Letter to the Editor
Diagnostic Hematology

Ann Lab Med 2023;43:208-210
https://doi.org/10.3343/alm.2023.43.2.208
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Whole-mount Electron Microscopy to Quantitate Platelet Dense Granules: Reference Intervals for Healthy Controls in Korea

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Dear Editor,

Platelets are metabolically active cells that have numerous functional organelles, such as endoplasmic reticulum, a Golgi apparatus, and mitochondria. In addition, they contain three major types of secretory granules: α-granules, dense granules (or δ-granules), and lysosomes [1-3]. Compared to α-granules, dense granules have relatively few types of small molecules, such as serotonin, adenine nucleotides (ADP and ATP), calcium, and polyphosphate. Platelet dense granule deficiency may result in mild to moderate bleeding tendency associated with mucocutaneous bleeding and has a similar prevalence as von Willebrand disorder [1-3]. α-Granules can be observed as red dots in platelets under a light microscope, whereas dense granules need to be examined at the ultrastructural level, using electron microscopy (EM) [4-6]. The whole-mount technique is simple, and EM remains the best and standard laboratory method to quantitate platelet dense granules. We quantitated platelet dense granules in a healthy population through transmission EM using whole-mount preparations. To our knowledge, this is the first study to establish and verify reference intervals for platelet dense granules for healthy controls in Korea.

Fresh specimens from healthy individuals (aged >18 years) who were referred to the Department of Laboratory Medicine in Dong-A University Hospital, Busan, Korea, between April 2020 and March 2021 for routine health checkups were used. We assessed their medical histories, bleeding or thrombotic disorders, and any medications that may affect platelet function. All hematologic and hemostasis results were normal. Peripheral blood specimens were collected in 3.2% sodium citrate VACUETTE blood collection tubes (Greiner Bio-One GmbH, Frickenhausen, Germany). Blood specimens were processed within 4 hours of phlebotomy. The Institutional Review Board of Dong-A University Hospital approved this study (DAUHIRB-20-197), and patient consent form was exempted.

To obtain platelet-rich plasma (PRP), the specimens were centrifuged at 200×*g* at room temperature for 10 minutes. The PRP specimens were transferred into microcentrifuge tubes. Ten microliters of PRP was placed onto Formvar-coated grids (Ted Pella, Redding, CA, USA), rapidly blotted with a filter paper, rinsed with 10 μL of distilled water three times, and air-dried [1, 2, 5]. The platelet whole-mount preparations were imaged using EM (Hitachi H-7650; Hitachi High-Tech Solutions, Tokyo, Japan). We prepared three grids per individual and determined the average number of dense granules per platelet. Thirty platelets in each

Received: July 4, 2022
Revision received: August 2, 2022
Accepted: September 11, 2022

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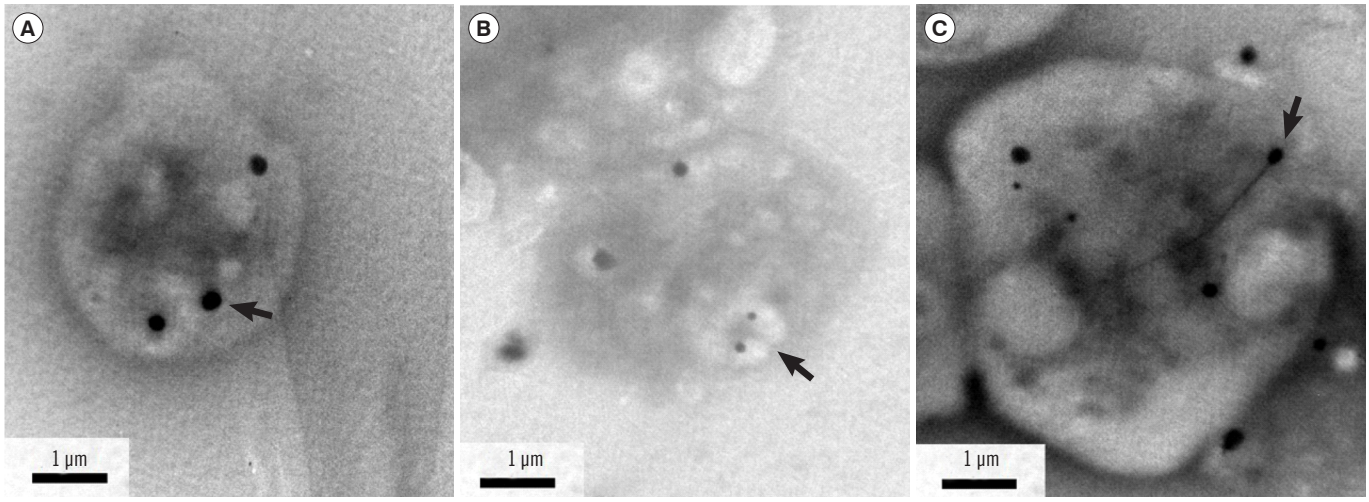

