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Himalayan Platelet Syndrome: Does it Have a Genetic Basis? A Hospital Based Study.

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ABSTRACT

Pursuant to observations that macro thrombocytopenia is prevalent in this Himalayan state and to strengthen and collaborate project to unmask this "Himalayan platelet syndrome", a study was undertaken to know the quantum and magnitude of this entity. Blood donors in a tertiary care hospital come from different parts of valley, thereby offer a representative character. This was coupled with asymptomatic thrombocytopenia cases seen on outdoor basis, which were also evaluated on similar and identical lines, after proper consent and approval from Hospital Ethics committee. Three familial pattern of asymptomatic macro thrombocytopenia from south and central Kashmir were identified. These subjects are on follow up for development of any neurosequelae, autoimmune disorder, cardiovascular event, nephritis that could explain current thrombocytopenia and its possible association with gene related macro thrombocytopenia, currently under way where some preliminary results have surfaced. Diagnosis of macro thrombocytopenia can help do away with unnecessary medications or spleenectomy.

KEYWORDS : WGAS, SNP, MPV, Giant forms.

Introduction

Platelets are one of the components of blood that play role in primary haemostasis. Normal count ranges from 150,000 to 450,000 per micro litre of blood. Thrombocytopenia is defined as a platelet count below 2.5th lower percentile of normal platelet count distribution. Results of third US National Health and Nutrition examination survey(NHANES 111) support traditional value of 1.5 $x10^9$ per /L as lower limit of normal. Thrombocytopenia is defined as a platelet count less than 150 $x10^3$ /µL and is a common haematological finding with a variable clinical expression [3, 4]. Nearly 2.5 % of population have Platelet count less than $150x10^9$ / L [1, 2, and 4]. However platelet count between 100-150 for more than six months and a cut-off value of < $100x10^9$ /litre may be more appropriate to identify a pathological condition. Further it is now appreciated that in non-western countries like India, the lower thresh hold of normal platelet count is lower than $150x10^9$ per litre. Cases are considered mild if counts range between 70 and 150 and severe if less than $20x10^9$ /L. It is equally important to differentiate pseudo thrombocytopenia because of clumping and aggregation in presence of EDTA anticoagulant from true thrombocytopenia.

Inherited thrombocytopenia's getting elucidated with each passing day; need to be taken cognisance of. Broadly classified as autosomal dominant like Epstein syndrome, Fetchner syndrome, May –Hegglin anamoly and Sebstein syndrome, autosomal recessive like Bernard – Souler syndrome,grey platelet syndrome, congenital a megakaryocytic thrombocytopenia and thrombocytopenia with absent radius(TAR) or X linked disorder Wiskot-Aldrich syndrome, GATAI related disease, FLNA related thrombocytopenia's. Peripheral blood picture and Mean platelet volume add to unmask further thrombocytopenias. Bernard soulier has mean platelet volume larger than average while Wiskot Aldrich has mean platelet volume smaller than average. But for Epstein syndrome, rest show granulocytic inclusion bodies in peripheral blood picture. The molecular defect in these disorders is located in the MYH9 gene encoding for non-muscle myosin heavy chain 11A. [9, 10]

Other less commonly seen forms of inherited macro thrombocytopenia include gray platelet syndrome, Mediterranean macro thrombocytopenia, platelet type vonWillibrand disease, and Montreal platelet syndrome, macro thrombocytopenia with mitral valve insufficiency and familial macro thrombocytopenia with glycoprotein (GP) 1V abnormality. All these disorders have specific clinical features. [24]

The aim of this study was to unmask the prevalence of asymptomatic and incidental macro thrombocytopenia and ascertain possible underlying cause including genetic basis.

