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Efficacy of the Measurement of 25-Hydroxyvitamin D_2 and D_3 Levels by Using PerkinElmer Liquid Chromatography-Tandem Mass Spectrometry Vitamin D Kit Compared With DiaSorin Radioimmunoassay Kit and Elecsys Vitamin D Total Assay

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Vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) can be procured from exogenous sources. These are then metabolized to 25-hydroxyvitamin D (25OHD_2 and 25OHD_3) in the liver. Measuring the levels of both 25OHD_2 and 25OHD_3 is imperative in assessing clinical nutritional status [1]. Vitamin D_2 or D_3 is provided as a vitamin D supplement in many countries.

Serum 25OHD levels can be measured by competitive binding assay, RIA, automated immunoassay, HPLC, and by the recently developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique. LC-MS/MS is considered as the "gold standard" for the detection and quantification of 25OHD_2 and 25OHD_3. The MS/MS Vitamin D kit from PerkinElmer (PerkinElmer, Waltham, MA, USA) is a commercial reagent kit, intended for the quantitative determination of 25OHD_2 and 25OHD_3. The MS/MS Vitamin D kit protocol was compared with the following assays: RIA from DiaSorin (DiaSorin, Stillwater, MN, USA) and automated electro-chemiluminescence immunoassay (ECLIA) from Roche (Roche Diagnostics GmbH, Mannheim, Germany).

After receiving approval by the Ethics Review Board of the Cheil General Hospital and Women's Healthcare Centre (Seoul, Korea), consecutive samples (n=50) sent for routine 25OHD analysis were used. The MS/MS Vitamin D kit was used along with the MS/MS Vitamin D Derivatization Box (PerkinElmer) on an LC-MS/MS system that included ACQUITY TQD tandem mass spectrometer (Waters, Milford, MA, USA). The MS/MS Vitamin D kit was compared with 25OHD 125I-based RIA kit and Elecsys Vitamin D Total assay. The MS/MS Vitamin D kit, RIA kit, and Elecsys Vitamin D Total assay were run according to the manufacturers' specifications. All three assays were compared by linear regression and Bland-Altman plot. The correlation between the methods was compared by using Pearson's correlation coefficient. Agreement in the assessment of the vitamin D status between methods was evaluated by using Cohen's kappa [2]. Statistical analysis was performed by SPSS software (version 18.0.0, SPSS Inc. Chicago, IL, USA).

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Precision of the LC-MS/MS method was evaluated by inter-assay CV (n=20) of quality control materials supplied by the manufacturer. At the three levels of 25OHD$_2$, CV was <4.0%. At the three levels of 25OHD$_3$, CV was <5.3%. Inter-assay CV for RIA and ECLIA were <13.0% and <9.8%, respectively.

A comparison of LC-MS/MS with ECLIA yielded the following regression equation: ECLIA=1.1325×LC-MS/MS+0.52. The corresponding equation for RIA was: RIA=1.0546×LC-MS/MS-0.8733. In comparison with LC-MS/MS, the ECLIA demonstrated an R^2 value of 0.8741 (Fig. 1A), with an average bias of +8.4 ng/mL (15.4%) (Fig. 1C), and the RIA demonstrated an R^2 value of 0.8976 (Fig. 1B), with an average bias of +0.6 ng/mL (1.9%) (Fig. 1D). This trend was also demonstrated in previous reports, with ECLIA showing positive bias compared with LC-MS/MS [2, 3]. The distribution of results for 25OHD$_2$ and 25OHD$_3$ is shown in Fig. 2. The 25OHD$_3$ levels showed no significant difference (Fig. 2A), while the 25OHD$_2$ levels were biased towards the lower end (Fig. 2B). Compared to LC-MS/MS, having a cutoff of 20 ng/mL (insufficiency vs. normal), 4% (1/25) of the samples were misclassified as normal with RIA and 12% (3/25) of the samples were misclassified as normal with ECLIA. Relatively, agreement of RIA was better (kappa=0.96) than that of ECLIA (kappa=0.88). RIA and ECLIA, which are currently employed in clinical laboratories for total 25OHD concentration measurement, showed an acceptable correlation with LC-MS/MS in the analytical range.

The MS/MS Vitamin D kit allows for the quantitative determination of the most clinically relevant metabolite forms of vitamin D (25OHD$_2$ and 25OHD$_3$). The 25OHD levels determined by MS/MS Vitamin D kit were in overall agreement with the levels determined by DiaSorin RIA and Roche ECLIA.

