Inherited Macrothrombocytopenia: Correlating Morphology, Epidemiology, Molecular Pathology and Clinical Features

Kanjaksha Ghosh¹ · Maitreyee Bhattacharya² · Ranjini Chowdhury² ·
Kanchan Mishra¹ · Malay Ghosh³

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Abstract Inherited macrothrombocytopenia is increasingly being recognized as a relatively common condition. This descriptive review aims at focusing on the different areas of advancement that have taken place with this condition with particular reference to India. A pubmed search of articles between January 1990 and October 2017 with the key words-macrothrombocytopenia, asymptomatic macrothrombocytopenia, macrothrombocytopenia India, syndromic macrothrombocytopenia, molecular pathology, megakaryopoiesis and platelet formation were searched. The shortlisted articles were then read. Review articles provided additional references and the articles thus obtained were also read. Special interest and research conducted by the authors provided further sources of information. A total of 487 articles were found of which 68 articles were related to our subject of review. Review articles were read and additional articles from the reference quoted. Forty-four percent of nonsyndromic Inherited macrothrombocytopenia showed mutations of MYH9, GP1BB, GP1Ba, GPIX, ABCG5 and 8, ACTN, FLI, TUBB and RUNX1 frequently in heterozygous state. All types of inheritance pattern namely autosomal dominant, recessive and sex linked patterns have been described. Syndromic causes of this phenomenon are well known and have been described. Many asymptomatic patients do have mild or

moderate bleeding history. Clinical algorithms to differentiate chronic ITP associated macrothrombocytopenia from inherited variety have been explored. Inherited macrothrombocytopenia is an emerging area of interest in platelet biology with its implication in diagnosis, prognosis, genetic counseling, management and in transfusion medicine.

Keywords Inherited macrothrombocytopenia · Chronic ITP · Syndromic macrothrombocytopenia · Nonsyndromic macrothrombocytopenia · Genetic pathology · Diagnostic algorithm · Bleeding complications · Review

Introduction

Primary hemostasis of the body is largely governed by the small sized platelets, derived from megakaryocytes. The phenomenon of reduced platelet count than normal (i.e. $< 150,000/\mu\text{L}$) with a significant increase in platelet size ($> 12 \text{ fl}$) [2] is termed as Macrothrombocytopenia [1, 2]. This clinical phenomenon can be either acquired or inherited. Reports show that the cases of inherited macrothrombocytopenia (IMTP) are comparatively less than that of the acquired pattern of the disease. Clinical spectrum of IMTP exhibits remarkable heterogeneity with respect to its varying mutations and clinical manifestations [1–4]. Clear diagnosis of this clinical condition is a major challenge as chances leading to inaccurate diagnosis as immune thrombocytopenia are common [3].

IMTPs is considered a rare clinical condition however identification of several new forms has shown an increasing frequency of this disorder affecting at least 2.7 per 100,000 individuals [4, 5], with mild to absent bleeding phenotype.

✉ Kanjaksha Ghosh
kanjakshaghosh@hotmail.com

¹ Surat Raktadan Kendra and Research Centre, Udhna Magdalla Road, Near Chosath Jogania Mataji Temple, Surat 395002, India

² Institute of Haematology and Transfusion Medicine, Medical College, Kolkata, 12 College Street, Kolkata 700078, India

³ P-78 Green View, Garia-P.O., Kolkata 700084, India

Certain populations in the world and in India may be affected by a high frequency of this condition.

Materials and Methods

A pubmed search of articles with key words e.g. macrothrombocytopenia, asymptomatic macrothrombocytopenia, macrothrombocytopenia India, syndromic macrothrombocytopenia, Molecular pathology megakaryopoiesis, platelet formation was done. Articles between January, 1990 and October, 2017, both years inclusive were searched. Review articles provided additional references. Special interest and research conducted by the authors provided additional sources of information. Each of the authors read those articles and shortlisted the articles of interest related to the field. Indian references were given preference as one of the interests in this review was with Indian data. Recent reviews in high impact journals were given extra weightage as these articles not only provided additional references but also provided insight into the current thought in this rapidly emerging field. Out of a total of 487 articles 68 articles were shortlisted and were read by all the authors. The authors had regular meetings to develop the final outline plan and composition of the review.

