Reviewer's report

Title: Endothelin-1 plasma levels in patients with osteoporosis

Version: 1 Date: 25 February 2005

Reviewer: Miryoung L Lee

Reviewer's report:

Major Compulsory Revisions

This is an interesting manuscript that investigates whether there is any difference in endothelin-1 (ET-1) levels among normal, low bone density (osteopenic), and osteoporotic study participants. In recent years, the ET-1 has been recognized as a physiologic regulator of bone remodeling as authors acknowledged in the text. However, there are a number of issues that the authors need to address. One of major concerns is a small number of study participants, because osteoporosis is complex disease, known to be affected by many factors, such as age, adiposity, menopause, and hormone replacement therapy.

Title and Abstract

1) The title should include the nature of current study design.
2) Since authors only measured Endothelin-1 not other isomers (i.e., endothelin-2, endothelin-3), authors should state the endothelin-1 (ET-1), not endothelins, throughout the manuscript where the authors explained the study results. Also, once the abbreviation of endothelin-1 was introduced as ET-1, authors need to use it consistently.
3) Conclusion statement: The final sentence should be more conclusive, and reflect the characteristics of study design. Because authors performed the investigation using cross-sectional study design, the authors should not conclude or generalize that ET-1 does not have a role in the physiopathology of osteoporosis. Their negative findings may be results of small samples sizes, or measurement errors in estimating plasma ET-1 levels. It should read "We did not find any significant differences in plasma ET-1 levels among three groups of study participants.".

Background

1) The authors should mention in more depth whether there are any epidemiologic studies available, in particular to investigate the associations between ET-1 and bone mineral density.
2) What was the rational to study between selecting osteoporosis disease status in this manuscript, and not bone mineral density in the hip or lumbar spine in participants?

Methods

1) Please note how many patients (participants) were invited to the present study, and how many were excluded.
2) Did authors have chances to measure any of biochemical markers related to osteoporosis, which are mentioned in background and discussion (e.g., osteopontin, osteocalcin, or calcitropic hormones, 1,25 (OH)2D3)?
3) Since authors mentioned that they had biochemical profiles or laboratory results to distinguish that none of the participants had any chronic diseases, it would be interesting to see whether there are any relationships between ET-1 and other biochemical profiles.
4) What was the coefficient of variation (CV) of the assay for ET-1?
5) Data analysis
The authors need to explain the statistical analyses more explicitly.

¿ Were the endothelin-1 levels normally distributed to perform linear regression analysis (Analysis of Variance, T-tests)?
¿ What variables/measures they used in the analysis? The authors need to clarify the dependent variable (e.g., adjusted ET-1 levels), independent variables, or covariates in the model.
¿ Was there any analysis regarding power?
¿ Which statistical program/software was used for analysis?
¿ Replace length with weight on page 7 line 7 height, length, T scores 

Results
The authors need to specify whether plasma ET-1 levels are crude or adjusted values independent of sex (as explained in Method section), and its standard error (Figure 1 and Table 1). Statistical significance levels, as mentioned in the text, need to be added in the Table 1 (footnote).

Discussion
1) The authors discussed that the estrogens may explain the sex difference, especially in men, among three groups. In addition, the authors need to discuss the influence of menopausal status among female participants since the hormone profiles and bone remodeling differ in pre- and post-menopausal women.
2) On second paragraph on page 11, the authors mentioned of significant correlation between T-scores and ET-1 levels. Can authors give any information regarding the correlation between bone mineral density and ET-1 levels in all participants? Did authors try to analyze bone mineral density as a continuous variable, not as a categorical variable (i.e., osteoporosis status by WHO criteria) in association with ET-1 levels?
3) The authors provided detailed information regarding the relationships between ET-1 and hormones and cytokines from in vivo and in vitro studies. However, the discussion and conclusions was not adequately supported by the provided data since the authors need to provide more information to conclude. In addition, some of the sentences are not clear in the context and need to be re-written. For example, on page 12, line 4, Matching of the parathyroid hormone at first leaded to decreasing of ET-A and ET-B mRNA levels. Overall, the authors need to organize and shorten the discussion to be more relevant to study hypothesis and results.
4) Please state any study limitations, such as selection bias of study participants, and generalization of study findings in other populations.

Reference
Reference No. 26 was not referred in manuscript.

List of abbreviations
The authors need to define the following abbreviations in the text where these are first used, or a list of abbreviations needs to be provided.
IL-6: interleukin 6
NO: nitric oxide
PGE-1: prostaglandin E1

Quality of written English
Reviewer’s decision: Need some language corrections and spell check before being published. The authors need to check standard scientific notations throughout the manuscript.

Examples
Consistent notation for ET-1 levels:
pg/ml (page 1), pg/mlt (page 6), pgr/ml (page 8), pgr/mlt (Figure 1)
Spell check:
1,25 dihidroksivitamin D3 (page 3, 4) Æ 1,25 dihydroxyvitamin D3
Prostasicline (page 11) Æ prostacyclin
Endoperoksid G/H synthaz (page 12) Æ endoperoxide G/H synthase

Statistical review
Reviewerâ€™s decision: Yes

Reason: The authors need to be more explicit in explaining and writing the statistical methods and models. For examples, the rationale of Studentâ€™s t-test was not clear since the comparison between sexes can be made in the analysis of variance.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes

Declaration of competing interests:
I declare that I have no competing interests.