

Megaloblastic Erythropoiesis- An Incidental Finding in Idiopathic Thrombocytopenic Purpura

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Abstract : Idiopathic thrombocytopenic purpura (ITP) presents as a primary form characterized by isolated thrombocytopenia (platelet count $< 100 \times 10^9/L$) in the absence of other causes or disorders that may be associated with thrombocytopenia, or a secondary form in which immune thrombocytopenia develops in association with another disorder that is usually immune or infectious. The diagnosis of ITP is usually a diagnosis by exclusion based on a demonstration of peripheral thrombocytopenia, with a history, physical examination, and complete blood count that do not suggest another cause for the thrombocytopenia. ITP may affect individuals of all ages, with peaks during childhood and middle aged. Pallor was the most common clinical manifestation of ITP. The pathogenesis of ITP is complex, involving alterations in humoral and cellular immunity. Thrombocytopenia is caused by antibodies that react with glycoproteins expressed on platelets and megakaryocytes (glycoprotein IIb/IIIa, Ib/IX and others), causing shortened survival of circulating platelets and impairing platelet production. Bone marrow examination done in most of the patients. Micro- normoblastic erythropoiesis found to predominate. Megaloblastic erythropoiesis found in 20.3% cases. Megaloblastic erythropoiesis- A finding which can be seen in vitamin B12 deficiency, Anti intrinsic factor or Anti gastric mural cell antibodies and may be associated with other disorders of autoimmunity, the most usual being autoimmune thyroid disorders, type I diabetes mellitus, hypoparathyroidism, Addison's disease, post-partum hypophyseal necrosis, ulcerative colitis and vitiligo. The aim of this study is to correlate Bone marrow aspiration findings and possibility of autoimmunity with Idiopathic thrombocytopenic purpura (ITP).

Keywords: Idiopathic thrombocytopenic purpura, autoimmunity, antiplatelet antibodies, megaloblastic erythropoiesis,

I. Introduction

ITP is a syndrome of various disorders that have in common immune mediated thrombocytopenia but differs in pathogenesis, natural history, comorbidities and responsiveness to therapy. It can be suspected if thrombocytopenia fails to respond to cobalamin therapy or is out of proportion to degree of anaemia.

The diagnosis of ITP is usually a diagnosis by exclusion based on a demonstration of peripheral thrombocytopenia, with a history, physical examination, and complete blood count that do not suggest another cause for the thrombocytopenia. The definition of immune thrombocytopenic purpura (ITP) is modified from that proposed by the Practice Guidelines of the American Society of Hematology for ITP. ITP is defined as thrombocytopenia: (a) in the presence of a normal hemoglobin level, white blood cell count and differential, and blood smear and (b) the absence of other causes of thrombocytopenia.

Slight splenomegaly may be found in patients with ITP. During the last several years, immune thrombocytopenia was also reported in patients with Helicobacter pylori, and platelet counts may or may not normalize with treatment directed only at Helicobacter.

Thrombocytopenia in patients with a variety of solid tumors has also been thought to most likely be immune mediated. Thrombocytopenia may accompany Graves disease and Hashimoto thyroiditis, but it is not certain if it is immunologically mediated. Platelet-associated IgG has been increased when studied, but there may also be enhanced reticuloendothelial phagocytosis. The pathogenesis of ITP is complex, involving alterations in humoral and cellular immunity. Thrombocytopenia is caused by antibodies that react with glycoproteins expressed on platelets and megakaryocytes (glycoprotein IIb/IIIa, Ib/IX and others), causing shortened survival of circulating platelets and impairing platelet production.

Immune complexes have been detected in patients with chronic ITP, but their role in immune platelet destruction remains controversial. The concomitant role of the antibody binding to the same antigens on megakaryocytes and the resultant effect of this binding on platelet production are still unknown.

The pathogenesis of ITP involves loss of tolerance to glycoproteins expressed on platelets and megakaryocytes. Epitope spreading may explain the fact that many patients have antibodies against more than one glycoprotein.

- Anti-platelet glycoprotein antibodies cause thrombocytopenia through two mechanisms: 1) reducing the survival of circulating platelets, and 2) inhibiting the production of new platelets by bone marrow megakaryocytes.
- Cellular immunity contributes significantly to the pathogenesis of ITP. Alterations in T cell subsets and decreased numbers and activity of regulatory T cells are common.

