Impaired platelet function and peripartum bleeding in women with Gaucher disease

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Summary

The risk of bleeding during delivery may be increased in women with Gaucher disease. We aimed to evaluate potential predictors for peripartum haemorrhage (PPH) during childbirth in these patients, while focusing upon coagulation tests and platelet function assays. Women with type 1 Gaucher disease who gave birth at Sheba Medical Center between 1999–2009 comprised the study cohort. Data collected included disease history, enzyme treatment, platelet counts, delivery and pregnancy outcome. PPH was defined as excessive bleeding during or immediately following delivery. Coagulation studies and platelet function tests, including aggregometry and cone and platelet (CPA) analyses, were performed on all women. We compared women with PPH (bleeders) and non-bleeders. Furthermore, women with abnormal CPA platelet function tests were compared with those with normal CPA platelet function with regards to the risk for PPH in at least one pregnancy. Forty-five pregnancies of 20 women were studied. Six women received enzyme replacement therapy during pregnancy. Mean platelet count prior to delivery was 83,000/μl ± 35,000/μl. Fourteen out of 45 (31%) deliveries were complicated by PPH. Neither thrombocytopenia nor enzyme therapy predicted PPH. Twelve out of 13 women with PPH (92.3%) versus 2/7 non-bleeders (28.6%) had impaired platelet aggregation (less than the 3rd percentile of normal average aggregate size values), when tested by CPA, (odds ratio [OR] 17.8, 95% confidence interval [CI] 2.5; 126.2; p=0.007). Notably, 78.6% of women with impaired CPA aggregation developed PPH during at least one delivery, as opposed to 16.7% of those with normal CPA platelet function tests (OR 11.6, 95% CI 1.7–77.7, p=0.018). In conclusion, women with type 1 Gaucher disease who have abnormal platelet function tests may have an increased risk of PPH.

Keywords

Type 1 Gaucher disease, peripartum bleeding, PPH, platelet function, Cone and platelet analyzer (CPA)

Introduction

Gaucher disease is an autosomal recessive lysosomal storage disorder in which there is a deficiency in the enzyme β-glucocerebrosidase, causing glucocerebroside accumulation in the cells of the reticulo-endothelial system. The disease has a particularly high prevalence among Ashkenazi Jews, wherein one of 17 is a carrier, and one of 850 has the disease (1).

Gaucher disease is marked by great phenotypic heterogeneity, ranging from asymptomatic status to a life-threatening disease with severe central nervous system involvement and shortened life span (3). There is a traditional sub-classification of the disease into three clinical types. Type 1, the most common form, is defined as chronic non-neuronopathic and is characterised by hepatosplenomegaly, anaemia, thrombocytopenia, and varying degrees of bone involvement. Haematological manifestations are common at presentation and usually manifest as a bleeding tendency due to thrombocytopenia, caused by hypersplenism and bone marrow involvement (4). The impact of the disease has been extensively modified by the introduction of effective enzyme replacement therapy, resulting in increased haemoglobin and platelet counts and a commensurate reduction in spleen and liver size (2).

Nevertheless, reports of patients with a bleeding tendency despite relatively high platelet counts and normal coagulation profiles, have led to the discovery of abnormal platelet function in a rather significant number of Gaucher patients (5, 6).

Since the risk of forthright bleeding is greatest at childbirth, the purpose of the present study was to investigate whether among Gaucher patients any clinical data or laboratory coagulation tests may predict PPH, and whether in pregnant patients with Gaucher type 1, platelet function tests performed during pregnancy may aid in identifying women at risk of excessive peripartum bleeding.
Materials and methods

The Gaucher Clinic at Shaare Zedek Medical Center, Jerusalem, is a national referral center for women with Gaucher. Pregnant women with type 1 Gaucher disease who reside in the center of Israel are encouraged to undergo antenatal care and postnatal follow-up at the high-risk pregnancy unit at the Sheba Medical Center in Tel Hashomer. Medical records at both these institutions were called for the purpose of this study.

