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An Investigation of ABO Blood Type and the Platelet Delta Granule Storage Pool

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Abstract

Individuals with bleeding tendencies are more likely to have blood type O than blood types A, B, or AB. Platelet storage pool deficiencies are a lesser-known group of bleeding disorders which often go undiagnosed and may account for a significant number of patients with unexplained bleeding defects. We hypothesized that patients with platelet δ-storage pool deficiency might also have a predominance of type O blood. A retrospective review of medical records of 2,020 patients with unexplained bleeding and evaluated for δ-storage pool deficiency was performed. Correlations between dense granule numbers, blood type, and von Willebrand factor were analyzed for statistical differences. 51.5% of blood samples were blood type O compared to an incidence of 44.0% in the U.S. population. There was a significant association of vWF and blood type O but not with the delta storage pool. There is a preponderance of blood type O in the study population compared to the U.S. population. There is no statistically significant link between blood type O and lower dense granule numbers in this study.

Keywords

platelets, blood type, ABO, bleeding, storage pool deficiency

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Introduction

Bleeding disorders, including defects in platelet function and the coagulation cascade, are a common cause of morbidity and mortality. Bleeding diatheses typically present with epistaxis, heavy menses, easy bruising, or other forms of mucocutaneous bleeding. Development of bleeding assessment tools (BAT) to determine relative severity of mucocutaneous bleeding symptoms, originally designed for initial assessment of potential von Willebrand disease (vWD), are known to have low sensitivity for distinguishing etiologies of bleeding for females with heavy menstrual bleeding (HMS).[1,2]

Laboratory evaluation of patients with presumed platelet function defects usually involves obtaining a complete blood cell count (CBC), partial thromboplastin time (PTT), prothrombin time (PT), platelet function testing, and von Willebrand factor (VWF) activity, VWF antigen, and factor VIII coagulant activity assays.[3–5] While many bleeding diatheses can be identified based on the results of these initial analyses, the results may be completely normal in some patients, frequently leading to a clinical diagnostic label of unexplained bleeding. Platelet storage pool deficiencies (SPDs) are an underappreciated group of bleeding disorders which often go undiagnosed and may account for a large portion of patients with apparently unexplained bleeding defects.[6] For instance, Amesse et al. found that unexplained bleeding of HMB in adolescent females was associated with platelet dense granule (delta) SPD (δ-SPD); many of these patients had normal platelet function study results.[7]

Platelet storage pool deficiencies include low numbers of specific platelet granules (ie, alpha or delta granules), a deficiency of a certain granule constituent, or a transport defect in which the granules' contents are not secreted into the blood; these disorders may be inherited as in Hermansky-Pudlak,

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Chediak-Higashi, and Wiskott-Aldrich syndromes or acquired such as in myeloproliferative or autoimmune disorders.[8] Platelet δ-SPD may be suspected with abnormal responses to ADP and/or epinephrine agonists in platelet light transmission aggregometry (LTA) assays, but the sensitivity and specificity of platelet LTA for δ-SPD is rather low.[9]

Blood type and bleeding tendencies related to Type O have long been known and some studies have also reported a that blood Type A is frequently seen in patients with thrombotic events.[10–12] The tendency to clot easily may be partially explained by higher plasma levels of vWF in non-O blood types than in type O.[13] This observation implies that patients with vWD have predominantly type O blood. In 2008, Wu et al. concluded the difference between O and non-O blood groups is similar to that predicted based solely on plasma vWF levels.[14] A correlation has also been found between H glycan density, which is partially determined by blood type, and vWF levels.[15,16]

The purpose of this study was intended to determine whether δ-SPD might also be prominent in Type O blood. We hypothesized that more patients with δ-SPD would have Type O blood than any other ABO type. The specific aim of our retrospective study was to compare laboratory data obtained during assessment of blood of patients with unexplained bleeding including ABO type, dense platelet dense granule numbers, and respective CBC.

