Evaluation of inherited and acquired platelet function disorders in iron deficient women with menorrhagia by whole blood lumi-aggregometer

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ABSTRACT

The commonest cause of iron deficiency anemia (IDA) in premenopausal women is often menstrual blood loss. However, no organic pathology is identified in more than 50% of menorrhagic women. We therefore investigated inherited and acquired bleeding disorders among women with unexplained menorrhagia who developed IDA. In vitro whole blood platelet aggregation (PA) with ADP, arachidonic acid (AA), ristocetin and collagen was studied in addition to full blood count, serum iron levels, serum iron binding capacity, transferrin saturation, ferritin, prothrombin and activated partial thromboplastin time, fibrinogen, D-Dimer, Factor VIII, Factor IX, Factor XI, ristocetin cofactor activity, blood type and bleeding time in 67 women before and after therapy. Before therapy; decreased agonist induced PA was observed in 20% of women by ADP, in 12% by AA, in 2% by ristocetin and in 6% by collagen. After oral iron therapy, decreased platelet aggregation was shown in 8% of women with ADP and 2% of women with AA while initial abnormal ristocetin and collagen induced platelet aggregation responses became normal. Also there was a statistically significant increase of ristocetin cofactor activities and FXI levels after iron repletion. We conclude that; rather than von Willebrand disease, platelet function abnormalities and FXI deficiency are the most common hemostatic disorders in women with unexplained menorrhagia and significant portion of these disorders can be reversed by iron therapy.

Key Words: Anemia, Menorrhagia, Platelet aggregation.

ÖZET

Demir eksikliği anemisi gelişen menorajili kadınlarda konjenital veya edinsel trombosit fonksiyon bozukluklarının tam kan trombosit agregasyonu ile karşılaştırılması

Premenopozal kadınlarda demir eksikliği anemisinin en sık nedeni çoğunlukla menstrual kan kayıplarıdır. Ancak menorajik kadınların %50'sinden fazlasında organik bir patoloji saptanamamaktadır. Bu sebeple biz; DEA gelişen sebebi bilinmeyen menorajili kadınlarda konjenital veya edinsel kanama bozukluklarını inceledik. Çalışmaya dahil edilen 67 kadında tedavi öncesi ve sonrası tam kan sayımı, serum Fe, TDBK, % saturasyon, ferritin, PT, aPTT, fibrinojen, D-Dimer, FVIII, FIX, FXI, RCof aktivitesi, kan grubu ve kanama zamanına ek olarak in vitro tam kan trombosit agregasyonu (ADP, araşidonik asit, ristosetin, kollajen ile) çalışıldı. Tedavi öncesi olguların %20'sinde ADP, %12'sinde AA (araşidonik asit), %2'sinde ristosetin ve %6'sında kollajen ile indüklenen trombosit agregasyonunda azalma gözlenmiştir. Oral demir tedavisi sonrası %8 ADP ve %2 AA ile indüklenen trombosit agregasyon bozukluğu saptanırken başlangıçta ristosetin ve kollajen ile indüklenen anormal trombosit agregasyon yanıtları normale dönmüştür. Ayrıca RCof ve FXI düzeyinde demir replasmanı sonrası istatistiksel olarak anlamlı bir düzelme bulunmuştur. Sonuç olarak; sebebi bilinmeyen menorajisi olan kadınlarda von Willebrand hastalığının aksine, trombosit fonksiyon bozuklukları ve FXI eksikliğinin en sık görülen hemostatik anormallikler olduğu ve demir tedavisi ile bu anormalliklerin önemli bir kısmının normale döndüğü ortaya konmuştur.

Anahtar Kelimeler: Anemi, Menoraji, Trombosit agregasyonu.

INTRODUCTION

Iron deficieny anemia (IDA) is the most common form of nutritional anemia worldwide. It occurs most often as a result of blood loss. While blood loss from the gastrointestinal tract is the commonest cause in adult men and postmenopausal women, menstrual blood loss is the commonest cause in premenoposal women[1].