The records of pregnant patients with Gaucher disease during the 10-year period of 1999–2009 who have been referred for antenatal care in Sheba Medical were included. The study design was a retrospective cohort study of prospectively collected data. Data collected from medical records, during pregnancy clinic visits and post delivery follow-up included: obstetric outcome data (i.e. gestational age and birth weight at delivery, mode of delivery, bleeding complications during labour, estimated blood loss, and other obstetric complications which may contribute to the incidence of bleeding such as pregnancy-induced hypertension and preeclampsia); platelet and haemoglobin counts prior to, at, and after delivery; the need for blood product transfusion at delivery; platelet counts during pregnancy; and coagulation studies results (see below) including platelet function studies, performed repeatedly during pregnancy and prior to delivery. All patients were referred for coagulation assays and platelet function tests to the Thrombosis Unit at the national Hemophilia Center at Sheba Medical Center. Since platelet function may wax and wane throughout pregnancy, the assays utilised for analysis were those performed at third trimester, prior to delivery.

Primary peripartum haemorrhage (PPH) was defined as excessive bleeding during or immediately following delivery as reported in the medical records by the physician attending the delivery, and/or necessitating blood product transfusion due to haemoglobin drop (7).

Coagulation studies

Blood samples were obtained at the first referral visit to the Coagulation Clinic and repeated during the last trimester of pregnancy to confirm any abnormalities. Blood samples were collected into 3.8% trisodium citrate anti-coagulant in a 9:1 blood/citrate ratio. Citrated blood was centrifuged and plasma aliquots stored at −35°C until analysis. Prothrombin, partial thromboplastin, and thrombin times as well as fibrinogen assays were performed using standard techniques. Because of relatively high prevalence of FXI deficiency in Ashkenazi Jewish women, all patients were also assessed for factor (F)XI deficiency (using a standard one-stage clotting assay of FXI activity). Von Willebrand factor (VWF) antigen and Ristocetin Co-factor (RiCoF) were measured using standard techniques.

Platelet function studies

Platelet aggregation was performed by standard technique using citrated platelet-rich plasma (PRP), as previously described (5). Epinephrine 5.0 μM, ADP 10.0 μM, collagen 10.0 μg/mL, and ristocetin 1.5 mg/ml were used as agonists. Normal PRP, obtained from healthy individuals, was used as a parallel control in each experiment. Aggregation was considered abnormal if maximum aggregation was less than 40% of control. Results were expressed as % aggregation relative to control. As platelet aggregation studies in the setting of low platelet counts (such as those presented by Gaucher patients) are often non-responsive, platelet function was also measured in whole citrated blood under shear force and flow conditions, using the cone and platelet analyser (CPA- Impact R, Diamed, Cresier, Switzerland) as previously described (8, 9). Two quantifiable parameters of platelet function were evaluated: platelet adhesion, defined as % total area covered by platelets (surface coverage; SC), and platelet aggregation, defined as the average size (AS) of aggregates in μm². The normal adult values in our laboratory were derived from 98 healthy adult controls and values were SC= 11.72 ± 3.1% (range: 6–22%), and AS= 44.93 ± 16.4 μm² (range: 24–94 μm²). We defined abnormal SC or AS values if results were 2 standard deviations (SD) below the mean values obtained from our normal controls, i.e. less than the 3rd percentile of normal values.

To minimise interdependency between variables, specifically due to several women undergoing more than one pregnancy each, we compared laboratory findings, including platelet function studies, as well as obstetric outcomes between two groups: “PPH-women” – women who bled excessively during at least one pregnancy, and “non-PPH women” – women who had no PPH episodes during pregnancy and delivery. Furthermore, in order to evaluate the specific contribution of platelet function studies to the risk of excessive bleeding during childbirth, we compared women with abnormal (as defined above) platelet function by CPA (group 1) to women with predefined normal platelet function by CPA during pregnancy (group 2).

The study was approved by the local Ethics Committee according to the declaration of Helsinki, and informed consent was obtained from all women tested.

