A clinico-hematological study of pancytopenia: An experience of a tertiary care teaching hospital, Jammu, India

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Abstract

Introduction: Pancytopenia is not a disease entity but a triad of findings that may result from a number of disease processes and is defined as simultaneous presence of anaemia, leucopenia and thrombocytopenia. Bone marrow aspiration along with trephine biopsy is important to find out the cause of pancytopenia. The present study was essentially a pilot study which was undertaken over a period of 2 years at a tertiary care teaching hospital. The aims and objectives of this study was to find out the epidemiological spectrum and prevalence of pancytopenia according to age and sex along with the correlation of the clinical, haematological and bone marrow findings of such patients. An attempt was also made to determine the etiopathogenesis of pancytopenia for ascertaining the individual pathological pattern (spectrum).

Materials and Method: The present study was conducted in the Postgraduate Department of Pathology, Government Medical College and Associated Hospitals, Jammu, India over a period of 2 years. A total of 100 patients were prospectively analyzed and were subjected to bone marrow examination.

Results: The prevalence of pancytopenia in our haematological set-up was observed to be 0.76%. Pancytopenia was seen to be more prevalent in males (62%) in comparison to females (38%) with male to female ratio of 1.64:1. In the present study, majority of cases were diagnosed as megaloblastic anemia (60%) followed by subleukemic acute lymphoblastic leukemia (11%), subleukemic acute myeloblastic leukaemia (4%) and Kala azar (3%). Myelopthisic due to metastatic carcinomatous deposits was the least common etiological factor accounting for one case only.

Conclusion: Detailed examination of peripheral smear reveals important information regarding etiology e.g. macro-ovalocytes with hypersegmented neutrophils in megaloblastic anemia, occasional blast cell in subleukemic leukemia, absence of any abnormal/immature cell in aplastic anemia, leucoerythroblastic picture in myelofibrosis, pelgeroid neutrophils in MDS and nRBC with abnormal cells in metastatic malignancies. However, the diagnostic accuracy is increased manifolds when detailed clinical history and examination are combined with complete blood counts and peripheral smear evaluation. Although, bone marrow study (bone marrow aspiration/bone marrow trephine biopsy) provides the definite diagnosis in cases of pancytopenia.

Keywords: Pancytopenia, Peripheral smear, Bone marrow, Spectrum, Etiology

Introduction

Pancytopenia is an important clinico-pathological entity encountered in our day to day clinical practice. It is not a disease entity but a triad of findings that may result from a number of disease processes. These disorders may affect bone marrow either primarily or secondarily, resulting in manifestations of pancytopenia and makes the patient prone to anaemic symptomatology, infection and haemorrhagic diathesis.[1,2]

According to the accepted definition by haematologists pancytopenia is defined as simultaneous presence of anaemia, leucopenia and thrombocytopenia. Therefore, it exists in human beings when the haemoglobin level is less than 10g/dl, the leucocyte count is less than 4x10/l and platelet count is less than 150 x10/l.[3]

Bone marrow examination (bone marrow aspiration and trephine biopsy) is considered essential and a cornerstone for diagnosis and management of most of the haematological disorders including pancytopenia.[4] This evaluation is done on the basis of cellularity, Myeloid: Erythroid (M:E) ratio,

abnormalities seen in erythroid, myeloid or megakaryocytic series, distribution of cells along with any infiltration amongst others. Depending upon the findings various etiological factors are determined e.g. nuclear cytoplasmic asynchrony, hyperplasia with presence of megaloblasts having open chromatin (maturation arrest at basophilic erythroblast), abnormal mitosis, large atypical giant myelocytes and metamyelocytes as seen in megaloblastic anemia.[5,6]

Although bone marrow aspiration is conclusive in many cases however, it's the trephine biopsy which is essential for diagnosis not only when a dry tap occurs as a consequence of the marrow being fibrotic or very densely cellular but it also allows a complete assessment of marrow architecture, the pattern of distribution and presence of any abnormal infiltrate.[7] Bone marrow biopsy in addition can be used for special stains and immunohistochemistry for further studies and in certain cases for further typing especially in lymphomas.[8]

The present study was essentially a pilot study which was undertaken over a period of 2 years at a tertiary care teaching hospital (northern most part of

India, Jammu) so as to take a glimpse of the pattern of pancytopenia in an area which is geographically demarcated from the rest of India having its own unique dietary pattern and customs. A comparison was also drawn with areas in and around Indian subcontinent and rest of the world. Such demographic case studies are often helpful in defining the burden of disease and to ascertain the spectrum of disease in population under study. They in many instances impart valuable knowledge and information to plan and implement various health programmes and remedial measures to limit the disease burden.

