Serum Osteoprotegerin, RANKL, and Dkk-1 Levels in Adults with Langerhans Cell Histiocytosis


Department of Endocrinology and Diabetes (P.M.), 251 Hellenic Air Force and VA General Hospital, 11525 Athens, Greece; Second Medical Clinic (S.A.P.), Aristotle University of Thessaloniki, Ippokration Hospital, 54655 Thessaloniki, Greece; Department of Endocrinology (A.D.A.), 424 Military Hospital, 56510 Thessaloniki, Greece; Departments of Clinical Therapeutics (E.T.) and Pathophysiology (G.K., M.S., G.A.K.), University of Athens School of Medicine, 11527 Athens, Greece; and Department of Medical Research (E.T., A.P.), 251 Hellenic Air Force and VA General Hospital, 11525 Athens, Greece

Context: Langerhans cell histiocytosis (LCH) is a rare disease of unknown etiology with a strong evidence of immunological dysfunction secondary to cytokine dysregulation.

Objective: This study aimed to evaluate serum receptor activator of nuclear factor κB ligand (RANKL), osteoprotegerin (OPG), and Dickkopf-1 (Dkk-1) levels in adult patients with LCH at various stages of the disease.

Design: This was a cross-sectional study in an adult LCH cohort followed for 12.2 ± 1.1 yr.

Setting: The study was conducted in an outpatient clinic.

Subjects: Twenty-five adult patients with a definitive LCH diagnosis and 50 matched controls participated in the study.

Interventions: Early morning, fasting, venous sampling was conducted in all subjects.

Main Outcome Measure: We compared RANKL, OPG, and Dkk-1 serum levels between patients and controls, as well as their association with disease parameters.

Results: Serum OPG levels were significantly higher (3.0 ± 0.2 vs. 1.7 ± 0.1 pmol/liter; P < 0.001), whereas RANKL/OPG ratio was significantly lower (0.201 ± 0.041 vs. 0.471 ± 0.072; P = 0.02) in LCH patients compared to controls. Both higher OPG (adjusted odds ratio, 3.431; 95% confidence interval, 1.329–8.924) and lower RANKL (adjusted odds ratio, 0.144; 95% confidence interval, 0.034–0.605) levels were independently associated with LCH in logistic regression analysis, after adjustment for all other parameters. Dkk-1 did not differ among patients and controls.

Conclusions: Adults with LCH have high serum OPG levels and low serum RANKL levels. In contrast with other disorders involving the skeleton, serum Dkk-1 levels are similar between LCH patients and controls. (J Clin Endocrinol Metab 97: E0000–E0000, 2012)
dealing with different disease manifestations (2, 3). Although LCH is a clonal disorder with an obscure pathogenesis, it exhibits features of an inflammatory disorder, and cytokines are considered important for both the migration and homing of LC and for the localized and systemic pathology of the disease (4, 5).

One of the features presenting most frequently in adults is skeletal involvement, which occurs in approximately 40% of patients, mainly in the form of osteolytic lesions (3). It has recently been shown that adults with active disease have lower bone mineral density (6). It was suggested that this could occur either as a consequence of the disease or from previously administered treatment, particularly chemotherapy, that was specifically associated with reduced bone turnover (6). At the cellular level, osteoclast-like multinucleated giant cells are present both at bone and at nonostotic lesions and can be activated by macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANKL) expressed on LCH cells or T cells (7). Although serum RANKL/osteoprotegerin (OPG) ratios have been shown to represent markers of osteolytic activity in children with LCH (5), this has not been studied in adult LCH patients. Dickkopf-1 (Dkk-1) is an inhibitor of the Wnt signaling pathway and may lead to bone resorption through an enhanced osteoblast-dependent osteoclastogenesis, by upregulating RANKL and/or down-regulating OPG (8). However, to date it has never been measured in patients with LCH.

In the present study, we opted to test the hypothesis that serum RANKL, OPG, and Dkk-1 levels could be affected in adults at various LCH disease activity, particularly in patients with osseous disease. We thus evaluated these markers in the same adult group that was found to have reduced bone mineral density (6).