Material and Methods

This was a single centre study where blood samples from incidental low platelet count cases either routine check up or donors referred or reporting to SKIMS, Soura Srinagar India from June 2012 to May 2014.Blood samples were taken by 18 gauge needle under all aseptic precautions and stored in an EDTA anticoagulant tube after taking proper consent from the subjects and analysed using 5part differential (Sysmax XT 2000i) in the Department of Haematology. A peripheral blood film examination to find out prevalence of asymptomatic thrombocytopenia in the aforementioned populace was also aimed at. In addition these subjects were screened for viral serology (HBV, HCV, HIV), H.pylori serology, ANA, TSH. History of nephritis, deafness was explored as well. Samples for genomic assay were processed under project protocol 72/2013.

Inclusion criteria

Incidental asymptomatic cases of low platelet count referred to SKIMS, Srinagar regardless of age or sex.

Exclusion criteria

Cases of wet or dry bleeding.

The ethics committee of sher-i-Kashmir institute of medical sciences cleared the study bearing protocol 72/2013 for unmasking "Himalayan platelet syndrome".

Results

1. A total of 456 cases participated in the study.
2. The median age of subjects reporting to O.P.D. was 14 years with a range of 5 to 34 years and numbered 456.
3. Out of 456, 198 were male, 210 were females and 48 were in paediatric age group.
4. Out of 456 subjects, 124(27.19%) had thrombocytopenia (platelet count $<150x10^9$) with mean platelet count of $80x10^{9\,p}$.
5. Thrombocytopenia was more pronounced in the age group of 35 to 45 years.
6. Mean body mass index of (BMI) of blood donors with thrombocytopenia was 28.00± 5.09k.

7. Mean platelet volume (MPV) in subjects with thrombocytopenia was 12.53±0.78Fl (10.2 to 13.6Fl) compared to 9.82±1.03Fl (8.1 to 12.4fL) in subjects with normal platelet count, which was statistically significant (p<0.05).
8. The increase in mean platelet volume was more pronounced at lower platelet count.
9. Two families from Charari-i-sharief District Budgam and one family from District Anantnag were identified with fair degree of confidence.
10. One of the family members of Charisharief group at the younger age of seven years was subjected to splenectomy.
11. SNP array analysis of these samples was carried out using two approaches, shared genome segment and whole genome association study. We were able to rule out some of our target genes.

Discussion

Given the reflection of high prevalence of thrombocytopenia in the community which has been our observation and to unmask the same, a project bearing Protocol No. 72/2013 was undertaken after ethical clearance. In our populace consanguinity is prevalent we thought it is all the more relevant to look at this issue. Briefly as we know May – Hagglin anomaly is characterized by autosomal dominant inheritance of giant platelets, characteristic inclusion bodies in neutrophils, oesinophils, and monocytes. These resemble Dohle bodies seen in acute infection but have a different ultra structure. Thrombocytopenia is common but rarely severe. Fetchner, Sebstein and Epstein syndromes are quite similar to May –Hagglin anomaly, but also manifest high tone sensoneural deafness, nephritis and cataracts. Mutation located in chromosome 22q12-13. This gene encodes non muscle myosin heavy chain (NMMHC) IIA, which is also expressed in platelets, kidneys, leucocytes and cochlea [13].

As a part of project SNP array analysis of these identified samples was carried out using two approaches, shared genome segment(SGS) and whole genome association study(WGAS).We were able to rule out some of our target genes into 3 chromosome regions (chr 5,chr,9,chr 10) primarily. None the less this being a large region in genome warrants further elucidation and perhaps a new entity thus far not defined which could very well earn the name of Himalayan platelet syndrome.