Megakaryopoiesis and Platelet Formation

Platelets are liberated by mature megakaryocytes (MKs) and circulate in blood with an average lifespan of 7–10 days. The hematopoietic growth factor, thrombopoietin (TPO), is the primary regulator of MK growth and platelet numbers. Thrombopoiesis can be illustrated in discrete steps along a continuum where the uncommitted haemopoietic stem cells become specialized to platelet-producing MKs directed by the presence of several megakaryocytic transcription factors namely *GATA-1*, *FOG-1*, *FLI-1*, *RUNX1*, *ETV6*, *HOXA11*, *MECOM* and *GFI1* [6–12].

Secondly, the committed megakaryoblast turns into a progenitor cell, capable of executing a finite number of cell divisions. Under proper conditions, megakaryocyte burst-forming units (BFU-MKs) and megakaryocyte colony-forming units (CFU-MKs) are formed. The ultimate stage of thrombopoiesis includes a massive rearrangement of the megakaryocyte cytoskeleton, in which long, branching cytoplasmic processes are extruded from the cell body, followed by its fragmentation into platelets at their termini. These “proplatelet processes” exhibit significant motility and trafficking of the granules and proteins characterizing the mature platelet [13].

$\beta 1$ tubulin influences the structure of the proplatelet and several spirals of tubulin is involved in the formation of mature platelet cytoskeleton. Actin dynamics also have an influential role in this process, as the highly structured maturing megakaryocyte submembrane actin skeleton is disrupted that permits the growing tubulin structures to “push” the cytoplasmic membrane into the highly elongated proplatelet process [14].

Several IPD (Inherited platelet disorders) genes—*MYH9*, *ACTN1*, *FLNA*, *TUBB1* and *DIAPH1* are associated with the regulation of megakaryocyte cytoskeleton, and the principal effect of deleterious variants of macrothrombocytopenia is due to aberrant proplatelet formation [15–19].

The differentiation of MKs and the subsequent shedding of platelets are regulated by a large number of genes, that includes several transcription factors, cytokines, cell surface receptors, signaling molecules, cytoskeletal proteins, molecular motors, and cell cycle regulators. The diminished production or shortened lifespan of platelets may be a consequence of mutations present in any of the genes. (Figure 1 depicts the regulation of megakaryopoiesis).

Molecular Mechanisms; Major Genes Involved

Despite the identification of a substantial list of mutations associated with congenital (inherited) thrombocytopenias till date, it is likely that many additional rare conditions are yet to be deciphered. The major IMTP causing mutations have been reported in more than 12 genetic mutations. The genetic mutations of this clinical disorder can further be grouped according to their inheritance pattern viz autosomal dominant, autosomal recessive and X-linked.

Autosomal Dominant Thrombocytopenias

Among these, the most frequently encountered IMTPs, autosomal-dominant *MYH9* disorders or *MYH9*-related disease (*MYH9RD*). *MYH9 related thrombocytopenias* involve mutations in Myosin heavy chain 9 gene leading to premature release of platelets from the bone marrow, manifested by macrothrombocytopenia and cytoplasmic inclusion bodies within leukocytes.

The history of *MYH9* related thrombocytopenias dates back to 1909 when May described a family in which several members had enlarged platelets but minor if any bleeding symptoms. In 1945, Hegglin found Dohle body like inclusions within the leukocytes of affected individuals with a dominantly-inherited giant platelet disorder. This led to the introduction of the term May-Hegglin anomaly (MHA) that describes the triad of thrombocytopenia, giant platelets, and leukocyte inclusion bodies [10]. The syndrome occurs due to a deletion in region 11 of the long arm of chromosome 22 giving it an autosomal dominant pattern

Fig. 1 The regulation of megakaryopoiesis. Formation of megakaryocytes by the hematopoietic stem cells (HSC) is tightly regulated by both cytokines and chemokines (in the left) as well as transcription factors (in the right). [Pang and Poncz]

across generations. The syndromes MHA, Epstein syndrome (EPS), Fechtner Syndrome (FS), and Sebastian platelet syndrome (SPS) are recognized to be related disorders, associated with the mutations in the MYH9 gene [20].