Aims and Objectives

1. To find out the epidemiological spectrum and prevalence of pancytopenia according to age and sex.
2. To correlate the clinical, haematological and bone marrow findings.
3. To find out the etiopathogenesis of pancytopenia for ascertaining the individual pathological pattern (spectrum).

Materials and Method

This study was carried out in the Postgraduate Department of Pathology, Government Medical College and Associated Hospitals, Jammu, India. The study was prospective in nature extending over a period of 2 years.

Patients with history of clinical features like pallor, fatigue, bleeding in the form of bruising or petechiae, persistent fever, bone or joints pains with or without organomegaly and /or lymphadenopathy, were taken up for detailed haematological analysis. Of these patients presenting with combinations of cytopenias and/or leucoerythroblastic blood picture and those who fulfilled the criterion of pancytopenia were also finally taken up for the study. In total 100 patients were selected.

All the patients were subjected to bone marrow examination. The technique of bone marrow aspiration and trephine biopsy and the methods of preparation, staining of slides and marrow sections used were those mentioned by Lewis.[9] The biopsy sample was then fixed overnight in 10% buffered saline and then decalcified biopsy core was processed in automatic tissue processor followed by embedding and staining. All the aspirate smears were stained by May-Grunwald-Giemsa (MGG) stain while trephine biopsy were stained by routine Haematoxylin & Eosin (H&E). The findings of trephine biopsy sections were studied for comprehensive details including marrow architecture, overall cellularity, pattern of involvement, metastatic foci or any abnormal cells, dysmegakaryopoiesis, granulomas, abnormal localization of immature precursors (ALIP), reticulin fiber density and thickness. Relevant clinical details were noted as per the clinical case sheets from the respective wards /units/departments where the patients were admitted or from outdoor tickets of the patient. In all the cases a detailed clinical history regarding nature and duration of illness, loss of weight, significant family history and drug history, if any, was taken. History of any chronic disease like tuberculosis, diabetes as well as hypothyroidism, was also noted. Both biopsy and aspirate were compared to each other for establishing a final diagnosis and to infer the cause of pancytopenia. The findings were also compared with other haematological parameters including peripheral smear examination. Observations thus obtained were recorded and analysed by means of descriptive statistics.

Results

In the present study, 100 patients who presented with pancytopenia during the period of 2 years were included after fulfilling the criterion designed at the start of the study. A total number of samples received in the Central Haematology Laboratory were 85,264. Out of these 13,158 cases who presented with clinical features like pallor, fatigue, bleeding in the form of bruising or petechiae, persistent fever, bone or joint pains, organomegaly and /or lymphadenopathy were taken up for detailed haematological analysis for evaluation of pancytopenia. Out of these, 100 cases which were diagnosed as pancytopenia and met the inclusion criterion were included in the study. Thus, the prevalence of pancytopenia in our haematological set-up was observed to be 0.76%.

Pancytopenia was seen more prevalent in males (62%) in comparison to females (38%) with male to female ratio of 1.64:1. In the current study, maximum number of cases were observed in the age group of 31-40 years (22%), followed by age group of 11- 20 years (17%). Least number of cases of pancytopenia were observed in the 70-80 years group (5%).

In the present study, majority of cases were diagnosed as megaloblastic anemia (60%) followed by aplastic anemia (16%), subleukemic acute lymphoblastic leukemia (ALL) (11%), subleukemic acute myeloblastic leukaemia (AML) (4%) and Kala azar (3%). Myelopthisic due to metastatic carcinomatous deposits was the least common etiological factor accounting for one case only. (Table 1)

The clinical presentation of generalised weakness and pallor was seen in all the cases (100%) followed by fatiguability (65%) and dyspnoea (52%). Lymphadenopathy and hepatomegaly which were the last two least common presentations were present in significant number of pancytopenic patients (14% and 12%).

In this study, megaloblastic anemia was the commonest cause of pancytopenia which was seen almost exclusively in vegetarians with most common presenting symptom being generalised weakness followed by dyspnoea, fever, splenomegaly and

bleeding manifestation. Lymphadenopathy or icterus were absent in these patients.

