

Open Access
Full Length Article

ORIGINAL ARTICLE

Frequency of Common Chromosomal Abnormalities in Patients with Idiopathic Acquired Aplastic Anemia

Imran Khalid¹, Nisar Ahmed², Abid Sohail³, Tariq Masood,⁴

¹ Assistant Consultant, Haematology Department, Akbar Niazi Hospital, Islamabad

² Professor/Head of Haematology Department, Children Hospital, Lahore

³ Professor/Head of Haematology Department, Khyber Medical University, Peshawar

⁴ Assistant Professor, Haematology Department, North West Hospital, Peshawar

ABSTRACT

Objective: To determine the frequency of common chromosomal aberrations in local population of idiopathic acquired aplastic anemia at the time of diagnosis, using G-banding cytogenetic analysis.

Patients and Methods: This cross sectional study was conducted in Department of Haematology, Pakistan Institute of Medical Sciences, and Islamabad and Department of Genetics, Children Hospital, Lahore from June 2015 to July 2017. Sample size was calculated using WHO sample size calculator. A total of sixty cases of peripheral blood pancytopenia having clinical suspicion of acquired aplastic anemia and diagnosed on bone marrow examination with aplastic anemia were included in the study. Bone marrow or peripheral blood samples were also processed for cytogenetics by G-banding and karyotyping according to International System for Human Cytogenetic Nomenclature (ISCN) to determine frequency of chromosomal abnormalities in the patients of acquired aplastic anaemia.

Results: Sixty cases diagnosed to have acquired aplastic anaemia using bone marrow examination as gold standard were included in the study based on inclusion criteria. Forty-five out of 60 patients (75%) had successful karyotyping whereas 15 out of 60 patients (25%) had inconclusive cytogenetics due to culture failure, inadequate metaphase cells and contamination. G-banding revealed normal karyotyping in 40 out of 45 patients (88.9%) while 5 out of 45 patients (11.1%) were found to have abnormal karyotyping. Chromosomal abnormalities revealed by abnormal karyotyping included three numerical abnormalities i.e. monosomy 7, trisomy 8, trisomy 14 and two structural abnormalities i.e. deletion of 11q, deletion of 13q. The frequency of chromosomal abnormalities in patients with acquired aplastic anaemia in this study was found to be 11.1%.

Conclusion: Cytogenetic analysis may be beneficial in differentiating acquired AA from other haemopoietic disorders of bone marrow failure, which may be missed, based on cell morphology alone. It also guides in deciding appropriate mode of treatment earlier and predicting prognosis of the disease.

Key words: Aplastic anemia, Chromosomal, Cytogenetic Nomenclature

Author's Contribution

¹ Conception, synthesis, planning of research and manuscript writing Interpretation and discussion

^{2,3} Data analysis, interpretation and manuscript writing, ⁴ Active participation in data collection.

Address of Correspondence

Imran Khalid

Email: doctorimrankhalid@gmail.com

Article info.

Received: September 20, 2018

Accepted: November 10, 2018

Cite this article. Khalid I, Ahmed N, Sohail A, Masood T. Frequency of Common Chromosomal Abnormalities in Patients with Idiopathic Acquired Aplastic Anemia. *JIMDC*.2018; 7(4):239-245

Funding Source: Nil

Conflict of Interest: Nil

Introduction

Aplastic anaemia is an immune mediated haemopoietic stem cell disorder characterized by pancytopenia with a hypocellular bone marrow in the absence of abnormal infiltrate and no increase in bone marrow reticulin.¹ Aplastic anemia is considered if at least two of the following defining criteria are fulfilled that includes haemoglobin level less than 10g/dl, neutrophil count less than $1.5 \times 10^9/L$, platelet count less than $50 \times 10^9/L$ and bone marrow cellularity less than 25% or 25 to 50% with less than 30% residual haemopoietic cells.² Disease severity of aplastic anemia is based on the criteria given by Camitta et al. in 1975.³ According to these criteria the severity of aplastic anemia is graded into very severe aplastic anaemia (VSAA), severe aplastic anemia (SAA) and non-severe aplastic anemia (NSAA). Severity of aplastic anaemia according Camitta criteria is based on blood cell counts and bone marrow cellularity.⁴ Grading of disease is significant in management decisions but has less prognostic value in terms of response to immunosuppressive therapy.⁵