Di George Syndrome

This syndrome occurs due to a deletion in region 11 of the long arm of chromosome 22 giving it an autosomal dominant pattern across generations. The disorder is manifested by decreased platelet count, increased MPV, and reduced expression of platelet surface *GP1b/IX/V* crucial for platelet adhesion and important for thrombin aggregation and activation. It occurs because the vast majority (> 90%) of patients with 22q11DS are heterozygous for a deletion of the *GP1b* gene [21, 22] and so are heterozygotes for Bernard–Soulier syndrome (BSS), a rare autosomal recessive bleeding disorder [23].

IMTP arising from *GFI1B* mutations is inherited in an autosomal dominant fashion. *GFI1B* regulates MEPs as a transcriptional repressor in the process of the growth and differentiation from HSCs, and critically affects the terminal maturation of megakaryocytes [24].

Autosomal recessive thrombocytopenias include Bernard Soulier syndrome, Mediterranean macrothrombocytopenia and Gray platelet syndrome (GPS).

Bernard Soulier syndrome (BSS)

More than 50 years ago Bernard and Soulier recognized an inherited bleeding disorder characterized by lifelong macrothrombocytopenia associated with marked platelet dysfunction [25]. The decreased/absent expression of the von Willebrand factor (VWF) receptor on platelets results in the BSS syndrome. The VWF receptor consists of 4 distinct polypeptide chains: *GP1b-α*, *GP1b-β*, *GPV*, and *GP1X*. The proposed molecular background behind this

giant platelet syndrome suggests the presence of defective linkage between *GP1b/IX/V* and cytoskeleton [26, 27].

Mediterranean macrothrombocytopenia, also known as autosomal dominant benign BSS [28] has been described among Mediterranean population such as Greeks and Italians but is absent in the north of Europe. Heterozygous mutations in *GP1BA* or *GP1BB* genes are common in individuals with Mediterranean macrothrombocytopenia, emphasizing that the genotypic and phenotypic expression of Mediterranean macrothrombocytopenia and BSS are similar. The most commonly occurring genotypic abnormality involves *GP1BA* missense mutation that leads to an amino acid substitution (Val156Ala), known as the Bolzano variant. BSS has been extensively studied in India and it has been reported that a larger fraction of people in Kerala are affected by this syndrome. Cases of BSS continue to be reported from different parts of India and molecular pathology of BSS has also been studied from this country recently [28].

ABCG5 & *8* are genes coding proteins which apparently ejects unwanted sterols from the body, including both cholesterol itself and the plant-derived sterol molecules such as sitosterol. The condition is associated with stomatocytosis and the entity is designated as Mediterranean stomatocytosis [29] or Mediterranean macrothrombocytopenia [30]. Recently one such patient with analysis of the family members has been reported from this country. The patient presented with stomatocytosis with macrothrombocytopenia [31].

Gray Platelet Syndrome

The basic defect in gray platelet syndrome (GPS) is thought to be the inability of the megakaryocytes to pack endogeneously synthesized secretory proteins into developing α -granules [32, 33]. Most GPS patients have macrothrombocytopenia. The reduced lifespan of α -granule-deficient platelets and the resulting abnormal

maturation of the megakaryocytes are thought to be an active contributor to the thrombocytopenia of GPS [34].

Myelofibrosis and splenomegaly occur in GPS [35]. Most GPS families are found to have cases of single or multiple affected siblings that are born to unaffected, often consanguineous parents, demonstrating the autosomal recessive (AR) inheritance pattern of the disorder. Rare families with autosomal dominant (AD) [36] and X-linked variants of GPS have been reported. The genetic cause behind the AR type of GPS is yet to be understood.

HZF was suggested as a candidate gene because of homology of the *Hzf*-null mouse model to GPS; however, there were no identification of mutations in the human *HZF* gene in 5 patients from 3 GPS families [37].

