

ORIGINAL RESEARCH

Exploring the Relationship Between ABO Blood Groups and Hemostasis Parameters: A Observational Study Conducted Among Medical Students

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ABSTRACT

Background:Bleeding time (BT), clotting time (CT), and blood grouping constitute standard procedures in hematology experiments within physiology labs. In the medical realm, these tests are pivotal in conditions such as thrombosis and epistaxis prior to surgical interventions. Achieving effective hemostasis holds paramount significance for both surgeons and anesthetists. While numerous investigations have explored the correlation between blood groups and various diseases, limited research has delved into the connection between blood groups and BT, CT. The study's primary objective is to evaluate the interrelationship between BT and CT and different ABO blood groups.**Methods:**Conducted within the Department of Physiology, this investigation involved 400 students as participants. The determination of blood groups was executed through the mixing of blood samples with antisera A, B, and antisera Anti D. **Results:**The study outcomes revealed that blood group O (37%) exhibited higher prevalence, followed by blood groups B (30.5%), A (23.5%), and AB (9%). Notably, bleeding time was observed to be prolonged (>3 minutes) in the AB blood group (37.5%), with group O (25%), A (25%), and B (12.5%) following suit. This difference was found to be statistically significant ($p=0.03$), emphasizing a noteworthy association between blood groups and prolonged bleeding time.**Conclusion:**The findings from our study highlight that blood group O emerged as the most prevalent, whereas AB stood as the least common. Notably, bleeding time (BT) was prolonged in the AB blood group, contrasting with clotting time (CT) being extended in the O blood group. Furthermore, our results indicate that clotting time was more prolonged in females compared to males.

Keywords:Bleeding Time, Clotting Time, Blood Groups

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INTRODUCTION

In the pivotal year of 1900, the eminent scientist Karl Landsteiner made an epochal contribution to the field of hematology by unraveling the mysteries of the ABO blood group system. This groundbreaking discovery not only marked a watershed moment in the understanding of blood composition but also laid the cornerstone for the inception of blood banks and the evolution of modern blood transfusion practices.¹The ABO blood group classification, now an integral part of medical science, is centered on the distinctive antigenic properties exhibited by red blood cells. Individuals with type A blood showcase the presence of the A antigen, while those with type B blood harbor the B antigen. Remarkably, individuals with type AB blood possess both A and B antigens, creating a

unique classification. On the other hand, individuals with type O blood lack both A and B antigens, setting them apart in this comprehensive categorization.²Delving into the molecular intricacies, the A and B antigens manifest as complex oligosaccharides on the surfaces of red blood cells. What distinguishes these antigens are the nuances in their terminal sugars, providing a molecular signature unique to each blood type. The transformative role in this intricate process is played by A and B glycosyltransferase enzymes, which catalyze the conversion of the H antigen into the A and B determinants. Notably, individuals belonging to the O blood group exhibit a distinctive characteristic – the deficiency of the A and B glycosyltransferase enzymes. Consequently, despite lacking A and B

antigens, they continue to express the H antigen. This biochemical phenomenon underscores the fascinating complexity of blood types and the intricate interplay of antigens and enzymes.

Landsteiner's seminal discovery not only unveiled the inherent diversity in blood composition but also paved the way for life-saving practices.³ The establishment of blood banks and the meticulous matching of blood types in transfusions became possible, transforming the landscape of medicine and ensuring safer and more effective medical interventions. The legacy of Landsteiner's work endures as an indelible mark on the history of medical science, contributing immeasurably to our understanding of human biology and the advancement of life-saving medical procedures.