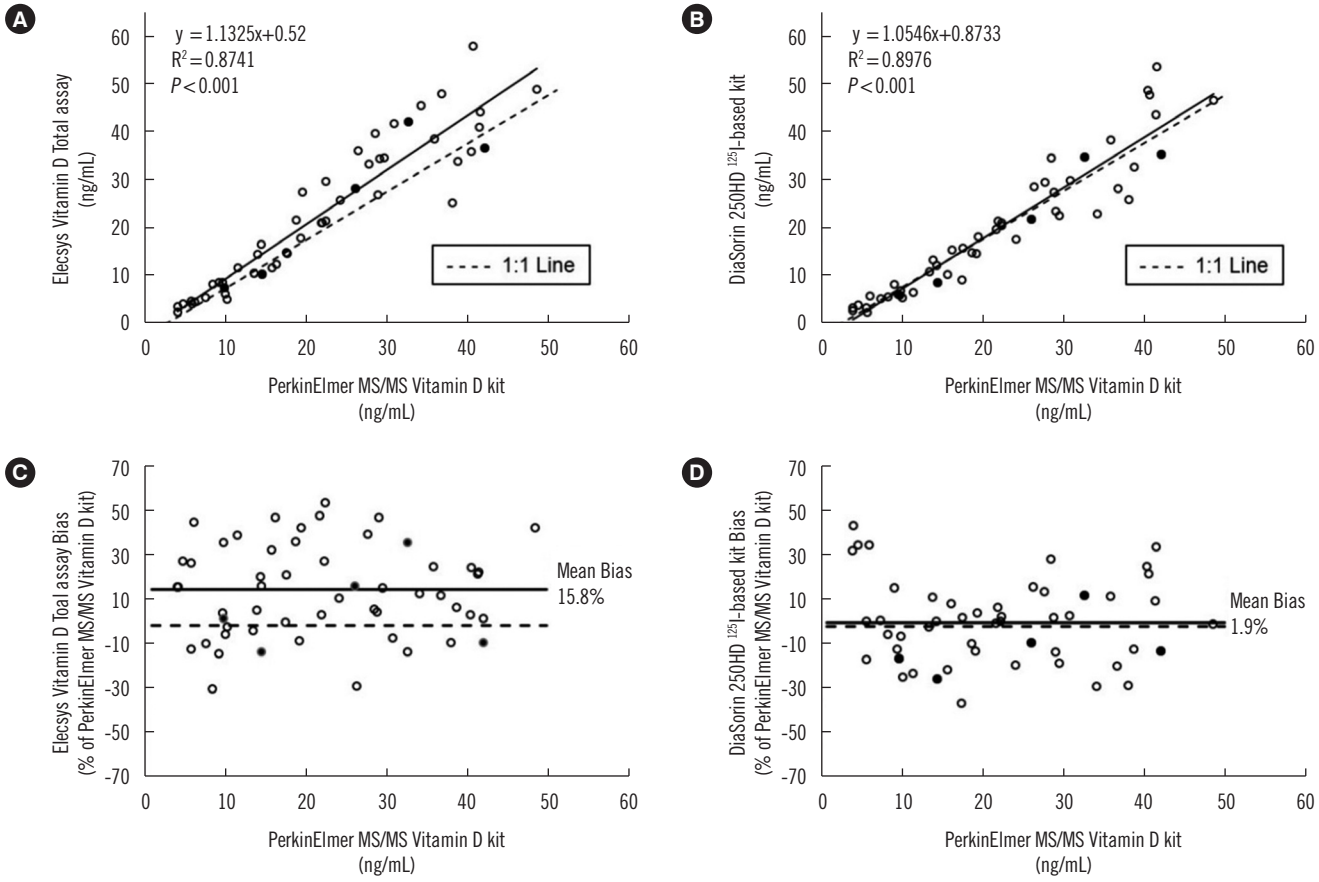

Fig. 1. Comparison between immunometric assays and LC-MS/MS (PerkinElmer MS/MS Vitamin D kit) for 25-hydroxyvitamin D quantification: (A, B) Linear regression between LC-MS/MS and ECLIA (Elecsys Vitamin D total assay), and LC-MS/MS and RIA (DiaSorin RIA kit), respectively. (C, D) Bland-Altman plot between LC-MS/MS and ECLIA, and LC-MS/MS and RIA, respectively. Open circles represent samples containing relatively low concentrations of 25-hydroxyvitamin D_2 (<1 ng/mL), and black circles represent samples containing relatively high concentrations of 25-hydroxyvitamin D_2 (≥1 ng/mL).
Abbreviations: ECLIA, electrochemiluminescence immunoassay; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

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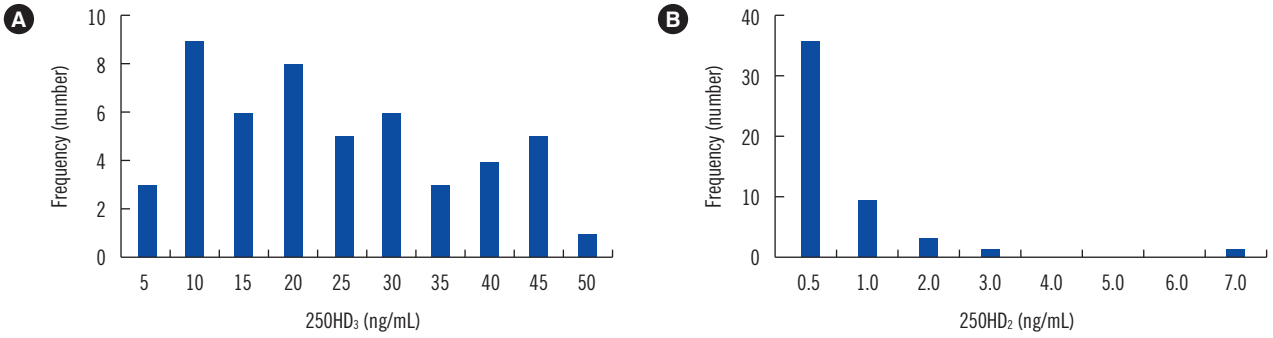

Fig. 2. Distribution for 25-hydroxyvitamin D_3 ($25OHD_3$) (A) and 25-hydroxyvitamin D_2 ($25OHD_2$) (B). Results were obtained by analyzing serum samples provided by 50 volunteers.

Author's Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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