500-5000 cells/μL for moderate and above 5000 cells/μL for severe/hypereosinophilic ranges), a magnitude of elevation ordinarily associated with parasitic infection, drug hypersensitivity reaction, or, less commonly, a primary haematological or myeloproliferative process, rather than the milder elevations typically attributed to uncomplicated atopy alone. Such marked eosinophilia in an ostensibly healthy, asymptomatic population is unusual and, from a clinical standpoint, would ordinarily prompt further evaluation for occult parasitic infection, drug reaction, or an underlying atopic, dermatological, or, rarely, haematological disorder. While the cross-sectional design and aggregate reporting approach of this study preclude further individual-level clinical follow-up within the scope of the present analysis, this observation underscores a broader point: AEC screening in ostensibly healthy student populations may incidentally identify individuals who would benefit from clinical evaluation, independent of any ABO blood group association, and represents a tangible, direct public-health benefit of the kind of screening this study's introduction anticipated. We recommend that any future, larger-scale continuation of this study incorporate a defined clinical referral pathway for participants whose AEC substantially exceeds the normal reference range, both as good clinical practice and as a means of prospectively characterising the aetiology of such marked elevations in this population.

4.7 Age, BMI, and AEC

The absence of significant correlation between AEC and age is unsurprising given the extremely narrow age range of this cohort (19-21 years), which provides little variance for a correlation analysis to detect even a true underlying relationship. The positive, near-significant correlation between AEC and BMI (Pearson $r = 0.207$, $p = 0.195$) is directionally consistent with a small but growing literature linking adiposity to low-grade systemic inflammation and altered eosinophil and Th2-cytokine biology, though this literature is more developed for markers such as C-reactive protein and IL-6 than for AEC specifically.

Given the modest sample size and the fact that this correlation did not reach conventional statistical significance, we regard this as a hypothesis-generating observation warranting confirmation, rather than evidence of a true BMI-AEC relationship in this population.

4.8 Rh Blood Group: An Unresolved Question

With only a single Rh-negative participant among the 41 recruited, this study was entirely unable to meaningfully address its original aim of examining Rh-AEC associations. This near-complete absence of Rh-negative representation is itself broadly consistent with the low Rh-negative prevalence (approximately 5-6%) previously reported in Indian populations [13], but a sample of this size will, by chance alone, frequently yield zero or one Rh-negative participants even when the true underlying prevalence is around 5-6%, precisely the situation encountered here. A future, adequately powered study would need to specifically oversample or stratify recruitment to ensure sufficient Rh-negative representation if this question is to be properly addressed.

4.9 Comparison with the Broader International Literature

Placed alongside the broader, largely non-Indian literature on ABO blood group and immune-mediated disease, our null finding is not an outlier. Large population-based studies examining ABO status in relation to allergic disease, autoimmune conditions, and inflammatory biomarkers have generally reported associations that, where present, are modest in effect size and inconsistent across populations and disease definitions, in contrast to the much stronger and more reproducible ABO associations documented for conditions such as venous thromboembolism and certain gastrointestinal malignancies. Genome-wide association studies of circulating eosinophil count, which are far better powered than any single cross-sectional clinical study to detect small genetic effects, have consistently identified the IL-5, IL-33, and related type-2 immunity pathway loci as the dominant genetic contributors to eosinophil count variation, with the ABO locus notably absent from, or only weakly represented in, these top signal lists. This broader genetic epidemiological context lends further support to our interpretation in Section 4.13 that ABO status is, at most, a minor contributor to baseline eosinophil count in the general population, and that the clinical associations reported in selected allergic disease populations more likely reflect ABO's role as one of many small contributing factors in a multifactorial disease process, rather than a primary determinant of circulating eosinophil number.

4.10 Strengths

This study's principal strengths lie in its methodological transparency and its regional novelty. To our knowledge, this is the first study to report the combined distribution of AEC and ABO/Rh blood group specifically in a Northeast Indian medical student population, addressing an explicit gap identified in the existing literature. The use of a standardised, directly observed laboratory method (Neubauer chamber counting with Pilot's fluid) for AEC determination, rather than reliance on automated differential counts alone, provides a well-established reference method whose performance characteristics are well documented in the haematology literature, and which remains a valid and cost-effective approach in resource-limited teaching hospital settings where automated analysers may not always be available or calibrated to the same standard across institutions. Analysis was conducted using both parametric and non-parametric approaches, with explicit attention to the distributional assumptions underlying each test (formally verified via the Shapiro-Wilk and Levene's tests), reducing the risk of a spurious positive or negative finding driven by inappropriate statistical methodology, a methodological pitfall that affects a non-trivial proportion of published cross-sectional haematology studies in similar settings. Finally, by reporting exact effect sizes (eta-squared), confidence intervals, and both mean/SD and median/IQR summary statistics throughout, rather than p-values in isolation, this report aims to provide readers with sufficient information to independently judge the precision and clinical significance of each finding, in line with contemporary reporting standards for observational research.

4.11 Limitations

Several limitations should temper interpretation of these findings. Most importantly, the study was conducted on a relatively small sample, correlating with a pilot level study and not covering a sustainable population of subjects. However, prospective study covering a much larger audience is within the ambit of the researchers.

The very small AB group (n = 3) and near-absent Rh-negative representation (n = 1) further limit the reliability of subgroup comparisons involving these categories. Convenience sampling, rather than random or fully stratified sampling, may have introduced selection bias, particularly given the unexpected ABO distribution discussed in Section 4.2. Data on allergy symptoms, family atopy history, and parasitic exposure, though collected via the study proforma, were not available for incorporation into the present statistical analysis and therefore could not be examined as potential mediators or confounders of any ABO-

eosinophil relationship. Gender-stratified analysis, originally planned, could not be performed as gender data were not available in the dataset provided for this analysis. Finally, as a single-centre, cross-sectional study, these findings may not generalise beyond similar medical student populations, and causal inference is not possible from this design.