The haematological parameters of RBC morphology on peripheral smear examination in relation to various etiologies of pancytopenia in the present study have been detailed. (Table 2) Parameters such as polychromasia (44%) followed by ovalocytosis were the most commonly recorded. (Fig. 1A)

The total haemoglobin concentration in different causes of pancytopenia were graded in three categories (according to severity) as 8 to 10 (category I), 6 to 8 (category II) and less than 6 (category III). Most of the etiological causes were seen in category III (43%). The individualized haemoglobin concentration according to various etiologies have been detailed. (Table 3)

In similar way, total leucocyte count (TLC) in different causes of pancytopenia was graded as 3000 to 4000/cumm (category I), 2000 to 3000/cumm (category II) and less than 2000/cumm (category III). Most of the causes were categorized under category I (59%), category III accounted for least number of cases (6%) with all the cases diagnosed as aplastic anemia. (Table 4, Fig. 1B)

Distribution of platelet count in different causes of pancytopenia was again categorised as; 1 to 1.5 lakh (category I), 50,000 to 1 lakh (category II) and less than 50,000 (category III).

Majority of the cases of pancytopenia were seen in category I (41%) while Category III (21%) was seen in least number of cases. (Table 5) More than half cases in category III were those of aplastic anemia.

Similarly reticulocyte count was also categorised in different causes of pancytopenia as Category I decreased < 0.5%, Category II –Normal (0.5 to 2.5%) and Category III –Increased (2.5%). Expectedly most of the cases of pancytopenia (67%) were seen in category II and only 8 cases out of 100 showed an increased reticulocyte count of which megaloblastic anaemia accounted for 2/3rd of these cases and the remaining increase in count was evident in patients of Leishmaniasis. (Table 6, Fig. 1C)
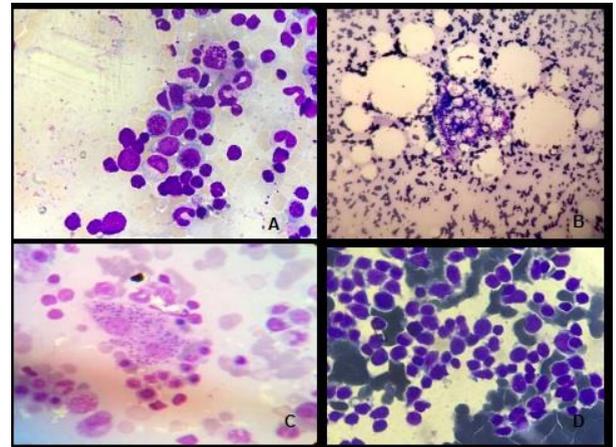

Fig. 1A: Smears prepared showing megaloblasts. (MGG, 400X]; Fig. 1B: Hypoplastic fragments in aplastic anemia. (MGG, 200X); Fig. 1C: Leishmania donovani bodies in macrophages of marrow aspirate (MGG, 400X); Fig. 1D: Blasts of acute leukemia (MGG, 400X)

Table 1: Etiopathogenesis of Pancytopenia

Causes	No. of cases	Percentage (%)
Megaloblastic anemia	60	60.00
Aplastic anemia	16	16.00
ALL (subleukemic)	11	11.00
AML (subleukemic)	4	4.00
Kala –azar	3	3.00
Multilple myeloma	2	2.00
Myelofibrosis	2	2.00
MDS	1	1.00
Metastasis	1	1.00
Total	100	100.00

Table 2: RBC morphology in different causes of pancytopenia (n=100)

RBC morphology	MA	AA	ALL	AML	Kala azar	MM	MF	MDS	Mets
Normocytic normochromic	-	2	1	1	-	-	-	-	-
Normocytic hyperchromic	-	4	6	2	-	2	-	-	-
Microcytic hypochromic	-	1	1	2	-	-	-	-	1
Macrocytic predominance	38	8	1	-	1	-	-	1	-
Dimorphic blood picture	22	-	2	-	2	-	2	-	-
Ovalocytes	40	1	-	-	-	-	1	-	-
Tear drop cells	2	-	-	-	-	-	2	-	-
Poly chromasia	44	-	-	-	-	-	-	1	-
Cabot rings	15	1	-	-	-	-	-	1	-
Basophilic stipling	12	1	-	-	-	-	-	1	-
Fragmented RBCs	2	-	3	1	-	-	-	-	-
nRBCS/100 WBCS	26	-	7	4	1	2	2	1	1

*- MA: Megaloblastic Anemia; AA: Aplastic Anemia; ALL: Acute Lymphoblastic Leukemia; AML: Acute Myeloiud Leukemia; MM: Multiple Myeloma; MF: Myelofibrosis; MDS: Myelodysplastic Syndrome; Mets: Metastasis, nRBCs: Nucleated red blood cells.