Autoantibodies are readily found in plasma or platelet elute in patients with active disease but are infrequently found in patients in remission. Platelet-associated autoantibodies have been detected in 75% of patients. Serum antiplatelet IgG autoantibodies are detected in approximately 50 to 85% of patients. IgA serum antiplatelet antibodies appear to be as frequent as IgG, and in approximately 50% of cases, both Ig subtypes occur in the same patient. IgM antibodies are also appear. ITP sera have also been demonstrated to bind to glycosphingolipids and cardiolipin.

Etiology: Infections have a clear role in the initiation of many cases of ITP, at least in childhood. *Helicobacter pylori* has an important role in the etiology or persistence of ITP. It has been hypothesized that individuals may have an inherited predisposition to ITP. An infection or medication may result in the development of ITP in these individuals.

A component of the etiology of ITP may include immune response genes that predispose patients to develop ITP. A common theme of immune dysregulation, not defined in its specifics, is associated with many of the diseases linked to ITP. The most obvious example is an inability to make antibodies normally. The mechanism of development of ITP may vary, but one study suggested that autoantibodies may be restricted to only one Vh gene family. The hypothesis is that an inability to make antibodies normally (i.e., to use the full Vh gene repertoire) may result in overutilization of this autoantibody-generating Vh gene family. These etiologies of ITP could be the result of abnormalities of B cells, but they could equally be explained by abnormal T cell interactions with B cells.

Defects in the classical pathway of complement may operate via failure to rapidly clear immune complexes, resulting in longer persistence of an "enhanced" immunogen (part of an immune complex). Low C4 has been associated with ITP and low levels of all of the components of the classical pathway have been linked to systemic lupus erythematosus (SLE).

The presence of megaloblastic anaemia can usually be suspected from examination of the peripheral blood and, if features are totally typical, bone marrow aspiration is often not done. The ready availability of accurate assays for vitamin B 12 and folic acid has lessened the importance of bone marrow examination. However, if typical peripheral blood features of megaloblastic erythropoiesis are lacking or if atypical features are present, bone marrow aspiration should be performed. Further tests indicated in patients with megaloblastic anaemia are assays of serum vitamin B 12 and red cell folate followed, when appropriate, by tests for autoantibodies and a Schilling test. If pernicious anaemia is suspected, tests for parietal cell and intrinsic factor antibodies are indicated; the former is more sensitive but less specific.

In summary, a number of possible etiologies, postinfectious and immune predispositions, may underlie the apparent considerable heterogeneity among patients with ITP.

II. Material And Methods

A retrospective study conducted at Osmania General Hospital over a period of 3 years, starting from May 2012 to April 2015. Age group included was 15-45 years. A total of 376 cases were selected and 325 cases were studied. All patients were having Hemoglobin Range of 4-10g%. Platelet count less than 1 lakh per microlitres. Out of the 325 ITP cases studied, splenomegaly in 15 patients of non leukemic, non neoplastic origin (symptomatic hypersplenic thrombocytopenia). In 295 cases, bone marrow aspiration was done using Salah's needle, site of aspiration being iliac crest or sternum to rule the cause of anaemia. Complete blood count obtained using 3 part automated hematology analyzer, Sysmex. Peripheral blood smear examination was done for presence of anaemia, leucocyte abnormalities. Coomb's test to exclude autoimmune hemolytic anaemia.

Thrombocytopenia secondary to other causes such as pseudo thrombocytopenia due to EDTA, Liver diseases, Acute or chronic leukemia, Myelodysplasia, Aplastic anaemia, Drugs intake, Lack of sufficient data & irregular follow up were excluded from the study.