GATA1 mutation accounts for the cause of X-linked GPS-like disease resulting in generalized hypogranularity of platelets and erythroid abnormalities [38]. The localization of the GPS gene to a 9.4-Mb interval on 3p21.1–3p22.1 was confirmed using genome-wide linkage analysis and homozygosity mapping, which includes the presence of 197 protein-coding genes and excluded 1423 (69%) of the 2075 exons in the interval. Mutation in NBEAL2 gene has recently been identified in AD variety of GPS [39].

The maintenance of normal platelet morphology is governed by the linkage between the membrane skeleton and cytoskeletal actin filaments as well as the marginal microtubule band. Defects in these systems may lead to macrothrombocytopenia. TUBB1 (tubulin beta 1 chain) mutation has been found to be associated with macrothrombocytopenia. A novel conserved p.F260S (c.779T > C) mutation located at the α - and β -tubulin intradimer interface was detected in macrothrombocytopenia. [17, 40] p.D249N and p.R318W, at or near the interface, has also been reported to cause macrothrombocytopenia [17].

X-linked macrothrombocytopenias include *GATA1* mutation and ACTN1 mutations.

The *GATA1* gene is a critical transcription factor responsible both MK and erythroid development [41]. The mutations are clustered in the highly conserved N-zinc finger domain, introducing single amino acid substitutions at the positions: Val205Met, Gly208Ser, Arg216Gly, Asp218Gly, and Asp218Tyr. In all these mutations, the amino acid substitution interferes with the association of GATA-1 with its transcriptional cofactor, FOG-1 (friend of GATA-1). The severity of the thrombocytopenia correlates with the degree to which the GATA-1/FOG-1 interacts [42]. Mutations of the *FOG1* gene might exhibit similar phenotype expression, which needs further investigation. Defective GATA-1 causes reduced transcription and protein expression of its target genes, including GPIIb, GPIIb, GPIIX, and GPV. The concomitant reduction in the

expression of GPIIb/IX/V and other platelet specific gene products is related to the macrothrombocytopenia and bleeding tendency in this disorder [6].

ACTN1 Mutations

α -actinin is a member of the actin crosslinking protein superfamily (4 isoforms: ACTN1, ACTN2, ACTN3 and ACTN4) that participates in the organization of the cytoskeleton. Platelets and megakaryocytes mainly express ACTN1 isoform of the α -actinin superfamily. Individuals with mutant ACTN1 alleles have approximately half the normal platelet counts and show an increase of 30% in platelet size [15].

Paris Trousseau Syndrome

Paris Trousseau/Jacobsen syndrome patients have deletion of FII1, a key transcription factor important in megakaryopoiesis on chromosome 11q24.3 [43]. Characteristic platelet abnormalities include giant platelets with large alpha granules visible on electron microscopy [44].

Other less frequent mutations have been reported in TPM4 [45], *PRKACG* [46] *FLNA* [16]. However, this array of mutations only accounts for approximately half the IMTP cases; in the remaining half, IMTP-causing mutations are unknown, which prevents proper diagnosis and treatment.

Clinical Characteristics

Majority of the patients are either asymptomatic or have minor bleeding manifestations. In many patients it is a chance detection. In some patients CMTP is associated with other phenotypic abnormalities which help in its early diagnosis. Associated phenotypic abnormalities are described extensively in Table 1 [16, 17, 47–61].

Diagnostic Approach

The presence of syndromic features can help in easy recognition of this condition, but the diagnosis remains challenging for majority of IMTPs, which often have a mild platelet type bleeding phenotype and are clinically heterogeneous. Establishment of a conclusive molecular diagnosis approach will help to predict the course of disease and in genetic counseling as well. A tiered approach is preferable.

