Blood Cells, Molecules and Diseases 60 (2016) 3–6

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Bengal macrothrombocytopenia is not totally an innocuous condition

Shahnaz Ali *, Shrimati Shetty, Kanjaksha Ghosh [1]

National Institute of Immunohaematology (ICMR), 13th Floor, KEM Hospital, Parel, Mumbai 400 012, India

ARTICLE INFO

Article history:
Submitted 8 April 2016
Revised 26 May 2016
Accepted 26 May 2016
Available online 27 May 2016

Editor: Mohandas Narla

Keywords:
Bengal macrothrombocytopenia
Platelet count
Mean platelet volume
Bleeding manifestations
Giant platelets

ABSTRACT

Inherited macrothrombocytopenia is a subgroup of thrombocytopenias, and is characterised by the presence of giant platelets and decreased platelet count with variable bleeding manifestations. Bengal macrothrombocytopenia is a newly described entity, previously called asymptomatic constitutional macrothrombocytopenia (ACMT), presented with variable bleeding tendencies; with mild to severe thrombocytopenia and macro-platelets in their peripheral blood smear and it is not totally an innocuous condition as described previously.

1. Introduction

Abnormal giant platelets are sometimes seen as an incidental finding in routine blood examinations, many of them are associated with acquired disorder such as idiopathic thrombocytopenic purpura (ITP) and myelodysplasia. In contrast inherited macrothrombocytopenia comprises a heterogeneous group of rare disorders, characterised by abnormal giant platelets, thrombocytopenia and bleeding tendency with variable severity; from no or mild bleeding tendency to severe bleeding diathesis and sometimes associated with syndromic features like renal failure, hearing loss and presenile cataracts. Many of these disorders share common clinical and laboratory features, making accurate diagnosis difficult and patients are often misdiagnosed and treated for idiopathic thrombocytopenic purpura (ITP). Bleeding syndromes that arise through an inherited defect of platelet production and giant platelets constitute a heterogeneous group of platelet disorders [1,2], some including the Bernard-Soulier syndrome (BSS) [3], MYH9 related macrothrombocytopenia (MYH9-RD) [4,5] and Mediterranean macrothrombocytopenias (MM) [6], sitosteroleamia/phytosteroleamia [7,8], the disease is caused by a mutation in either of the ABCG5 or ABCG8 genes which encode an ATP-binding cassette protein called Sterolin [9,10], a rare X linked GATA-1 associated macrothrombocytopenia has been associated with dyserythropoiesis and thalassemia [11] and Harris platelet syndrome (HPS) in healthy donors from West Bengal [12].

Inherited macrothrombocytopenias were previously considered to be relatively rare, but their prevalence is likely under estimated from complexities of diagnosis and spectrum of subclinical phenotype and limited knowledge is available about them, recent progress in the elucidation of the responsible genes for several inherited macrothrombocytopenias led to advances in understanding the pathophysiology and pathogenesis of the disease, however, in approximately half of the cases with inherited macrothrombocytopenia, the molecular cause remain unknown [13].

One of the first inherited giant platelet disorder associated with abnormal red cell morphology was found in Mediterranean Macrothrombocytopenia (MM) reported in healthy Mediterranean immigrants to Australia, which was associated with stomatocytosis, macrothrombocytopenia and splenomegaly. Harris platelet syndrome (HPS) is the most common giant platelet disorder to be described [14] so far in healthy blood donors from the north-eastern part of the Indian subcontinent (Bhutan, Nepal and Bangladesh) and is characterised by mild to severe thrombocytopenia, with giant platelets, absent bleeding symptoms and normal platelet aggregation studies.