Recent investigations have brought to light a compelling association between individuals with blood group 'O' and a heightened susceptibility to epistaxis, or nosebleeds.⁴ This intriguing link is believed to be attributed to the lower expression of Von Willebrand factor in individuals carrying the blood group 'O'. Von Willebrand factor is a crucial component in the blood clotting process, and its reduced levels in blood group 'O' individuals may contribute to a predisposition for nosebleeds. Moreover, the tapestry of research has woven a narrative connecting ABO blood groups with an array of health conditions, presenting a comprehensive panorama of their impact on human health. Beyond the conventional understanding of blood groups solely in the context of blood compatibility, emerging data suggests that ABO blood groups may exert influence on various physiological processes and disease susceptibilities. Intriguingly, data from multiple studies point towards ABO blood groups as potential determinants in conditions such as duodenal ulcer, gastric carcinoma, diabetes mellitus, urinary tract infections, and venous thrombosis. This expanding knowledge underscores the far-reaching implications of blood group variations, extending beyond transfusion medicine into the realms of systemic health.^{5,6} At the heart of these physiological intricacies lies the concept of hemostasis, the body's innate ability to spontaneously arrest bleeding from injured capillaries and vessels. This intricate process involves a cascade of events, including vasoconstriction to reduce blood flow, the formation of a platelet plug to seal the breach, clot retraction for stabilization, and finally, clot lysis for tissue repair. The orchestration of these stages showcases the complexity of the body's response to vascular injuries and emphasizes the importance of maintaining a delicate balance in hemostatic mechanisms. As our understanding of the interplay between ABO blood groups and health conditions deepens, these revelations hold promise for personalized medicine. Tailoring medical interventions based on an individual's blood type could become a targeted approach for disease prevention and management. The

intersection between blood groups and diverse health conditions illuminates the intricate tapestry of human biology, offering new avenues for research and potential breakthroughs in healthcare.⁷

Bleeding Time (BT) is precisely defined as the temporal span between the initiation of bleeding and the subsequent arrest of bleeding, a cessation attributed to the formation of a temporary platelet plug. Typically lasting between 3 to 4 minutes, BT serves as a valuable metric influenced primarily by platelet function. The intricacies of this process underscore the dynamic nature of hemostasis, where platelets play a pivotal role in mitigating bleeding episodes. On the other hand, Clotting Time (CT) is delineated as the elapsed time from the onset of bleeding to the formation of the inaugural fibrin thread⁸. A normal CT value falls within the range of 5 to 8 minutes. CT, in contrast to BT, is intricately linked to clotting factors. The presence, efficiency, or deficiencies of these clotting factors significantly impact the duration of CT. Notably, any defects or absence of these factors can result in a prolonged clotting time, highlighting the critical role these factors play in the coagulation cascade. Drawing inspiration from the understanding that ABO blood groups exhibit associations with various diseases, this particular study embarked on a quest to unravel the intricate relationships between different ABO blood groups and two crucial hemostatic parameters—BT and CT. By focusing on students as the study cohort, the research aimed to shed light on potential variations in bleeding and clotting times among distinct ABO blood groups, thus contributing to our broader comprehension of the multifaceted interplay between blood types and hemostatic processes. The endeavor seeks to uncover potential insights into the physiological nuances that underlie the observed associations between blood groups and specific health conditions.⁹

MATERIALS AND METHODS

A cross-sectional investigation was undertaken within the confines of the Physiology Department, marking a concerted effort to explore and understand specific aspects of the subject matter. In adherence to ethical standards and guidelines, the study received the formal approval of the institutional ethical committee, ensuring that the research protocol aligns with ethical principles and safeguards the welfare and rights of the participants involved. The approval from the institutional ethical committee underscores the commitment to conducting the study in a manner that upholds integrity, transparency, and respect for ethical standards in research.

This cross-sectional study, carried out in the Physiology Department and ethically approved by the institutional committee, was designed to investigate the interplay between ABO blood groups, bleeding time (BT), and clotting time (CT). The study enrolled a cohort of 400 participants, specifically targeting

medical and dental students aged between 17 and 20 years. In the pursuit of a comprehensive understanding, the inclusion criteria involved securing informed consent from willing participants within the specified age range. This deliberate selection aimed to provide insights into a particular demographic group and minimize confounding variables. Conversely, exclusion criteria were meticulously applied to ensure the study's specificity. Individuals with a documented history of bleeding and clotting disorders were excluded, recognizing the potential influence of pre-existing conditions on the study outcomes. Moreover, participants currently undergoing drug regimens involving non-steroidal anti-inflammatory drugs (NSAIDs) were excluded, as were smokers, acknowledging the potential impact of these factors on hemostatic parameters. These refined criteria were implemented with the intention of creating a more homogenous study population, facilitating a targeted exploration of the relationship between ABO blood groups, bleeding time, and clotting time within the selected age group of medical and dental students. The careful consideration of inclusion and exclusion criteria serves to enhance the internal validity of the study, enabling a more accurate interpretation of the results and contributing valuable insights to the broader understanding of hemostasis in this specific demographic context.

