Platelet dysfunction and other hemostatic disorders in women with menorrhagia: the utility of whole blood lumi-aggregometer

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Menorrhagia is a very common clinical problem among women of reproductive age and is unexplained in more than 50% of cases [1]. The widely accepted clinical definition of menorrhagia is blood loss of 80 ml, or more, per period [2]. Underlying bleeding disorders, such as von Willebrand disease (VWD) and platelet abnormalities may present as menorrhagia. However, the relevance of a systemic screening for hemostasis in women with menorrhagia remains controversial [3]. Here, we describe the findings of a prospective study investigating the frequency of platelet dysfunction and other hemostatic defects among women with unexplained menorrhagia using whole blood lumi-aggregometer.

Sixty-seven Turkish white women aged between 17 and 50 years, with a physician diagnosis of menorrhagia were screened after the study was approved by the local ethics committee. Women with known bleeding or other systemic disorders such as renal, hepatic and endocrine diseases were excluded initially. All cases were examined at the gynecology department and had pelvic ultrasonography; those with submucous uterine fibroids, fibroids more than 2 cm in diameter, uterine polyps, ovarian tumors and intrauterine device were excluded. Women were required to have not ingested combined oral contraceptives and other hormonal based therapy at least one cycle prior to sampling. We also attempted to prevent any medication especially non-steroidal anti-inflammatory drugs, aspirin as well as alcohol in the last 10 days prior to the aggregation studies. All subjects were evaluated on days 3–7 of their menstrual cycle to minimize interindividual variation. Sixteen age-matched women with no menorrhagia served as controls. Both patients and controls underwent the following laboratory tests: hemoglobin, platelet count, activated partial thromboplastin time (aPTT), factors (F) VIII, IX, XI, ristocetin cofactor activity (RCof), platelet aggregation and ATP release.

Platelet function testing was performed on whole blood platelet lumi-aggregometer (Chronolog Corporation, Model 560-Ca) using luminescence method in diluted blood (1:1 blood normal saline ratio). Citrated blood was collected under light tourniquet through 19 gauge needles into 4.5-ml vacutainers (Becton Dickinson) containing 3.2% trisodium citrate in a 9:1 blood anticoagulant ratio. The citrate tubes were thoroughly mixed by gentle inversion before dispensing 450 μl of citrated whole blood into cuvettes (chronolog No: 367) each containing 450 μl normal saline and a disposable siliconized stir bar. After the tubes were warmed at 37°C and stirred in the incubation wells, 100 μl luciferin (chronolog No: 395 chrono Lume Reagent) was added to the cuvette and the luminescence calibrated 5 min later; the impedance electrode was placed into the cuvette and calibration checked. After agonist addition platelet aggregation and ATP release tracings were measured over 6 min. The agonist used and their final concentrations were; ADP (Chrono Par 384) 5.0 mM, Arachidonic acid (AA) (Chrono Par 390) 0.5 mM, Ristocetin (Chrono Par 396) 1.0 mg/ml, and Collagen (Chrono Par 385) 2 μg/ml. Calculated platelet aggregation (ohms) and ATP release (nmol) normal ranges (as mean ± standard deviation) were 8–21 ohms and 0.4–2.9 nmol for ADP, 13–23 ohms and 0.8–3.5 nmol for AA, 17–28 ohms and 0.5–2.7 nmol for collagen, 5–16 ohms for ristocetin.

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Platelet aggregation and ATP release responses were considered decreased if below the reference range (Fig. 1). Test for Platelet functioning, on all samples was completed within 2 h of blood collection.