Incidence of aplastic anaemia in West is around 2 per million populations in a year, that is twofold higher (3 to 4 per million population) in Asia.⁶ Aplastic anaemia can be congenital or acquired.⁷ Presence of somatic abnormalities and characteristic sensitivity of haemopoietic cells to chromosomal breakage on exposure to clastogenic agents such as diepoxybutane (DEB) and mitomycin c (MMC) is suggestive of congenital aplastic anemia.⁸ Some cases of congenital aplastic anemia may lack the characteristic phenotypic abnormalities.⁹ Constitutional mutations result in increased genomic instability and reduced cell survival in congenital aplastic anaemia that leads to increased chromosomal DNA damage by DNA crosslinking agents due to aberration in BRCA pathway.¹⁰ BRCA gene (a tumor suppressor gene) is involved in DNA damage response pathway; cells lacking BRCA protein are susceptible to chromosomal breakage after exposure to DEB or MMC.¹¹ Acquired aplastic anaemia is associated with various etiological factors that include different drugs, chemicals, ionizing radiations, pregnancy, autoimmune diseases, graft versus host disease and viral infections.¹² No definitive causative factor is found in majority of the

cases of acquired aplastic anaemia.¹³ Various drugs, chemicals, viral infections such as Hepatitis virus, Varicella Virus, Parvo virus, CMV, EBV, HIV and other factors such as Ionizing radiations, Smoking, Graft versus host disease etc. are associated with aplastic anemia.

Cytogenetic abnormalities have been described infrequently worldwide in few patients (12%) with otherwise typical aplastic anaemia at diagnosis.¹⁴ Common cytogenetic abnormalities related with idiopathic acquired aplastic anaemia include trisomy 6, trisomy 8, trisomy 14, trisomy 15, monosomy 7, monosomy 19, del 5q and del 7q.¹⁵

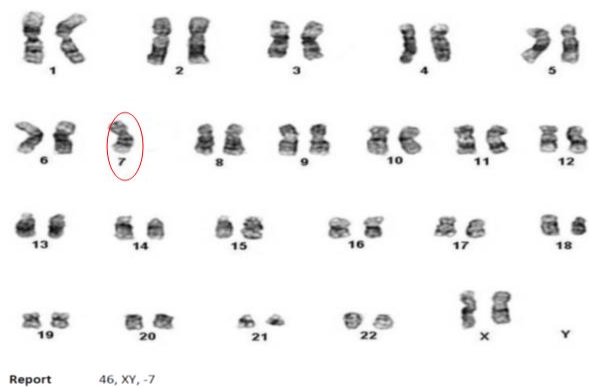


Figure 1: G-Banding Chromosomal analysis of peripheral blood cell culture reveals male karyotypewith a missing chromosome no.7 in 20 metaphase cells examined. This finding is consistent with diagnosis of Monosomy 7

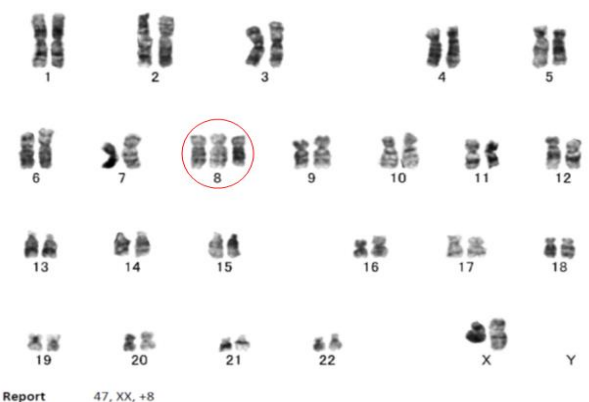


Figure 2: G-Banding Chromosomal analysis of bone marrow cell culture reveals female karyotype with an extra chromosome no.8 in 20 metaphase cells examined. This finding is consistent with diagnosis of Trisomy 8