Table 3: Total Haemoglobin concentration in different causes of pancytopenia (n=100)

Diagnosis	8-10 gm /dl	6-8gm/dl	<6gm/dl
Megaloblastic anemia	15 (25.00)	20(33.33)	25 (41.67)
Aplastic anemia	2 (12.50)	6 (37.50)	8 (50.00)
ALL (sub leukemic)	-	6 (54.54)	5 (45.45)
AML (sub leukemic)	-	2(50.00)	2 (50.00)
Kala – azar	-	2 (66.67)	1 (33.33)
Multiple myeloma	-	2 (100.00)	-
Myelofibrosis	-	1 (100.00)	1 (50.00)
MDS	-	1 (100.00)	-
Metastasis	-	-	1 (100)
Total	17 (17.00)	40 (40.00)	43 (43.00)

Table 4: Total leucocyte count in different causes of pancytopenia (n=100)

Diagnosis	3000-400	2000-3000	<2000
Megaloblastic anemia	42(70.00)	18 (30.00)	-
Aplastic Anemia	6(37.50)	4 (25.00)	6 (37.50)
ALL (sub leukemic)	4 (36.37)	7 (63.63)	-
AML (sub leukemic)	1 (25.00)	3 (75.00)	-
Kala-azar	3 (100.00)	-	-
Multiple myeloma	2 (100.00)	-	-
Myelofibrosis	-	2 (100.00)	-
MDS	1 (100.00)	-	-
Metastasis	-	1 (100.00)	-
Total	59 (59.00)	35 (35.00)	6 (6.00)

Table 5: Distribution of platelet count in different causes of pancytopenia (n=100)

Diagnosis	100,000-1,50,000	50,000-100,000	<50,000
Megaloblastic anemia	34 (56.67)	20 (33.33)	6 (10.00)
Aplastic anemia	-	5 (31.25)	11(68.75)
ALL (sub leukemic)	2(18.18)	6 (54.54)	3 (27.28)
AML (sub leukemic)	-	2 (50.00)	2 (50.00)
Kala -azar	2 (66.67)	1(33.33)	-
Multiple myeloma	1 (50.00)	1(50.00)	-
Myelofibrosis	1(50.00)	1 (50.00)	-
MDS	-	1 (100.00)	-
Metastasis	-	1 (100.00)	-

Table 6: Distribution of reticulocyte count in different causes of pancytopenia (n=100)

Diagnosis	Decreased (<0.5%)	Normal (0.5-2.5%)	Increased (>2.5%)
Megaloblastic anemia	-	55 (91.70)	5 (8.30)
Aplastic anemia	16 (100.00)	-	-
ALL (subleukemic)	6 (54.54)	5 (45.46)	-
AML (sub leukemic)	3 (75.00)	1 (25.00)	-
Kala -azar	-	-	3(100.00)
Multiple myeloma	-	2 (100.00)	-
Myelofibrosis	-	2 (100.00)	-
MDS	-	1 (100.00)	-
Metastasis	-	1 (100.00)	-

Discussion

Pancytopenia is either an expression of bone marrow against antigens/tissue destructive agents or replacement of the normal bone marrow elements by abnormal or malignant tissue. In many cases, there is idiopathic suppression of bone marrow's normal cellular proliferation and differentiation of apparently normal hematopoietic elements.

Many underlying mechanisms in these conditions are ineffective haematopoiesis with cell death in the marrow, formation of defective cells that are rapidly removed from circulation, antibody mediated sequestration and destruction of cells and trapping of normal cells in a hypertrophied and over-active reticuloendethelial system.

Although in India, there have been many studies on the frequency of various causes of pancytopenia but not much is documented from northern plains of Jammu. It is thought that various local, cultural, socioeconomic factors including dietary parameters play a significant role in pathogenesis of pancytopenia.

In the present study of pancytopenic patients, the maximum prevalence of pancytopenia was seen in the age group 31-40 years (22%), followed by 11-20 years age group (17%). Likewise, Khodke et al[5] found the maximum number of pancytopenia in the age group of 12-30 years. However, Nazi and Razi[10] in their study found most common age group of pancytopenia in the range from 21 to 30 years.

The male to female ratio in the present study was 1.64:1 which is approximately the same as in the study by Jha et al[11] while the study of Khodke et al[5] found male to female ratio of 2:1.

