Morphological Changes of Megakariocytes in Diseases That Cause Thrombocytopenia

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Abstract

Background: Morphological changes of megakariocytes are common and they are different according to the cause of thrombocytopenia.
Aim: To differentiate the morphological changes of megakariocytes according to the cause of thrombocytopenia.
Materials and methods: A total number of 88 cases of symptomatic thrombocytopenia (in which bone marrow examination was indicated) were included. The study was done in a legal private laboratory for hematological investigations (Zaid Bin Ali private laboratory) in karbala city. Patients with thrombocytopenia were referred from different specialists. Blood samples were taken for complete blood picture and platelet count. Thrombocytopenia was defined as platelet number below 100 $X10^9$/L. Bone marrow aspirate samples were taken, bone marrow smear slides were made, fixed and stained by Leishman stain, dried and examined under the light microscope for various abnormal morphological changes of megakariocytes. Normal megakariocytes were defined as the largest marrow cells which retain lobulated nuclei (4-16 lobes) while abnormal morphological changes include large magakariocytes with dispersed nuclear lobes, dwarf micromegakario- cytes, juvenile megakariocytes, emperipolesis, megakariocytes with bare nuclei, presence of cytoplasmic vacuoles, hyperpolyploidy (defined as nuclear lobes more than 16) and hypopolyploidy (defined as nuclear lobes less than 4). Using Fisher's exact test, P value of less than 0.05 was considered to be significant.
Results: Large magakariocytes with dispersed nuclear lobes, dwarf micromegakariocytes, juvenile megakariocytes and emperipolesis were 31% (9 out of 29), 34.48% (10 out of 29), 96.55% (28 out of 29) and 72.41% (21 out of 29), respectively in cases of thrombocytopenia which are caused by ITP.
Large magakariocytes with dispersed nuclear lobes, dwarf micromegakariocytes, juvenile megakariocytes and emperipolesis were 71.42% (5 out of 7), 85.71% (6 out of 7), 57.14% (4 out of 7) and 57.14% (4 out of 7), respectively in cases of thrombocytopenia which are caused by MDS. In MDS, the presence of large magakariocytes with dispersed nuclear lobes and dwarf micromegakariocytes was statistically significant when compared with other diseases that cause thrombocytopenia ($P<0.05$).
The other abnormal morphological changes of megakariocytes in cases of thrombocytopenia are shown in table 1. While the presence of one or more megakaryocytic changes or absence of such changes in various hematological cases of thrombocytopenia are shown in table 2.
Conclusion: There are great interactions and similarities in morphological changes of magakariocytes among different hematological diseases; however, the diagnostic approach will vary when detailed knowledge about morphological changes of megakariocytes is available.
Key words: dwarf micromegakariocytes, bone marrow smears, thrombocytopenia, myelodysplastic syndrome.

الخلاصة

الخلفية: التغيرات الشكلية للخلايا المولدة لاقراص الدم شائعة وتختلف هذه التغيرات حسب السبب المرضي الذي يؤدي الى نقصان عدد الاقراص الدموية.

الغرض: للتمييز بين التغيرات الشكلية للخلايا المولدة للاقراص الدموية حسب الامراض المسببة لنقص الاقراص الدموية.

المواد والطرق: تمت دراسة 88 حالة مرضية لنقص عدد الاقراص الدموية التي تحتاج الى عملية اجراء فحص نخاع العظم للوصول الى التشخيص الطبي. تم جمع عينات الدم وعينات نخاع العظم . تم فحص عينات الدم وتم اعتبار نقص اقراص الدم في حالة وجود اقل من 100.000 قرص دموي/ملم^3

تم فحص عينات نخاع العظم بعد اجراء مسحات وصبغ المسحات بصبغة الليشمن وتم التركيز على اشكال الخلايا المولدة للاقراص الدموية .

النتائج: وجدت نسبة 31% (9 من 29)من الخلايا المولدة للاقراص الدموية التي تمتاز بكبر حجمها مع ضرورة عدم وجود الترابط بين فصوص النواة ونسبة 34.48% (10 من 29) تمتاز بكونها صغيرة جدا(قزم) ونسبة 96.55%(28 من 29) تمتاز بعدم النضج لدى المرضى المصابين بنقصان الاقراص الدموية بسبب مرض النزف الجلدي الحاصل بسبب نقص الاقراص الدموية المبهم(ما يسمى بتكسر الاقراص الدموية).

وجدت نسبة 71.42%(5 من 7) من الخلايا المولدة للاقراص الدموية التي تمتاز بكبر حجمها مع ضرورة عدم وجود الترابط بين فصوص النواة ونسبة 85.71% (6 من 7) تمتاز بكونها صغيرة جدا(قزم) ونسبة 57.14%(4 من 7) تمتاز بعدم النضج لدى المرضى المصابين بنقصان الاقراص الدموية بسبب متلازمة اعتلال الخلايا المولدة للدم. الجدول 1 يحتوي على نسب وجود الاشكال المختلفة للخلايا المولدة للاقراص الدموية وفقا للمرض المسبب لنقص الاقراص الدموية.