Statistical analysis

Statistical analysis was performed with SigmaStat 1.0 software (Jandel Engineering Ltd, Linslade, Bedfordshire, UK). Continuous variables were compared using the Student’s t-test when the data were normally distributed, and the Mann-Whitney rank sum test when not normally distributed. Categorical data were compared using the Pearson chi-square test and Fisher’s exact test, as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated when appropriate and considered significant if the confidence interval excluded unity. A p-value <0.05 was considered statistically significant.
Results

Twenty women undergoing 45 pregnancies and deliveries were included in the analysis. Mean pregnancies number per woman was 2.25. Six women received enzyme replacement therapy (ERT) with imiglucerase (Cerezyme™, Genzyme Corp., Framingham, MA, USA) throughout their pregnancy. Indications for enzyme therapy included significant persistent thrombocytopenia and significant hepatosplenomegaly.

Mean gestational age at delivery was 39.1 ± 1.72 weeks, with a mean birth weight of 3,186 ± 524 grams. Mean platelet count during antenatal follow-up was 93,200 ± 33/mm³ (range: 49–165,000/mm³); mean platelet count at delivery was 91,200 ± 38/mm³. Caesarean section was performed in 11/45 (24.4%) deliveries for various obstetric indications, and the remaining pregnancies culminated with vaginal deliveries.

Of 45 deliveries in the study, 14 (31%) were complicated by PPH, and 11 of these (78.6%) required blood product transfusions. Not surprisingly, the documented post delivery haemoglobin concentration was significantly lower (7.97 g/dl) in PPH pregnancies, as opposed to 10.5 g/dl in pregnancies uncomplicated by PPH (p<0.001). The risk of excessive bleeding was higher in deliveries performed by caesarean section (6 PPH cases of 11 caesarean sections, 54.5%) when compared with vaginal deliveries (8 PPH cases of 34 vaginal deliveries, 23.5%), although this difference was not statistically significant (p=0.07).

Of the 20 women participating in our study, 13 (65%) experienced PPH in at least one pregnancy, and were defined as “PPH-women”, or “bleeders”, and the rest were defined as “non-PPH women”. In an effort to define risk factors for an increased risk of PPH, we compared obstetric parameters, between bleeders and non-bleeders. There was no difference in the mean number of pregnancies, birth weight, mode of delivery and presence of recombinant enzyme therapy between PPH-women and non-PPH women. In addition, mean platelet counts at delivery were low, and similar, between deliveries with and without PPH (85,500 ± 26,800/μl vs. 94,700 ± 44,200/μl, respectively, p=0.74), and between PPH women and non-PPH women (87,200 ± 27,900/μl vs. 107,000 ± 65,900/μl, respectively, p=0.86).

As outlined in Materials and methods, we evaluated platelet function both by standard techniques of platelet aggregation and under shear force and flow (CPA). Standard platelet aggregation studies were consistently impaired with ADP and epinephrine. Abnormal standard aggregation (non-responsive or impaired epinephrine-/ADP-induced aggregation), that may be found in around 20% of blood donors, was noted in 5/13 PPH women (38.5%) and in 3/7 non-PPH women (42.9%, p=1.0), and were therefore not useful for defining bleeding risk.

As platelet aggregation studies in the setting of low platelet counts (such as those presented by Gaucher patients) are often non-responsive, platelet function was also evaluated using the CPA, under conditions of shear force and flow, as outlined in Material and methods.