Monosomy 7 is associated with a high risk to develop haematological malignancies (MDS or AML) and has poor response to immunosuppressive therapy (IST) with poor prognosis.¹⁶ In contrast, trisomy 8 is associated with good response to IST and has better prognosis.¹⁷ Cytogenetic analysis is attempted to detect cytogenetic abnormalities related with acquired aplastic anaemia, although it is difficult to perform because of inability to obtain sufficient metaphase cells for chromosomal analysis in a hypocellular bone marrow.¹⁸ In such situation, molecular cytogenetics by fluorescent in situ hybridization (FISH) can be attempted which is limited to few centers only and has a higher cost constraint.¹⁹ Clinical significance of cytogenetic abnormalities may also be that hypo plastic MDS or hypo plastic AML may present as aplastic anaemia and it can be difficult to distinguish two conditions on basis of morphology alone, treatment option and response to treatment is also different in such condition.²⁰

Cytogenetic analysis is limited to a few centers in Pakistan. Incidence of aplastic anemia is relatively more common in Southeast Asian countries. No study has been conducted before, to find association of cytogenetic abnormalities with acquired AA in Pakistan. The objective of this study was to determine frequency of common chromosomal aberrations among the patients of idiopathic acquired aplastic anemia in local population at the time of diagnosis by G-banding cytogenetic analysis.



Figure 3: G-Banding Chromosomal analysis of peripheral blood cell culture reveals female karyotype with an extra chromosome no.14 in 16 metaphase cells examined. This finding is consistent with diagnosis of Trisomy 14



Figure 4: G-Banding chromosomal analysis of bone marrow cell culture reveals male karyotype with deletion of q-arm of chromosome no.11 in 16 metaphase cells examined. (del 11q)

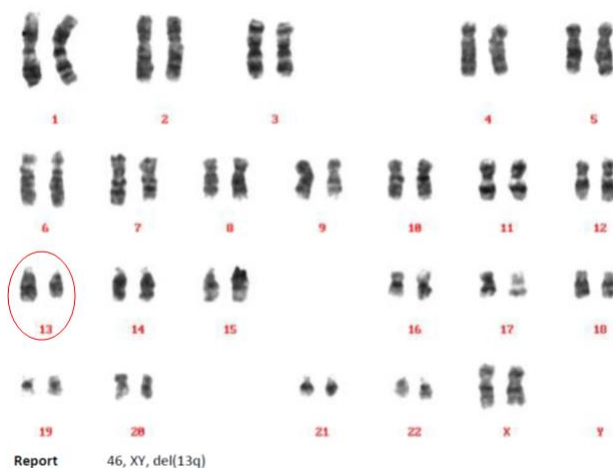


Figure 5: G-Banding chromosomal analysis of peripheral blood cell culture reveals female karyotype with deletion of q-arm of chromosome no.13 in 16 metaphase cells examined. (del 13q)

Patients and Methods

This cross-sectional study was conducted in Department of Genetics, Department of Haematology, Children Hospital, Lahore and Department of Haematology, Pakistan Institute of Medical Sciences (PIMS), Islamabad from June 2015 to July 2017. In total 64 participants (both male and female patients of all age groups) who presented to the Department of Haematology with peripheral blood pancytopenia having a clinical suspicion of acquired aplastic anemia., with features of bone marrow aplasia were included in the study. Patients with

congenital aplastic anaemia, patients with features of bone marrow dysplasia and abnormal infiltrates and patients with post chemotherapy and radiotherapy aplasia were excluded from the study. The study was approved by ethical board of each participating hospital. Informed consent was taken from the patients or their parents. Sample size was calculated according to WHO sample size calculator with 95% confidence interval, prevalence of 12% cases of AA with chromosomal abnormalities and 8% margin of error and sample size calculated was 60 and sampling was done by convenient sampling technique.

