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The Prevalence of Laboratory Abnormalities of Hemostasis in Women with Endometriosis: A Case-Control Study

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ABSTRACT

Objective: To assess the frequency of inherited bleeding disorders in women with endometriosis and to establish if there is an association between coagulation parameters and severity of endometriosis.

Design, Setting and Patients: A case-control study including women with endometriosis (n=84) and age-matched controls (n=30) was conducted at the Royal Free Hospital. All participants were interviewed to complete Pain Impact Questionnaire (PIQ), and Pictorial Blood Assessment Chart (PBAC). Laparoscopic revised American Society of Reproductive Medicine (rASRM) stage of endometriosis was documented where recorded. Laboratory investigations of hemostasis included platelet aggregation, and coagulation factor levels (VIII, IX, XI, XIII, Von Willebrand Factor (VWF).

Main outcome measure(s): Frequency of laboratory abnormalities of hemostasis among case and control group. Correlation of hemostatic variables with PIQ score, PBAC score and laparoscopic staging.

Results: Women with endometriosis had significantly more defects of platelet aggregation to one and multiple agonists compared to controls (31% vs 4%, p = 0.005 and 15% vs 4%, p< 0.05, respectively). VWF level demonstrated a significant downward trend with increasing laparoscopic stage (r = -0.35, p = 0.01). Five women with severe endometriosis had a VWF level < 50 IU dL-1.

Conclusion: Endometriosis is associated with platelet aggregation defects. This may have important implications in the treatment of endometriosis.

Keywords: Endometriosis, Bleeding disorders, Platelet function disorder, Platelet aggregation, Von Willebrand factor

INTRODUCTION

Endometriosis is a complex gynecological condition that affects around 10% of women of reproductive age. It is characterized by the presence and growth of ectopic endometrial tissue outside of the uterus resulting in dysmenorrhea, dyspareunia, and sub fertility. The chronicity of the condition has a significant impact on women's lives including work, social functioning and sexual relationships.

The pathogenesis of endometriosis is still not fully understood. The most commonly accepted hypothesis is the retrograde menstruation theory; viable endometrial fragments pass back through the fallopian tubes, possibly due to a pressure gradient originating from dysynergic uterine contractions. Once in the peritoneal cavity they can implant, grow and invade pelvic structures [1]. The likelihood of this occurrence is influenced epidemiologically by any menstrual, reproductive or personal factor that increases pelvic contamination to regurgitated endometrium, such as duration and heaviness of menstrual blood flow [2]. The risk of developing endometriosis is increased in women with a duration of menses > 6 days (OR; 2.5, 95% CI; 1.1-5.9) [3].

Aberrant immunological mechanisms are also implicated in the pathogenesis with increased activation of circulating monocytes and macrophages and release of inflammatory cytokines into the peritoneal fluid, which stimulate ectopic endometrial cell proliferation [4,5]. Another theory is that endometriosis is derived from a metaplastic process occurring in the peritoneal mesothelium [6].

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The retrograde menstruation/implantation and metaplasia theories do not explain the wide variation in clinical manifestations associated with this condition. A well-described paradox of the condition is the lack of correlation between symptom severity and staging of the disease found at laparoscopy [7,8].

The aim of this study is to assess whether laboratory abnormalities of hemostasis are increased in women with endometriosis, which may in turn be implicated in the pathogenesis of endometriosis. In addition, the correlation between symptom severity, disease staging and bleeding tendency is investigated.

MATERIALS AND METHODS

The study was conducted from July 2013 until July 2014 at the Royal Free Hospital in north London. The research protocol was reviewed and ethical approval granted by a National Research Ethics Service (NRES) committee. Written informed consent was obtained from each participant.

Study population

Case participants were identified through a local hospital database that provided diagnostic information regarding laparoscopic procedures. Women aged 18-55 years with a laparoscopically confirmed diagnosis of endometriosis were invited to attend the Royal Free Hospital for an interview and laboratory investigations of hemostasis. Participants were excluded if they had a known inherited bleeding disorder such as Von Willebrand disease (VWD), platelet function disorder, carriers of hemophilia or other rare inherited factor deficiency, or if they were taking anticoagulant medication.