Platelet adhesion, as measured by CPA, was significantly lower among the study cohort (SC= 2.76 ± 1.5%) as compared with control values obtained from health volunteers (SC=11.72 ± 3.1%, p<0.001). Mean platelet aggregate size as measured by CPA was also significantly lower in the study cohort compared with control values (AS 21.7 ± 4.8 μM² vs. 44.93 ± 16.4 μM², respectively, p<0.001). As previously outlined, we defined abnormal results as values below 2 SD below mean control values. When comparing PPH women with non-PPH women, the risk of bleeding was significantly associated with evidence of abnormal platelet function. Specifically, 12/13 (92.3%) women who bled excessively during at least one delivery demonstrated impaired (below 3rd percentile) platelet aggregation (AS) by CPA, while only 2/7 (28.6%) women with no PPH events demonstrated abnormal platelet aggregation (OR 17.8, 95%CI 2.5; 126.2, p=0.007). Platelet adhesion (SC) CPA studies were not useful in defining women at risk of bleeding excessively during delivery, as they were almost uniformly impaired (below 3rd percentile) in our study cohort. The comparison of platelet function studies between PPH women and non-PPH women is presented in ▶ Table 1. Interestingly, lower platelet counts were not significantly associated with impaired platelet function tests.

Coagulation parameters (PT, PTT, thrombin time, fibrinogen levels, von Willebrand antigen and ristocetin cofactor) were normal for all women studied. However, two women of the PPH group presented with mild FXI deficiency (levels of 45–51% vs. normal activity defined as above 60%) whereas all non-PPH women and the remaining 11/13 bleeders had normal FXI activity.

Finally, the risk of bleeding was also similar between enzyme-treated women (i.e. more severe disease) and non-treated women (3/6, or 50%, compared with 10/14, or 71.4%, p=0.6).

Since impaired platelet function, as tested by CPA, prevailed among women with PPH, we further analysed the women with normal vs. pre-defined abnormal platelet aggregation, as tested by CPA.

Of the 20 women with Gaucher type 1 participating in our study, 14 women had evidence of impaired platelet aggregate size measured by CPA analysis and were defined as group 1. The remaining six women had normal platelet aggregate size by CPA and were termed group 2.

Table 1: Platelet counts and function studies in PPH women and non-PPH women.

<table>
<thead>
<tr>
<th></th>
<th>PPH women</th>
<th>Non-PPH women</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean platelet counts at delivery (x1,000/μl)</td>
<td>87.2 ± 27.9</td>
<td>107 ± 65.7</td>
<td>0.86</td>
</tr>
<tr>
<td>Abnormal SC</td>
<td>13 (100%)</td>
<td>6 (85.7%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Abnormal AS</td>
<td>12 (92.3%)</td>
<td>2 (28.6%)</td>
<td>0.007</td>
</tr>
<tr>
<td>FXI deficiency</td>
<td>2 (15.4%)</td>
<td>0</td>
<td>0.52</td>
</tr>
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</table>

Platelet function studies were measured in whole citrated blood under shear force and flow conditions, using the cone and platelet analyzer (CPA), as outlined in the Materials and Methods section. SC – surface coverage, represents platelet adhesion, defined as the percentage of total area covered by platelets; AS – average size of the bound aggregates, denotes platelet aggregation.
Table 2: Obstetric characteristics of Gaucher type 1 women with abnormal platelet function (group 1) and women with normal platelet function (group 2).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Mean number of pregnancies per woman</td>
<td>2.21</td>
<td>2.33</td>
<td>0.86</td>
</tr>
<tr>
<td>At least one cesarean delivery</td>
<td>6 (42.8%)</td>
<td>1 (16.7%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Cesarean sections of total deliveries</td>
<td>6/31 (19.4%)</td>
<td>5/14 (35.7%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks) (mean ± SD)</td>
<td>39.3 ± 1.4</td>
<td>38.5 ± 2.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Birth weight at delivery (grams) (mean ± SD)</td>
<td>3196 ± 556</td>
<td>3164 ± 466</td>
<td>0.86</td>
</tr>
<tr>
<td>Recombinant enzyme treatment (%)</td>
<td>3 (21.4%)</td>
<td>3 (50%)</td>
<td>0.30</td>
</tr>
<tr>
<td>PPH during at least one delivery (%)</td>
<td>11 (78.6%)</td>
<td>1 (16.7%)</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Table 3: Platelet counts and function studies in Gaucher type 1 women with abnormal platelet function (group 1) and women with normal platelet function (group 2). Platelet function studies were measured in whole citrated blood under shear force and flow conditions, using standard aggregometry * and the cone and platelet analyser (CPA), as outlined in Materials and methods.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean platelet counts at delivery (x1,000/μL) (mean ± SD)</td>
<td>82.8 ± 25</td>
<td>104.5 ± 54</td>
<td>0.25</td>
</tr>
<tr>
<td>Abnormal aggregation *</td>
<td>6 (42.8%)</td>
<td>2 (33.3%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean platelet SC by CPA</td>
<td>2.45%</td>
<td>3.96%</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean platelet AS by CPA (μM²)</td>
<td>19.99</td>
<td>28.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FXI deficiency</td>
<td>1 (7.1%)</td>
<td>1 (16.7%)</td>
<td>0.52</td>
</tr>
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</table>