Demographic profile and clinical data of the patients was taken. Systemic examination was performed to find positive signs of anemia, haemorrhage, infections, lymphadenopathy, hepatomegaly, splenomegaly and especially any dysmorphic features to exclude congenital AA. Biochemical and radiological findings, CBC, reticulocyte count, peripheral blood smear and bone marrow (aspiration and trephine biopsy was done to confirm diagnosis of aplastic anemia Cytogenetics or chromosomal analysis was done by Giemsa Trypsin banding prior to induction of immunosuppressive therapy. Blood or Bone marrow samples were collected under aseptic measures in sterile sample collection tubes (green top vacutainers) containing sodium heparin anticoagulant to prevent coagulation. Samples were transported to genetics lab. at room temperature within 24 hours for cytogenetic analysis where they were processed for cell culture, culture harvesting, slide preparation and slide staining. Karyotyping was done according to (ISCN) International System for Human Cytogenetic Nomenclature²¹ metaphase and karyotype images were seen using cytovision system (microscope, camera, monitor, computer, software Macktype 5.6) for chromosome analysis. At least 16 to 20 metaphase cells were analyzed at each microscopic examination for a successful result. Cytogenetic analysis was labelled inconclusive if metaphase cells were less than 16. A cytogenetic abnormality was considered to exist when 2 or more cells had the same structural or numerical chromosomal abnormality (86). Data was recorded and analyzed using Statistical Package for Social Sciences (SPSS) Version 20. Median was calculated for Age of the study participants and Frequency (%) of chromosomal

abnormalities was calculated in the study patients diagnosed to have acquired aplastic anemia.

Results

Four out of 64 cases were excluded from the study as per exclusion criteria, among those two cases (3.1%) were diagnosed to have Myelodysplastic syndrome based on peripheral blood and bone marrow findings while two cases (3.1%) were diagnosed as Congenital aplastic anemia on chromosomal breakage analysis. Sixty out of 64 cases (93.8%) diagnosed to have acquired aplastic anemia on the basis of clinical, peripheral blood and bone marrow findings, were included in this study as per inclusion criteria. Diagnosis of aplastic anemia was confirmed by considering bone marrow findings as gold standard. Among 60 patients of acquired aplastic anemia, 34 were male and 26 were female. Age distribution of the study participants ranged from 1 to 84 years with a median age of 10 years. Most of the study participants were children as 52 patients (81%) were in age group of 1 - 18 years whereas 12 patients (19%) were in age group of 19 - 84 years.

Forty five out of 60 patients (75%) had successful karyotyping among those 23 were male and 22 were female whereas 15 out of 60 patients (25%) had inconclusive cytogenetics.

Table 1: Severity distribution of aplastic anemia among study patients

Severity	NSAA	SAA	VSAA
Male	27	5	2
Female	17	8	1
Total	44	13	3
Percentage	73.3%	21.7%	5%

Table 2: Frequency breakup of Cytogenetics results among study patients

Cytogenetics	Normal	Abnormal
Male	24	2
Female	16	3
Total	40	5
Percentage	89.9%	11.1%

Table 3: Categories of Chromosomal abnormalities in acquired aplastic anemia

Numerical abnormalities (N=3)	Monosomy 7
	Trisomy 8
	Trisomy 14
Structural abnormalities (N=2)	Deletion 11q
	Deletion 13q