2. Materials and methods

One hundred and twelve unrelated cases of macrothrombocytopenia were taken up for the study, who were referred to the Department of Haemostasis, National institute of Immunohaematology, Mumbai and Department of Haematology, NRS Medical College from 2009–2014, a few family members were also included. Diagnosis was confirmed on

* Corresponding author.
E-mail address: alishahna@gmail.com (S. Ali).
[1] Surat Raktadan Kendra & Research centre, 1st Floor Khatodara Health Centre, Chosath Joganio Mata Mandir, Udhna Magdalla Road, Surat-395002, Gujrat, India.

http://dx.doi.org/10.1016/j.bcmd.2016.05.011
1079-9796/

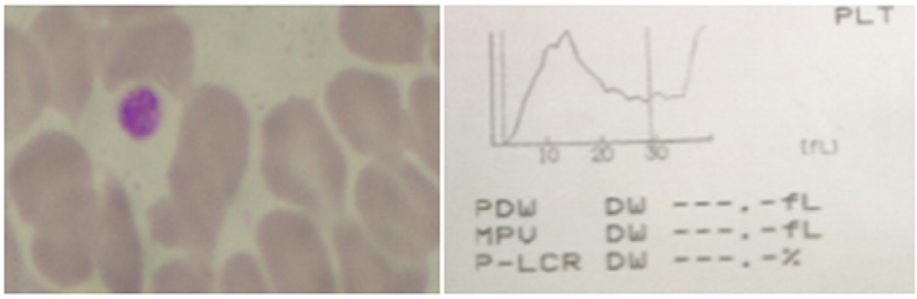

Fig. 1. Peripheral Blood Smear and platelet histogram showing the presence of giant platelets.

the basis of reduced platelet count ($<150 \times 10^9$/L) and mean platelet volume (MPV) which was greater than 10fl. Final diagnosis was confirmed by the presence of giant platelets in their peripheral blood smear evaluated by light microscopy (Fig. 1). All cases had normal anti-platelet antibody and ferritin levels [15]. The cases were grouped into three categories based on degree of thrombocytopenia i.e. mild ($100–150 \times 10^9$/L), moderate ($50–100 \times 10^9$/L) and severe ($<50 \times 10^9$/L). A detailed clinical history was taken for each case including the age of onset, marriage type, family history and type of bleeding, drug history etc. Pregnant females were excluded from the study.

Based on WHO bleeding scale, the severity of bleeding symptoms were segregated into grade 0 (no bleeding), grade 1 (petechiae), grade 2 (mild blood loss), grade 3 (gross blood loss) and grade 4 (debilitating blood loss). The study was approved by the ethics committee and undertaken in accordance with the ethical guidance of the institution involved. Written consent was obtained from all the cases before the collection of blood sample. Complete blood counts (CBC) were determined on blood samples collected in EDTA tubes using XT-2000i cell counter (Transasia Bio-Medical Ltd., Mumbai, India) within 30–120 min of collection. Repeated complete blood counts were performed on each case for at least four times in different times to see the reproducibility of platelet count and MPV. Peripheral blood smears were stained with modified Leishman's stain and evaluated by light microscopy.

2.1. Statistical analysis

Data were analysed using Prism 5 analyser (graphpad.com/scientific-software/prism/) using Spearman's rank correlation method. *P* values <0.05 were considered significant.

3. Results

All the cases had low platelet count (median, range) 89 (27–147 $\times 10^9$/L) and with high MPV 13.25 (12–18.5 fl), and showed the presence giant platelets without any inclusion bodies in their peripheral blood smear. The cases had low platelet biomass 0.84 (0.27–1.3), low haemoglobin 13.1 (10.1–16) and higher platelet distribution width 17.5 (14.1–18.9) as compared to controls (Table 1). There was no significant difference in MCV, MCHC, MCH and WBC between the cases and controls (Table 1).

The study included 55 females (Range: 8–70 years, Median age: 25 years) and 57 male (Range: 8–70 years, Median age: 30 years).

The distribution of cases were across India, the maximum numbers of cases were from West Bengal followed by other states, Maharashtra, Uttar Pradesh, Bihar, Orissa, Assam, Meghalaya, Kerala and one case was from neighbouring area Nepal (Fig. 2).

Severity of thrombocytopenia was varied among the cases; 45 had mild, 54 had moderate and 12 had severe thrombocytopenia, and only one had normal platelet count but was presented with giant platelets in peripheral blood smear.

According WHO bleeding scale 68 had grade 0 (Mild-30, Moderate-33, Severe-5); 6 had grade 1 (mild-2 and moderate 4); 18 had grade 2 (mild-10 and moderate-8); 16 had grade 3 (mild-2, moderate-9 and severe-5) and only 4 had grade 4 (moderate-1 and severe-3) bleeding score.