III. Results

Our study on 325 patients showed the most common age group involved was 15-30 years (Figure no 1). Female preponderance was noticed with an average of 75%. (Figure no. 2) In our study, we could find that pallor was the most common clinical manifestation (Table no 1). Peripheral blood examination (Figure no 3) was done in all patients. 20.3% of the cases showed anisopoikilocytosis, with predominantly macrocytes, ovalocytes, thrombocytopenia, hypersegmented neutrophils in few. In all cases in whom anaemia was seen, bone marrow aspiration was carried out to rule out other causes. Bone marrow

examination revealed normoblastic, microblastic, megaloblastic erythropoiesis. Few showing micro-normoblastic picture(Figure no4). Microcytic picture was predominant, amounting to 38 %. Iron deficiency anaemia could be attributed to it. However , Megaloblastic erythropoiesis was identified in 20.3% cases. Bone marrow examination in patients with megaloblastic picture showed increased megakaryocytes(figure no.5), immature megakaryocytes with large non lobulated nuclei (Figure no6), megaloblasts (Figure no7&8), giant metamyelocytes were seen along with cells of erythroid lineage. The bone marrow in megaloblastic anemia is hypercellular, with erythroid precursors predominating. Iron stores were evaluated by Perl's stain, which was either normal or decreased.

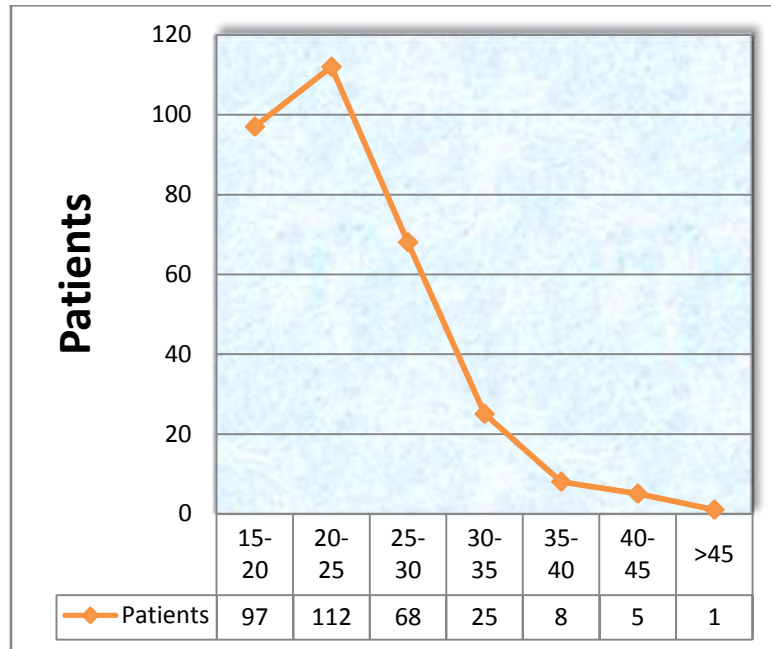


Figure no.1 : Age vs number of patients in all age groups.

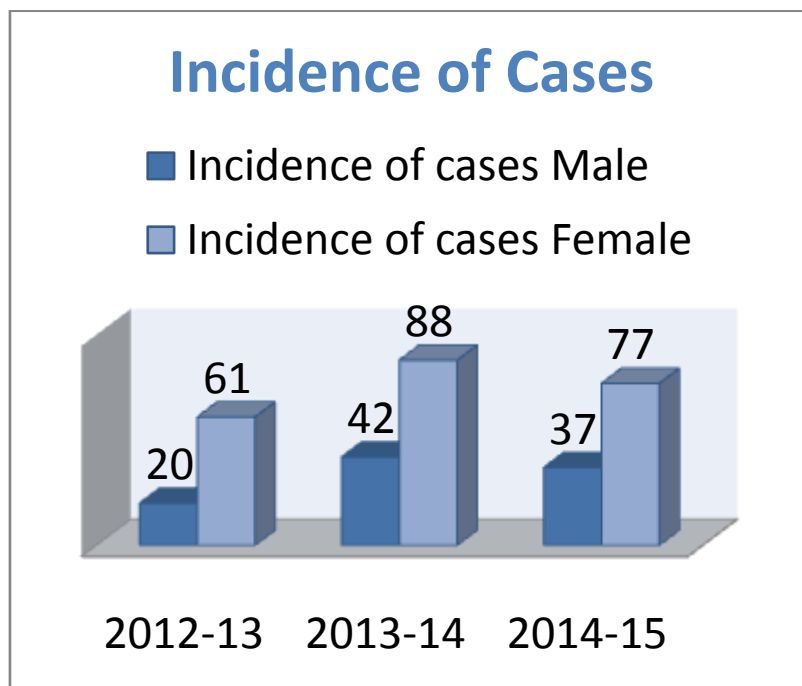


Figure no 2: Incidence of ITP in females and males during 3years study.