Materials and Methods

Ethical Issues

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by prior approval from the Institutional Review Board of The University of Toledo Medical Center. All relevant patient information obtained from medical records was de-identified prior to assessment. All methodologies employed to analyze blood samples followed guidelines established by the College of American Pathologist (CAP, Chicago, IL, USA) and the Clinical Laboratory Improvement Amendments (CLIA, CDC, Atlanta, GA, USA).

Study Design

This is a retrospective study comparing data generated during evaluation of patients with unexplained bleeding for a possible δ-SPD. As standard operating procedure in our laboratory, we obtain a CBC for quality assurance and ABO type prior to processing of whole blood for electron microscopic examination to determine the mean numbers of dense granules per platelet. All patient samples included in this study had been submitted for evaluation due to unexplained bleeding and suspicions of an underlying platelet dysfunction disorder based upon clinical symptoms and other laboratory screening tests. A total of 2020 patient samples evaluated during 2015 to 2020 were included in this study. We did not have patient drug histories available to determine whether bleeding could be the result agents such as acetylsalicylic acid. Information for each patient included age, sex, platelet dense granule count, blood type, and CBC with differential. The study population consisted of 29.4% males and 70.6% females. Patients' ages ranged from 6 weeks to 94 years (Figure 1). The study population was heterogeneous, other than all had been evaluated for unexplained bleeding.

Complete Blood Cell Counts

The CBC, with leukocyte differential percentages, was performed using a Sysmex XN-3100™ Automated Hematology System (Lincolnshire, IL, USA). Results obtained included platelet count, mean platelet volume, white blood cell count, percentage of neutrophils, percentage of lymphocytes, percentage of monocytes, percentage of eosinophils, percentage of basophils, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, and red cell distribution width.

ABO Blood Typing

Blood type was determined using a standard blood typing card (Lab-Aids™ Basic Blood Typing Kit, Fisher Scientific, Cat # S67356). Several drops of each patient's whole blood were mixed with anti-A serum and separately with anti-B serum. Hemagglutination of blood with anti-A serum identified the presence of A antigen in the patient's blood, while clumping with anti-B serum indicated B antigen. Accordingly, if both A and B anti-sera induced agglutination, the sample was blood Type AB and if no clumping was observed, the sample was classified as Type O.

Electron Microscopy

Criteria for submission of peripheral blood required samples collected in acid citrate dextrose Vacutainer® blood collection vials (yellow top ACD tubes, solution A or B, Becton Dickinson, Franklin Lakes, NJ). Platelets were obtained via centrifugation of whole blood at room temperature for 15 min at 200 g, creating platelet rich plasma (PRP) for preparation of platelet whole cell mounts.[17,18] Our application of the whole mount technique uses twenty µl of PRP placed upon parlodion coated copper EM grids and incubated for 10 min. Grids are then briefly washed with deionized water for 2 to 3 s, blotted gently with filter paper, and air-dried. Preparations were assessed using a FEI Tecnai G2 Spirit BioTwin transmission electron microscope (Hillsboro, OR) at 80 KV. The mean number of DG/PL was determined by enumeration of the total number of DGs from 100 consecutive PLs observed (Figure 2). Platelets partially obscured by a grid bar or that exhibited preparation artifacts were excluded. The normal range of dense granules per platelet (DG/PL) used in our lab has been used for more than 25 years and vetted against verified control subjects. Control subjects were screened by medical history for clinical bleeding symptoms and by laboratory