Age-matched female control subjects were staff members recruited from the Royal Free Hospital without a diagnosis of endometriosis, or symptoms suggestive of the condition (including sub fertility). Control subjects were matched, as far as possible, in ethnicity, blood group, and smoking status. Participants were only included if they were willing to abstain from taking medication that interfered with platelet function for seven days prior to laboratory testing. In addition, they were asked to avoid herbal preparations, caffeine, and excessive exercise on the day of testing. Participants found to have abnormal results were invited back for repeat testing and consultation with a specialist in hemostasis.

Participants meeting the inclusion criteria were asked to complete the Pain Impact Questionnaire (PIQ-6), a six-question health survey designed to subjectively measure severity and impact of pain on an individual's functional health and wellbeing [9]. Each participant completed a pictorial blood assessment chart (PBAC) to quantify menstrual blood loss [10]. The stage of endometriosis was recorded according to laparoscopic findings, where documented, using the revised American Society for Reproductive Medicine (rASRM) classification system to define disease severity **(Figure 1)** [11].

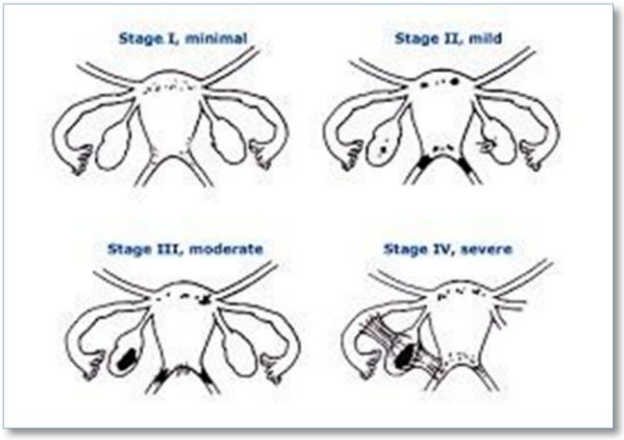

Figure 1. Revised American Society for Reproductive Medicine (rASRM) classification system for endometriosis.

Endometriosis is classified into four stages: stage I (minimal), stage II (mild), stage III (moderate), and stage IV (severe) depending on the extent, and depth of endometriosis implants; presence and severity of adhesions; and the presence and size of ovarian endometriomas.

Laboratory methods

A sample of 30 ml venous blood was collected from each participant using a 19-gauge butterfly with minimal occlusion of the antecubital fossa vein into blood collection tubes containing 106 mol/L sodium citrate [Sarstedt monovettes, Sarstedt, Leicester, UK]. Platelet rich plasma [PRP] was prepared by centrifuging whole citrated blood samples at 150 x g for 15 minutes at room temperature and then transferring the PRP to a separate container, and allowing to stand for 30 minutes prior to testing. Platelet function was assessed by light transmission aggregometry (LTA) and was carried out using a dual channel Payton aggregometer 600B (Payton Associates Ltd, Scarborough, Ontario, Canada). The maximum percentage of aggregation at three and five minutes were recorded following the addition of various platelet agonists including [final concentrations of each agonist used stated in brackets]: adenosine diphosphate (ADP) [2, 3 µm], epinephrine [2, 3 µm], collagen [1µg/mL], ristocetin [0.5 and 1.5 mg/mL], arachidonic acid [1 µm] and U46619, a synthetic thromboxane analogue [1 µm]. The remaining PRP was further centrifuged at 2000g for 12 minutes, aliquoted and then re-centrifuged again to make platelet poor plasma (PPP), which was then aliquoted and stored at -70ºC until undergoing further testing.

The prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time and Clauss fibrinogen tests were assayed on an ACL TOP coagulometer

[Instrumentation Laboratory, USA]. VWF antigen (VWF: Ag) was measured by an in-house ELISA, and VWF: Ristocetin cofactor (VWF: RCo) was measured by methods previously described [12] Clotting factors FVIII, FIX, and FXI were assayed on thawed PPP by one-stage clotting methods as previously described [13] FXIII antigen levels were measured using a FXIII ELISA assay [Affinity Biologicals, Quadratech Diagnostics Ltd, UK].