Inconclusive cytogenetics can be due to cell culture failure, inadequate metaphase cells or contamination. G-banding revealed normal cytogenetics results in 40 out of 45 patients (89.9%) among those 24 were male and 16 were female, while five out of 45 patients (11.1%) had cytogenetic abnormalities among those two were male and three were female, table 2. Majority of the patients of acquired aplastic anemia (n=40) included in this study had normal cytogenetics. In total 44 patients had NSAA, 13 SAA and 3 patients had VSAA (Table 1). Among five patients with chromosomal abnormalities, three patients had severe aplastic anaemia (SAA) while two patients were diagnosed to have non-severe aplastic anaemia (NSAA). Chromosomal abnormalities revealed by G-banding in five patients of acquired aplastic anemia were numerical and structural. Three out of five patients had numerical chromosomal abnormalities among those one was male and two were female having monosomy 7, trisomy 8 and trisomy 14 respectively whereas two out of five patients had structural chromosomal abnormalities among those one was male and one was female having deletion of 11q and deletion of 13q respectively. All five patients of acquired aplastic anemia found to have abnormal cytogenetics in this study were in age group of 1 - 18 years. The frequency breakup of chromosomal abnormalities is depicted in table 3.

Discussion

Aplastic anaemia is haematological disorder of bone marrow failure characterized by T cell mediated destruction of haemopoietic cells.²² In the current study, cytogenetic abnormalities were observed in 11.1% patients of acquired aplastic anemia. Trisomy was the commonest numerical cytogenetic

abnormality seen in two patients followed by monosomy in one patient. Deletions were the structural cytogenetic abnormalities found in two patients. Clinical and haematological profile of the patients with abnormal cytogenetics were similar to those with normal cytogenetics.²³ Some of these characteristic cytogenetic abnormalities are also seen in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), however the morphological diagnosis was consistent with aplastic anemia. It may be difficult to distinguish two conditions on the basis of cell morphology alone therefore in this situation abnormal karyotype may suggest diagnosis of hypoplastic MDS or hypoplastic AML. Cytogenetic analysis can play a significant role in management of patients with cytogenetic interpretation, which can reduce risk of misdiagnosing hypoplastic MDS and hypoplastic AML as aplastic anemia, monosomy and trisomy are mostly seen in aplastic anemia, deletions favor diagnosis of MDS whereas translocations and inversions are commonly found in AML. Cytogenetic analysis in this way may be most beneficial in differentiating aplastic anemia from other haemopoietic disorders of bone marrow hypoplasia/aplasia, therefore guides in suggesting appropriate mode of treatment that helps in avoiding possibility of mistreating the patients. Some authorities exclude diagnosis of aplastic anaemia in patients with abnormal cytogenetics regardless of bone marrow morphology, however in some research centers patients with abnormal cytogenetics are diagnosed as aplastic anemia on morphological grounds.²⁴ Response to therapy and survival in patients of aplastic anaemia with normal and abnormal cytogenetics was also compared in some studies.²⁵ Patients without cytogenetic abnormalities have good response to immunosuppressive therapy (IST) and better survival therefore cytogenetic analysis helps in predicting prognosis of aplastic anemia. Patients with persistent cytogenetic abnormalities after treatment are at higher risk of developing haematological malignancies (AML or MDS). Patients of MDS presents with clinical

manifestations of bone marrow failure which are related to anaemia, neutropenia and thrombocytopenia.²⁶ Immunosuppressive therapy (IST) improves pancytopenia but response is relatively poor in patients of MDS.²⁷ In few patients, acquired aplastic anaemia may develop into acute leukemia.²⁸ Patients of AML presents with clinical features of bone marrow failure and organ infiltration by leukemic cells. Chemotherapy regimens have limited role in treating patients with AML.²⁹

In this study cytogenetic abnormalities in patients of acquired aplastic anemia were found to be 11.1% in our region. These findings are compatible with the study conducted by Vineeta Gupta et al. at Institute of Medical Sciences, Banaras University, India who found cytogenetic abnormalities to be 11.9% in patients of AA.³⁰ Similar figures are reported in various other studies, demonstrating chromosomal abnormalities in acquired AA.³¹ Some patients with acquired aplastic anaemia are at higher risk of evolving into MDS. It is sometimes difficult to distinguish between the two conditions.³²

Prognosis of the patients with acquired aplastic has improved with better supportive care of anaemia, infections and bleeding.³³ Estimated 10-years survival is 68% in patients receiving IST and 73% in patients with HSCT. Mode of treatment in patients with aplastic anaemia depends on age of the patients, children with HLA matched sibling donor are preferably treated with HSCT whereas adults without HLA matched sibling donor are generally treated with IST along with supportive therapy.