The process of determining blood groups and bleeding time involved meticulous procedures within the Physiology lab. For blood group determination, samples were collected aseptically through finger pricks, ensuring a sterile environment. Subsequently, red cell suspensions were prepared by mixing blood with normal saline. The red cell suspension underwent a detailed process: it was combined with antisera anti-A and anti-B, covered with a Petri dish for an incubation period of 8 minutes. The determination of blood groups was then based on the presence or absence of agglutination, with confirmation achieved by observing agglutination under a low-power objective of a compound microscope. Moving to the determination of bleeding time (BT), the Duke's filter paper method was employed. Under strict aseptic conditions, a deep finger prick was made using a lancet. The length of time required for bleeding to cease was meticulously recorded by blotting the drop of blood emerging from the incision every 30 seconds with blotting paper. The computation of BT involved multiplying the number of blood spots on the filter

paper by 30 seconds. The standard normal range for BT, as determined by the Duke's filter paper method, falls within the range of 1 to 5 minutes. These methods underscore the precision and attention to detail employed in the laboratory processes, ensuring accurate and reliable results for the subsequent analysis of the relationship between ABO blood groups and bleeding time. The utilization of established methodologies and adherence to standard procedures further enhance the credibility of the study's findings.

RESULTS

In this study encompassing 400 participants aged 17 to 20 years, a gender-diverse group consisting of 256 males and 144 females was examined. Upon scrutinizing the collected data, it emerged that blood group O held the highest prevalence, succeeded by blood groups B, A, and AB, with percentage distributions in the order of O (37%) > B (30.5%) > A (23.5%) > AB (9%) as outlined in Table 1.

Table 2 further delves into the distribution of Bleeding Time (BT) and Clotting Time (CT) in correlation with the observed blood groups. Notably, BT exceeding 3 minutes was more prevalent in blood group AB (37.5%), followed by group O (25%), A (25%), and B (12.5%). The chi-square test revealed a statistically significant difference ($p=0.03$). Meanwhile, Table 2 also illustrates that CT surpassing 4 minutes is more prominent in blood group O (46.67%), followed by A (33.33%), B (13.33%), and AB (6.67%). However, the chi-square test did not yield a statistically significant result ($p = 0.433$).

Gender-wise comparisons presented in Table 3 brought forth intriguing findings. It was observed that BT tended to be prolonged in males compared to females, whereas CT exhibited a more prolonged duration in females. Specifically, BT exceeding 3 minutes was noted in 87.5% of males compared to 12.5% in females, although the statistical analysis deemed this difference statistically insignificant ($p=0.157$). In contrast, CT exceeding 4 minutes was prevalent in 60% of females compared to 40% in males, and this difference was statistically significant ($p=0.04$).

These outcomes not only provide valuable insights into the distribution of ABO blood groups and hemostatic parameters but also shed light on potential gender-related variations in bleeding and clotting times within the studied population.

Table 1: Gender-wise distribution of ABO blood group

Gender	A	B	AB	O	Total
Male	64 (16%)	74 (18.5%)	20 (5%)	98 (24.5)	256 (64%)
Female	30 (7.5%)	48 (12%)	16 (4%)	50 (12.5)	144 (36%)
Total	94 (23.5%)	122 (30.5%)	36 (9%)	148 (37%)	400

Figure 1: Gender-wise distribution of ABO blood group

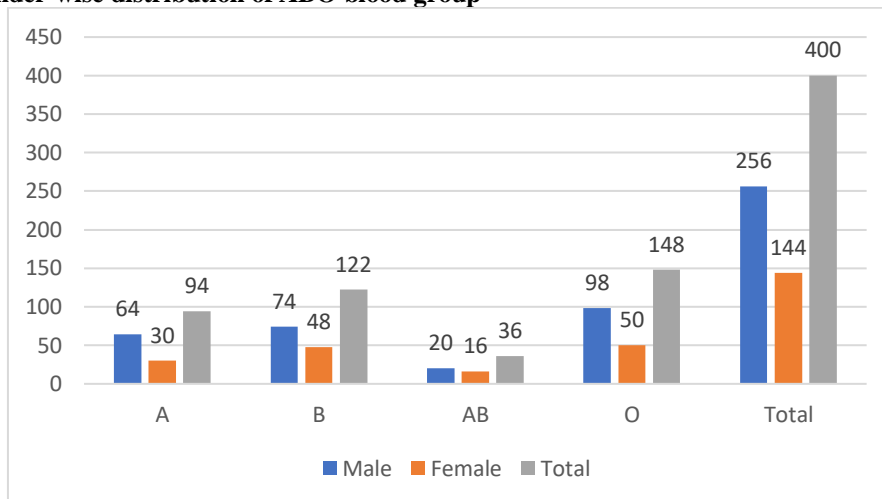


Table 2: Distribution of bleeding time and clotting time among ABO blood group with Chi-square analysis