Fig. 1. Representative electron micrographs of platelet whole-mount preparations from healthy individuals. (A) Electron-opaque dense bodies. (B) Granules enclosed by membranes in hyaloplasm. (C) Dense granules with long tails (arrows, ×12,000 magnification).

specimen were examined for dense granules by a hematopathologist using digital EM images at ×12,000 magnification. Statistical analyses were performed using MedCalc software version 20.011 (MedCalc Software Ltd, Ostend, Belgium). $P<0.05$ was considered significant.

In total, 10 women (age range, 28–82 years) and 10 men (34–86 years) were included. The mean platelet count was 256.05×10^9/L. Granules of different sizes and shapes were observed, usually appearing round and dense black. Some dense granules were enclosed by membranes in the hyaloplasm. Dense granules with long tails were occasionally observed. Representative EM images from three individuals are shown in Fig. 1.

The average numbers of dense granules ranged from 2.87 to 5.7 per platelet (mean, 4.06; median, 4.0; Fig. 2). There was no significant difference in the number of platelet dense granules between men and women (4.03±1.62 in women, 4.09±1.48 in men, $P>0.05$). The 95% confidence interval was 2.34–5.79, and the overall mean±SD of was 4.06±1.55 (Fig. 2).

In the literature, relatively broad ranges are reported for dense granules, e.g., >3.68 per platelet, 1.95–4.37 in young people, 3.07±0.12 in the pediatric age group, 1.2–4.0 in adults, 4.9–8.8 and 4.9–8.2 in men, 0.83±1.62 in fetuses, and 2.28±2.15 in neonates [2, 4, 6-9]. In line with our results, Brunet, *et al.* [2] reported that dense granule counts were similar between pediatric and adult patients and were not significantly different between women and men. Sorokin, *et al.* [4] found no significant variability according to ethnicity, sex, or age. Tian, *et al.* [9] found no significant differences among young-, middle-, and old-age groups.

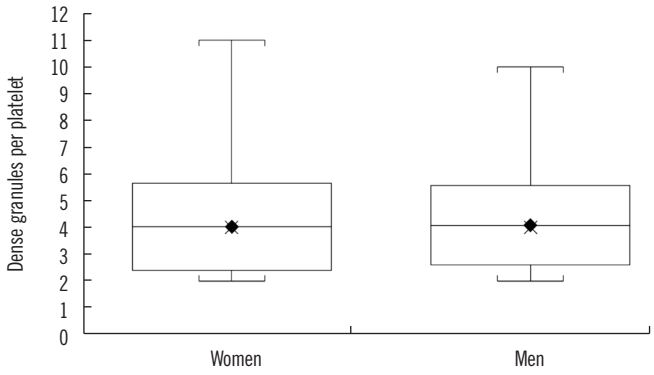

Fig. 2. Dense granule counts in platelets from healthy adults. Box plots showing midspreads and total ranges in women and men. The vertical axis indicates the number of dense granules per platelet.

In summary, we established and verified reference intervals for quantitating platelet dense granules for healthy controls in Korea, which will aid in the diagnosis of platelet storage pool diseases.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Han JY, Woo KS, and Rha SH designed the study and wrote the manuscript. Shim JR, Kang M, and Han JY prepared the platelet slides. Rha SH and Han JY conducted electron microscopy for the quantitation of dense granules. Woo KS conducted the

statistical analysis. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

RESEARCH FUNDING

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2022R1A2C1009739).

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