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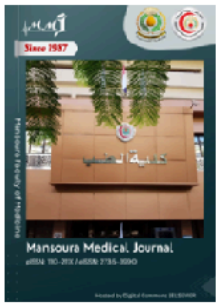


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# COEXISTANCE OF ACUTE MYELOID LEUKEMIA (AML-M4) AND HODGKIN'S LYMPHOMA: IMMUNOHISTOCHEMICAL, SKY AND INTERPHASE FISH ANALYSIS.

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# COEXISTANCE OF ACUTE MYELOID LEUKEMIA (AML-M4) AND HODGKIN'S LYMPHOMA: IMMUNOHISTOCHEMICAL, SKY AND INTERPHASE FISH ANALYSIS.

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## ABSTRACT

We describe a case of 61-year-old man presented with multiple constitutional symptoms and found to have simultaneous occurrence of acute myeloblastic leukemia (AML-M4) and Hodgkin's lymphoma in the cervical lymph node. Peripheral blood (PB) and bone marrow (BM) smears showed typical AML-4 features. Karyotyping of the bone marrow revealed many cytogenetic changes including t(16;17) and –Y. SKY analysis show the following clonal pattern 42,X,-Y,der(2)t(2;11)(q37;?), der(5;17)(p10;p10), der(16)t(16;21)(q24;q11),-18, -21. To prove that the Hodgkin's lymphoma of the cervical lymph node is not a leukemic infiltrate and that it is from different clone, interphase fluorescence in situ hybridization (IFISH) was applied on the lymph node biopsy using X and Y centrosome probes revealed absence of loss of chromosome Y. These findings indicated that this patient had a coexistence of AML and Hodgkin's lymphoma. Up to our knowledge this is the first cytogenetically proved case report of a simultaneous occurrence of these two diseases.

*CLINICAL HISTORY :*

The patient was a 61-year-old man presented with multiple costitutional symptoms including weight loss, shortness of breath, night sweat and fever. On admission he was found to

have cervical lymphadenopathy and macrocytic anaemia with blast seen on peripheral blood film. A bone marrow biopsy was interpreted as a myelodysplastic syndrom, probably CMML in transformation to AML-M4. His past medical history includes Charcot-Marie-Tooth disease and exposure of tuberculosis. On admission a surgical lymph node biopsy showed a classical Hodgkin's disease. Chest x-ray and CT scan showed scarring in the apices and multiple nodule as well as lesion in the vertebral body of T10 consistent with a bony metastasis. The patient has been treated for AML and this was accompanied by INH, rifampin and pyridoxine. He become persistently neutropenic and developed mucositis secondary to candida and chemotherapy. He also developed profcund hyponatremia and elevated liver enzymes and jaundice and finally developed septic shock and passed a way.

## MATERIAL AND METHODS:

*Immunohistochemistry :*

Section of the tumor were fixed in 10% neutral buffered formalin and processed for light microscopic examination. Immunohistochemical studies were performed on formalin fixed paraffin embedded tissue using avidin-biotin peroxidase complex method as directed by the manufacturer. The panel of antibodies used was as follows: CD45 (DAKO, 1/80 dilution), CD15, CD30, CD68 (DAKO, 1/100), CD3 (DAKO, 1/50), CD20 (DAKO, 1/50, CD79a (DAKO, 1/50), CD45ROA6 (DAKO, 1/400), Myeloperoxidase (DAKO, 1/5000), muramidase (DAKO, 1/3000), and Low molecular weight cytokeratin (Becton Dickinson, 1/10), and vimentin (American Research Product Inc, 1/300).

*Interphase FISH :*

Interphase FISH has been performed on 5 micron unstained tissue sections using adjacent H&E stained sections as guidance. The appropriate section was chosen. The standard technique for FISH on paraffin sections was applied 1-3, with some modification as we previously described 4, 5.

*SKY analysis and G-banding :*

Cytogenetic analysis was performed on bone marrow samples using standard Trypsin Giemsa G-banding 6. ISCN criteria were used to define abnormal cell clones 7. Slides for SKY involved were rehydrated in a descending ethanol series and fixed

in a 1% formalin solution followed by a 1X PBS (phosphate-buffered saline) wash. Slides were dehydrated and then denatured at 75C in 70% formamide/2X SSC (saline sodium citrate) for 40 seconds followed by a final dehydration. The SKY paints (Applied Spectral Imaging, Carlsbad CA) were allowed to hybridize for hours to the denatured slides. Post hybridization washes and hapten detections were carried out using established techniques 8 and as per the manufacturer's instructions. Ten metaphase images were captured using Applied Spectral Imaging's software v1.2 and analyzed using SKYVIEW v3.1.