Variables	Time (minutes)	A	B	AB	O	Total (Percentage)	p Value
Bleeding Time	<= 3	90 (23.43%)	120 (31.25%)	30 (7.81%)	144 (37.5%)	384 (96%)	0.03
	>3	4 (25%)	2 (12.5%)	6 (37.5%)	4 (25%)	16 (4%)	
Clotting Time	<= 4	84 (22.70%)	118 (31.89%)	34 (9.19%)	134 (36.21%)	370 (92.5%)	0.43
	> 4	10 (33.33%)	4 (13.33%)	2 (6.67%)	14 (46.67%)	30 (7.5%)	

Figure 2: Distribution of bleeding time and clotting time among ABO blood group with Chi-square analysis

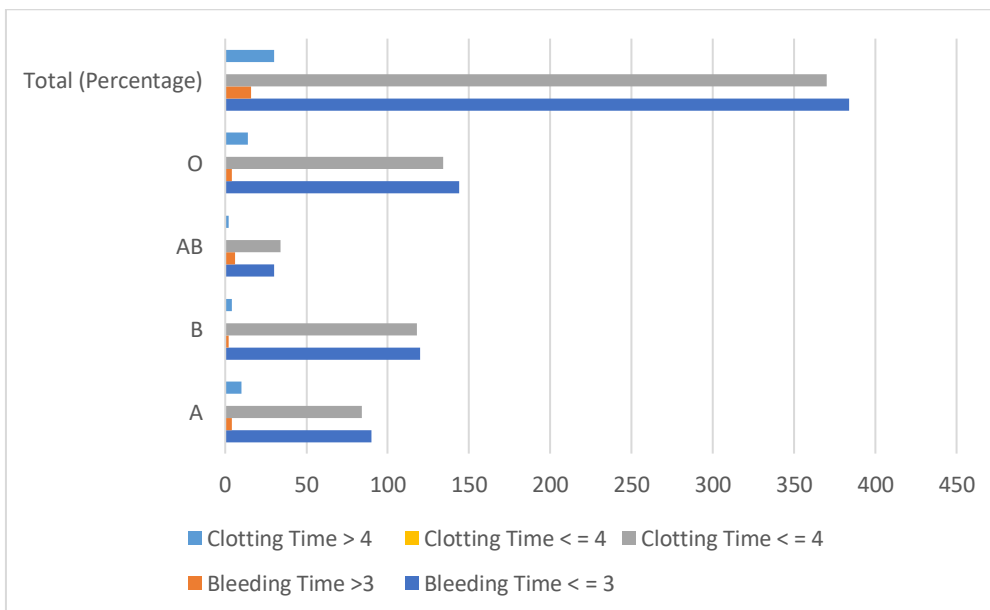
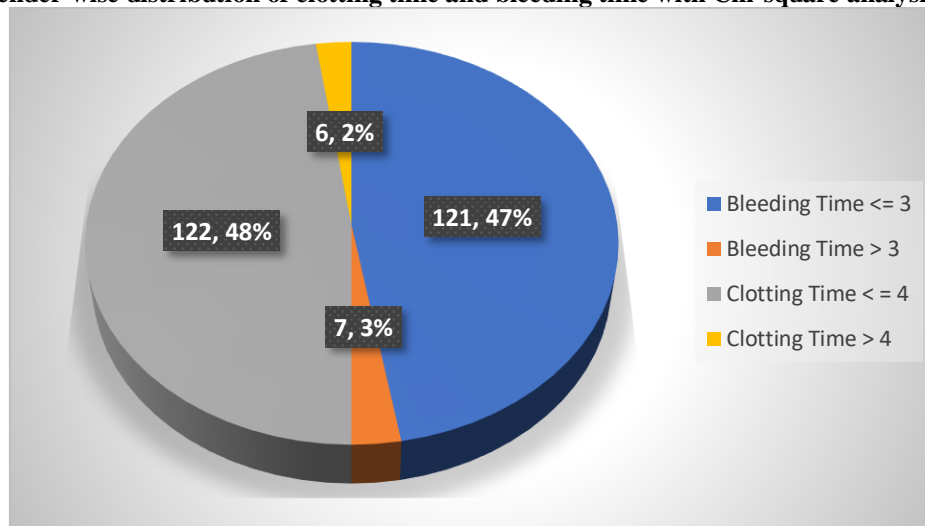