Abnormal aggregation * results define impaired aggregation with epinephrine and/or ADP, below 40% of normal control values. SC – surface coverage, AS – average size of the bound aggregates.

Discussion

Postpartum haemorrhage is a life-threatening event that has serious consequences for mother and child if not recognised and dealt with promptly (10). It is estimated that 150,000 maternal deaths worldwide annually result from obstetric haemorrhage, with the majority dying from postpartum bleeding (11). Peripartum blood loss sufficient to cause symptoms of hypovolaemia, a significant drop in haematocrit or requiring blood product transfusion is estimated to occur in approximately 4% of vaginal deliveries (12) and 6% of caesarean deliveries (13). Other obstetrical risk factors include induced labour, placenta previa, placental abruption, macrosomia, and multifetal pregnancy (14).

Women with Gaucher disease are at an increased risk of excessive bleeding at delivery although the underlying cause appears to be neither severity of Gaucher disease nor the lack of specific ERT (15). Thrombocytopenia is probably a contributing factor to the very high risk of peripartum bleeding in Gaucher patients, yet impaired platelet function, coagulation factor deficiencies, and other idiosyncratic co-morbidities may also play a role. In the current study, we found that 65% (!) of patients experienced PPH in at least one pregnancy. Therefore, the identification of risk factors in this group of patients is of utmost importance in preparing for, and dealing with the PPH event.

As previously reported (12, 13), there was a trend (p=0.07) for excessive bleeding in deliveries through caesarean section (54.5%) when compared with vaginal deliveries (23.5%). Interestingly, lower platelet counts did not correlate with bleeding risk and with platelets’ impaired aggregation in our cohort (Table 1), although all counts were below the normal range and the sample size may be too small for extrapolation.

ERT by Cerzyme™, applied to severe cases of Gaucher disease, has been shown to result in improved platelet counts in Gaucher patients (16, 17). In our cohort, ERT may have had a mitigating effect on bleeding, since women receiving ERT were less prone to PPH, presumably due to improved platelet counts and function due to therapy.
In our study, impaired platelet aggregation (as tested by CPA) prevailed among Gaucher patients with PPH and significantly differentiated “bleeders” from “non-bleeders” (Table 1). Furthermore, AS below the 3rd percentile of normal controls was a predictor of PPH occurrence, regardless of platelet counts (Table 3).

Since it is difficult to assess platelet function in the presence of low platelet counts, we used whole blood shear force induced platelet deposition with Impact-R™ (CPA), a rapid, feasible, point-of-care tool, available to assess whole blood platelet adhesion and aggregation in various conditions (9). Interestingly, platelet adhesion (manifested by SC) tested by CPA was impaired in our cohort as a whole, thus being too sensitive to detect the differences among bleeders and non-bleeders with Gaucher disease. Nevertheless, lower than 3rd percentile aggregation (AS) certainly differentiated women with or without PPH (Table 3). CPA is tested in whole blood, therefore, both SC and AS may be affected by haematocrit levels (9). This fact does not detract from the value of our findings, as low haematocrit is clearly a risk for bleeding. The association of impaired platelet function in pregnant Gaucher patients with an increased risk of peripartum bleeding has not been previously reported. On the contrary, Sagdilek et al. (18) have demonstrated platelet function to be preserved in normal pregnancy when compared with non-pregnant controls, while other authors have shown platelet activation during specific pregnancy-related conditions such as preeclampsia (19, 20) and pregnancy-induced hypertension (21).