Statistical analysis

Student's *t* test was used to assess difference in age, and mean hemostatic variables between cases and controls. Chi squared or Fisher's exact test was used to compare nominal demographic data, frequency of factor deficiencies, and abnormal platelet aggregation responses between cases and controls. Multiple logistic regression analysis was used to determine if there were any correlation between PIQ-6 score, PBAC score and hemostatic variables. Laparoscopic staging, PIQ-6 score and hemostatic variables were analyzed by a two-tailed Spearman's Rho test. All statistical tests were carried out using SPPS version 22.0.

RESULTS

A total of 84 women with a diagnosis of endometriosis were recruited. Three women were excluded from the final analysis as they had a confirmed diagnosis of an inherited bleeding disorder. Thirty asymptomatic women without a diagnosis of endometriosis were recruited as controls. There was no significant difference in age, ethnicity, blood group O, or smoking status between cases and controls **(Table 1).**

Table 1. Baseline characteristics of cases and controls.

Characteristic	Cases (n = 81) N (%)	Controls (n = 30) N (%)	P value
Median age (range)	39 (22-55)	35 (23-53)	0.174
Ethnicity			
White	57 (70)	22 (73)	0.742
Black	4 (5)	1 (7)	1.00*
Asian	20 (25)	7 (23)	0.716
Smoking status			
Current smoker	4 (5)	1 (3.3)	1.00*
Non-smoker	77 (85)	29 (97)	1.00*
Blood group 'O'**	19/42 (45)	6/12 (50)	0.972

p value determined by Chi squared test for parametric data (frequency > 5) or Fisher's exact test (*) for non-parametric data (frequency < 5).

Missing data (**)

The primary indication for undergoing laparoscopy in the women with endometriosis was 51/81 (63%) for dysmenorrhea/pelvic pain, 10/81 (12%) for sub fertility, 8/51 (10%) for ovarian cysts, and 6/81 (7%) for heavy menstrual bleeding. Seven women (8.6%) had undergone hysterectomy for treatment of endometriosis. rASRM laparoscopic staging was available for 65 case participants and were distributed as follows: 18 (28%) women with stage I, 15 (23%) women with stage II, 14 (22%) women with stage III, and 18 (28%) with stage IV endometriosis.

There were significantly more defects of platelet aggregation with one agonist among the women with endometriosis compared to the control group (31% versus 4%, $p = 0.005$). A significantly higher frequency of abnormal platelet aggregation response to epinephrine was detected in women with endometriosis compared to controls (25% versus 4%, $p = 0.02$) **(Table 2).** In addition, there were significantly more abnormal aggregation responses to multiple agonists (≥ 2) in women with endometriosis (15% versus 4%, $p < 0.05$). Three women (4%) in the endometriosis group were diagnosed with a platelet function disorder following retesting. Two women had reduced aggregation response to weak agonists ADP and epinephrine. Another woman had an inappropriate response to ristocetin at low concentration. Only one woman in the control group had abnormal aggregation to multiple agonists, which was normal on repeat testing.

Among the women with endometriosis, two had low VWF. One woman had both VWF: Ag and VWF: RCo level below 45 IU dL-1 and another had a VWF: RCo level below 45 IU dL-1. The woman with low VWF: RCo was diagnosed with type 2 VWD, whilst the other woman had normal VWF on repeat testing. Among the control group, there was one woman with low VWF: Ag and VWF: RCo level, which was normal on repeat testing. Thus, the frequency of VWF below our laboratory reference range did not differ significantly between cases and controls ($p = 0.57$ and 1.0 for VWF: Ag and VWF: RCo, respectively **(Table 2).**

Deficiencies in coagulation factors XI or XIII (below our laboratory reference range) were detected in both cases and controls. Four women (5%) with endometriosis and one woman (3%) in the control group had FXI level below 70 dL-1. These women were tested for the common FXI gene mutations and no abnormalities were detected. Similarly, five women (6%) with endometriosis and three women (10%) in the control group had FXIII below 70 IU dL-1. The isolated deficiencies in either FXI or FXIII levels were between 48-67 IU dL-1 and were thought to represent >2 standard deviations from the mean in the general population.

No significant differences in the frequency of abnormalities were detected between the groups (p = 1.00 and 0.68 for FXI and FXIII, respectively **(Table 3).**

Table 2. Frequency of platelet aggregation abnormalities in cases and controls.