Patients and Methods

In this cross-sectional study, 25 patients with LCH (13 males) aged 37.08 ± 2.74 yr (range, 20–68 yr), with a body mass index (BMI) of 25.6 ± 1.06 kg/m², were recruited following strict inclusion criteria regarding confounding parameters (metabolic bone disorders, malignant diseases, impaired renal function), as previously described (6). The mean duration of the disease was 12.2 ± 2.11 yr (range, 0.1–40 yr; total follow-up, 305.15 yr). Nineteen patients had multisystem disease (seven active and 12 inactive) and six had single system disease (solely bone involvement; four active and two inactive). Multisystem involvement included bones (n = 13), lungs (n = 13), pituitary (n = 14), skin (n = 9), lymph nodes (n = 5), gingiva (n = 3), central nervous system (n = 2), ear (n = 2), genitalia (n = 2), liver (n = 1), and thyroid (n = 1). Nineteen patients had bone involvement either within the context of multisystem involvement (n = 13) or single-system disease (n = 6). Fourteen patients were receiving or had received at least one course of chemotherapy for LCH before the evaluation. Thirteen patients had received steroids for at least one period of time during the course of their disease (mean total exposure to steroids, 8.3 ± 0.84 months; range, 4–16 months). Eleven of the 13 patients with a history of previous treatment with steroids had also received chemotherapy. Five patients were currently receiving bisphosphonates (three alendronate, one risedronate, one zoledronate) for osteoporosis and/or the disease itself, and one had received alendronate up to 4 yr before evaluation.

Fifty healthy subjects (27 males) matched for age (mean, 37.4 ± 1.9 yr; range, 20–71 yr) and BMI (26.2 ± 0.8 kg/m²) participated as controls. Controls were not receiving any medication known to affect bone metabolism.

Early morning fasting blood samples were collected from all subjects. RANKL, OPG, and Dkk-1 were measured with commercial ELISA kits (Biomedica Medizinprodukte GmbH and Co. KG, Wien, Austria) on samples stored at −80 C. PTH was also measured in patients who had 25-hydroxyvitamin D of 50 nmol/liter or less for the exclusion of possible secondary hyperparathyroidism.

The study has been approved by the local institutional committee and is in accordance with the guidelines of the Declaration of Helsinki; informed consent was obtained from all subjects.

Results

Serum OPG levels were significantly higher, whereas the RANKL/OPG ratio was significantly lower in patients with LCH compared with controls. There were no differences in serum RANKL and DKK1 levels among the two groups (Table 1). Similar results were obtained when LCH patients with bone involvement were compared with their matched controls (OPG patients 3.1 ± 0.2 vs. controls 1.7 ± 0.2 pmol/liter; P < 0.001; RANKL/OPG ratio, patients 0.448 ± 0.092 vs. controls 0.482 ± 0.087; P = 0.024). Although unadjusted RANKL levels were nonsignificantly lower in patients than controls (Table 1), in logistic regression analysis (Table 2), both higher OPG (adjusted OR, 3.431; 95% CI, 1.329–8.924) and lower

<table>
<thead>
<tr>
<th>TABLE 1. Comparative data between LCH and control group</th>
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<tr>
<td>No. of patients/ women</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>RANKL (pmol/liter)</td>
</tr>
<tr>
<td>OPG (pmol/liter)</td>
</tr>
<tr>
<td>RANKL/OPG ratio</td>
</tr>
<tr>
<td>DKK1 (pmol/liter)</td>
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Data are presented as mean ± SEM or number.

* Between-group comparison (independent sample t test or Mann-Whitney test for continuous and χ² or Fisher’s exact test for categorical variables).
RANKL (adjusted OR, 0.144; 95% CI, 0.034–0.605) levels were independently associated with LCH after adjustment for age, gender, and BMI (model $R^2 = 0.456$; $P = 0.001$). The same pattern was observed in patients with LCH and bone involvement when compared with their matched controls (OPG adjusted OR, 6.735; 95% CI, 1.346–33.708; RANKL adjusted OR, 0.025; 95% CI, 0.001–0.368; model, $R^2 = 0.623$; $P < 0.001$).

Within LCH patients only, serum OPG, RANKL, and Dkk-1 levels and RANKL/OPG ratio were not different when patients were divided according to disease activity (active vs. inactive disease), extent and site of involvement (multi- vs. single-system disease), and treatment.

**Discussion**

In this study, it was demonstrated that adult patients with LCH have higher serum OPG and lower serum RANKL levels than controls after adjustment for age, gender, and BMI. Dkk-1 levels were not different between patients and controls. In addition, it was shown that serum OPG, RANKL, and Dkk-1 levels and RANKL/OPG ratio of LCH patients do not appear to correlate with disease activity, extent, duration, and/or treatment received.

LC is one of the most efficient presenters of antigens, but LC in LCH are immature, proliferating at a moderate rate, and presenting antigens poorly (9, 10). It is considered that the clinical manifestations in LCH result from immunological dysfunction secondary to a “cytokine storm” taking place within the lesions (4). Relevant cytokines, both at the lesional and systemic level, are granulocyte M-CSF, interferon-γ, IL-1, IL-10, TNF-α, soluble IL-2 receptor, RANKL, OPG, and IL-17 (4, 5, 11). More specifically, the implication of RANKL and OPG in the pathogenesis and course of LCH has been recently evaluated. M-CSF and RANKL expressed on LCH cells or T cells are able to activate osteoclast-like multinucleated giant cells at both osseous and nonosseous lesions (7). In addition, serum RANKL/OPG ratio was positively correlated with the overall osteolytic activity among children with active disease (5). Based on these findings, it seems reasonable to assume that serum RANKL and OPG levels may be altered among LCH patients due to either the disease itself or LCH-induced changes in bone metabolism.