## RESULT

The bone marrow biopsy showed features of acute myeloblastic leukemia (AML-M4). The flowcytometry finding was consistent with AML-M4. Cytogenetic analysis by G-banding showed that 17 out of 20 metaphases were hypodiploid. The following pattern was seen: 42,X-Y,add(2)(p25), add(2)(q37),del(5)(q13),der(16)t(16;17)(q22 or q24;q11.2 or q21),-17,-18,-21[17]/46,XY[3]. A hypodiploidy is characteristic with hematological malignancies. The del(5q) is associated with myeloid neoplasia. The (16;17) translocation occasionally encountered in AML-M4. SKY analysis (figure 1) on the same specimen was consistent with the G-banding findings. Fluorescence in situ hybridization (FISH) analysis of 200 metaphase preparation using the inversion 16 FISH DNA probe (Vysis inv 16 Coatasome) indicated that the inversion characteristic of AML was not present. SKY analysis showed the following clonal pattern: 42, X,-Y, der(2)t(2;11) (q37;?), der (5;17) (p10 ;p10), der(16)t(16;21)(q24;q11),-18,-21. The cervical lymph node biopsy show diffuse proliferation of large descohesive cells with prominent nucleoli and some mitotic figures. Many of these cells have the morphology of classical Reed-Sternberg and their mononuclear, mummified and lacunar cell variants. Immunohistochemistry showed that the large cells are strongly positive for CD15, CD30 and negative for CD45, CD20, Lysozyme, Myeloperoxidase, S-100 and low molecular weight cytokeratin. Occasional large cells are CD79a positive. There was a reactive background comprising CD3+ve, CD45RO (A6)+ve T-Lymphocytes, plasma cells and scattered histiocytes. So the morphologic and immunohistochemical features are those of classical Hodgkin's lymphoma. With respect to subtype the

differential diagnosis included the syncytial variant of nodular sclerosis (NSII) and mixed cellularity. Because the existence of AML-M4 diagnosis in this patient and that some of AML-M4 could express CD15 as well and to prove that the simultaneous classic Hodgkin lymphoma of the cervical lymph node is not a leukemic infiltrate and that it is from different clone we have performed an interphase FISH analysis (figure 2) of the cervical lymph node biopsy to role out leukemic infiltrate and we performed the FISH using dual centromere probes for Y (which was lost in the leukemia cells and X as internal control). Those sheets of mononuclear variant RS cells turned to have normal X and Y chromosomes pattern. 1X and 1Y signals were seen in 77.5% of the scored nuclei. The rest of the nuclei showed either only volvement by HD demonstrated that accepted range related to truncation of the nuclei.

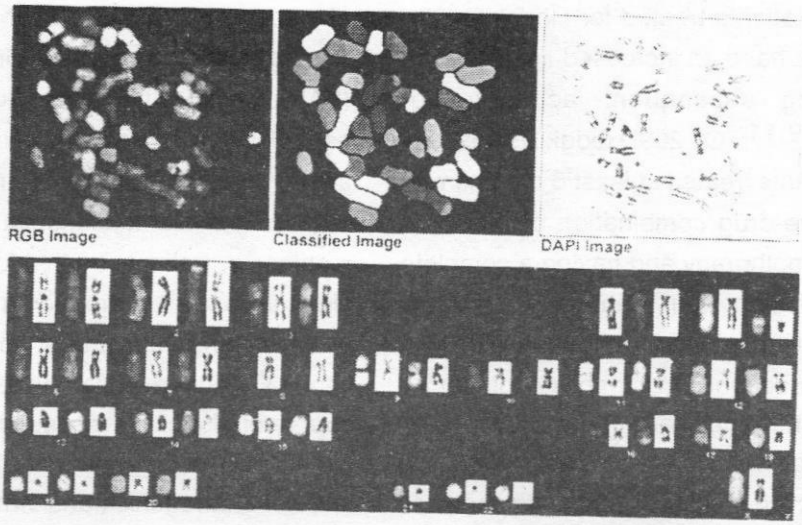


**FIGURE 1 :** Analysis by sequential SKY and G-banding. The sequential application of G-banding SKY showing RGB display image (upper right). SKY showing classified image using the same metaphase from the patient (upper middle). SKY analysis showed the following clonal pattern: 42, X, -Y, der(2)t(2;11)(q37;?), der(5;17) (p10;p10), der(16)t (16;21) (q24;q11), -18, -21.

**FIGURE 2 :** Interphase FISH analysis show that most of the cells reveal one red signal (chromosome X) and one green signal (chromosome Y). This pattern of signals was seen in 77.5% of the scored nuclei. The rest of the nuclei showed either only X or Y signal, which is considered with in the accepted range due to truncation of the nuclei.

