Factors to be Considered in Osteoprotegerin Measurement

Dear Editor;

I read with great interest the recently published article by Esteghamati, *et al*. in which the authors investigated the relationship between plasma osteoprotegerin (OPG), metabolic parameters, and the severity and extent of coronary artery calcification in patients with symptoms suggestive of coronary heart disease (CHD).[1] In conclusion, increased OPG was found to be independently associated with the severity and extent of CHD and suggested as a potential marker in assessing the risk of subsequent CHD, particularly in females. However, I think that there are some points that should be emphasized about this study.

First, as is known, OPG was identified a decade ago as a member of the tumor necrosis factor receptor superfamily. Since then, there have been many studies investigating association between serum osteoprotegerin levels and the presence/severity of cardiovascular disease.[2–3] As can be seen in Table 2 of the original study, serum triglyceride levels were found significantly different and higher in CHD patients with and without diabetes. In a study by Pérez de Ciriza, *et al*., the influence of pre-analytical and analytical factors on osteoprotegerin measurements was investigated and a positive concentration-dependent analytical interference was observed with increasing triglyceride concentrations. However, this issue has not been taken into account and discussed enough in the current study. This situation can also lead to misinterpretation of results.

Second, although it is specified that OPG levels in *serum* were evaluated in the title of the original study, the authors stated that they assessed the relationship between *plasma* OPG, metabolic parameters, and the severity and extent of coronary artery calcification in patients with symptoms suggestive of CAD in the last paragraph of the *Introduction* section. Herein, there is a contradiction whether OPG measurements were performed in serum or plasma samples. In addition, in the *Patients and Methods* section, the preferred sample type was not specified while specifying which kit was used to measure OPG levels. However, as noted in the aforementioned study, OPG concentrations were significantly lower in serum samples than all plasma samples.[4] Moreover, OPG measured in EDTA, heparin and citrate were found to be 29.5%, 19.1%, and 24.1% higher than in serum, respectively. This is important when comparing OPG concentration from different studies due to the differences observed among sample type and standard used. Therefore, it should be better to specify sample type while evaluating OPG levels.

In conclusion, detailing factors that may affect the OPG results during the analysis phase would improve the value of the study when interpreting OPG levels among participants with the risk of subsequent CHD.

Conflict of interest/financial disclosure statement: The author states that there is no conflict of interests regarding the publication of this paper. This paper received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Reply;

We read with great interest the comments made by Dr.Sertoglu on our paper.[1]

The letter raises an interesting point regarding the clinical importance of pre-analytical and analytical measurement errors. Recently Pérez de Ciriza, *et al.* evaluated the analytical interference of endogenous substances on osteoprotegerin (OPG) measurements.[2] OPG concentrations were measured in each sample after adding different levels of triglycerides (TG) [Level 1 (76 mg/dL), level 2 (190 mg/dL), level 3 (314 mg/dL), level 4 (432 mg/dL), and level5 (551 mg/dL)].[2] They showed a positive concentration-dependent analytical interference with increasing TG concentrations from levels 1 to 5 (P value < 0.05). In our study, although the median serum TG in coronary heart disease patients without diabetes was higher than those with diabetes (P value = 0.05), the interquartile ranges of TG were broadly overlapping between the two groups. Also, the TG varied within a narrower range in our study (110 mg/dL to 231 mg/dL) compared with the study by Pérez de Ciriza, *et al.*[2] Therefore, while we agree that higher serum TG may interfere with OPG measurement, the above reasons show that the risk of analytical interference errors in our study is negligible. Furthermore, step-wise adjustments for potential confounding factors including serum TG were aimed at reducing the risk of inaccuracy where possible.

Also, we would like to clarify that in our study,[1] the concentrations of OPG were determined on serum samples of patients. After 12 hours of fasting, venous blood samples were collected and centrifuged immediately to extract sera. The specimens were then kept in freezing temperature (-20°C) until laboratory measurement. OPG concentrations were measured using EnzymeLinked Immunosorbent Assay (ELISA) method with commercially

available kits (BiomedicaMedizinprodukte GmbH & Co. KG) following the manufacturer's instructions.

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