Platelet count Mean (± SD)	**Cases**		**Controls**		***p* value**
	(*n* = 81)	***n* (%)**	**(*n* = 30)**	***n* (%)**	
	384	(102)	344	(81)	0.060
Agonist					
Adenosine diphosphate	12	(14.8)	1	(3.6)	0.179
Epinephrine	20	(24.7)	1	(3.6)	0.023
Collagen	3	(3.7)	1	(3.3)	1.000
Arachidonic acid	4	(4.9)	0	(0)	0.578
Ristocetin	3	(3.7)	0	(0)	0.497
U46619	0	(0)	0	(0)	
One agonist	25	(30.9)	1	(3.6)	0.005
Multiple agonists (2 or more)	12	(14.8)	1	(3.6)	0.047
Platelet function disorder	3	(3.7)	0	(0)	0.385

p value computed by Fisher's exact test.

Table demonstrating frequency of abnormalities in cases and controls. The variables highlighted in grey are significantly different (p < 0.5).

Table 3. Abnormal hemostatic variables.

	Reference range	**Cases (n = 81)**		**Controls (n = 30)**		***p* value**
		***n* (%)**	**Level**	***n* (%)**	**Level**	
Factor XI	70-150 IU dL-1	4 (4.9)	48, 61, 65, 67,	1 (3.3)	64	1.000
Factor XIII	70-175 IU dL-1	5 (6.2)	59, 62, 63, 64, 66	3 (10)	64, 65, 67	0.680
VWF: Ag	45-145 IU dL-1	1 (1.2)	44	1 (3.3)	41	0.566
VWF: RCo	45-145 IU dL-1	2 (2.4)	38, 27	1 (3.3)	39	1.000

p value computed by Fisher's exact test

VWF: Ag, von Willebrand factor antigen; VWF: RCo, von Willebrand factor ristocetin cofactor activity level.

Table demonstrating the mean and standard deviation in hemostatic variables in cases with severe endometriosis compared with controls. The variables highlighted in grey are significantly different.

Overall, no significant difference was detected in the mean hemostatic variables between women with endometriosis and controls **(Table 4).**

The only hemostatic variable that approached clinical significance (p = 0.06) was difference in mean platelet count (384 x 109/L in cases versus 344 x 109/L in controls). VWF: RCo level demonstrated a significant downward trend with increasing rASRM stage (r = -0.35, p = 0.01) **(Figure 2).**

Patients with severe (stage IV) endometriosis (n = 18) had a significantly reduced mean VWF: RCo level compared to controls (60 IU dL-1 in severe cases versus 77 IU dL-1 in controls, p = 0.02). Five women out of 18 with severe endometriosis had a VWF: RCo level < 50 IU dL-1. This is

in contrast to among the control group where only two women had VWF: RCo level < 50 IU dL-1. Patient with severe endometriosis had an increased platelet count compared to controls (429 x 109/L in severe cases versus 344 x 109/L in controls, $p = 0.01$ **(Table 5).**

Table 4. Difference in hemostatic variables between cases and controls.

	Reference range	Cases Mean (± SD) (n = 81)	Controls Mean (± SD) (n = 30)	*p* value
PT	9-13.5 secs	11.3 (0.9)	11.3 (0.8)	0.810
INR	0.9-1.2	0.9 (0.1)	1.0 (0.1)	0.362
APTT	28-36 secs	30.9 (3.0)	30.8 (3.0)	0.820
Fibrinogen	1.5-4.0 g L-1	2.6 (0.6)	2.5 (0.5)	0.852
Platelet count	150-450x109/L	384 (102)	344 (81)	0.060
Factor VIII	50-150 IU dL-1	98 (28.0)	97 (30.0)	0.887
Factor IX	50-150 IU dL-1	104 (20.7)	104 (16.6)	0.924
Factor XI	70-150 IU dL-1	91 (14.8)	88 (14.2)	0.563
Factor XIII	70-175 IU dL-1	103 (30.1)	104 (24.3)	0.974
VWF: Ag	45-145 IU dL-1	92 (34.3)	91 (32.6)	0.904

p value determined by student's t test

PT; prothrombin time, INR; internal normalized ratio, APTT; activated partial thromboplastin time, VWF: Ag, von Willebrand factor antigen; VWF: RCo, von Willebrand factor ristocetin cofactor activity level.

