Utility of blood smear and bone marrow smear examination in autopsy practice: A preliminary observation

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Abstract

Autopsy diagnosis rests on observation of gross anatomical features, microscopic examination and toxicological analysis. However, certain diseases need additional laboratory procedures for confirming diagnosis or ruling out diagnosis. Examination of blood film and bone marrow smear has considerable diagnostic and therapeutic importance in clinical setup. In the present study we have found encouraging morphological findings in blood and bone marrow smears for arriving at diagnosis in few conditions and the method can be utilized as supplementary laboratory procedure for confirming or ruling out diagnosis.

Key words: autopsy, death, bone marrow, blood smear, diagnosis

Introduction

Traditionally, autopsy diagnosis primarily rests on observation of gross anatomical features, microscopic examination and toxicological analysis. On certain instances, radiological examination may be needed and now many centers in India are taking help of such modality (1). However, certain diseases need additional laboratory procedures for confirming diagnosis or ruling out diagnosis. In recent times, many researchers have employed variety of laboratory procedures to compliment autopsy diagnosis and include application of immunohistochemical methods, DNA detection of malaria by polymerase chain reaction & immunochromatographic rapid test, diagnosis of sickle cell disease by molecular analysis of the β globin gene, detection specific meningococcal polysaccharide by latex agglutination assay, ELISA, detection of specific IgE in snake bite or wasp/bee sting envenomation, various biochemical methods, enzyme study, cytological methods and so on (2-10). Examination of blood smear and bone marrow smear is also an additional investigation that can be tried to reach the cause of death. This short communication aimed to highlight the importance of these conventional laboratory means in autopsy practice.

Case report 1

A 30-year male was brought for forensic autopsy as a case of sudden death. He was known case of chronic myeloid leukemia. While traveling to Mumbai for treatment he sustained sudden cardiac arrest. On external examination, the skin was having multiple purpuras. No injury was evident. On internal examination, brain was congested and oedematous. White matter showed multiple petechial hemorrhages. Lungs were congested and oedematous. Heart was unremarkable

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except petechial hemorrhages over epicardium. Liver and kidneys were congested. Spleen was enlarged weighing 1160 gm. Microscopic examination revealed leukemic cells in cerebral vessels and pulmonary vessels. Myocardium, liver, spleen and kidney showed infiltration by lukemic cells.

Case report 2

A 37-year male was admitted at Govt. Medical College Nagpur for 5 days. A probable diagnosis of aplastic anemia with pancytopenia with bleeding tendency was kept by treating doctors. Being jail inmate, a forensic autopsy was conducted. At autopsy, external examination showed multiple ecchymotic areas over trunk and limbs. Brain was congested. Both pleural cavities contain 1000 ml blood tinged fluid. Lungs were congested and patchy consolidation was noted. Heart was unremarkable. Peritoneal cavity contains 1500 ml blood tinged fluid. Spleen was enlarged and weighing 400 gm. Other abdominal organs were congested. Microscopic examination of cerebrum and cerebellum revealed congestion. Myocardium showed congestion and lymphocytic infiltrate. Lung showed pulmonary edema, congestion, patchy consolidation and caseating granulomas. Special stains for acid-fast bacilli (AFB) were positive (Fig 1). Liver showed multiple caseating granulomas and special stains for AFB were positive. Spleen was congested with hemorrhages and foci of necrosis were noted. Kidney showed glomerular sclerosis, congestion, tubular atrophy, interstitial fibrosis and multiple foci of chronic inflammatory infiltrate with multiple granulomas. The blood smear and bone marrow smears were prepared in both cases and the findings are presented in table 1.

Table 1. showing blood smear and bone marrow smear findings along with postmortem interval

Case no.	Postmortem interval	Blood smear	Bone marrow
1	4 hours	RBCs normocytic to microcytic, mild to moderate hypochromia, DLC – blast 70%, promyelocytes and myelocytes 25%, lymphocytes 5%. Sudan Black stain – blasts were positive indicating myeloid nature	Hypercellular marrow; marrow show mostly blast cells. Erythropoiesis and megakaryopoisis was suppressed
2	7 hours	RBCs showed microcytic hypochromic, anisocytosis present, WBCs showed leucopenia with predominantly lymphocytes, platlets depleted	Cellular marrow with bone marrow predominantly occupied by degenerative (morphology not clear for interpretation). Erythropoiesis – few erythroid precursors i.e. normoblast seen. Granulopoiesis – few mature cells seen. Megakaryocytes not seen. Slight increase of normal plasma cells seen