Table 3: Gender-wise distribution of clotting time and bleeding time with Chi-square analysis

Variables	Bleeding Time		Clotting Time	
	<= 3	> 3	<= 4	> 4
Male	121	7	122	6
Female	71	1	63	9

Figure3: Gender-wise distribution of clotting time and bleeding time with Chi-square analysis

DISCUSSION

Conducted with precision and meticulousness, this study engaged a cohort of 400 students aged between 17 and 20 years, aiming to unravel the intricate relationships between ABO blood groups and hemostatic parameters.¹⁰ The resultant data brought forth intriguing observations that not only shed light on the prevalence of specific blood groups but also hinted at potential associations with bleeding and clotting times. The distribution of ABO blood groups within the participant pool revealed a clear dominance of blood group O, encompassing 37% of the studied population. Following closely were blood groups B at 30.5%, A at 23.5%, and AB at 9%. These figures provided a comprehensive snapshot of the ABO blood group landscape among the student demographic. Diving deeper into the association between ABO blood groups and bleeding times, it was compellingly established that a significant proportion of students with a bleeding time exceeding 3 minutes belonged to the AB blood group. This correlation was deemed statistically significant ($p = 0.03$), suggesting a unique hemostatic profile for individuals with blood group AB, particularly in the context of prolonged bleeding times. Conversely, the exploration of clotting times revealed that a notable portion of students with clotting times surpassing 4 minutes were in the O blood group.¹¹ While this association did not reach statistical significance ($p = 0.43$), the observation prompts further inquiry into the potential factors influencing clotting times in individuals with blood group O. Adding another layer to the analysis, a gender-based comparison uncovered that clotting times were significantly prolonged in females compared to males ($p = 0.04$). This gender-related variation in clotting times introduces an additional dimension to the study, emphasizing the importance of considering demographic factors in understanding hemostatic processes. In essence, this comprehensive study not only provides a nuanced portrayal of ABO blood group distributions within the specified age

group but also highlights intriguing associations with bleeding and clotting times. The findings beckon further research endeavors to elucidate the underlying mechanisms contributing to these observed patterns and their potential implications for personalized healthcare.

The outcomes of our study brought to light a distinct prevalence order of ABO blood groups, with the sequence being $O > B > A > AB$. This particular distribution aligns with findings from various studies conducted by different researchers, showcasing a consensus on the prevalence hierarchy of ABO blood groups. However, it is noteworthy that our results diverged from those reported by certain researchers, where the predominant group was identified as B, followed by O, A, and AB. This discrepancy in findings underscores the variability that can exist in different populations and geographical regions, potentially influenced by genetic, environmental, or demographic factors.^{12,13} The nuanced understanding of such variations contributes to the rich tapestry of knowledge in the field of hematology. Moreover, our study delved into the implications of blood group differences in terms of thrombosis risk, drawing parallels with the work of Franchini et al. According to their findings, non-O group individuals were identified as having a higher likelihood of developing thrombosis compared to O group individuals. This heightened risk was attributed to the increased presence of von Willebrand factor (vWF) in non-O group individuals. Building upon this insight, a study by Jenkins and O'Donnell emphasized that non-O group individuals exhibited a 25% higher concentration of vWF compared to group O individuals. This discrepancy in vWF levels is implicated in influencing both bleeding time (BT) and clotting time (CT), suggesting that individuals with blood group O may manifest shorter BT and CT durations in comparison to non-O group individuals. These findings contribute to a more nuanced understanding of the intricate relationship

between ABO blood groups, von Willebrand factor levels, and hemostatic parameters. They highlight the need for a comprehensive exploration of these associations across diverse populations to elucidate the multifaceted nature of hemostasis and its potential clinical implications.