Since the development of OPG and RANKL assays, there has been considerable interest regarding their use as markers of metabolic bone diseases and neoplastic disorders. However, it is quite possible that potential changes at the cellular level cannot always promote a reciprocal alteration in serum levels (12). Specifically, serum OPG and RANKL may not reflect their levels and activity in the bone microenvironment because only a small amount may reach the systemic circulation, whereas part of the serum OPG and RANKL concentrations may originate from nonskeletal sources (13). In addition, serum RANKL reflects only a small part of its total production because the majority is cell-bound and can be only assessed in vivo by flow cytometry after bone biopsy (14). Moreover, the current commercially available assays for OPG cannot exclusively detect the biologically active dimeric form because they have been designed to detect all forms of OPG (monomer, dimer, RANKL/OPG complex) (15).

In our study, we found high serum levels of OPG among LCH patients with or without bone involvement, whereas low levels of serum RANKL were independently associated with the disease. A plausible explanation for these findings could be a shift of circulating RANKL to LCH lesions with a concomitant increase in cell-bound concentrations, whereas OPG, acting as a circulating decoy receptor, could be compensatorily increased in a self-defense manner. Because high OPG levels have previously been reported in children with LCH, it was suggested that serum OPG may operate as a protective mechanism, acting as a decoy receptor for TNF-related apoptosis-inducing ligand (5). Although the hypothesis of the increased cell-bound RANKL and the compensatory increment of OPG seems more reasonable from a pathophysiological point of view, future studies determining the exact role of RANKL in LCH biology are urgently needed. If the latter hypothesis is proved, it may have therapeutic implications because denosumab, a human antibody against RANKL, is currently available and already being used in the treatment of osteoporosis (16). Based on this hypothesis, such a treatment could potentially be used to target lesional cell-bound RANKL.

Serum levels of Dkk-1 are assumed to reflect its expression in bone microenvironment, and serum Dkk-1 levels can be used as predictors of the extent of bone disease in patients with multiple myeloma, breast cancer, and thalassemia (17–19). Additionally, Dkk-1 production by multiple myeloma cells was regarded as a contributing factor to the reduced bone formation within the lytic bone lesions.

**TABLE 2. Logistic regression analysis between LCH group (n = 25) and control group (n = 50)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted OR</th>
<th>95% CI for adjusted OR</th>
<th>$P$ value</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>0.930</td>
<td>0.866–0.998</td>
<td>0.045</td>
</tr>
<tr>
<td>Gender$^b$</td>
<td>0.575</td>
<td>0.133–2.478</td>
<td>0.458</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.976</td>
<td>0.815–1.169</td>
<td>0.793</td>
</tr>
<tr>
<td>OPG</td>
<td>3.431</td>
<td>1.319–8.924</td>
<td>0.011</td>
</tr>
<tr>
<td>RANKL</td>
<td>0.144</td>
<td>0.034–0.605</td>
<td>0.008</td>
</tr>
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</table>

$^a$ Control group had rated as zero and LCH group as 1 within the dependent variable.

$^b$ Female had rated as zero and male as 1 within gender.
We found no difference in Dkk-1 levels between LCH patients and controls or any correlations between Dkk-1 and RANKL and/or OPG levels. According to our results, Dkk-1 is neither implicated in the pathogenesis of LCH nor related to alterations of the RANKL and OPG levels during the evolution of bone lesions.

Our study has several limitations, with the first being the small number of patients enrolled. However, large numbers of patients cannot be easily accrued from a single center in a disease that has such a low prevalence. Because LCH exerts periods of relapses and remissions, it is very difficult to define whether the disease is active or not, especially in the absence of severe symptoms or signs. Specifically, the inclusion of patients with subclinical disease in our cohort can potentially explain the absence of differences between serum RANKL and OPG levels between patients with active and inactive disease. Finally, the results of chemotherapy cannot be easily interpreted in patients receiving different kinds of treatment and at different time intervals before the evaluation.

In conclusion, increased adjusted serum OPG and decreased adjusted serum RANKL levels were observed in adult LCH patients irrespective of the disease’s activity, extent, duration, and/or treatment applied. This could represent a compensatory mechanism that increases the circulating decoy receptor (OPG) against the lesional acrual of cell-bound RANKL. LCH has no effect in serum Dkk-1 levels at any stage of the disease.

Acknowledgments
Address all correspondence and requests for reprints to: Dr. Polyzois Makras, M.D., Ph.D., Department of Endocrinology, Diabetes, 251 Hellenic Air Force and Veterans General Hospital, 3 Kanellopoulou st, 115 25 Goudi, Athens, Greece. E-mail: makras@internet.gr.

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References