Menorrhagia is defined as a menstrual loss of 80 mL or more per period. It is seen in 5% of women of reproductive age and in 12% of gynecological referrals. Menorrhagia has been attributed to a number of different general and local causes but in more than 50% of cases no organic pathology is found[2].

Increased menstrual blood loss has been reported in women with inherited bleeding disorders especially von Willebrand disease (vWD) and carriers of haemophilia. Other less common inherited bleeding disorders including deficiencies of prothrombin, fibrinogen, FV, FVII, FX and FXI may also be associated with menorrhagia. However the prevalence of bleeding disorders is not known with menorrhagia. In addition screening of disorders is not part of clinical investigations for menorrhagia[3].

Unexplained menorrhagia is a common problem among women of reproductive age. Although vWD is presumed to be the most common hemostatic disorder in women with menorrhagia, the prevalance of platelet disorders and other hemostatic disorders is uncertain in menorrhagic women with unknown origin. We have therefore carried out this present study to asses the frequency of inherited and acquired bleeding disorders among women with unexplained menorrhagia who developed IDA. Since seperation of platelets from their natural environment may influence their function which has been described to be dependent on presence of erythrocytes and leukocytes, we performed all platelet aggregation studies in whole blood rather than platelet rich plasma.

MATERIALS and METHODS

Patients

67 women with IDA and a history of heavy periods (mean age 32.6 ± 1) were screened. Sixteen healthy women (mean age 28.8 ± 2) constituted the control group. A detailed

menstrual history and history about other bleeding symptoms was taken. In the patient population; erythrocyte indices (Hb↓, Htc↓, MCV↓, MCHC↓, RDW↑) and tissue iron status (serum iron↓, serum iron binding capacity↑, transferrin saturation↓ , ferritin↓) were compatible with IDA. The initial exclusion criteria were known bleeding or other systemic disorders such as renal, hepatic and endocrine diseases; use of any intrauterine device in past 2 months and treatment with anticoagulants, antifibrinolytics, non-steroidal antiinflammatory agents, combined oral contraceptives or progesterone. All patients had a gynecological examination and pelvic ultrasonography; those with submucous uterine fibroids, fibroids more than 2 cm in diameter, uterine polyps, ovarian tumors or endometriomas were excluded. Women in the study group were given a 12-week course of iron as ferroglycine sulfate with a daily dose of 200 mg in two doses. Platelet aggregation (PA) was studied before and after oral iron therapy in the patient and once in the control group at the study entry. We attempted to prevent any medication especially non-sterodial anti-inflammatory drugs, aspirin, iron formulation as well as alcohol in the last ten days prior to the aggregation studies, considering their variable effects on platelet function testing. Women were also evaluated on days 3-9 of their menstrual cycle to minimize interindividual variation.

The study was approved by the local ethics committee.

Sample Collection and Laboratory Methods

Fasting venous blood was collected under light tourniquet through 19 gauge needles into 4.5 mL vacutainers (Becton Dickinson) containing 1/10 volume of 3.2% trisodium citrate with 0.105 molarity. The collection was performed early in the morning following a light breakfast. Platelet aggregation was measured by whole blood aggregometer using luminescence method in diluted blood; in the ratio of 1 part physiological saline to 1 part blood (Chronolog Corporation, Model 500-Ca). ADP (5 µM, Chrono Par 384) , AA (0.5 mM, Chrono Par 390), ristocetin (1.0 mg/mL, Chrono Par 396) and collagen (2 µg/mL, Chrono Par 385) were used as agonists.

Twenty-two normal female subjects were studied to establish a reference range. The reference interval represents the mean ± 2 standard deviations (Table 1). Calculated platelet aggregation (ohms) and ATP release (nmole) ranges were 4-21 ohms and 0.5-2 nmole for ADP, 14-39 ohms and 0.5-3.5 nmole for AA, 17-44 ohms and 0.5-2 nmole for collagen, 3-10 ohms for ristocetin. Platelet aggregation and ATP release responses were considered decreased if below the reference range.

Platelet functing testing on all samples was completed within 2 hours of collection.

Ristocetin cofactor activity was studied on the same aggregometer by the use of optical method in platelet rich plasma.