The use of CPA has been previously suggested as potential screening for impaired platelet function in patients treated by clopidogrel (22), nevertheless, it should be specified that currently none of the platelet function tests available (including classical aggregometry) are well standardised with generally acceptable cut-off values (23).

Platelet adhesion may correlate with bleeding symptoms in patients with immune thrombocytopenia, while the size of aggregates that are formed under high shear was reported to correlate with in vivo platelet activation (24). Thus, the use of CPA analysis for assessing bleeding risk in cases when PPH may be anticipated is a novel use of this modality and should be further studied in a population of women with and without bleeding tendencies.

Two women in our cohort had mild FXI deficiency, comparable to the reported prevalence among Ashkenazi Jews (25). One of the two women belonged to the platelet dysfunction group (group 1). Since bleeding tendency is variable, even among subjects with severe FXI deficiency, blood component replacement therapy for surgeries and medical interventions, including child-birth, is not always mandatory (26, 27). Nevertheless, the combined, potentially synergistic impact of thrombocytopenia, platelet dysfunction and decreased FXI activity in patients with Gaucher disease should be considered. Meticulous local haemostatic measures may be recommended to avoid excessive bleeding in such patients.

Our study has several limitations, such as the selection bias of patients because of referral by the primary Gaucher clinic, potentially because of specific concerns. Moreover, the reported results, obtained in a potentially hypercoagulable state, are applicable to future pregnancies only and should not be relied upon in cases of future surgical procedures in these patients. The lack of normal pregnancy controls for CPA studies is another flaw, yet since other studies have reported improved whole blood platelet function in pregnancy (18–21), the use of normal population controls (whose whole blood platelet aggregation may be even lower than anticipated for normal pregnancy controls) as comparator for the Gaucher cohort, should not have affected our study’s results and conclusion.

Indeed, normal pregnancies may be complicated by thrombocytopenia (28), however, since Gaucher patients are already thrombocytopenic this point probably does not detract from our results. Moreover, the women studied by us showed no worsening of baseline thrombocytopenia throughout pregnancy. Since platelet function is generally improved during pregnancy due to the hypercoagulable state, we believe that platelet function assayed at 3rd trimester truly represents bleeding risk.

Further studies are required in order to predict whether in vitro correction of whole blood clotting assays (e.g. CPA tests or whole blood thromboelastography) may be feasible, with a potential future clinical correlation.

In summary, this study has shown that platelet function testing by CPA may be useful in defining the increased risk of peripartum bleeding in Gaucher disease. Impaired CPA tested platelet aggregation (AS) may serve as a potential predictor of PPH; however, our findings should undoubtedly be further validated.

Future application of whole blood clotting and platelet function assays in high risk populations (e.g. women with congenital and acquired bleeding disorders) may allow for safer deliveries, by preparation with adequate blood products, as well as considering (if optional) avoidance of procedures which may carry an increased risk in patients (such as epidural analgesia or caesarean section) with impaired platelet function.

Funding and acknowledgement
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What is known about this topic?
- Gaucher patients are at increased risk of bleeding due to thrombocytopenia and platelet dysfunction.
- Peripartum haemorrhage (PPH) incidence may be increased among women with Gaucher disease.

What does this paper add?
- Platelet function of Gaucher patients, even while thrombocytopenic, can be assessed by whole blood shear force induced aggregation using the Cone and platelet analyser (CPA).
- Women with Gaucher and PPH show significantly lower aggregation tested by CPA as compared to Gaucher patients who did not suffer PPH.
- Low aggregation (below 3rd percentile of normal) may independently predict PPH occurrence in women with Gaucher.
References