Majority of cases (61%) were asymptomatic and the major clinical manifestations were; 4% easy bruisability, 7% ecchymosis, 16% epistaxis, 5% frequent gum bleed, 10% menorrhagia and 6% had history of prolonged bleeding time after trauma. 12 had family history bleeding and 3 had transfusion history in the past. The laboratory findings which include complete blood count, screening coagulation, platelet aggregation and receptor study of 112 cases are included in Table 1.

4. Discussion

Platelet related bleeding disorders are either inherited or acquired, giving rise to bleeding manifestations of varying severity. Taking medical/drug history is the first step of diagnosis of any bleeding disorder and the best screening method for any platelet related disorder. Family history and age of onset of bleeding play a major role in differentiating inherited from acquired platelet function disorders, consanguineous partnerships increases the likelihood of a recessive platelet disorder. Screening coagulation tests; Prothrombin time (PT), activated partial

Table 1
Laboratory findings in cases with Bengal macrothrombocytopenia (BM).

Red blood cell indices median (range)		Platelet indices median (range)		Screening coagulation median (range)		Platelet aggregometry (%) median (range)		Receptor study (%) median (range)	
Hb gm/dL	A:13.1 (10.1–16) B: 14.1 (13–18.1)	Platelet Count ($\times 10^9$/L)	A: 89 (27–147) B: 186 (177–304)	PT (s)	A: 12.5 (11–13.9) B: 12.9 (12–14)	ADP (6 μM)	A: 63 (17–78) B: 89 (50–98)	GPIb (CD42b)	A: 75.3 (42.9–98.6) B: 90 (50–98)
MCV	A: 87.3 (64.4–95.6) B: 85.6 (65.1–94.8)	MPV (fl)	A: 13.25 (12–18.5) B: 10 (7.9–10.4)	APTT (s)	A: 28.6 (25–32) B: 29 (28–33)	Risto (1.25 mg/ml)	A: 75.5 (15–99) B: 80 (50–100)	GPIIb/IIIa (CD41a)	A: 80.5 (34.6–98.8) B: 89 (50–99)
MCH	A: 29.2 (19.6–32.0) B: 28.9 (20–31.8)	Platelet Biomass (%)	A: 0.84 (0.27–1.3) B: 0.98 (0.52–1.6)	TT (s)	A: 15.1 (12.9–18) B: 15.1 (15–19)	AA (0.75 mM)	A: 81.5 (10–98) B: 89 (50–100)	GPIX (CD42a)	A: 79.3 (27.3–98) B: 91 (50–98.2)
MCHC	A: 33.3 (30.1–35.4) B: 31.2 (31–34.8)	PDW (fl)	A: 17.5 (14.1–18.9) B: 16.5 (11.6–19.8)			Coll (4 μg/ml)	A:64 (47–90) B: 90 (50–99)		
WBC	A: 6.1 (3.4–9.8) B: 6.4 (3.5–9.5)								

A: Cases; B: Controls; PT: Prothrombin Time, APTT: Activated Prothrombin Time, TT: Thrombin Time, ADP: Adenosine diphosphate, Risto: Ristocetin, AA: Arachidonic Acid, Coll: Collagen.

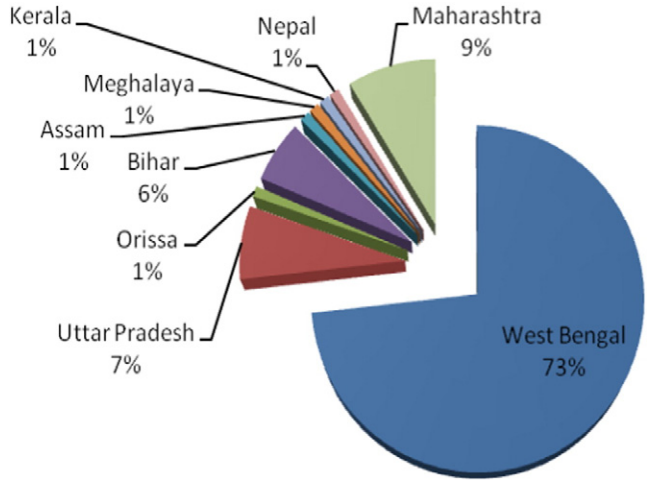

Fig. 2. Geographical distribution of Bengal macrothrombocytopenia cases.

thromboplastin time (APTT) and thrombin time (TT) was normal ruling out factor related deficiency. Platelet aggregation study with different agonists and receptor study of GPIb/IX/V and GPIb/IIIa was normal which helped in differential diagnosis with Bernard Soulier Syndrome (BSS), Glanzmann Trombasthenia (GT) and von Willibrand Disease (vWD) (Table 1).