The investigation should start with a detailed bleeding history, family history, past history of immune thrombocytopenia, drug history, nutritional history to suspect

Table 1 Phenotypic abnormalities associated with CMTP

Disease	Inheritance	Associated features
MYH9-related disease	AD	Risk of sensorineural deafness, nephropathy, cataract. Elevated liver enzymes in some cases. Döhle-like inclusions in granulocytes [47]
Bernard–Soulier syndrome (BSS)	AD/AR	None [48, 49]
Gray platelet syndrome	AR	Platelet count decreases over time. Development of progressive bone marrow fibrosis and splenomegaly. Elevated serum vitamin B12 levels [50]
ACTN1-related thrombocytopenia	AD	The macrothrombocytopenia was accompanied by anisocytosis. Flow cytometry showed increased expression of platelet GPIb/IX and GPIIb/IIIa [51]
TUBB1-related thrombocytopenia		None [17]
Digeorge syndrome	AD	Neuropsychiatry problem, cardiac anomalies, hypocalcemia, and immune defects [52]
GATA1-related diseases	XL	Hemolytic anemia with laboratory abnormalities resembling beta-thalassemia, splenomegaly, dyserythropoietic anemia. May present with congenital erythropoietic porphyria [53]
GF11B mutation	AD	Decreased number of a-granules on the giant platelets [54]
Mediterranean macrothrombocytopenia	AR	Stomatocytosis, haemolytic anemia, phytosterolemia [55]
Paris Trousseau type thrombocytopenia	AD	Cardiac and facial anomalies, mental retardation, large platelet granules [56]

vitamin B12/folate deficiency and detailed clinical examination of the patient. This should be followed by:

1. Complete hemogram including MPV (normal 7–10 fl) [62] and PDW. Mean MPV in Inherited Macrothrombocytopenia (IMTP) is 12.5 fl but it may be as high as 20 fl.
2. Many modern haematology counters have now additional capability in addition to give more accurate platelet count (impedance counters underestimate platelet counts in macrothrombocytopenia and exaggerates its prevalence), MPV and immature platelet fraction. As immature platelets are often bigger in size and they are produced in various pathologic states. This parameter along with platelet histogram and platelet scatter plot could be important in discriminating secondary macrothrombocytopenia from inherited one.
3. Platelet assessment and peripheral smear examination in parents and first degree relatives to establish the inherited nature of the condition.
4. Peripheral smear examination to confirm both thrombocytopenia and presence of macrothrombocytes. On the average 20% platelets are found to have a diameter larger than 4 μm and 12% more than 8 μm (average diameter of normal platelet is 1.5–3 μm) [63]. Abnormalities detected at *PBS* examination that raise suspicion of inherited forms of macrothrombocytopenia are listed in Table 2.
5. Platelet function study (Fig. 2 shows the diagrammatic algorithm of the study).
6. Immunofluorescence using antibody against non-muscle myosin heavy chain detects a spotty abnormal distribution of these molecules in all pts with MYH9 related diseases [63].
7. Flow cytometry to detect lack of GPIb IX in BSS [64].
8. SDS PAGE This method is used to diagnose BSS, GPS, X linked thrombocytopenias [65, 66]. (Not done now as simpler tests are available).
9. Electron microscopy—to see ultra structure of platelets, to detect lack of alpha granules, inclusions in MYH9 disorders (microfilaments and ribosomes in an amorphous area of neutrophil, cytoplasm devoid of specific granules) [67, 68].
10. DNA analysis and Targetted sequencing including NGS (Next Generation Sequencing).

Finally, due to the presence of potential pitfalls during the interpretation of the role of novel rare variants, it is important to apply rigorous standards when assigning pathogenicity. Providing a molecular diagnosis to patients is highly recommended, but making false assumptions about variants could be harmful.

Management

No definite guideline is available for management of inherited asymptomatic or mildly symptomatic macrothrombocytopenia. However in patients with bleeding manifestations following approach can be taken.

Table 2 Peripheral smear correlated with associated molecular pathology in macrothrombocytopenia

Cell type	Disease
Platelets	
Giant platelets	MYH9-RD, Biallelic BSS, GPS, FLNA-related, TUBB1-related
Large platelets	Monoallelic BSS, Paris-Trousseau (P-T)/Jacobson [57] syndr, GATA1-RD, ITGA2B/ITGB3-related
Pale platelets' (α -granule def)	GPS [58]
Vacuolated platelets	GATA1-related disease [53]
Erythrocytes	
Anisocytosis	GATA1-related disease [59]
Stomatocytosis	Mediterranean macrothrombocytopenia [60]
Neutrophils	
Dohle-like bodies	MYH9-RD [61]

Fig. 2 Diagrammatic algorithm of the study

Platelet Transfusion

Platelet transfusion may be required to check significant bleeding. To overcome the risk of alloimmunization, performing HLA typing in patients with these severe disorders and transfusing them with HLA-matched platelets, if available is of significant importance.