In the present study the disease processes resulting in pancytopenia in the peripheral blood in order of decreasing frequency were megaloblastic anemia (60%), aplastic anemia (16%), subleukemic ALL (11%), subleukemic AML (4%), Kala-azar (3%), multiple myeloma (2%), MDS (1%) and metastasis (1%). This finding corresponded with the findings of studies done by Tilak and Jain,[12] Khodke et al[5] and Khunger et al[13] who in their studies found megaloblastic anemia in 68%, 44% and 72% respectively as the most common cause of pancytopenia. The second most common cause of pancytopenia in the present study was aplastic anemia which was similar to the study done by Khunger et al.[13] Subleukemic ALL (11%) and subleukemic AML (5%) were the third and fourth most common cause of pancytopenia in the present study. This finding was in concordance with the studies done by Menon et al[14] and Kumar et al[15] who described subleukemic leukemia as the third most common cause of pancytopenia. (Fig. 1D)

Kala-azar was fifth common cause of pancytopenia in the present study. Tilak and Jain,[12] Khodke et al[5] mentioned leishmaniasis as an important cause of pancytopenia. Most of the patients in the present study

had migrated from endemic area and presented with fever and hepatosplenomegaly.

Multiple myeloma, myelofibrosis, MDS and metastasis to bone marrow were other relatively less common causes of pancytopenia in the present study. Nevertheless, they do occur as is mentioned in the various studies enumerated above.

The high prevalence of nutritional anemia in India has been cited for increase in frequency of megaloblastic anemia. The geographical, socio-economic and food habits we share with our neighbouring countries may be responsible for the high incidence of megaloblastic anemia in our country. Chronically ill patients have been reported to develop acute folic acid deficiency which lead to megaloblastic changes in the marrow and present as pancytopenia.

In this study, 15 (25%) and 8 (13.4%) cases of megaloblastic anemia presented with splenomegaly and hepatomegaly. Ishtiaq et al[16] in their study found 6 (15.4%) and 7(17.9%) of megaloblastic anemia with splenomegaly and hepatomegaly respectively. It is important to evaluate other possible causes of pancytopenia such as acute malignancy besides megaloblastic anaemia when there is associated lymphadenopathy along with hepatosplenomegaly.

Epidemiologically, aplastic anemia has a pattern of geographic variation opposite to that of leukemia with higher frequency in the developing world than in the industrialised west.

Four patients in this group were farmers and 2 were painters by profession. Taking this into consideration, the likelihood of the farmers being exposed to insecticides and painters to chemical like benzene. These should be considered as possibilities for causing aplastic anemia as shown by the work of Snyder et al[17] in which they have found association with aplastic anemia.

The incidence of haematological malignancies presenting with pancytopenia in pediatric age group and adults in the study by Jha et al[11] was 21.42%. It was 15% in our study. However, Gupta et al[18] in their study found that acute leukemia was the second most common cause of pancytopenia in cases of children.

The incidence of visceral leishmaniasis can be due to the changes in the animal reservoir and vectors, and in the immunity status of part of the population exposed to leishmania. The World Health Organization estimates that approximately 500,000 new cases of this potentially fatal disease occur each year, more than 90% of which are acquired in parts of the Indian subcontinent, Sudan and Brazil. CDC[19] and Sudeck[20] in their studies described that if a person was exposed to an endemic area and gives the history of fever, hepatosplenomegaly with pancytopenia then one should consider the possibility of Kala-azar. The cases in this studies were exposed to endemic area (Bihar) and they had the history of fever with hepatosplenomegaly and presented as pancytopenia. Sud et al[21] and Sever-

Prebilic et al[22] have reported the presence of visceral leishmaniasis in non-endemic area also. In this study 3% of the total cases of pancytopenia were diagnosed to have visceral leishmaniasis.

Conclusion

Detailed examination of peripheral smear reveals important information regarding etiology e.g. macro-ovalocytes with hypersegmented neutrophils in megaloblastic anemia, occasional blast cell in subleukemic leukemia, absence of any abnormal/immature cell in aplastic anemia, leucoerythroblastic picture in myelofibrosis, pelgeroid neutrophils in MDS and nRBC with abnormal cells in metastatic malignancy. The diagnostic accuracy is increased manifolds when detailed clinical history and examination are combined with complete blood counts and peripheral smear evaluation. Bone marrow study (bone marrow aspiration/bone marrow trephine biopsy) provides the confirmation of the diagnosis. Clinical discussion by the haematopathologist with the treating clinician prior to an invasive procedure like bone marrow aspiration/biopsy shall go a long way in overall management of these patients. This interaction shall also guide us for further action that may include not only identifying the underlying cause of pancytopenia but also giving targeted investigations and therapy in such patients. This will in turn save time, avoid unnecessary investigations, reduce hospital stay of the patient and ensue prompt treatment.