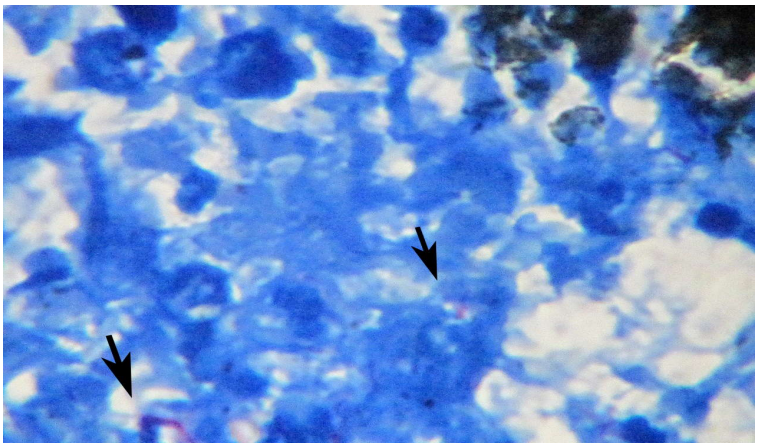

Figure 1. microphotograph showing acid fast bacilli in section of lung (arrow); anthracotic pigment seen at right upper corner (AFB, X 100)

Material and methods

Thin blood smears were prepared by blood obtained from heart. The smears were air dried and stained with Leishman's stain in standard way. Bone marrow was aspirated from the sternum. The smears were prepared immediately from the marrow aspirate and were fixed in 95% methanol after air-drying. Using standard procedure, Leishman's stain was used for staining. In addition, in case no. 1, Sudan Black stain was used as special stain. Additional sets were prepared and sent to Department of Pathology for confirming the findings.

Results

The results obtained from blood film and bone marrow smears are presented in table no. 1 against the postmortem interval (Fig 2, 3 & 4). In case no. 1, considering the splenomegaly, the diagnosis of blast crisis in chronic myeloid leukemia was kept. In case no. 2, a diagnosis of disseminated tuberculosis with pancytopenia secondary to tuberculosis was considered.

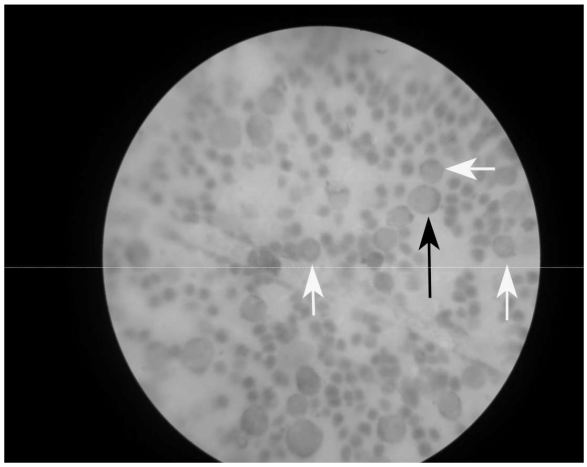

Figure 2. microphotograph showing blood smear with promyelocytes (black arrow) and myeloblast (white arrow) (Leishman's, X 100)

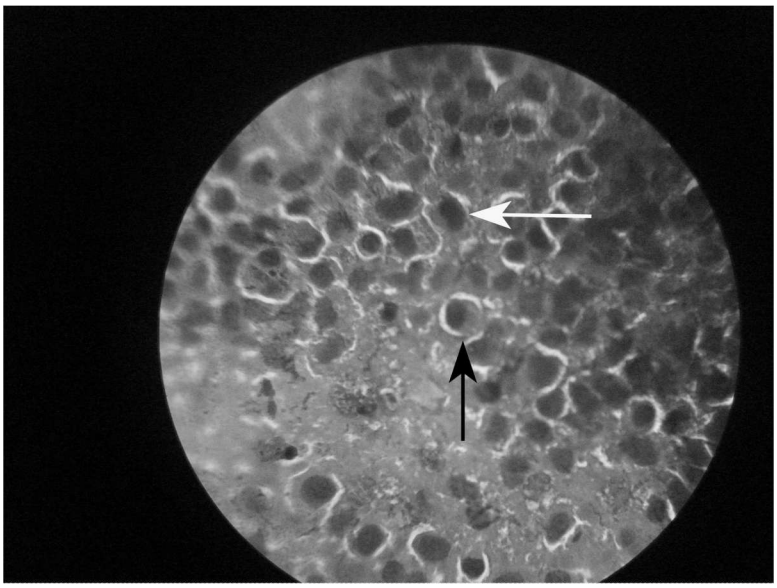

Figure 3. microphotograph showing bone marrow smear with myelocytes (white arrow), metamyelocytes (black arrow) and blast cells (Leishman's, X 45)