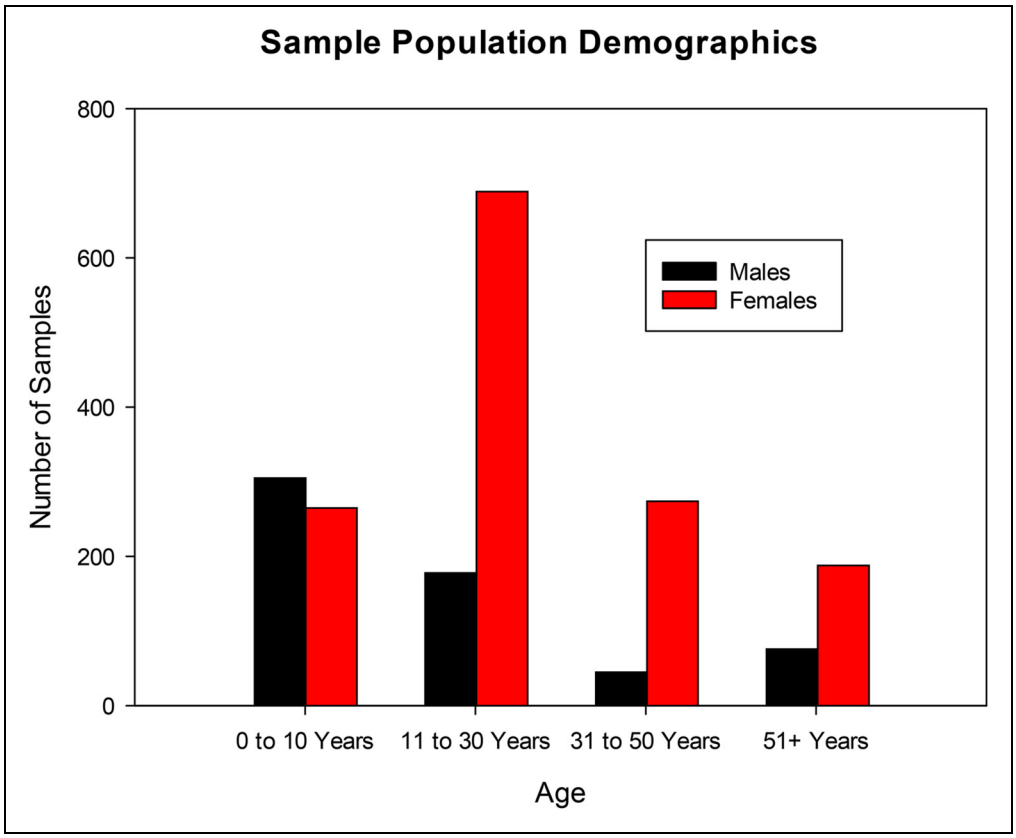

Figure 1. The graph demonstrates significantly more females were evaluated for platelet storage pool deficiency than men. In the prepubescent ages, more males were evaluated. The basis of the observation is unknown but one might postulate a relationship of higher estrogen levels and bleeding symptoms ($n = 2020$).

assessment of platelet function with von Willebrand profile testing, luminescence transmission aggregometry (LTA), biochemical determination of ATP:ADP ratios extracted from platelets, and electron microscopy to determine a mean number of platelet dense granules, granule diameter, and dense granule volume per platelet.[19,20]

Statistics

Descriptive statistics were calculated using R statistical software (R Core Team, R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) to characterize subject demographics. Univariate analysis of variance, Tukey HSD, and linear discriminant analysis were also utilized to compare blood types, mean dense granule number per platelet and vWF. SigmaPlot software (version 14.5, Systat Software, Inc. Palo Alto, CA) was also used to produce graphs for the manuscript.

Results

This study's subjects were categorized by DG/PL values into five groups: less than 1.00 DG/PL, 1.01 to 2.0 DG/PL, 2.01 to 3.00 DG/PL, 3.01 to 3.68 DG/PL, and greater than 3.68 DG/PL. Tables 1 and 2 shows the population demographics with respect to blood type and dense granule count. The frequency of blood types in our study demonstrated more Type O patients and fewer Type A patients than that of the general U.S. population (Table 1). Of 2020 subjects included in this study, 51.5% had blood type O, whereas the frequency of type O is prevalent in 45% of the US population (web database Statista.com, 2021). The prevalence of Type O for our patients was 13% higher than the general US population; conversely, Type A blood was found to be 11.5% less than that of the general population. There was statistical significance between blood type and the dependent variable vWF in the study population ($P < .001$) but no difference for blood type and the dependent variable mean number of dense granules. There was also no significant difference of CBC results for any group.