References

1. Gayathri BN, Rao KS. Pancytopenia: A clinico hematological study. J Lab Physicians. 2011;3(1):15-20.
2. Dasgupta S, Mandal PK, Chakrabarti S. Etiology of Pancytopenia: An Observation from a Referral Medical Institution of Eastern Region of India. J Lab Physicians. 2015;7(2):90-5.
3. de Gruchy's Clinical Haematology in Medical Practice, 5th edition. F Firkin, C Chesterman, D Penington, B Rush. Wiley-Blackwell scientific publications. Oxford University Press, 1989.
4. Mannan R, Manjari M, Sharma S, Bhatia K, Singh G, Bhasin T. Documentation of Myeloproliferative Disorder as the Commonest Hematological Malignancy in Predominant Rural Based Pilot Study at Punjab (India): An Incidental Finding or Association. Annals of Pathology and Laboratory Medicine. 2016;3(3):A237-43.
5. Khodke K, Marwah S, Buxi G, Yadav RB, Chaturvedi NK. Bone marrow examination in cases of pancytopenia. J Indian Acad Clin Med. 2001;2:55-9.
6. Dasgupta A, Padma SK, Sajitha K, Shetty J. Etiological Evaluation of Pancytopenia in a Tertiary Care Hospital. Annals of Pathology and Laboratory Medicine. 2016;3(5):A441-50.
7. Safaei A, Shokripour M, Omidifar N. Bone Marrow and Karyotype Findings of Patients with Pancytopenia in Southern Iran. Iran J Med Sci. 2014;39(4):333-40.
8. Azaad M A, Li Y P, Zhang Q R, Wang H X. Detection of pancytopenia associated with clinical manifestation and their final diagnosis. Open J Blood Dis. 2015;5:17-30.
9. Lewis M. Miscellaneous test. In: Lewis SM, Bain BJ and Bates I, editors. Dacie and Lewis Practical Haematology, 10th edition. Philadelphia: Churchill Livingstone, 2006:595-7.
10. Nazi M, Raziq F. The incidence of underlying pathology in pancytopenia. JPMI 2004;18 (1):76-9.
11. Jha A, Sayami G, Adhikari RC, Panta AD, Jha R. Bone marrow examination in cases of pancytopenia. JNMA J Nepal Med Assoc 2008; 47 (169); 12-7.
12. Tilak V, Jain R. Pancytopenia--a clinico-hematologic analysis of 77 cases. Indian J Pathol Microbiol.1999;42(4):399-404.
13. Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia--a clinico haematological study of 200 cases. Indian J Pathol Microbiol. 2002;45(3):375-9.
14. Memon S, Shaikh S, Nizamani MA. Etiological spectrum of pancytopenia based on bone marrow examination in children. J Coll Physicians Surg Pak. 2008;18(3):163-7.
15. Kumar R, Kalra SP, Kumar H, Anand AC, Madan H. Pancytopenia-a six year study. J Assoc Physicians India. 2001;49:1078-81.
16. Ishtiaq O, Baqai HZ, Anwer F, Hussai N. Patterns of pancytopenia patients in a general medical ward and a proposed diagnostic approach. J Ayub Med Coll Abbottabad. 2004;16(1) :8-13.
17. Snyder R. Leukemia and benzene. Int J Environ Res Public Health. 2012;9:2875-93.
18. Gupta V, Tripathi S, Tilak V, Bhatia BD. A study of clinico-haematological profiles of pancytopenia in children. Trop Doct. 2008;38(4):241-3.
19. Centers for Disease Control (CDC) and Prevention. Two cases of visceral leishmaniasis in U.S. military personnel--Afghanistan, 2002-2004. MMWR. 2004;53(12):265-8.
20. Sudeck H. Kala Azar. Rare import and significant differential diagnosis. Internist (Berl). 2006;47(8):825-8.
21. Sud A, Varma N, Marwaha RK, Patel FM, Trehan A, Singh S, et al. Visceral leishmaniasis in a non-endemic area of India. Trop Doct. 2004;34(4):247-9.
22. Sever-Prebilić M, Prebilić I, Seili-Bekafigo I, Dokić S, Ivaniš N, Načinović-Duletić A, et al. A case of visceral leishmaniasis in the Northern Adriatic region. Coll Antropol. 2002;26(2):545-50.