Clinical Report

Positive Expression of Macrophage Inflammatory Protein-1α and -1β in a Patient with Diffuse Large B-cell Lymphoma Accompanied by Hypercalcemia and Multiple Osteolysis

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Multiple osteolysis and hypercalcemia are rare conditions accompanying diffuse large B-cell lymphoma (DLBCL). There have been no reports demonstrating the protein expression of osteoclast-activating factors in such cases. Herein, we report a case of a 38-year-old man with DLBCL, who initially presented with hypercalcemia, multiple osteolysis and renal insufficiency that mimicked multiple myeloma with poor outcome. Immunohistochemical analysis was conducted to examine the expression of macrophage inflammatory protein (MIP)-1α, MIP-1β and receptor activator of NF-kappa B ligand (RANKL) in a lymph node that was obtained at diagnosis. Compared with a control DLBCL sample, we observed increased expression of MIP-1α in lymphoma and vascular endothelial cells and MIP-1β in stromal cells in our case. RANKL expression was not observed in either our case or control cases. MIP is likely associated with the development of osteolysis and hypercalcemia in some lymphoma cases.

Key wards: diffuse large B-cell lymphoma, osteoclast activating factor, macrophage inflammatory protein, receptor activator of nuclear factor-kappa B ligand, hypercalcemia

Introduction

Macrophage inflammatory protein (MIP)-1α and MIP-1β as well as C-C chemokines derived from myeloma cells are not only bone-resorbing factors but also major osteoclast-activating factors. There are some associations between the levels of MIP and disease activity in myeloma patients. In contrast, there have been few reports of positive MIP expressions in non-Hodgkin lymphoma (NHL). In addition, although humoral hypercalcemia occasionally occurs in NHL, multiple osteolysis with hypercalcemia in association with MIP is rare in NHL. Here, we describe a case of diffuse large B-cell lymphoma (DLBCL) with marked hypercalcemia, multiple osteolytic lesions and renal insufficiency that resembled myeloma at diagnosis without any humoral abnormality.

Case report

A previously healthy 38-year-old man was admitted to the hospital because of hypercalcemia and renal impairment with one-month history of general fatigue and fever. Physical examination revealed bilateral cervical lymphadenopathies and a solitary soft tissue tumor on an occipital lesion. Laboratory tests showed the following results. Peripheral blood analysis showed a white blood cell of 4.5 × 10^9/l without atypical lymphocytes or blasts. Haemoglobin was 13.6 g/dl and platelet count was 28.0 × 10^9/l. Serum lactate dehydrogenase was 228 IU/l (normal range, 106–220), serum urea 31.1 mg/dl, serum creatinine 3.22 mg/dl, calcium 16.1 mg/dl and phosphate 4.8 mg/dl. Intact parathyroid hormone (PTH) was 8 pg/ml (10–65), PTH-related peptide (PTH-rP) < 1.0 pmol/ml (< 1.1) and calcitriol (1, 25-(OH)2 D3) 9.2 pg/ml (20–60). Soluble interleukin-2 receptor was 27,500 U/ml (220–530) and β2 microglobulin 16.6 mg/l (1.0–1.9). Serum IgG, IgA and IgM were 1504, 83 and 33 mg/dl, respectively. Serum total protein was 6.8 g/dl and albumin 3.6 g/dl, with a mild M-peak. Immunelectrophoresis showed M proteins of the IgG-κ type, but Bence Jones proteins were absent.

X-rays showed a punched-out lesion on the scalp (Fig. 1). CT scan of the neck, chest and abdomen showed generalized lymphadenopathies without bulky masses. Bone scintigraphy showed multiple uptakes. Biopsy of a left cervical lymph node and bone marrow was performed for diagnosis.

Pathological findings indicated monotonous proliferation of lymphomatoid tumor cells without follicular...
formation (Fig. 2). These cells had abundant nucleoli with dysplasia but no appearance of spindle-shaped, fibrosis, or starry-sky appearance. There were also no findings of nucleus with clock-face, spoke wheel chromatin or duchter body. Tumor cells were positive for CD20, CD79a, bcl-6, MUM-1, but negative for CD3, CD10, CD23, CD30, CD56, CD138, CD246, bcl-2, cyclin D1, and terminal deoxynucleotidyl transferase (TdT). More than 60% of the tumor cells were positive for Ki-67. Tumor cells represented negative for kappa and lambda chains. Flow cytometric analysis of biopsy lymph node revealed negativity of neither CD38 nor CD138. The diagnosis of CD20-positive DLBCL of non germinal center (non-GC) type rather than anaplastic myeloma was given, because tumor cells showed a DLBCL morphology and were negative

Fig. 1  Bone X-ray shows a punched-out lesion on an occipital lesion (arrow).

Fig. 2  Haematoxylin and eosin staining shows monotonous proliferation of small to mid-sized round tumor cells, which is consistent with non-Hodgkin lymphoma (Original magnification 400 ×).

Fig. 3  Chromosomal analysis by G-banding. Complex karyotype was documented from a lymph node specimen, including deletion of the 13q14 region, 48, XY, ins (1) (q11? q42? q21?), + 5, inv (9), +12, del (13) (q14), add (14) (q32).
from not only both kappa and lambda chains, but CD38, CD56, and CD138, a common surface marker of myeloma. Results of cytogenetic analysis using G-banding of the lymph node indicated a complex karyotype, including deletion of chromosome 13q14 (Fig. 3).