As stated previously, all samples had been submitted for evaluation of platelet δ-SPD to explain unexplained bleeding symptoms. Of the 2020 patients evaluated, 1296/2020 (64.3%) were diagnosed with δ-SPD with a mean of 2.57 DG/PL. Considering only the patients who had been diagnosed with δ-SPD, Type O blood was found to be 51.3% (666/1296), Type A was 35.6% (462/1296), Type B was 10% (130/1296) and Type AB 2.9% (38/296). Patients with Type O blood and diagnosed with δ-SPD had essentially an identical frequency

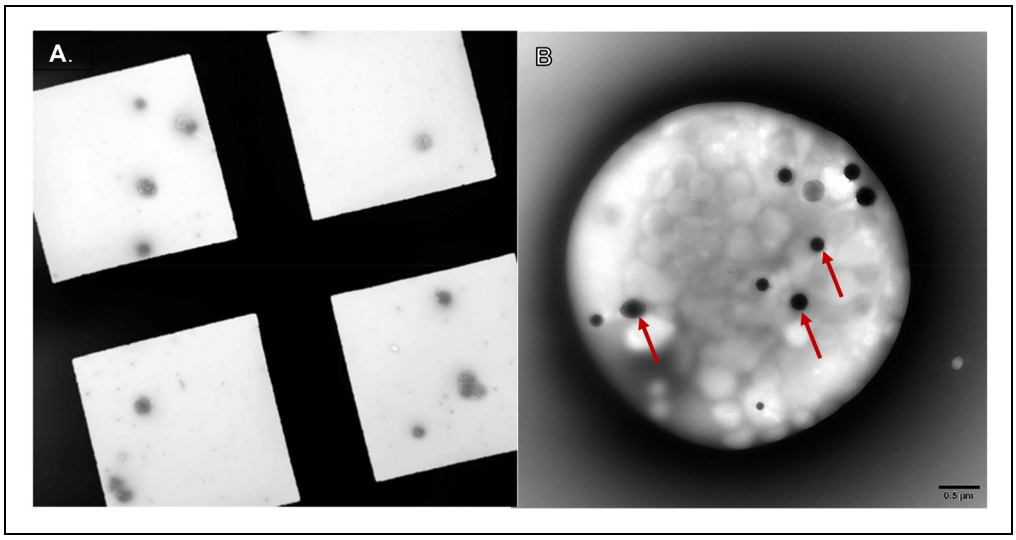

Figure 2. Air-dried whole mounted platelets viewed with an electron microscope. (A) Low magnification demonstrates platelets upon a thin support film (black bars are copper mesh to support the thin film). (B) A platelet appears translucent in the microscope; platelet dense granules stand out a dense opaque circles (arrows).

to the overall frequency of total blood types in our study. All data is available for review in Supplemental Tables 1 and 2 within this manuscript.

Discussion

The objective of this study was to test the hypothesis that δ-SPD is more prevalent in patients with blood type O than other blood types. Statistical analysis demonstrates a preponderance of blood type O in our patients which was expected given the published literature that type O subjects have a tendency to bleed and type A subjects have a higher risk for thrombotic events than reported for other blood types. Likewise, the percentage of patients with type A blood was lower than the general population. It must be emphasized that our study has inherent bias; all blood samples assessed were from patients suspected of having a bleeding diathesis. To our knowledge, all patients had had clinical symptoms suggesting a potential platelet function disorder and that they had been sufficiently evaluated by laboratory tests such as vWD profile analysis and platelet aggregation and/or secretion analysis without an established diagnosis: therefore "labeled" as having unexplained bleeding. The majority of samples we receive are referred from institutions throughout the US; unfortunately, many are submitted with a test order to rule out δ-SPD and limited clinical information.