In our study we found a statistically high mean platelet volume (11.4Fl), red cell distribution width (17.0) and a low platelet count in subjects with thrombocytopenia compared to those with normal platelet count. Majority of subjects with thrombocytopenia had giant platelets with a high mean platelet volume which was comparable with a study carried out by Harris et al[12,11] who described a syndrome called as "asymptomatic constitutional macro thrombocytopenia" in north east states of India characterised by absent bleeding symptoms, platelet count on an average of 50 thousand. The same set of subjects at one of prime Haematology centres in India at Vellore where author had privilege of being associated with, would earn a label of "Bengal platelet"[11]. Giles C [21] in an analysis of 5000 unselected blood specimens showed an inverse relationship between number of circulating platelets and their mean platelet volume. In this study nearly 95% of normal adults the platelet count varied from 150 to 450 x10^9 per L and MPV from 7.0 to 10.5 Fl. Lamparelli RD etal[19] studied platelet count and mean platelet volume in 564 normal subjects using a Coulter Model S-Plus electronic counter. The mean platelet count was 283x10^9 per litre while mean platelet volume was 9.32Fl.A non linear inverse correlation between platelet count and number was documented (r =0.38; p< 0.0001). Grahm SS et al [22] studied effects of age and sex in a study population of 447 normal persons. There was an inverse, nonlinear relationship between MPV and platelet count, with no statistical difference (p>0.05) seen between males and females. Bess man JD et al [23,3] measured whole blood mean platelet volume and count by Coulter counter model S-plus, in 683 normal subjects. There was no linear inverse relation between MPV and count; change in MPV was most pronounced at lower platelet count. The mean platelet volume is dependent on a number of variables, including time of analysis after venepuncture, method of analysis, anticoagulant used and specimen storage temperature. The influence of these laboratory variables is significant and reproducible mean platelet volumes are dependent on standardised laboratory methodology. When pre-analysis factors are controlled, alterations in platelet volume can be demonstrated in a number of disease states and assessment of platelet volume can be useful in monitoring and diagnosis of patients. An understanding of the pathophysiology of alterations in platelet volume and of the inverse relationship between platelet volume and count is a prerequisite for the successful clinical application of platelet volume measurements rather platelet indices similar and identical to red cell indices. [23]

Platelet count has been found different in different seasons.Peng et al[18] studied that the effects of biological variations on platelet counts in 694 healthy subjects aged 18 to 60 years living in three cities including Chengdu) Sichuan province), Suzhou (Jiangsu province) and Harbin (Heilongjiang province) in china. Platelet counts in healthy subjects were significantly lower in Chengdu(52to202x10^9 per L) and Suzhou(60x 259x10^9 per L) than in Harbin(154 to 348x10^9per L)(p<0.0001), but the mean platelet volume(MPV) determined concurrently was negatively correlated with platelet count, the MPV values were significantly higher in Chengdu(11.8 to 15.6Fl) and Suzhou (10.9 to 15.8fL) than in Harbin(9.5to 12.9fL)(p<0.0001). Platelet counts were significantly higher in summer (73 to 289x10^9 per L) than in winter (52x202x$10^{9\ per}$ L) (P<0.0001), but the MPV values were lower in summer (11.2 to 14.7fL) than in winter (11.8 to 15.6fL) (p< 0.05) in Chengdu. These findings suggest that the platelet count could be greatly influenced in healthy subjects by biological variations such as geographical, seasonal ethnic and genetic variations.

Norris et al studied MPV and mean platelet diameter (MPD) i 35 patients with inherited macrothrombocytopenias and 56 patients with ITP. They reported that platelets were larger in inherited macrothrombocytopenias than in ITP. Combining MPD with MPV increased sensitivity and specificity of macro thrombocytopenia diagnosis to 0.97 and 0.89 respectively [25].This compares well with our observations.

Many cases of thrombocytopenia in children and adults are diagnosed incidentally after a routine blood count. With automation and refinement in laboratory haematology more cases are being picked up with no identifiable cause, and with absent bleeding. These subjects need to be followed for development of any subsequent autoimmune disorder that could explain current thrombocytopenia. Therefore a detailed workup including priming Resident staff about macrothrombocytopenias, history ,examination, CBC with MPV and platelet indices akin to red cell indices,relevent baseline investigations and genetic assay can surely lay hands on correct diagnosis and further unmask this entity which in our parlays is "Himalayan platelet syndrome". Awareness and Unmasking this diagnostic dilemma would certainly save unnecessary use of steroids, splenectomy and their associated side effects on quality of life moreso in growing paediatric population. Genetic counselling in future for these disorders perhaps may not be a distant dream.

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