الاستنتاج: الدراسة المتانية والدقيقة للاشكال المختلفة للخلايا المولدة للاقراص الدموية في نخاع العظم تؤدي الى قدرة التمييز.

Introduction

Morphological changes in megakariocytes in thrombocytopenia due to myelodysplastic changes are well known, reported and documented; however, such dysplastic or dysmegakariocytic changes can be seen in cases of thrombocytopenia which are not related or not caused by myelodysplastic syndrome, but only few limited and old studies provides the significance of morphological changes in megakariocytes in cases of thrombocytopenia which are due to non myelodysplastic causes [(1)]. Poor sources of information can be explained since the hematology sciences are detecting more advanced ways of dysmegakariopoiesis like cultural characteristics, specific marker studies, electron microscope megakariocytes changes and flow cytometry rather than morphological changes using the ordinary light microscope which is the only one existing facility for diagnosis and research in developing countries . In 2009 M. Muhory et al study showed that the specificity of micromegakariocytes is 83% in cases of thrombocytopenia due to myelodysplastic syndrome compared to non myelodysplastic causes of thrombocytopenia [(2)]. Thrombocytopenia can be a primary (idiopathic) disorder as in Idiopathic Thrombocytopenic Purpura (ITP) or it can be secondary to other diseases as in Myelodysplastic Syndrome (MDS), acute leukemias, megaloblastic anemias, hypersplenism, chronic leukemias, secondary to other autoimmune diseases (as in systemic lupus erythematosis), aplastic anemias and Paroxysmal Nocturnal Hemoglobinurea (PNH) [(3, 4)]. Dysplastic changes in megakariocytes are typically seen in cases of MDS like dwarf micromegakariocytes, large mononuclear or binuclear megakariocytes and large megakariocytes with widely dispersed (disconnected) nuclear lobes; however, such changes may be seen in other hematological disorders[(5)].

Materials and methods

A total number of 88 cases of symptomatic thrombocytopenia (in which bone marrow examination was indicated) were included. . The study was done in a

legal private laboratory for hematological investigations (Zaid Bin Ali private laboratory) in karbala city. Patients with thrombocytopenia were referred from different specialists Blood samples were taken for complete blood picture and platelet count. Thrombocytopenia was defined as platelet number below 100 $X10^9$/L. Bone marrow aspirate samples were taken, bone marrow smear slides were made, fixed and stained by Leishman stain, dried and examined under the light microscope for various abnormal morphological changes of megakariocytes. Normal megakariocytes were defined as the largest marrow cells which retain lobulated nuclei (4-16 lobes) while abnormal morphological changes include large magakariocytes with dispersed nuclear lobes, dwarf micromegakariocytes, juvenile megakariocytes, emperipolesis, megakariocytes with bare nuclei, presence of cytoplasmic vacuoles, hyperpolyploidy (defined as nuclear lobes more than 16) and hypopolyploidy (defined as nuclear lobes less than 4). Large megakariocytes with dispersed nuclear lobes are defined as large megakariocytes with clearly disconnected nuclear lobes. Dwarf micromegakariocytes are defined as megakariocytes whcich retain the size of normal monocyte. Juvenile megakariocytes are defined as megakariocytes which are larger than normal megakariocytes with very high nuclear/cytoplasmic ratio, open chromatin pattern and scanty deeply bluish cytoplasm. Using Fisher's exact test, P value of less than 0.05 was considered to be significant.

Results

Out of 88 cases of thrombocytopenia, 68.18% (n=60) were below the age of 18 years, 31.81% (n=24) were 18-42 years old and 4.54% (n=4) were 50-60 years old. Out of 88 cases of thrombocytopenia, 59.1% (n=52) were males and 40.9% (n=36) were females. Out of 88 cases of thrombocytopenia, 44.31% (n=39) had acute leukemia, 32.95% (n=29) had Idiopathic Thrombocytopenic Purpura (ITP), 7.95% (n=7) had Myelodyplastic Syndrome (MDS), 7.95% (n=7) had megaloblastic anemia, 4.54% (n=4) had thalassemia, and 2.27% (n=2) had bone marrow secondary metastatic tumors.

Among different abnormal morphological changes of megakariocytes, there is no statistically significant relation in cases of thrombocytopenia which are caused by acute leukemias as shown in table 1.

large magakariocytes with dispersed nuclear lobes, dwarf micromegakariocytes, juvenile megakariocytes and emperipolesis were 31% (9 out of 29), 34.48% (10 out of 29), 96.55% (28 out of 29) and 72.41% (21 out of 29), respectively in cases of thrombocytopenia which are caused by ITP i.e several abnormal morphological changes of megakariocytes can be seen in a single case of ITP.

large magakariocytes with dispersed nuclear lobes, dwarf micromegakariocytes, juvenile megakariocytes and emperipolesis were 71.42% (5 out of 7), 85.71% (6 out of 7), 57.14% (4 out of 7) and 57.14% (4 out of 7), respectively in cases of thrombocytopenia which are caused by MDS. In MDS, the presence of large magakariocytes with dispersed nuclear lobes and dwarf micromegakariocytes was statistically significant when compared with other diseases that cause thrombocytopenia ($P<0.05$).