The prevalence of δ-SPD in the US is unknown. It has been reported to have an autosomal dominant inheritance pattern but may also be acquired.[21,22] VWD is reported to be the most common platelet function disorder affecting approximately 1% of the US population.[23,24] Plasma vWF concentrations are lower in subjects with blood type O in contrast to non-O blood groups and may be indirectly attributed to blood type.[16,25,26] This may be due to an association of higher levels of vWF clearance in subjects with blood type O and non-O blood types have lower levels of clearance. The exact mechanism by which the protein is eliminated more rapidly in blood group O is not known, but it may related to an interaction between glycan structures and clearance receptors.[27] The post-translational glycosylation of proteins mediated by A and B antigens may decrease elimination of circulating vWF from the blood.[11,25]

In addition to platelets and serum levels of vWF, several studies have found erythrocytes to be an essential component of the blood clotting system.[11] Erythrocytes affect platelet

Table 1. Study Subjects Categorized by Blood Type and Number of Dense Granules per Platelet.

Group	% O	% A	% B	% AB	Total %
All subjects	51.5	35.4	9.8	3.3	100.0
<3.68 DG/PL	33.0	22.9	6.4	1.9	64.2
<1.00 DG/PL	0.8	0.3	0.1	0.0	1.3
1.01 to 2.00 DG/PL	6.1	3.1	1.2	0.1	10.5
2.01 to 3.00 DG/PL	16.1	13.1	3.3	1.2	33.7
3.01 to 3.68 DG/PL	9.9	6.4	1.8	0.6	18.7
>3.68 DG/PL	18.6	12.6	3.3	1.4	35.8
U.S. population	44.0	42.0	10.0	4.0	100.0

Table 2. Average Number of Dense Granules for Blood Type in the Study Population.

Blood type	Frequency	Mean # DG/PL
O	971	3.41 ± 1.37
A	670	3.51 ± 1.39
B	192	3.25 ± 1.19
AB	61	3.61 ± 1.21

activity through a variety of mechanisms, including the release of adenosine diphosphate and thromboxane A2 from alpha granules, the formation of erythrocyte-platelet aggregates via adhesive molecules, and the absorption of nitric oxide, a vasodilator, by free hemoglobin.[11] Zhong et al. has postulated this may deal with the fact that, in addition to red blood cells, ABO antigens are present on some platelet surface glycoproteins and glycosphingolipids.[28] Variable glycosylation of these structures may influence platelet function, leading to differences in the risk of thrombosis between blood groups. Our study provides additional support that there is a relationship between blood type (ie, unique glycoproteins on the red blood cells surface) and storage pool deficiencies.

Limitations of our study include many that are common for retrospective investigations. As stated previously, most of our samples are referred with limited clinical data. We were limited to a total of 255 samples with vWF results, only 11% of our samples evaluated for blood type and the platelet dense granule storage pool. Regardless, we did find significant differences for vWF and blood type by Tukey's HSD test. Our study was not a case control study, another frequent limitation of retrospective investigations. All patients included in our study were presumed to have unexplained bleeding.

Conclusion

It is intuitive that δ-SPD would have a relatively high prevalence in our study, as all subjects were referred for unexplained bleeding. From this data, it can be inferred that subjects with Blood Type O are prone to bleeding diatheses related to lower levels of vWF than other blood types and that platelet δ-SPD is significantly more common in Type O than other blood types.

Abbreviations and Acronyms

CBC	Complete Blood Cell Count
DG	Dense Granule
LTA	Light Transmission Aggregometry
PL	Platelet
PRP	Platelet Rich Plasma
SPD	Storage Pool Deficiency
vWD	von Willebrand Disease
vWF	von Willebrand Factor.

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Author Contributions

RR analyzed the data and wrote the manuscript, PK and JC processed samples and collected data, WG designed and directed the project, collected and analyzed data, and wrote the manuscript.

Declaration of Conflicting Interests

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Informed Consent

Not applicable.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author. All data is available in the manuscript as Supplemental Material.

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Supplemental Material

Supplemental material for this article is available online.

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