Percentage Incidence of Clinical Feature		
Clinical Feature	Total number of Incident cases	% of Incidence
Pallor	295	90
Gum Bleeding	238	73.6
Petechial Hemorrhage	178	54.7
Epistaxis	58	17.8
Bleeding P/V	28	8.6
Splenomegaly	15	4.6
Spontaneous Abortions	12	3.6
Hypertension	6	1.6
Malena	5	1.5
Hematuria	4	1.2
Jaundice	2	0.6

Table no 1: Percentage incidence of clinical cases in ITP

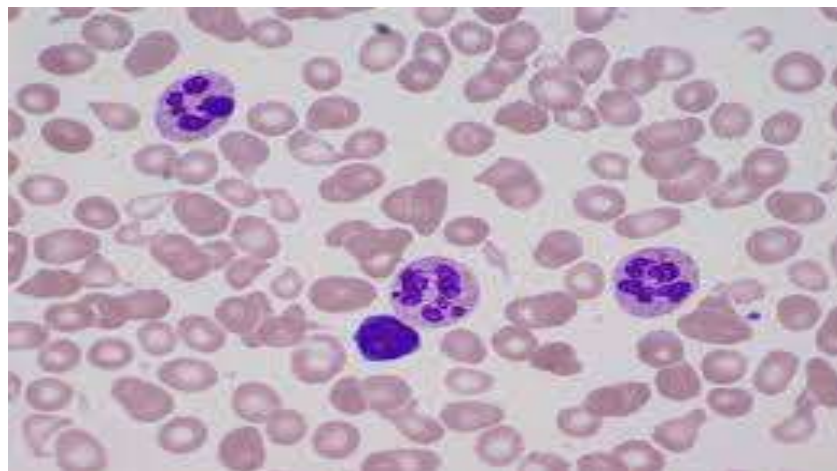


Figure no 3: Peripheral blood smear examination showing Anisocytosis with predominant macrocytes, ovalocytes, hypersegmented neutrophils, thrombocytopenia.

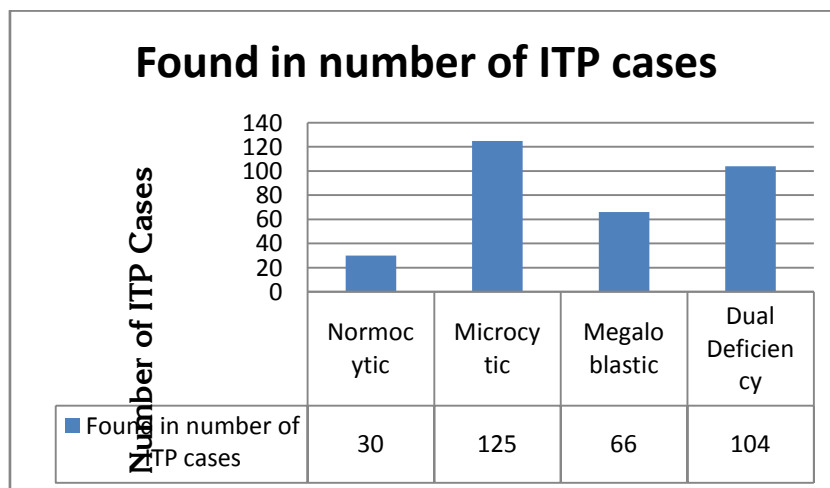


Figure no 4: Incidence of Bone marrow findings in ITP

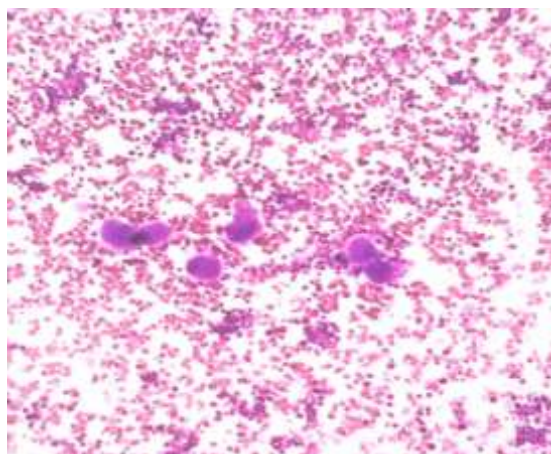


Figure no 5: Bone marrow examination, 10x Magnification showing Increased megakaryocytes