Approximately 25% patients of AA have inadequate response to therapy.³⁴ HSCT is the curative treatment option, however may be at additional risk of developing graft versus host disease (GVHD) and potential for graft rejection.³⁵

Conclusion

Cytogenetic analysis may be beneficial in differentiating acquired AA from other haemopoietic disorders of bone marrow failure, which may be

missed, based on cell morphology alone. It also guides in deciding appropriate mode of treatment earlier and predicting prognosis of the disease.

References

1. Victor Hoffbrand, Daniel Catovsky, Edward GD Tuddenham. Acquired aplastic anemia. In: Postgraduate Haematology, 6th edition. Wiley Blackwell, 2011.
2. Dezern AE, Brodsky RA. Clinical management of aplastic anemia. *Expert Rev Hematol.* 2011; 4(2):221 - 230.
3. Kulasekararaj AG, Jiang J, Smith AE, Mohamedali AM, Mian S, Gandhi S, et al. Somatic mutations identify a subgroup of aplastic anemia patients that progress to myelodysplastic syndrome. *Blood.* 2014; 124(17):2698 - 704.
4. Victor Hoffbrand, Paul Moss. Aplastic anaemia and bone marrow failure. In: Essential Haematology, 6th edition. John Wiley & Sons, 2011.
5. Wali R, Adil S, Naqvi MA. Aplastic anemia clinicohaematological features, treatment and outcome analysis. *J Coll Physicians Surg Pak.* 2011; 21(4):219 - 222.
6. Biswajit H, Pratim PP, Kumar ST, et al. Aplastic anemia a common hematological abnormality among peripheral pancytopenia. *N Am J Med Sci.* 2012; 4(9):384 - 88.
7. Niemeyer C, Baumann I. Classification of childhood aplastic anemia and myelodysplastic syndrome. *Am Soc Hematol Educ Program.* 2011; 2011:84 - 89.
8. Dokal I, Vulliamy T. Inherited bone marrow failure syndromes. *Haematologica.* 2010, 95: 1236 - 1240.
9. Bacigalupo A, Passweg J. Diagnosis and treatment of acquired aplastic anemia. *Hematol Oncol Clin North Am.* 2009, 23:159 - 170.
10. Victor Hoffbrand, Daniel Catovsky, Edward GD Tuddenham. Inherited aplastic anemia and bone marrow failure syndromes. In: Postgraduate Haematology, 6th edition. Wiley Blackwell, 2011.
11. Scheinberg P, Wu CO, Nunez O, Young NS. Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. *British Journal of Haematology.* 2009, 144(2): 206 - 216.
12. Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet.* 2012, 44(11):1179 - 1181.
13. Marsh JCW, Gordon Smith EC. Aplastic anaemia and other causes of bone marrow failure. In: Oxford Textbook of Medicine, 5th Edition. Oxford University Press, 2009.
14. Hussein K, Tefferi A. Conventional cytogenetics in haematological disorders. *European Journal of Haematology.* 2009, 82:329 - 338.
15. Afable MG, Wlodarski M, Makishima H, Shaik M, Sekeres MA, Tiu RV, et al. SNP array based karyotyping, differences and similarities between aplastic anaemia and hypocellular myelodysplastic syndromes. *Blood.* 2011, 117:6876 - 6884.