The outcomes of our study pertaining to bleeding time (BT) underscored a compelling association, revealing that BT exceeding 3 minutes was significantly prolonged in individuals with blood group AB when compared to counterparts with other ABO blood groups. This intriguing finding aligns with similar observations reported in various studies, collectively reinforcing the notion that blood group AB may indeed be linked to an extended bleeding time.^{14,15} However, it is crucial to note the presence of conflicting results across different investigations, with some studies suggesting a more prolonged BT in individuals with blood group O, albeit without reaching statistical significance. The divergence in findings emphasizes the complex and multifaceted nature of the relationship between ABO blood groups and hemostatic parameters. Shifting the focus to clotting time (CT), our study revealed that CT exceeding 4 minutes was more prolonged in individuals with blood group O, followed by blood groups A, B, and AB. Despite the apparent trends, this difference did not achieve statistical significance in our study. These results echo the findings of Mirdha and Jena, where CT was prolonged in the O blood group and reached statistical significance. However, it's noteworthy that the literature presents conflicting perspectives, with certain studies suggesting prolonged CT in individuals with blood group B, while others propose a statistically significant elongation of CT in blood group B compared to other ABO groups.

These nuanced and sometimes contradictory outcomes highlight the intricate interplay of genetic, environmental, and demographic factors that contribute to hemostatic processes. The variation in results emphasizes the need for a comprehensive understanding of the mechanisms underpinning these associations, taking into account the diverse contexts in which they manifest.¹⁶ The collective body of research not only adds layers to our comprehension of hemostasis but also prompts ongoing inquiries to unravel the intricacies of ABO blood group influences on bleeding and clotting times. Continued exploration holds the potential to uncover novel insights with clinical implications, enhancing our ability to tailor medical interventions based on individual characteristics. The gender-wise comparison in our study yielded noteworthy results, indicating that clotting time (CT) is significantly prolonged in females compared to males, while bleeding time (BT) values did not show statistical significance. This finding aligns with some studies that have reported a significant prolongation in both bleeding and clotting times in females when compared to males.¹⁷ However,

it is important to acknowledge that our study contrasts with other investigations that reported no significant variation in BT and CT between genders. An intriguing perspective from the study conducted by Ercan et al sheds light on a potential contributing factor. According to their findings, female individuals may exhibit prolonged bleeding time and clotting time due to the presence of the hormone estrogen. Estrogen, in this context, is suggested to lower the plasma level of fibrinogen, a key component in the clotting process, consequently leading to increased clotting time. This hormonal influence provides a mechanistic insight into the observed gender-related variations in hemostatic parameters. The nuanced nature of these gender-related differences in bleeding and clotting times underscores the complexity of hemostatic regulation.^{18,19} Hormonal influences, genetic factors, and other physiological variables contribute to the intricate interplay observed in different populations. The collective evidence from various studies emphasizes the need for a comprehensive understanding of these gender-specific variations in hemostasis, with potential implications for personalized healthcare and clinical interventions. Continued research in this realm holds the promise of unraveling additional intricacies and refining our understanding of hemostatic processes in different demographic groups.

CONCLUSION

In terms of blood group prevalence, our findings indicated that blood group O emerged as the most common, while AB stood out as the least common within the studied population. This distribution aligns with general trends observed in ABO blood group frequencies. Examining bleeding time (BT), it was compellingly revealed that individuals with blood group AB exhibited a significantly prolonged bleeding time, surpassing 3 minutes more frequently compared to other ABO blood groups. This statistically significant association sheds light on the potential impact of blood group AB on hemostatic parameters, suggesting a unique profile in terms of bleeding time. On the other hand, clotting time (CT) presented a distinct trend, with individuals in blood group O demonstrating a higher prevalence of CT exceeding 4 minutes. However, this association did not reach statistical significance, indicating a trend without a statistically confirmed difference. The variation in CT among blood groups implies potential complexities in the clotting cascade influenced by different ABO blood types. Moreover, our study delved into the gender-specific aspects of hemostasis, revealing that clotting time was significantly higher in females compared to males. This gender-related difference in clotting time adds another layer of complexity to the study's findings, suggesting that hormonal or physiological variations between genders may contribute to the observed disparities in clotting times. In summary, our study contributes to the

growing body of knowledge on ABO blood group distributions and their potential impact on bleeding and clotting times. The findings underscore the intricate interplay between genetic factors, blood type variations, and hemostatic parameters, paving the way for further exploration into personalized healthcare approaches and tailored interventions based on individual characteristics.

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