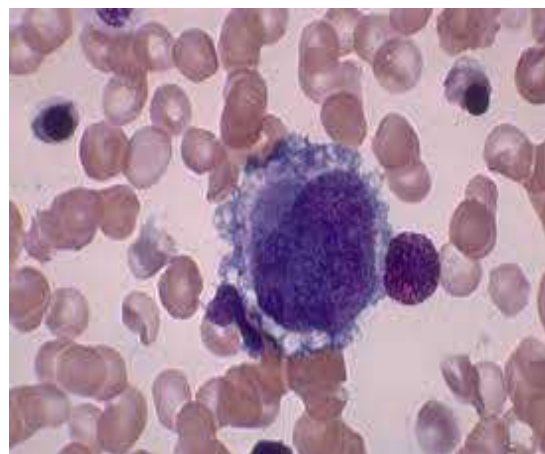


Figure no 6: Bone marrow examination, 100x magnification showing immature megakaryocyte

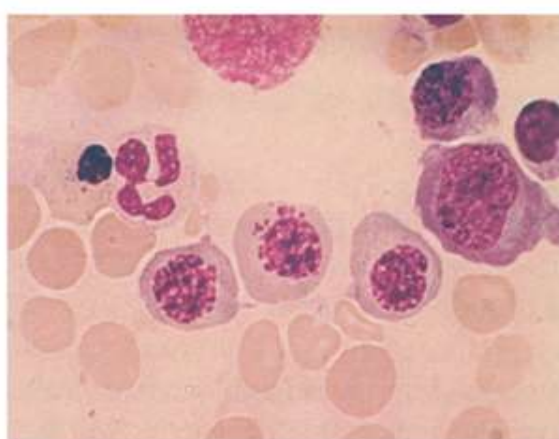


Figure no 7 : Bone marrow examination, 100x

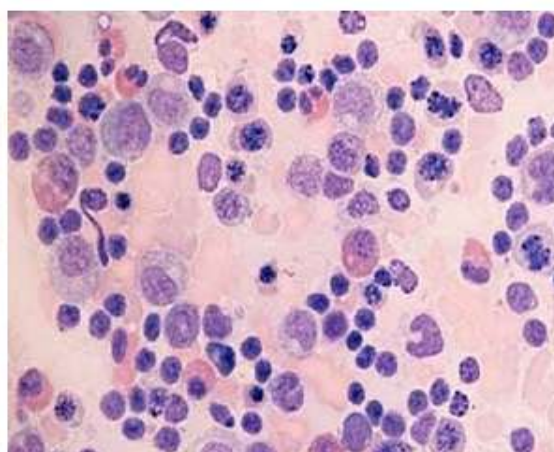


Figure no 8: Bone marrow examination, 40 x Magnification showing megaloblasts and giant metamyelocyte.

Attributes	Autoimmune cytopenia in Pernicious Anaemia Arthur P Rabinowitz et al (Department of Medicine, Los Angeles, USA, 1990)	Relationship between ITP and Pernicious Anaemia Tordi junca, Alonso Flores et al (Department of Hematology, Barcelona, Spain, 2000)	CURRENT STUDY (Upgraded Department of Pathology, Osmania General Hospital, Hyderabad, India)
Cases under study	4	231	325
Age Group (Years)	25-58	25-65	15-45
Sex Incidence (Females, Males)	3 Females, 1 Male	120 Females, 49 Males	226 Females, 99 Males
Hemoglobin Range (g%)	3.5-9.5	<10	<10
Platelet Count	20000-1.2 Lakh	<1.2 Lakh	<1 Lakh
Megakaryocyte count	Normal or increased	Normal or increased	Normal or increased
Bone Marrow Aspiration Findings	Megaloblastic erythropoiesis - Found in all cases (100%)	Megaloblastic erythropoiesis-found in 139 cases (60.1%)	Megaloblastic erythropoiesis-found in 66 cases (20.3%)
Iron Stores	Decreased	Normal or Decreased	Normal or Decreased
Coomb's Test	Negative	Not performed	Negative
Presenting Symptoms	Epistaxis, Bleeding PV, Mucosal bleeding, Fatigue, Dyspnoea	Epistaxis, Bleeding PV, Mucosal bleeding, Gum bleeding	Pallor, Epistaxis, Gum bleeding, Petechial hemorrhages, Spontaneous abortions, Bleeding plv, Malena
Other Investigations	None	Serum pepsinogen, Anti intrinsic factor Antibodies, Anti parietal cell antibodies	None