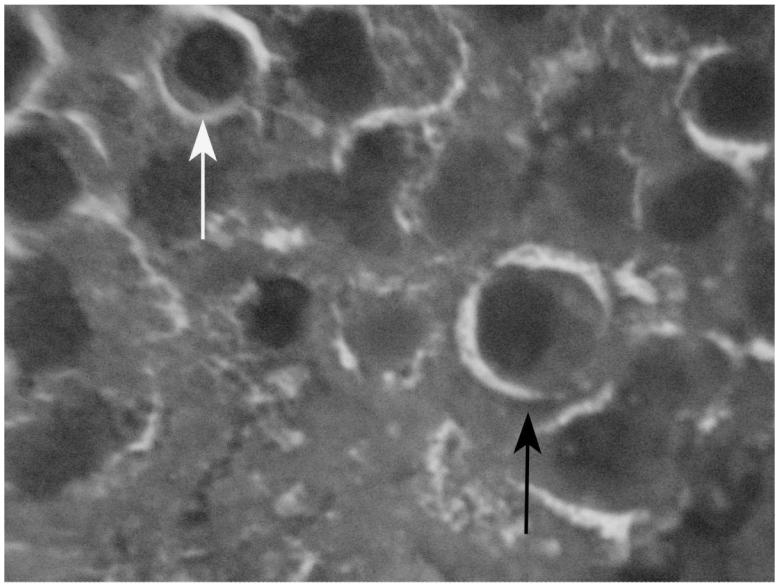

Figure 4. microphotograph showing bone marrow smear with blasts (white arrow) and promyelocyte (black arrow) (Leishman's, X 100)

Discussion

It is well known that sudden deaths have varied presentation. These deaths are referred to forensic doctors for autopsy examination and formulating the cause of death. The sudden death may be due to cardiovascular, respiratory, gastrointestinal, nervous system or genitourinary aliments or may be due to disseminated malignancies, hemolymphatic malignancies, infective diseases like malaria, leptospirosis, dengue, hepatitis etc (11, 12). Under such circumstances, in addition to conventional histopathological examination, the autopsy surgeon need additional laboratory means.

However, unlike clinical laboratory investigation, postmortem laboratory investigations have inherent limitations due to ongoing postmortem changes in body fluid and tissues.

Examination of blood film and bone marrow smear has considerable diagnostic and therapeutic importance in clinical setup however, the same do not hold true in autopsy practice. The postmortem changes occurring after death affects the cellular elements making difficult to read or interpret these films (13, 14). Moreover, the hostile climatic condition of our country, especially summer season adds woes. Still, if these films are interpreted with caution, they may have considerable diagnostic importance. In a recent study conducted for ascertaining postmortem interval from the morphology of blood cells, it was observed that red blood cells (RBCs) could be identified up to 18 hour, platelets up to 20 hours and lymphocytes up to 24-27 hours in postmortem period (15). Similarly Laiho et al demonstrated that about 30% of the bone marrow cells are viable with a postmortem time of over 200 hours (16).

The results of present study are encouraging and up to 7-hour postmortem interval, the blood and marrow smears can be interpreted. Further studies are warranted to ascertain the effect of lengthening postmortem interval over blood and marrow smears and up to what postmortem interval the smears could be interpreted?

In case no. 1, the patient was known case of chronic myeloid leukemia and autopsy revealed presence of leukemic cells in cerebral and pulmonary vessels. The myocardium, liver, spleen and kidneys showed infiltration by leukemic cells. Bone marrow revealed blast cells. In this case diagnosis of blast crisis in chronic myeloid leukemia was considered and was straightforward. However, in case no. 2, clinically the diagnosis of aplastic anemia was kept. Histopathological examination revealed disseminated tuberculosis. The deceased was having splenomegaly and microscopic examination showed hemorrhage and foci of necrosis. Antemortem peripheral smear revealed features of pancytopenia. At autopsy, the bone marrow was cellular and infiltrated by mononuclear cells. Therefore a diagnosis of disseminated tuberculosis with suppressed marrow activity due to tubercular infection was considered. Pancytopenia is a state characterized by the simultaneous presence of anemia, leucopenia and thrombocytopenia. In case of aplastic anemia, the aspirated bone marrow will be dilute on smear. The specimen will reveal stromal cells, residual lymphocytes and fatty cells. Apart from aplastic anemia, hypersplenism can also cause pancytopenia. But in this condition, the marrow does not show any specific changes rather it is hypercellular due to active erythropoiesis and leucopoiesis (17). Similarly the condition has to be differentiated from multiple myeloma. In multiple myeloma, abnormal plasma cells in the marrow are noted along with pancytopenia (11). Overwhelming infection like disseminated tuberculosis can produce pancytopenia and clinically the condition is indistinguishable from aplastic anemia. Bone marrow examination is capable of differentiating between these conditions, as cellularity in disseminated tuberculosis is greater than aplastic state. Furthermore, for additional confirmation, the bone marrow can be subjected to Ziehl-Nielsen staining for presence of tubercular bacilli (17). In most of the condition, the bone marrow is usually readily aspirated and will be sufficient to make smears. However, a dry tap suggests fibrosis, myelophthisis or aplasia. Under such circumstances a trephine biopsy is generally recommended for making appropriate diagnosis.