The bleeding time was performed by the Ivy method.

Sample for blood counts were drawn into Becton Dickinson anticoagulated tubes and complete counts were made by Beckman Coulter Gen-S SM,USA automated blood counting device.

Blood for serum iron, total iron binding capacity, transferrin saturation and ferritin was taken after 12 hours of fasting into BD vacutainers. Serum iron was studied by

Table 1. Reference interval for impedance and release by whole blood lumi-aggregometer (mean ± 2 SD)

Agonist	Impedance (ohms)	Release (nmole)
ADP (5.0 mM)	4-21	0.5-2
AA (0.5 mM)	14-39	0.5-3.5
Rist (1.0 mg/mL)	3-10	0.5-2
Collagen (2.0 mg/mL)	17-44	0.8-4.9

Schimadzu UV 120-02 spectrofotometer using BioMerieux Ferrimat kit. Total iron binding capacity was calculated after transferrin studied by Beckmann Coulter Image nefelometer. Ferritin was studied by ACS: 180 plus full automated chemiluminescence device owned to Ciba-Corning Company.

Full automated STA compact device of Diagnostica STAGO was used to measure FVIII, FIX, FXI and D-Dimer through 0.105 molarity, 3.8% sodium citrate containing tubes by using manifacturer's original kits. In the 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: FVIII (50-150%), FIX (50-150%), FXI (50-150%) and D-Dimer (0.00-0.50 μg/mL).

Statistical Analysis

Data before and after therapy were analyzed using SPSS (Statistical Package for Social Sciences).

Differences in means of platelet function studies between pretreatment and posttreatment were evaluated by paired samples T-test and Wilcoxon signed ranks test. Median values were given for Wilcoxon signed ranks test and T-test results were given as mean ± SE.

Differences in means of platelet function studies between cases and controls were evaluated by Mann-Whitney test and independent samples T-test. Median values were given for Mann-Whitney test while T-test results were given as mean ± SE. For variable distribution Ryon-Joiner normality test was used.

Mann-Whitney test was also used to analyze influence of patients' blood type on RCof levels.

A value of $p< 0.05$ was accepted as statistically significant.

RESULTS

Characteristics of Study Population

Study group subjects were found to have heavy and/or prolonged periods with passage of frank blood clots and increased number of pads per cycle compared with control group subjects ($p< 0.001$), while age distribution was similar in both. Bleeding time was significantly longer in the test group than the control group ($p< 0.001$) (Table 2).

Platelet Function Studies

Pre- and post-treatment platelet aggregation and secretion responses to 5 μM ADP, 0.5 mM AA, 1.0 mg/mL ristocetin, and 2 μg/mL collagen are shown in Table 3. There was a significant increase in AA induced PA ($p< 0.05$) and a decrease in ristocetin induced PA ($p< 0.01$) after treatment. Pre- and post-treatment platelet aggregation values induced by ADP and collagen in addition to platelet secretion values induced by all agonists were not significantly different ($p> 0.05$).

Platelet aggregation and secretion responses in the study group after treatment and in the control group are shown in Table 4. Platelet fuction studies disclosed no significant difference between the two groups ($p> 0.05$).

Table 2. Age, menstrual cycle features and bleeding time in the study and control group subjects

	Study group (n= 67)	Control group (n= 25)	p
Age	32.6 ± 1.2	29.4 ± 1.5	> 0.05
Day	6.3 ± 0.2	4.3 ± 0.2	< 0.001
Pad	4.2 ± 0.2	2.1 ± 0.1	< 0.001
Bleeding time	3.5 ± 0.3	2.2 ± 0.2	< 0.001

Table 3. Pretreatment and posttreatment values of platelet aggregation and secretion induced by agonists in the study group (n= 50)

	Pretreatment	Posttreatment	p
Platelet aggregation			
ADP (ohm)	10.76 ± 0.78	9.96 ± 0.74	> 0.05
AA (ohm)	20.6 ± 0.84	23.08 ± 0.79	< 0.05
Rist (ohm)	14	10	< 0.01
Collagen (ohm)	28	28	> 0.05
Platelet secretion			
ADP (nm)	0.95	0.85	> 0.05
AA (nm)	1.45	1.4	> 0.05
Collagen (nm)	1.61 ± 0.11	1.33 ± 0.09	> 0.05

Median; Mean ± SE.