Table no 2 : Comparative study of our study with Rabinowitz et al and Tordi junca et al study.

IV. Discussion

Our study was compared with the study done by Rabinowitz et al (Department of medicine, Los Angeles, USA, 1990) and Tordi junca et al (Department of hematology, Barcelona, Spain, 2000), which also revealed female preponderance. Other studies have suggested that there is a 2 to 3 : 1 female to male predominance, consistent with the generally recognized increased incidence of autoimmune disease in women and a peak age of onset of ITP in women of child-bearing age.

In Rabinowitz et al and Tordi junca et al study, bleeding gums was most common. Bone marrow examination revealed Microcytic picture was predominant, amounting to 38%. Iron deficiency anaemia could be attributed to it. However, Megaloblastic erythropoiesis was identified in 20.3% cases. In the study by Rabinowitz et al and Tordi junca et al, Megaloblastic erythropoiesis was seen in 100% and 60% cases respectively (Table no 2). Bone marrow examination in patients with megaloblastic picture showed increased megakaryocytes (figure no.5), immature megakaryocytes with large non lobulated nuclei (Figure no6), megaloblasts (Figure no7&8), giant metamyelocytes were seen along with cells of erythroid lineage. The bone marrow in megaloblastic anemia is hypercellular, with erythroid precursors predominating. Iron stores evaluated by Perl's stain, was either normal or decreased, which coincided with Rabinowitz et al study and Tordi junca et al study.

V. Conclusion

There is no diagnostic blood test (e.g., antiplatelet antibodies) that reliably and consistently includes or excludes ITP. An algorithm for the diagnosis of ITP could proceed in three steps. A) Distinguish ITP from systemic disorders causing thrombocytopenia as part of their pathophysiology. B) Distinguish immune thrombocytopenia from nonimmune thrombocytopenia. C) Distinguish primary ITP from secondary ITP i.e., distinguishing primary, "idiopathic" ITP from ITP secondary to other diseases such as SLE, HIV, CLL, and COVID, drug induced thrombocytopenia.

Other tests, in addition to a bone marrow examination, include Reticulocyte count, to help exclude both Thrombotic thrombocytopenic purpura and Evans syndrome (autoimmune anemia in association with ITP). Antinuclear antibody (ANA) and antidouble stranded DNA antibody to investigate the possibility of SLE. Prothrombin time, partial thromboplastin time, fibrinogen, and D-dimer to consider DIC or other nonimmune condition. Radiologic studies (e.g., CT scan) to search for lymphoma or other malignancy.

In summary, ITP is usually an autoantibody-mediated disease that results in often severe thrombocytopenia. ITP has the virtue of having an easily obtainable and highly specific surrogate marker for hemorrhage: the platelet count. Fortunately, even at low platelet counts, serious hemorrhage (e.g., intracranial) is rare. Difficulties in diagnosis include the lack of a specific platelet antibody test. Other tests (e.g., plasma TPO levels, reticulated platelet counts, and the number of large platelets) are being developed to provide complementary pathophysiological information. Autoimmunity in case of megaloblastic picture should be considered. Current management is therefore largely based on an individual patient's degree of bleeding and response to previous treatments. There are a number of standard treatments. Methods to acutely increase the platelet count are numerous and generally effective, including high-dose steroids, IVIG, and higher dose, 75 µg/kg IV anti-D. In addition, there appears to be a clear benefit of combining agents in difficult patients. Maintenance of the platelet count prior to splenectomy, or when splenectomy (the only curative treatment in more than 50% of patients) has failed, is the subject of ongoing studies but the upfront treatments and especially the novel thrombopoietic agents seem useful.

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