The clinical presentations in inherited platelet disorders vary considerably and range from no symptoms to a severe bleeding tendency. Macrothrombocytopenia is of one such platelet related bleeding disorder which was previously considered rare but with the introduction of automated cell counters in recent time, they are being increasingly recognised during routine examination of complete blood counts and Bengal macrothrombocytopenia is one of the most common forms of macrothrombocytopenia which is an unrecognised and under diagnosed condition in India [16].

Measurement of platelets using automated cell counters is usually precise and accurate if it is not too low count [17] and utmost care has been taken to avoid time and temperature dependent fall of the platelet count and analysis of platelet aggregates and clumps by microscopic analysis and the reproducibility of platelet counts was authenticated by taking repeated CBC of each case in different times. Other bleeding disorders were excluded from the study depending on their clinical history/drug history and screening coagulation profile, platelet function and receptor study.

In total of 112 cases, 73% of them were from the state of West Bengal showing it prevalence in the Eastern region of India, the result was consistent with the published reports [12], and rest of the cases were from other states of India (Fig. 2) and neighbouring country Nepal suggesting that the disorder is not locus specific and has spread to other places due

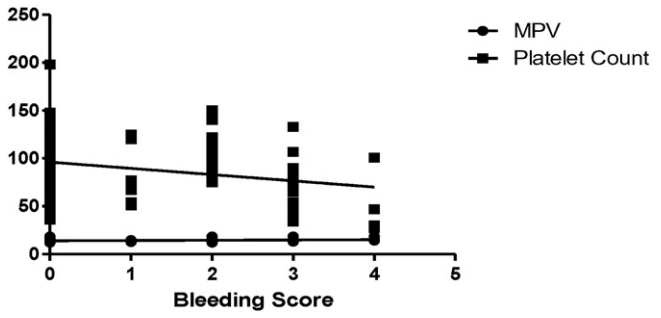

Fig. 3. Who bleeding score in relation to platelet count and mean platelet volume in Bengal macrothrombocytopenia cases.

global migration. It affects male and females equally and mostly diagnosed at the adult or later stage of their life and mostly diagnosed during health checkup for visa verification/pre-employment or when posted for surgery. Asymptomatic cases were mostly diagnosed during routine blood examination. Most of cases suffered mild or moderate thrombocytopenia and few had severe thrombocytopenia, and all were presented with giant platelets. The WHO bleeding score showed an inverse significant correlation with platelet count, direct significant correlation with MPV and severity of the bleeding manifestations ($p < 0.05$) (Fig. 3). There was no difference of bleeding scores in males and females when compared with platelet count and mean platelet volume (MPV).

Out of 112, 12% (13) of them had severe thrombocytopenia, 48% (54) had moderate thrombocytopenia and 40% (45) had mild thrombocytopenia and one subject (1%) had normal platelet count with large platelets in the blood smear without any inclusion bodies. Majority of the cases had mild or moderate platelet count with large platelets in their peripheral blood smear (MPV > 12 fl). The cases had low platelet biomass and haemoglobin and higher platelet distribution width; other parameters were normal as compared to controls (Table 1) which is consistent with earlier study [18].

61% (68) of the cases were asymptomatic without any bleeding manifestations; 11% had a family history of minor bleeding, most of the asymptomatic cases were detected during routine analysis of blood samples.

Predominant clinical manifestations when present were easy bruisability, ecchymosis, epistaxis, frequent gum bleed, menorrhagia and prolonged history of bleeding after trauma. Three of them were transfused at least once in their life time and 12 had family history of bleeding in the past. Pedigree analysis in one of the families showed dominant mode of inheritance [19]. Therefore Bengal macrothrombocytopenia is mostly represented with asymptomatic cases with low platelet count and high MPV but it is not totally an innocuous condition as they are also presented with other bleeding manifestation which varies in severity.