Table 4. Values of platelet function induced by agonists in the study group after treatment and in the control group

	Posttreatment (n= 50)	Control (n= 22)	p
Platelet aggregation			
ADP (ohm)	9.96 ± 0.74	12.4 ± 0.89	> 0.05
AA (ohm)	23.08 ± 0.80	23.64 ± 1.40	> 0.05
Rist (ohm)	10	10.5	> 0.05
Collagen (ohm)	29.32 ± 0.84	30.90 ± 1.44	> 0.05
Platelet secretion			
ADP (nm)	0.85	0.90	> 0.05
AA (nm)	1.40	1.25	> 0.05
Collagen (nm)	1.23	1.2	> 0.05

Median; Mean ± SE

Six of ten women with initial abnormal ADP induced PA and five of six women with initial abnormal AA induced PA became normal posttreatment while all abnormal platelet aggregation studies with ristocetin and collagen normalized after therapy. We could not detect any women with impaired platelet secretion in the study group, before and after therapy.

Hemostatic Studies

A statistically significant difference was seen in women with decreased FXI levels and RCof activities after iron therapy.

Of seven women with decreased FXI concentrations and nine women with decreased RCof activities pretreatment, four still had FXI deficiency posttreatment, while all abnormal RCof activities were normalized by iron therapy (Table 5).

Moreover, no statistically significant difference was found between vWF levels and patients' ABO blood type (Table 6).

DISCUSSION

We have observed significant differences between the study group and the control group with respect to menstrual cycle duration,

Table 5. RCof and FXI values of the study group

	Pretreatment median	Posttreatment median	p
RCof* (%)	49	100	< 0.01
FXI** (%)	40	44	< 0.05

* Patients with abnormal RCof activities before therapy (n= 9).

** Patients with abnormal FXI levels before therapy (n= 7).

Table 6. Influence of patients' ABO blood type on ristocetin cofactor levels

Blood type	n	RCof% median	p
O	25	84	> 0.05
Other (A, B, AB)	42	111	

passage of clots and number of pads per cycle. Study group subjects were found to have excessive hemorrhagia with passage of frank blood clots and were requiring increased number of pads per cycle compared with control group subjects (p< 0.001).

In the 1960's Taymor and associates claimed iron deficiency anemia to be an important cause of menorrhagia and postulated that in the presence of iron deficiency there was a relative inadequacy of the spiral arterioles of the endometrium which prolonged the period of heavy menstrual flow[4,5]. However, more recent hematologic studies have shown that rather than this anatomic factor described by Taymor and colleagues, undiagnosed qualitative platelet disorders may occur in the majority of women with unexplained menorrhagia[2,6-8]. Objectively confirmed menorrhagia using a pictorial bleeding assessment chart in women with von Willebrand's disease, carriers of haemophilia and FXI deficiency was reported in 73%, 57% and 59% of women, respectively[9]. In a study of iron deficient polymenorrheic adult females, platelet aggregation abnormalities in response to various aggregating agents was detected and it was suggested that this resulted from the hemostatic defect caused by anemia[10]. Recently, Philipp et al evaluated platelet functional defects and other inherited bleeding disorders in women with unexplained menorrhagia. In their study the most commonly found platelet function defects were reduced aggregation responses to epinefrine and ristocetin and abnormal ADP induced ATP release[11].

Regarding the prevelance of inherited and acquired platelet function disorders or other hemostatic disorders 9-11% in the general population, we suggest that; inherited and acquired platelet dysfunction and other hemostatic disorders may lead to excessive bleeding during periods of women and diminish their life quality[9].