16. Scheinberg P, Wu CO, Nunez O, Boss C, Sloand EM, Young NS. Treatment of severe aplastic anemia with a combination of horse antithymocyte globulin and cyclosporine, a prospective randomized study. *Haematologica*. 2009, 94:348 – 354.
17. Tichelli A, Schrezenmeier H, Socie G, Marsh J, Bacigalupo A, Dührsen U, et al. A randomized controlled study in patients with newly diagnosed severe aplastic anemia receiving antithymocyte globulin (ATG), cyclosporine, with or without G-CSF. *Blood*. 2011, 117:4434 – 4441.
18. Kim SY, Lee JW, Cho BS, Eom KS. The characteristics and clinical outcome of adult patients with aplastic anemia and abnormal cytogenetics at diagnosis. *Genes Chromosomes Cancer*. 2010, 49:844 – 850
19. Passweg JR, Marsh JCW. Aplastic Anemia, First line Treatment by Immunosuppression and Sibling Marrow Transplantation. *Am Soc Hematol Educ Program*. 2010, 153:36 – 42.
20. Eapen M, Horowitz M. Alternative donor transplantation for aplastic anemia. *Am Soc Hematol Educ Program*. 2010, 2010:43 – 46.
21. Shaffer LG, Slovak ML, Campbell LJ. An International System for Human Cytogenetic nomenclature ISCN, 2009.
22. Xing J, Zhang Y, Han K, Salem AH, Sen SK, Huff CD, Zhou Q, Kirkness EF, Levy S, Batzer MA and Jorde LB. Mobile elements create structural variation: Analysis of a complete human genome. *Genome Res*. 2009, 19:1516 - 1526.
23. Li J, Yang S, Lu S, et al. Differential gene expression profile associated with abnormality of bone marrow mesenchymal stem cells in aplastic anemia. *PLOS One*. 2012, 7(11): 47764.
24. Breakey VR, Meyn S, Ng V, Allen C, Dokal I, Lansdorp PM, et al. Hepatitis-associated aplastic anemia presenting as a familial bone marrow failure syndrome. *J Pediatr Hematol Oncol*. 2009; 31(11):884 - 887.
25. Dezern AE, Brodsky RA. Paroxysmal nocturnal hemoglobinuria: a complement mediated hemolytic anemia. *Hematol Oncol Clin North Am*. 2015 Jun. 29 (3):479 - 494.
26. Guidelines for the Diagnosis and Management of Adult Aplastic Anaemia. British Committee for Standards in Haematology 2015.
27. Rathore S, Pramanick A, Regi A, Lionel J. Aplastic Anemia in Pregnancy. *Journal of Obstetrics and Gynaecology of India*. 2014, 64(1):26 - 28.
28. Tschiedel E, Gierenz N, Wieland R, Wulff B, Ballauff A. Severe aplastic anemia in six children after non A E hepatitis. *Z Gastroenterol*. 2010, 48(8):825 - 828.
29. Ferrer RA, Wobus M, List C, et al. Mesenchymal stromal cells from patients with myelodysplastic syndrome display distinct functional alterations that are modulated by lenalidomide. *Haematologica*. 2013, 98(11):1677 – 1685.
30. Gupta V, Kumar A, Saini I, et al. Cytogenetic profile of aplastic anaemia in Indian children. *Indian J Med Res*. 2013; 137(3):502 – 506.
31. Marsh JC, Ball SE, Cavenagh J, Darbyshire P, Dokal I, Gordon Smith EC, Keidan J, Laurie A, Martin A, Mercieca J, Killick SB. Guidelines for the diagnosis and management of aplastic anaemia. *British journal of haematology*. 2009; 147(1):43 - 70.
32. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016; 127 (20):2391 - 2405.
33. Gonzalez Casas R, Garcia Buey L, Jones EA, Gisbert JP, Moreno-Otero R. Hepatitis associated aplastic anaemia, a syndrome associated with abnormal immunological function. *Aliment Pharmacol Ther*. 2009; 30(5):436 - 443.
34. Geyh S, Oz S, Cadeddu RP, et al. Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. *Leukaemia*. 2013, 27(9):1841 – 1851.
35. Killick SB, Bown N, Cavenagh J, Dokal I, Foukaneli T, Hill A, Hillmen P, Mufti G, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. *Br J Haematol*. 2016; 172(2):187 – 207.