Table demonstrating the mean and standard deviation in hemostatic variables between cases and controls. There were no significant differences

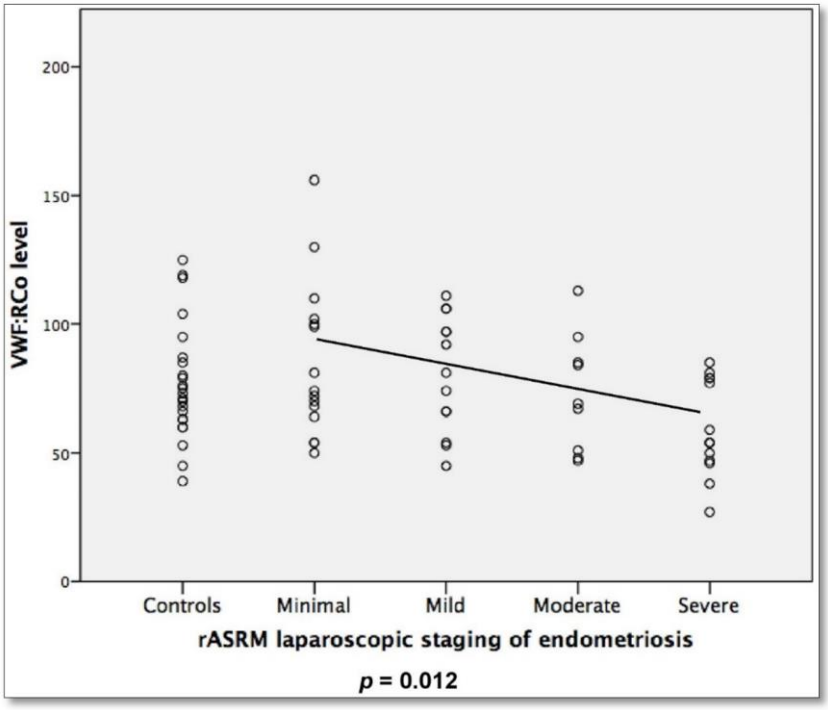

Figure 2. Scatter plot demonstrating the distribution of von Willebrand Factor Ristocetin Cofactor activity level (VWF: RCo) according to revised American Society for Reproductive Medicine (rASRM) stages of endometriosis.

Table 5. Difference in hemostatic variables in cases with severe endometriosis compared to controls.

	Reference range	Severe cases Mean (± SD) (n = 18)	Controls Mean (± SD) (n = 30)	*p* value
PT	9-13.5 secs	11.1 (0.6)	11.3 (0.8)	0.285
INR	0.9-1.2	0.9 (0.1)	1.0 (0.1)	0.317
APTT	28-36 secs	31.2 (2.5)	30.8 (3.0)	0.987
Fibrinogen	1.5-4.0 g L-1	2.6 (0.7)	2.5 (0.5)	0.797
Platelet count	150-450x109/L	429 (116)	344 (81)	0.005
Factor VIII	50-150 IU dL-1	93 (25.0)	98 (29.7)	0.569
Factor IX	50-150 IU dL-1	108 (21.8)	103 (16.4)	0.387
Factor XI	70-150 IU dL-1	92 (15.5)	91 (16.2)	0.820
Factor XIII	70-175 IU dL-1	112 (47.7)	104 (24.3)	0.535
VWF: Ag	45-145 IU dL-1	90 (37.9)	88 (30.6)	0.773
VWF: RCo	45-145 IU dL-1	60 (18.6)	77 (22.17)	0.024

p value determined by student's t test

PT; prothrombin time, INR; internal normalized ratio, APTT; activated partial thromboplastin time, VWF: Ag, von wille brand factor antigen; VWF: RCo, von Willebrand factor ristocetin cofactor activity level

Women with endometriosis had significantly increased mean PBAC score compared to controls (319, SD ± 366 in case group vs 147, SD ±166 in controls, p = 0.024). No statistically significant difference was detected between any hemostatic variable and the PBAC score. However, the mean PBAC score was significantly increased in women with platelet aggregation defects to one agonist (408, SD ± 418, p = 0.021), and multiple agonists (489, SD ±589, p = 0.015) compared to the mean PBAC score of women without platelet aggregation defects (266, SD ± 297).