Leukocyte-depleted transfusions should always be preferred. Platelets should only be considered for life threatening bleeds. Most of the asymptomatic macrothrombocytopenia being asymptomatic does not require any intervention except counseling. Patients who are likely to get platelet or blood product transfusion must

be vaccinated against hepatitis B infection if they have not already received it or are already infected with the virus.

Antifibrinolytic Therapy

Use of aminocaproic acid or tranexamic acid in the setting of mucocutaneous bleeding, menorrhagia, or gastrointestinal bleeding may be beneficial [69].

rFVIIa

For use in patients with bleeding refractory to conventional therapy, Recombinant activated factor VII (rFVIIa) has

been used. Several reports have been published examining the utility of this in platelet related bleeding disorders [70].

TPO-Receptor Agonists

These have shown some efficacy in elevating the platelet count in some disorders, including MYH9-related diseases [71].

Indian Experience

Naina et al. [72] screened a total of 203 blood donors to analyze the platelet and red blood cell indices in blood donors with MT. Among these, 101 blood donors were from northeastern part of India and the rest of the 102 blood donors hailed from southern part of India.

A significant difference was observed between platelet count (Mean $\pm$ SD) $136 \pm 40 \times 10^9/L$ versus $262 \pm 53 \times 10^9/L$ in southern India ($p < 0.000$). Thirty-three donors with MT had a normal platelet count with MPV more than 12 fl. MPV was 13.6 ± 0.13 fl (range 12–21.9 fl) in donors with MT and 7.3 ± 0.6 fl (range 6–9.2 fl) in southern Indian blood donors. The platelet distribution width (PDW) was 17.4 ± 0.8 in donors with MT and was 16.38 ± 0.5 in southern Indian blood donors ($p < 0.000$). There was no associated red blood cell abnormalities observed with MT [71].

A prospective study [73] was conducted over 8 months at the Christian Medical College and Hospital, Vellore, to determine the geographic distribution of MT in India and neighboring countries. Of the 10,200 blood donors screened over a period of 8 months; 1002 were randomly screened for MT. Nine hundred and thirty-one blood donors were residents of India, and remaining 71 were from Nepal, Bhutan and Bangladesh. There was no previous history of any abnormal bleeding episode in any of the donors. There were no abnormal inclusion bodies in the leukocytes. There was a statistically significant difference in platelet count ($p < 0.000$) and mean platelet volume ($p < 0.000$) between eastern states and all other states. 8.5% of blood donors who came to donate blood in CMC Vellore for their patients were found to have platelet count below $100 \times 10^9/L$ compared to 0% population from north and western part of India. They detected six abnormal profiles, all of which included single-nucleotide polymorphisms (SNPs) represented in the control population. Preliminary family studies have shown possible autosomal dominant mode of inheritance. MYH9A was not mutated in these samples.

Kakkar et al. detected macrothrombocytopenia in 75 patients, majority (96%) of North Indian origin. The MPV (fl) in the 75 patients ranged from 10.9 to 23.3 (mean 15.1 ± 3.1 fl) with a dispersed cytogram pattern distinct

from that seen in patients with normal platelet count, raised platelet count or low platelets due to secondary thrombocytopenia (MPV 10.9 ± 2.6 fl) or ITP (MPV 10.8 ± 3.5 fl). They concluded that macrothrombocytopenia is an under diagnosed condition and should be initially suspected on automated blood counts. [74].