Pretreatment platelet aggregation results were abnormal in ten women by ADP, in six women by AA, in one woman by ristocetin and in three women by collagen. After a twelve week course of iron therapy agonist induced platelet aggregation was found to be normal in six of ten women with initial abnormal ADP induced PA and five of six women with initial abnormal AA induced PA. Moreover, platelet aggregation defects by ristocetin and collagen were found to be normalized after therapy. There is similar data in the literature that includes examination of platelet function alterations in iron deficiency anemia and effect of iron therapy on platelet functions, especially in children. Kürekçi et al studied platelet aggregation induced by various concentrations of ADP and collagen with impedance aggregometry in 25 iron deficient children before and after therapy. The posttreatment mean maximum aggregation values were significantly higher in their study. Authors concluded that iron deficieny anemia in infants may cause defective ex vivo whole blood platelet aggregation and can be reversed by iron therapy[12]. In a similar study by Kabakuş and et al platelet aggregation tests were performed by impedance and optic methods in 47 children with IDA. A supression in collagen and ristocetin induced PA by the

impedance method was found in addition to ADP and ristocetin induced PA by the optic method. All defective PA responses were reversed by iron supplementation therapy and therefore it was advised that care should be taken when using anti-aggregant agents in IDA[13]. The study of Çalışkan et al evaluated platelet functions using impedance and optic method in 42 children with IDA during and after iron therapy. Decrease in collagen induced platelet aggregation by the two methods and ADP induced PA by the whole blood method were normalized after therapy[14]. Malhotra et al, in their series of 30 children with IDA, demonstrated in vitro diminished platelet aggregation in platelet-rich plasma, and they noted the return of this hyporeactivity to normal following the correction of anemia by a 12-week course of anemia[15].

In our study a significant normalization of platelet aggregation defects by agonists was found, suggesting that acquired platelet function disorders rather than inherited disorders are more likely to cause menorrhagia in iron deficient menstruating women.

The earlier literature yielded little information about how iron effects platelet functions. Polette and Blache have showed that iron, as a key element in lipid peroxidation, plays an important role in platelet aggregation by enhancing the release of arachidonic acid and thromboxane A_2 from platelet phospholipids through the production of oxygen radicals in an animal model. They have also shown that a well documented correlation between the increase in the activity of iron-containing enzymes such as cycloxygenase and lipoxygenase and the increase in plasma iron concentration was persisting[16]. In the study of Barradas et al with iron chelators such as deferoxamine, significant information about the interraction between iron and platelets was demostrated. They suggested that iron chelators inhibit platelet function since both cycloxygenase and lipoxygenase are iron containing enzymes[17]. Our findings highlight the need for further studies to determine the influence of iron in molecular mechanisms of platelet aggregation.

Abnormal FXI and ristocetin cofactor levels were diagnosed in 7/67 and 9/67 of our cases, respectively. Following iron therapy a statistically significant increase was seen in FXI and RCof levels. In the study of Philipp et al, 10/74 had vWF: RCof less than 60% and 2/74 had mild FXI deficiency[11]. Subnormal plasma vWF level was also reported in 42% of 122 menstruating women with IDA[18]. The authors suggested that subnormal vWF activity lead to excessive menstrual bleeding worsened by aspirin intake and intrauterin device, frequently with resulting IDA[18]. Our results demonstrate that in addition to platelet function abnormalities, FXI and RCof decreases can cause hemorrhagia in women with IDA which of some can be reversed by iron therapy. The influence of iron on FXI and RCof levels, if any, has not been reported to date, necessiating further studies to be carried out. The present study indicate that FXI level and ristocetin cofactor should be included in initial testing of the women with abnormal bleeding to avoid unnecessary surgical interventions.

No significant difference was observed between von Willebrand factor level and ABO blood type. Several things that may influence plasma concentrations of von Willebrand factor are reported in the literature; individual's ABO blood type is one of these. Persons with blood type AB have 60% to 70% higher levels of vWF than do those who have blood type O[19]. However our findings do not support this.

We conclude that;

1. Acquired platelet function disorders rather than congenital disorders may cause menorrhagia in iron deficient women.

2. vWD is not the commonest hemostatic disorder found in women with menorrhagia, in contrast to the literature.

3. Subnormal FXI levels and RCof activities are seen in substantial portion of women with IDA and menorrhagia.

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