Platelet count was the only hemostatic variable to demonstrate a weak positive correlation with PIQ-6 score in the logistic regression analysis ($r2$ = 0.031, p = 0.03). In addition, there was no significant difference in PIQ-6 scores in women with abnormal platelet aggregation compared to those without. (**Figure 3)** shows distribution of PIQ-6 scores across the rASRM laparoscopic stages. No significant trend in PIQ-6 score was detected across the different stages (p = 0.5).

DISCUSSION

Main findings

In our study, a significantly higher proportion of women with endometriosis had abnormal platelet aggregation response to one and multiple agonists compared to controls. Furthermore, the women with platelet aggregation defects were more symptomatic with heavy menstrual bleeding (HMB) and increased PBAC score (p = 0.024). A high frequency of HMB has been reported in women with platelet function disorders and VWD [14-19]. The increased frequency of abnormal platelet aggregation and low VWF: RCo seen in women with advanced disease indicate that primary hemostasis defects are associated with endometriosis.

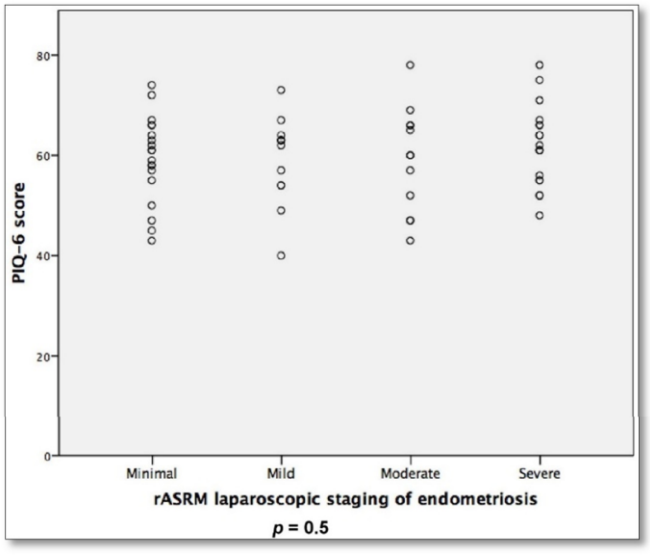

Figure 3. Scatter plot demonstrating the distribution of Pain Impact Questionnaire (PIQ-6) score according to revised American Society for Reproductive Medicine (rASRM) stages of endometriosis.

These findings could have implications in the pathogenesis of endometriosis. Firstly, with increased retrograde menstruation, which may in turn increase the rate of endometriosis formation. Secondly, impaired local hemostasis within endometriotic implants may result in recurrent cyclical internal bleeding, exacerbating the spread of the condition throughout the pelvic cavity. Endometrial tissue that implants ectopically continues to be under hormonal regulation and undergoes monthly proliferation and shedding [20]. The pain results from localized internal bleeding within or around the endometrial deposits.

Menstruation is a highly complex, regulated process that occurs following progesterone withdrawal, vasoconstriction of spiral arterioles, and shedding of the endometrium. Local factors within endometrial tissue promote a pro-hemorrhagic environment. Down regulation of tissue factor (TF) and plasminogen-activator inhibitor 1 (PAI-1) occurs following progesterone withdrawal [21]. Inducible nitrous oxide (i NO) results in inhibition of platelet aggregation, relaxation of smooth muscle and vasodilatation with increased menstrual blood flow [22]. VWF levels fluctuate during the menstrual cycle and are lowest (VWF: Ag and VWF: RCo) during the first four days of menses [23]. Hormonal fluctuations around the time of menstruation may impair both systemic and local primary hemostasis within endometriotic implants.

There was no increased frequency of low VWF detected in our patients with endometriosis compared to controls. However, the downward trend of VWF activity level (VWF: RCo) with increased disease severity indicates that endometriosis may be associated with a functional defect in primary hemostasis. In addition, severe endometriosis may be different from minimal/mild stages, and more likely to be associated with impaired local hemostasis. Alternatively, impaired systemic platelet aggregation may result in progression to more advanced disease. Other studies have suggested that there might be an association between VWD and endometriosis. In a study that assessed the reproductive experience of women with VWD, endometriosis was reported in 30% of cases compared to 13% of controls (p = 0.01) [17]. However, the increased detection of endometriosis may be higher in women with VWD, who suffer from excessive menstruation and therefore are more likely to consult with a gynecologist.