Sample for blood counts were drawn into Becton Dickinson anti-coagulated tubes and complete counts were made by Beckman Coulter Gen-S SM, USA automated blood counting device. The coagulation tests were performed on fully automated STA compact device of Diagnostica STAGO. Activated partial thromboplastin time (APTT) was performed using rabbit brain cephalin with kaolin as activator (PTT A, Diagnostica Stago, Asnieres, France). Prothrombin time (PT) was performed using Neoplastine CI Plus (Diagnostica Stago). Factors (F) VIII, IX and XI were measured by one-stage assay using kaolin as activator and partial thromboplastin. In all clotting factor assays, 1:10, 1:20, 1:40, 1:80 dilutions of each standard and 1:10 dilution of test sample were used as well as parallel line bioassay analysis of the data was done. The normal ranges for these tests in our laboratory are: APTT (26–36 s), PT (9.4–15.4 s), F VIII (50–150%), F IX (50–150%) and F XI (50–150%). The bleeding time was performed by the same experienced personnel using Ivy technique, with an upper limit of 8 min.

Comparison of data was performed using Pearson Chi-square and Fisher’s exact tests using SPSS for Windows package.

Study group subjects were found to have heavy and/or prolonged periods with passage of Frank blood clots and needed increased number of pads per day compared with control group subjects ($P < 0.001$). Bleeding time was significantly longer in the test group than the control group ($P < 0.001$). Mean hemoglobin values in the study group were significantly lower than the control group while mean platelet counts in the study group were significantly higher than the control group ($P < 0.001$) (Table 1).

In women with menorrhagia, the global prevalence of bleeding disorders was 36/67 (53.7%), including platelet dysfunction, von Willebrand factor and factor deficiencies (Table 2). Platelet dysfunction was the most commonly observed hemostatic abnormality. Platelet aggregation was decreased with one or more agonists in 20 (29.9%) women with menorrhagia. Thirteen women had decreased aggregation in response to ADP, 6 women had decreased aggregation in response to AA, 5 women had decreased aggregation in response to ristocetin and 3 women had decreased aggregation in response to collagen. Platelet ATP release was abnormal in 13 (19.4%) of women with AA. Fourteen women (20.9%) had ristocetin cofactor activity less than 60%. Of these, eight patients were found to have von Willebrand disease of mild severity. Mild F XI deficiency was detected in seven women and one woman with combined F XI and F IX deficiency was identified. Bleeding time was prolonged in 12/67 (18%). Twenty-five of the 67 women had blood type O while the rest had other groups (A, B, AB). No bleeding disorder was found in female controls.

In contrast to earlier literature [4], VWD was not the commonest hemostatic disorder among the participating women in this study. A great intraindividual variation in plasma concentrations of von Willebrand factor, which may, in part, be related to hormonal changes during various phases of the menstrual cycle has been described [5]. In the present study, we obtained a very low interindividual variation by restricting sampling to cycle days 3–7. Recently, platelet functional defects have been found to be prevalent in women with menorrhagia [1, 6–8]. Our data are in good agreement with these observations showing

![Fig. 1 A representative study of hypoactive platelets. In this diagram ristocetin (channels 1 and 3) and AA (channels 2 and 4) were added to one of two cuvettes. Impedance (channels 2 and 2) and release (channels 3 and 4) were measured by the extent of deflection from the baseline in the vertical axis. Impedance was measured in ohm and release was measured in nmol ATP. The results are as follows: ristocetin 7 ohm and 0.0 nmol; AA 8 ohm and 0.65 nmol. As a result, AA induced aggregation and ATP secretion of the test subject in channels 2 and 4 show lack of response.](image-url)
both reduced platelet aggregation and ATP release responses to different agonists. The utility of standard platelet aggregometry in screening for underlying bleeding disorders in women with menorrhagia is also demonstrated.

Despite the relatively small sample size, our findings emphasize the importance of screening for hemostatic defects including platelet function testing in women with unexplained menorrhagia. Increased awareness among obstetricians and gynaecologists and close collaboration with hematologists will be extremely helpful in diagnosing underlying bleeding disorders in these women. Since menorrhagia is a common health and social problem, discovering an undiagnosed hemostatic disorder that can be managed by targeted medical treatment options will improve quality of life of large numbers of women.

### References