Ali et al. studied [75] over one hundred and twelve unrelated cases of macrothrombocytopenia, who were referred to the Department of Haemostasis, National institute of Immunohaematology, Mumbai from Department of Haematology, NRS Medical College from 2009–2014; a few family members were also included. All the cases had low platelet count (median, range) 89 ($27\text{--}147 \times 10^9/L$), high MPV 13.25 fl (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 gm/dl (10.1–16 gm/dl) and higher platelet distribution width-17.5 (14.1–18.9) as compared to controls. There was no significant difference in MCV, MCHC, MCH and WBC between the cases and controls. Majority of cases (61%) were asymptomatic and the major clinical manifestations were: easy bruisability (4%), ecchymosis (7%), epistaxis (16%), frequent gum bleed (5%), menorrhagia (10%) and history of prolonged bleeding after trauma (6%). On genetic analysis of these cases, twenty-three candidate gene defects were identified in 48 of 112 cases, 20 of which were novel. Of the candidate defects identified, 91% were missense and 9% were nonsense variations. The missense variations were in *GP9*, *ABCG5*, *GP1BB*, *GP1BA*, and *MYH9* while the nonsense defects occurred in *MYH9* and *GP1BA* [62].

In a study from West Bengal, out of the 45 donors who were screened for apheresis, 30 (66.67%) were found to have giant platelets [76]. Post transfusion, all these patients with MT showed significantly higher fractional recovery of platelets in the recipients.

A high prevalence of an autosomal dominantly inherited form of MT with mild to severe thrombocytopenia was detected in the Muslim population in Kashmir Valley in the northern Indian subcontinent, 15% of the donors had thrombocytopenia (mean platelet count $109.6 \times 10^9/L$ compared to $189.9 \times 10^9/L$ in controls; $p \leq 0.0001$). The mean platelet volume MPV (12.53 ± 0.78 fl vs 9.52 ± 1.03 fl) and red cell distribution width (RDW) in thrombocytopenic subjects was found to be higher than in those with normal counts ($15.6 \pm 1.61\%$ vs $13.22 \pm 1.36\%$, $p \leq 0.001$). Hematocrit and other red cell indices were not different in the 2 groups. None of the participants had a history of bleeding, renal disease, sensorineural deafness, or leukocyte inclusion bodies. Peripheral blood platelet morphology revealed large

platelets in all subjects. The *MYH9* gene was sequenced and no mutation was found [77].

Implication of Large Prevalence of Asymptomatic or Mildly Symptomatic Inherited Macrothrombocytopenia in General Population

Inherited MT is no more an uncommon finding among Indian population as its prevalence varies from 15% in Kashmiri populations to higher values for population from West Bengal or North Eastern states of India.

While symptomatic and syndromic MTs should be extensively investigated and there is no consensus on how extensively asymptomatic MT should be investigated? The patient should be evaluated for bleeding manifestations primarily and then a prudent approach depending on available investigative technique should be adopted. Mildly symptomatic and asymptomatic cases may be mistaken as chronic ITP leading to unnecessary investigation and therapeutic misadventure. Platelet count done on an impedance based counter always underestimates the platelet number, hence a well stained peripheral blood smear should always be examined along with manual platelet count or platelet count done on alternative hematology counter working on non impedance based counting. Recently there is a renewed interest on detecting inherited platelet defects using peripheral smears with few immunostains [77]. Syndromic and nonsyndromic variety of MT should be clearly separated on the basis of clinical and laboratory presentation.

Questions arise whether we can distinguish between inherited asymptomatic macrothrombocytopenia from chronic ITP using simple means? Fiore et al. [78] in a study showed any three of the following six conditions if positive can discriminate inherited MT from Chronic ITP with 91% sensitivity and similar levels of specificity, the six items are (i) age at discovery < 34 years (ii) Positive family history (iii) MPV > 11 fl (iv) Excess of giant platelets in smear > 20% (v) Negative history of bleeding (vi) Mean platelet surface area of > 4 μm^2 . It is quite possible that some of these asymptomatic individuals could become blood donors and can even be considered for platelet apheresis. If platelets are prepared from such donors then two questions arise (i) Are these platelet hemostatically as good as normal platelets even though their recovery after transfusion is better? (ii) We will need a separate set of quality parameters (e.g. platelet count X MPV could be one) for quality control of such a collection.