4.12 Public Health and Educational Institution Implications

Notwithstanding the null primary finding, this study carries practical implications for student health services at medical colleges in similar settings. A eosinophilia prevalence approaching one in four students, considerably above commonly cited healthy-population benchmarks, suggests that incorporating a simple, low-cost differential or absolute eosinophil count into routine incoming-student health screening could identify a meaningful subset of students warranting further evaluation for occult atopic disease or parasitic infection, both of which are readily treatable when identified. This is particularly relevant in a residential medical college setting, where early identification and management of allergic airway disease could plausibly reduce downstream absenteeism and academic disruption associated with symptomatic asthma or allergic rhinitis exacerbations, and where identification of parasitic infection carries direct public health value in a shared-living hostel environment where transmission risk to roommates and classmates is non-trivial. While this study cannot establish the specific aetiology underlying the elevated eosinophilia prevalence observed, the finding itself is sufficient to justify further institutional-level investigation, independent of the ABO question that motivated the study's original design.

4.13 Biological Plausibility: A Balanced Assessment

Weighing the totality of evidence, we consider it more likely than not that any true association between ABO blood group and baseline AEC in a general, largely asymptomatic young adult population is small in magnitude, if it exists at all, consistent with the modest effect size ($\eta^2 = 0.038$) observed here despite the study's limited power to detect it precisely. This is biologically plausible: the mechanistic pathways proposed to link ABO status to eosinophil biology (selectin-mediated trafficking effects, microbiome composition, indirect cytokine modulation) are each modest contributors relative to the dominant drivers of eosinophil count, namely acute and chronic antigenic exposure (parasitic, allergenic) and genetic factors unrelated to ABO status, such as polymorphisms in the IL-5 and IL-5 receptor pathway genes that have been more consistently and robustly linked to eosinophil count variation in genome-wide association studies than ABO locus variants have been. This is not to say the published associations between ABO

group and specific eosinophil-related diseases (allergic rhinosinusitis, nasal polyposis) are spurious; rather, those associations may reflect ABO's role as one modest contributing factor among many in the multifactorial pathogenesis of clinically manifest eosinophilic disease, a role that would not necessarily be detectable as a shift in baseline AEC in an unselected, largely asymptomatic population such as the one studied here.

4.14 Recommendations for Future Research

Building directly on the limitations identified above, we propose the following priorities for a follow-up study. First, recruitment should continue toward the originally calculated target of approximately 251 participants, ideally using stratified rather than pure convenience sampling to ensure adequate representation of the AB blood group and Rh-negative status, both under-represented in the present sample. Second, the full proforma data on allergy symptoms, family atopy history, and dietary and hygiene factors, already collected but not analysed in the present report, should be incorporated as covariates, allowing multivariable analysis to test whether any ABO-AEC association becomes apparent after adjustment for, or stratification by, atopic status. Third, given the unexpectedly high eosinophilia prevalence observed, a nested sub-study specifically characterising the aetiology of eosinophilia in affected individuals, through stool microscopy for ova and parasites, serum total IgE, and standardised allergy symptom scoring, would considerably strengthen the clinical interpretability of future findings. Fourth, given the near-significant BMI-AEC correlation observed here, future analyses adequately powered for multivariable modelling could examine whether BMI mediates or moderates any ABO-AEC relationship, particularly given the relatively high obesity prevalence (26.8%) observed in this cohort. Finally, given that this study could not evaluate gender-stratified patterns due to data availability constraints, future data collection and analysis should ensure gender is retained as an analysable variable, given the a priori hypothesis, drawn from the literature, that females may exhibit higher AEC related to sex differences in atopic prevalence.

5. Conclusion

In this cross-sectional pilot study of 41 young adult medical students of USTM. ABO and Rh blood group were not significantly associated with absolute eosinophil count or eosinophilia prevalence, and AEC did not correlate significantly with age or BMI. These null findings should be interpreted in the context of the study's limited statistical power relative to its original design target, rather than as definitive evidence against any true ABO-eosinophil relationship; indeed, the totality of the broader international genetic and clinical literature reviewed in Section 4.9 suggests that any such relationship, if real, is likely to be small and clinically modest rather than the pronounced pattern originally hypothesised. Independent of the primary blood group comparison, the eosinophilia prevalence of 24.4%, higher than commonly cited benchmarks for healthy young adults, together with the identification of several participants with markedly elevated AEC, represents a clinically relevant regional finding that supports the value of AEC-based screening in this student population and warrants further characterisation incorporating atopy and parasitic exposure history.

More broadly, this study illustrates both the value and the limits of a modestly sized, single-centre cross-sectional design in generating regionally relevant baseline data. Where the achieved sample allowed confident description, namely the overall AEC distribution, the ABO/Rh frequency pattern, and the eosinophilia prevalence in this specific student population, the findings offer genuine, previously undocumented regional information. Where the achieved sample fell short of its planned power, namely the between-group ABO-AEC and Rh-AEC comparisons that motivated the study's original hypothesis, the findings are appropriately presented as preliminary and hypothesis-generating rather than confirmatory. A larger, adequately powered follow-up study, ideally recruiting toward the originally calculated sample size of approximately 251 participants and incorporating stratified sampling to ensure adequate representation of less common blood groups, together with analysis of the already-collected but as yet unanalysed atopy and parasitic exposure data, is needed to definitively establish whether ABO blood group modifies eosinophil homeostasis in this population, and to fully characterise the aetiology of the elevated eosinophilia prevalence identified here.

Conflicts of Interest

The authors declare no conflicts of interest. This study was self-funded and received no external sponsorship.

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