After diagnosis, one cycle of CHOP therapy was administered, and hypercalcemia and renal insufficiency were improved to normal. He received additional 3 courses of a dose-intensified CHOP regimen with rituximab and salvage regimens, then complete remission was obtained. Although he underwent autologous peripheral blood stem cell transplantation (APBSCT) with in vivo rituximab graft purging, relapse was documented three months after transplantation by FDG-PET scan. In spite of further treatment, the disease progressed and the patient died.

Tumor samples were obtained from a cervical lymph node at initial diagnosis. Lymph nodes from other patient with DLBCL but without hypercalcemia and osteolysis were used as controls. Immunohistochemical analysis of formalin-fixed, paraffin-embedded tissue sections was performed. Deparaffinised sections were quenched for endogenous peroxidase activity by immersing the sections in 0.3% hydrogen peroxide solution for 20 min at room temperature, and then washing several times in phosphate-buffered saline (PBS), pH 7.2. The sections were incubated for 2 h at 4°C with the following primary antibodies: anti-human CCL3/MIP-1α antibody (mouse anti-human 1α IgG; R&D Systems, Minneapolis, USA), anti-human CCL4/MIP-1β antibody (goat anti-human 1β IgG; R&D Systems) and RANKL (FL-317) antibody (rabbit polyclonal antibody; Santa Cruz Biotechnology, California, USA). After washing with PBS several times, biotinylated secondary antibodies were applied for 30 min at room temperature. After washing, the tissue sections were incubated with horseradish peroxidase (HRP)-labelled streptavidin for 30 min at room temperature. The tissue-bound HRP activity was visualized using 0.005% 3,3’-diaminobenzidine tetrachloride (DAB) in PBS containing hydrogen peroxide (10 µl/150 ml DAB solution). The sections were stained lightly with haematoxylin and mounted.

Increased expressions of MIP-1α in lymphoma cells and vascular endothelial cells were observed (Fig. 4A). MIP-1β was not observed in lymphoma cells, but vascular endothelial cells showed weak positive for it (Fig. 4B). RANKL expression was not detected in either tumor cells or stromal cells in the present case (Fig. 4C). Other DLBCL sample shows no expressions of MIP-1α (Fig. 2D), MIP-1β (Fig. 2E) or RANKL (Fig. 2F), respectively.

**Discussion**

Osteolysis and hypercalcemia are rare in lymphoma patients, in contrast to myeloma. Three major mechanisms that can induce hypercalcemia in cancer patients by increasing bone resorption and releasing calcium have been proposed: (1) osteolytic metastases with local release of cytokines, including osteoclast-activating factors, (2) tumor secretion of parathyroid hormone-related protein (PTHrP) and (3) tumor production of calcitriol. Few lymphomas are associated with elevations in serum PTHrP levels, except for...
adult T cell lymphoma/leukemia that causes hypercalcemia\(^9,10\). Typically, other mechanisms can often explain hypercalcemia associated with lymphoma, such as excess production of calcitriol or the release of cytokines that promote osteolysis\(^11\). Hypercalcemia in our case was considered to be due to osteolysis that caused secretions of some cytokines, since both calcitriol and PTHrP were within normal ranges.

MIP plays an important role in bone metabolism. MIP-1\(\alpha\) can directly enhance osteoclast formation, and both MIP-1\(\alpha\) and MIP-1\(\beta\) participate in osteoclast formation by inducing RANKL expression in stromal cells\(^2\). In myeloma patients, there are some associations between the expression level of MIP and disease activity\(^2,3\). In addition, MIP also plays a key role in adhesive interactions between myeloma cells and narrow stromal cells, which produce some additional cytokines, such as interleukin 6, and RANKL\(^12\). Therefore, in our case, we assumed that osteolysis and hypercalcemia resulted from MIP-RANKL interactions induced by tumor cells. However, no RANKL expression was observed in the present case. The lymph node biopsy sample might contain less stromal cells which produces RANKL in association with MIP-RANKL interaction.

From the immunohistochemistry results, lymphoma cells expressed MIP-1\(\alpha\) and stromal cells expressed both MIP-1\(\alpha\) and MIP-1\(\beta\). MIP-1\(\alpha\) probably activated osteoclasts in the present case, as has been reported in myeloma. Several inflammatory cytokines are associated with MIP upregulation in stromal and hematopoietic stem cells, such as lipopolysaccharide, certain interleukins and tumor necrosis factor\(^13\). Although no cytokines were examined in this case, it might be presumable that MIP production in the stroma in our case have been stimulated by these cytokines released from lymphoma cells.

It is known that myeloma patients with high MIP-1\(\alpha\) serum levels have poor prognosis\(^13\). However, as far as we know, only one case report of lymphoma patient presenting positive MIP expression with poor outcome. Previously, Matsushashi et al reported a case of DLBCL accompanied by hypercalcemia and multiple osteolysis similar to our case\(^14\). In their report, positive expressions of MIP-1\(\alpha\) and MIP-1\(\beta\) of the tumor samples were identified using reverse transcriptase-polymerase chain reaction. However, the protein expression of MIP has not been reported previously. To our knowledge, this is the first case reporting positive expressions of MIP-1\(\alpha\) and MIP-1\(\beta\) proteins in DLBCL.

In conclusion, MIP expression is probably associated with the development of osteolysis and hypercalcemia not only in myeloma but also in some cases of lymphoma. Because of the rarity of similar cases, additional investigations will be required.

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References