Declaration of interest statement

None.

Acknowledgements

We thank Department of Biotechnology, Govt. of India (BT/PR7125/MED/14/956/2006) for their financial support.

References

[1] J.G. Drachman, Inherited thrombocytopenia: when a low platelet count does not mean ITP, Blood 103 (2004) 390–398.
[2] A.E. Geddis, K. Kaushanky, Inherited thrombocytopenias: towards a molecular understanding of disorders of platelet production, Curr. Opin. Pediatr. 16 (2004) 15–22.
[3] J. Bernard, J.P. Soulier, Sur une nouvelle variete de dystrophie thrombocytaire-hemorragipare congenitale, Sem. Hôpitaux Paris 24 (1948) 3217–3223.
[4] B. Rocca, F.O. Ranelletti, N. Maggiano, et al., Inherited macrothrombocytopenia with distinctive platelet ultra-structure and functional features, Thromb. Haemost. 83 (2000) 35–41.
[5] M. Seri, A. Pecci, F. Di Bari, et al., MYH9 related diseases: May-Heggling anomaly, Sebastian syndrome, Fechtner syndrome and Epstein syndrome are not distinct entities but represent a variable expression of a single illness, Medicine (Baltimore) 82 (2003) 203–215.
[6] W.E. Behrens, Mediterranean macrothrombocytopenia, Blood 46 (1975) 199–208.
[7] A.K. Bhattacharya, W.E. Connor, Beta-sitosteroleamia and xanthomatosis. A newly described lipid storage disease in two sisters, J. Clin. Investig. 53 (1974) 1033–1043.
[8] D.C. Rees, M. Iolascon, S. Carella, et al., Stomatocytic haemolysis and macrothrombocytopenia Mediterranean stomatocytosis/ macrothrombocytopenia) is the haematological presentation of phytosteroleamia, Br. J. Haematol. 130 (2005) 297–309.
[9] S.B. Patel, G. Salen, H. Hidaka, et al., The sitosteroleamia locus is found at chromosome 2p21, J. Clin. Investig. 102 (1998) 1041–1044.

[10] K.E. Berge, H. Tian, G.A. Graf, L. Yu, et al., Accumulation of dietary cholesterol in sitosteroleamia caused by mutations in ABC transporters, Science 290 (2000) (1771–1775).
[11] K.E. Nichols, J.D. Crispino, M. Poncz, et al., Familial dyserythropoietic anaemia and Thrombo-cytopenia due to an inherited mutation in GATA-1, Nat. Genet. 24 (2000) 266–270.
[12] H.V. Naina, S.C. Nair, D. Danial, et al., Asymptomatic constitutional macrothrombocytopenia among West Bengal donors, Am. J. Med. 112 (2002) 742–743.
[13] R. Bottega, C. Marconi, M. Faleschini, et al., ACTN1-related thrombocytopenia: identification of novel families for phenotypic characterization, Blood 125 (5) (2015) 869–872.
[14] H.V. Naina, S.C. Nair, S. Harris, et al., Harris syndrome - a geographic perspective, J. Thromb. Haemost. 3 (2005) 2581–2582.
[15] Y.S. Mehta, K. Ghosh, S.S. Badakere, A.V. Pathare, D. Mohanty, Role of antiidiotypic antibodies on the clinical course of idiopathic thrombocytopenic purpura, J. Lab. Clin. Med. 142 (2) (2003) 113–120.
[16] N. Kakkar, M.J. John, A. Mathew, Macrothrombocytopenia in North India: role of automated platelet data in the detection of an under diagnosed entity, Indian J. Hematol. Blood Transfus. 31 (1) (2015) 61–67.
[17] M. Zandecki, F. Genevieve, J. Gerard, A. Godon, Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes, Int. J. Lab. Hematol. 29 (1) (2007) 21–41.
[18] H.V. Naina, S. Harris, Platelet and red blood cell indices in Harris platelet syndrome, Platelets 21 (4) (2010) 303–306.
[19] S. Ali, S. Shetty, K. Ghosh, A novel mutation in GP1BA gene leads to mono-allelic Bernard Soulier syndrome form of macrothrombocytopenia, Blood Coagul. Fibrinolysis 4 (2016 Feb) (Epub ahead of print).