We found an increased platelet count in women with severe endometriosis and a positive correlation with platelet count and PIQ-6 score. An increased platelet count in women with severe endometriosis has been confirmed previously [24]. Thrombocytosis is a marker of chronic inflammation, and inflammation is strongly implicated in the pathogenesis of endometriosis. In addition, platelet count increases with chronic active bleeding and iron deficiency state [25]. We were unable to demonstrate a correlation with bleeding tendency and symptom severity. This may indicate that a bleeding tendency does not exacerbate endometriosis symptoms. However, the women were asked to objectively assess symptom severity over the past four weeks, and thus the PIQ-6 score may not have reflected the woman's symptoms when they were at their peak of severity (i.e., prior to laparoscopic ablation).

Interpretation

Routine testing for a disorder of primary hemostasis in women with endometriosis is laborious, expensive and time consuming using traditional laboratory methods such as those utilized in this study. On the other hand, a diagnosis of platelet function disorder would aid treatment decisions in such cases; women with a positive diagnosis should be advised to avoid anti-platelet and non-steroidal anti-inflammatory medication, which further impairs platelet function and is commonly used to treat the pain of endometriosis. In addition, surgical intervention is commonly required for the management of endometriosis, therefore establishing a diagnosis of a bleeding disorder is important to enable preventative methods to avoid perioperative bleeding complications. A more cost-effective approach would include screening with a detailed bleeding history or bleeding score prior to laboratory testing. Women with endometriosis who are identified as having a co-existing platelet function disorder or VWD would potentially benefit from hemostatic therapy (i.e., desmopressin or tranexamic acid) during menstruation.

Strengths and limitations

Sample size was limited due to the complexity of laboratory investigations. Although LTA is considered the gold standard for testing platelet aggregation, it is a difficult and time-consuming process. Blood samples have to be obtained in such a way as to avoid prematurely activating the platelets, and the samples are required to be processed immediately. For this reason, only a limited number of participants could be recruited at any given time. The control population comprised of asymptomatic women who had never sought medical attention for symptoms of endometriosis. The prevalence of endometriosis in asymptomatic women ranges from 1-22%, depending on the diagnostic criteria and the population studied [26-29]. Thus, it was not feasible to fully exclude whether women in our control population had the condition. This may have affected the validity of our results due to bias towards the null hypothesis. This could be resolved by obtaining samples from women undergoing laparoscopic sterilization (i.e., with a surgically confirmed normal pelvis). However, as mentioned previously, due to the complexity of the laboratory investigations, this was not feasible, and we were unable to control for this potentially confounding factor. In addition, abnormalities in platelet aggregation detected with LTA do not always signify a bleeding disorder. An individual with suboptimal response to ephedrine only, and no bleeding history should not be considered as having a

functional platelet abnormality with current clinical testing [30]. All abnormalities in platelet aggregation found on initial testing were repeated with the addition of flow cytometry, nucleotide studies, and genetic testing if appropriate to establish or exclude a diagnosis of platelet function disorder. Further research is required to determine whether the finding of a high frequency of abnormal platelet aggregation in women with endometriosis is detected in a larger cohort, ideally with control subjects including women who attend for laparoscopic sterilization. The impact of hemostatic treatment during menstruation should then be investigated in women with endometriosis who are found to have a disorder of hemostasis.

CONCLUSION

Endometriosis is associated with platelet aggregation defects. A dysfunction in primary hemostasis may account for disease progression and thus contribute to the underlying pathogenesis of endometriosis. Selective screening of symptomatic women with a positive bleeding history could have important implications for treatment of endometriosis. Women found to have a co-existing platelet aggregation abnormality should be managed accordingly, and advised to avoid antiplatelet medication.

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DISCLOSURE OF INTERESTS

None of the listed authors have links to any biomedical commercial company or any disclosure of interests to declare.

CONTRIBUTION TO AUTHORSHIP

JD and RAK designed the research. JD, BH, AR, and OR conducted the research. JD analyzed the data and wrote the first draft of the paper. JD and RAK revised the paper. All authors approved the paper prior to submission.

DETAILS OF ETHICAL APPROVAL

The study was reviewed and ethical approval was granted on 22nd April 2013 from the National Research Ethics Service (NRES) Committee of London, Harrow.

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