## DISCUSSION

Patients treated for Hodgkin's disease have an increased risk of developing subsequent acute leukemia.[9,11]. Of 209 Hodgkin's disease patients treated at least 6 months with a five-drug combination of induction chemotherapy and having a complete remission, four patients developed acute myelogenous leukemia (AML) as a second malignant neoplasm[12]. On the other hand the occurrence of Hodgkin's disease as a second malignancy in childhood acute lymphoblastic leukemia is also described but still as rare occurrence.[13,14]. However the simultaneous occurrence of Hodgkin's disease and AML at the same time has not been described yet in the literature. We report a unique case of AML-M4 associated with Hodgkin's disease. The bone marrow samples showed the typical features of AML-M4 and the cytogenetic examination and SKY analysis revealed the presence of many clonal cytogenetic changes including 42, X, -Y, der(2) t(2;11) (q37;?), der(5;17) (p10;p10), der (16)t(16;21)(q24;q11),-18, -21.It is reported that long-term Hodgkin's disease treated with several courses of radiotherapy and chemotherapy and complicated with acute myeloblastic leukemia 11. Brusamolino et al reported the hematological and cytogenetic characteristics of 75 cases of therapy-related acute non-lymphoid leukemia (t-ANLL) occurring in Hodgkin's disease (HD) analysed in multi-institution study [10]. The median latent time from remission of HD to leukemia was 34 months.[10]. Patients treated for Hodgkin's disease have an increased risk of developing subsequent acute leukemia.[9] Leukemia complicating the course of Hodgkin's disease must be considered an independent disease and not a iatrogenic transformation of a preexisting lesion.[15]. There was a significant relationship between the intensity of the treatment and the appearance of leukemia.[16]. Reports of acute nonlymphoblastic leukemia occurring after successful treatment of Hodgkin and non-Hodgkin lymphoma (NHL) are appearing with increasing frequency[17]. On the other hand Hodgkin's disease can develop in patients with know leukemias. Hodgkin's disease as a second malignancy in childhood acute lymphoblastic leukemia is of rare occurrence and the therapy for acute lymphoblastic leukemia may alter the pattern of presentation of Hodgkin's disease and its histology, making diagnosis difficult [13]. In a study that was undertaken to examine the influence of various factors

on the occurrence of acute nonlymphocytic leukemia (ANLL) in a group of longterm survivors of Hodgkin's disease (HD), Maurizi Enrici et al reviewed patients with HD and demonstrated that splenic treatment does not lead to ANLL. Treatment with MOPP alone and with MOPP plus RT can increase the risk of ANLL. [18]. Despite the observed relatively high risk of secondary leukemia, the rate of death from progressive Hodgkin's disease, nonleukemic complications, and unrelated causes still far exceeds the rate of leukemia-related deaths in these patients.[19]. Secondary Hodgkin's disease in childhood acute lymphoblastic leukemia does not appear to have a poor prognosis and long-term survival and possible cure of both diseases may be achieved.[14]. It is suggested that the development of acute leukemia was related to irradiation in these patients, and that additional such cases could be expected with the use of intensive radiation and chemotherapy, a risk probably justified in view of the improved control of Hodgkin's disease achieved by these programs.[20]. Simultaneous occurrence of hairy cell leukemia and Hodgkin's disease in the same patient has been reported.[21]. Acute myelogenous leukemia may occur after long time of successful treatment of Hodgkin's disease and long-term follow-up of all patients treated with radiotherapy and/or polychemotherapy is necessary.[22]. In general the incidence of secondary tumors among patients with Hodgkin's disease turned out to be much higher than in general population and complications were more frequently observed in men after combined therapy (radio- and drug therapy).[23]. Myeloblastic leukemia has been reported even in a child with Hodgkin's disease[24]. Tura et al demonstrated that splenectomy and, as previously described by others, the number of courses of MOPP are prognostic factors that increase the risk of secondary ANLL in HD patients treated with combined modality therapy. These data raise interesting questions regarding the possible role of the spleen in leukemia development.[25]. Hodgkin's disease and acute leukemia can complicate each other, however up to our knowledge coexistence of AML and Hodgkin lymphoma is not described in the English literature. This is the first case report of a simultaneous occurrence of these two diseases that has been proved at the cytogenetic level. The lymph node biopsy, which reveals infiltration by these large cells, which are in keeping

with HD, it was difficult to rule out completely the possibility of leukemic infiltrate of the lymph node. Although the immunohistochemistry was helpful in supporting the diagnosis of HD, the use of SKY and interphase FISH was very helpful in confirming that those tumors are different and represent completely two different clones. Because the bone marrow sample which reveal the leukemia demonstrated the loss of chromosome Y, while interphase FISH analysis of the lymph node material which reveal the involvement by HD demonstrated that there is no loss of chromosome Y. We concludes that the coexistence of two different hematological malignancy may occur and should be kept in mind and that the use of SKY and IFISH some time is very helpful in proving the coexistence of these two malignancy.

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