Most of the automated blood cell counters in India are impedance based. As described they always underestimate platelet counts in MT by not counting large platelets. Hence in such a case MPV may not be obtained correctly and machine often does not produce any value [79] so

further requisite correction needs to be made. Another area of the concern for prevalence of macrothrombocytopenia includes how actually these patients behave with anti-platelet drugs and many new directly acting oral anticoagulants (DOACs) in addition to traditional and existing ones, few studies are available in this important area. ISTH scoring for DIC in such patients may also be altered [80] and may need recalibration.

Unanswered Questions on Inherited Macrothrombocytopenia in India

Number of publications and data related to overall prevalence and regional prevalence of this condition in our country is patchy and meagre. Hence extensive study of region wise prevalence of this condition is required. This disorder was found to be heterogenous in terms of distribution, epidemiology, MPV and molecular pathology wherever in the world it has been studied.

Recent reviews show that approximately 40–50% of such conditions can be explained by molecular pathology [81]. Pathological basis for others is still being worked out and every day newer genetic loci/allele are being discovered as cause for IMTP [82]. In this context the Indian study reaches similar conclusions [75]. One interesting finding in that series was finding heterozygous mutations in BSS causing gene in approximately 9% of the patients. In that case should we offer prenatal and pre-marriage counseling for this condition? Unfortunately we don't have adequate information to act and more studies are needed. Proteomic studies in this condition can give useful insight and only one such study from India has shown altered levels of actin binding proteins such as myosin light chain, coactosin like proteins and actin related protein 2/3 complex in affected platelets as important contributor in disorganizing platelet cytoskeleton, predisposing it to macrothrombocytopenia [83].

It is also important to know about the molecular pathogenesis of the other 50–60% patients or individuals with asymptomatic thrombocytopenia. Well collected family samples with GWAS based study and exome sequencing can give us valuable data. However one of the study on a small number of samples for which known mutations were absent could be studied by a combination of subtractive mRNA hybridization and RQ PCR of suspected mRNA species [84].

Informative results were obtained that reported the differential expression of 40 genes that could be classified into following categories (i) Cytoskeletal proteins (ii) ATP binding proteins (iii) Transcription factor genes (iv) Tyrosine Kinases and surprisingly (v) Several iron binding proteins. In addition to targeted sequencing, NGS and exome sequencing, in parts of the world where this

condition is common, genome wide sequencing of properly collected families and samples could throw new light in the pathogenesis of this disorder.

Further studies needs to be conducted to get a proper insight of this condition. In addition the general challenge of macrothrombocytopenia in Transfusion Medicine and their unknown behavior towards anti-platelet and anti coagulant drugs has already been discussed.

Conclusion

Inherited macrothrombocytopenia is not an uncommon condition. Limited studies from India have shown at least one percent of population to have this condition [74] and a much higher prevalence in Bengal [72, 76] and in Kashmiri population studied elsewhere [77]. As practicing hematologists we have all experienced and seen these cases in different parts of India. Hence there is a real need of exploring its prevalence in different parts of India. Though largely asymptomatic, many of them do have milder bleeding tendency [62] but it is a highly heterogenous disorder in India [62]. Nonsyndromic as well as syndromic inherited macrothrombocytopenias are interesting conditions with unique mutations on different genes that may provide answers to the jigsaw puzzle on genetic control of megakaryopoiesis and platelet formation. In syndromic form it also tells us how some platelet proteins have much wider tissue distribution and its wider import on disease biology.

Coming down to more mundane level, we need to have standardized diagnostic criteria of the condition i.e. the cut off MPV, platelet count, machines to be used for counting such platelets, type of history to be taken, which patients should go for more exhaustive investigation and the subsets who will need marriage counseling and prenatal diagnosis.

A major challenge in diagnosis of asymptomatic inherited macrothrombocytopenia is its differentiation from immune thrombocytopenia. The diagnosis must be clearly and unequivocally made as these asymptomatic patients has often been treated with corticosteroids and other immunosuppressants and even underwent splenectomy [85].

Compliance with Ethical Standards

Conflict of interest None declared.

Ethical Approval This article is a review article and follows all the ethical rule of publication.

Informed Consent Not applicable.

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