The other abnormal morphological changes of megakariocytes in cases of thrombocytopenia are shown in table 1. While the presence of one or more megakaryocytic changes or absence of such changes in various hematological cases of thrombocytopenia are shown in table 2.

Table 1. Morphological changes of megakariocytes in cases of thrombocytopenia due to various hematological diseases.

All (88) cases of thrombocyto-penia	Large mega.	Dwarf mega.	Juven. mega.	Emperip o-lesis	Bare nuclei	Cytoplasmic vacuoles	Hyperploid	Hypopolyploidy
Leukemias (39/88)	0	2 (5.12%)	3 (7.69%)	2 (5.12%)	2 (5.12%)	0	0	0
ITP (29/88)	9 (31%)	10 (34.48 %)	28 (96.55 %)	21 (72.41%)	4 (13.79 %)	4 (13.79%)	0	6 (20.68%)
MDS (7/88)	5 (71.42 %)	6 (85.71 %)	4 (57.14 %)	4 (57.14%)	3 (42.85 %)	1 (14.28%)	3 (42.85%)	2 (28.57%)
Megaloblastic anemia (7/88)	0	1 (14.28 %)	2 (28.57 %)	2 (28.57%)	1 (14.28 %)	0	3 (42.85%)	0
Thalassemia (4/88)	0	0	1 (25%)	1 (25%)	0	1 (25%)	0	0
Bone marrow metastasis (2/88)	0	0	0	1 (50%)	0	0	0	0

Table 2. Presence or absence of morphological changes of megakariocytes in cases of thrombocytopenia due to various hematological diseases.

All (88) cases of thrombocyto-penia	The presence of **one or more** morphological changes or absence of such changes	
	Present	Absent
Leukemias (39/88)	9	30
ITP (29/88)	29	0
MDS (7/88)	7	0
Megaloblastic anemia (7/88)	6	1
Thalassemia (4/88)	3	1
Bone marrow metastasis (2/88)	1	1

Conclusion

Morphological changes of megakariocytes in cases of thrombocytopenia due to myelodysplastic or non myelodysplastic causes are valuable and bring special interest especially in underdeveloped and developing countries due to the poor facitity for well sophisticated megakariocyte studies like cultural studies, specific marker studies, electron microscope and ultrastructural studies. From this study we concluded that the diagnostic accuracy will be improved and will be able to differentiate between hematological disorders that cause thrombocytopenia and so that therapeutic and prognostic values will be also improved.

Discussion

Dysplastic changes of megakariocytes which are seen in myelodysplastic syndrome (MDS) are well known[1]; however, full hematological knowledge can differentiate such changes from similar morphological changes of megakariocytes in other diseases like idiopathic thrombocytopenic purpura, megaloblastic anemia and paroxysmal nocturnal hemoglobinurea [3,5].

The presence of large megakariocytes with dispersed nuclear lobes and dwarf micromegakariocytes was 71.42% and 85.71%, respectively in cases of thrombocytopenia which are caused by myelodysplastic syndrome. These findings were statistically significant (P<0.05) when compared with other diseases which cause thrombocytopenia. Such findings are similar to findings of Tricot G et al study [6] which showed that the presence of large megakariocytes with dispersed nuclear lobes and dwarf micromegakariocytes was 76% and 83%, respectively in cases of myelodysplastic syndrome.

Juvenile megakariocytes were seen in 96.55% of cases of thrombocytopenia which are caused by idiopathic thrombocytopenic purpura (ITP), a finding which is consistent with Wang et al study[7] which showed a figure of 100%.

Juvenile megakariocytes are also present in diseases that cause thrombocytopenia other than idiopathic thrombocytopenic purpura as shown in table 1; however, a collection of information about the total morphological changes of megakariocytes can differentiate the causative disease of thrombocytopenia [3]. Not only the morphological changes of megakariocytes, but as well as the number of megakariocytes in bone marrow aspirates is important aid for the diagnosis of various hematological diseases that cause thrombocytopenia [8]. The presence of cytoplasmic vacuoles in megakariocytes is reported to be important aid for the diagnosis of infection associated thrombocytopenia [9]. This study showed that the presence of cytoplasmic vacuoles was 13.79% in cases of thrombocytopenia which are caused by ITP which might reflect associated infection, a finding which is similar to Chanarin et al study[10] which showed a figure of 16.2%.

Emperipolesis was present in 71.42% of cases of thrombocytopenia which are caused by ITP; however, and as shown in table 1, emperipolesis was also present in other diseases. In this study, findings were correlated with findings of a study on megakariocytes [11] which showed that emperipolesis was present in 68% and 66% of cases of thrombocytopenia which are caused by ITP and MDS, respectively.

Hyperpolyploidy was seen in 42.85% and, similarly, 42.85% of cases of thrombocytopenia which are caused by MDS and megaloblastic anemia. Such finding is correlated with a finding of a study on megakariocytes in MDS [12] which showed a figure of 49% for hyperpolyploidy in MDS.

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