Conclusion

The present morphological observation for blood and bone marrow smears are encouraging and help to arrive at diagnosis in few conditions. If the films are examined in early postmortem period and cautiously interpreted then the method could prove good supplementary laboratory mean for confirming or ruling out diagnosis.

References

1. Gauda HS, Mestri SC. Piecemeal or whole body X ray in firearm injuries – a case report. J Indian Acad Forensic Med 2008;30:231-4.
2. Becker K, Ortmann C, Bajanowski T, Brinkmann B, Peters G. Use of polymerase chain reaction for postmortem diagnosis of malaria. Diagn Mol Pathol 1999; 8: 211-5.
3. Bardale R. Diagnosis of malaria at autopsy: Preliminary observations with immunochromatographic rapid test. J Forensic Med Toxicol 2008; 25: available at www.indianjournals.com
4. Kutlar F, Mirmov D, Glendenning M, Holley L, Kutlar A. Postmortem molecular diagnosis of sickle β thalassaemia. J Clin Pathol 2005; 58: 548 – 9.
5. Challener RC, Morrissey AM, Jacobs MR. Postmortem diagnosis of meningococcemia by detection of capsular polysaccharides. J Forensic Sci 1988; 33: 336 – 46.
6. Selvanayagam ZE, Gnanavendhan SG, Ganesh KA, Rajagopal D, Rao PV. ELISA for the detection of venoms from four medically important snakes of India. Toxicon 1999; 37: 757 – 70.
7. Schwartz HJ, Squillace DL, Sher TH, Teigland JD, Yunginger JW. Studies in stinging insect hypersensitivity: postmortem demonstration of antivenom IgE antibody in possible sting-related sudden death. Am J Clin Pathol 1986; 85: 607 – 10.
8. Robert V, Stewart MD. Postmortem diagnosis of myocardial disease by enzyme analysis of pericardial fluid. Am J Clin Pathol 1984; 82: 411 – 7.
9. Luna A, Carmona A, Villanueva E. The postmortem determination of CK isoenzymes in the pericardial fluid in various causes of death. Forensic Sci Int 1983; 22: 23 – 30.
10. Luna A, Gomez Zapata M, Vicente V, Osuna E. A cytologic study of the sediments in pericardial fluid as it relates to a diagnosis of the mechanism of death. Z. Rechtsmed 1987;99:129-34.
11. Shah VB, Deokar MS, Modi CJ, Lad S, Rupani AB, Meenakshi B. The different facets of haemolymphatic malignancies – an autopsy study of 10 cases. Bombay Hosp J 2008;50:566-71.
12. Ost A, Nilsson-Ardnor S, Henter JI. Autopsy findings in 27 children with haemphagocytic lymphohistiocytosis. Histopathology 1998;32:310-16.
13. Babapulle CJ, Jayasundera NPK. Cellular changes and time since death. Med Sci Law 1993;33:213-22.

14. Penttila A, Laiho K. Autolytic changes in blood cells of human cadavers II. Morphological studies. Forensic Sci Int 1981;17:121-32.

15. Bardale R, Dixit PG. Evaluation of morphological changes in blood cells of human cadaver for the estimation of postmortem interval. Medicolegal Update 2007;7:35-9.

16. Laiho K, Penttila A. Autolytic changes in blood cells and other tissue cells of human cadavers. 1. Viability and ion studies. Forensic Sci Int 1981;17:109-20.

17. Firkin F, Chesterman C, Penington D, Rush B (eds). Pancytopenia and aplastic anemia. In: de Gruchy's Clinical Hematology in Medical Practice. 5th ed 1989. Blackwell Science Ltd. 119-36.