Research Article

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Correlation between serum 1,25-dihydroxy-vitamin D and 25-hydroxyvitamin D in response to analytical procedures; a systematic review and meta-analysis

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Abstract

Objectives: In this study, the aim is to provide a more detailed understanding of vitamin D metabolism by evaluating the correlation between 1,25-dihydroxyvitamin D (1,25(OH)2D) and 25-hydroxyvitamin D (25(OH)D) according to the variations in measurement methods and clinical conditions.

Methods: We searched PubMed, Embase, and Web of Science for studies reporting correlation results between 1,25(OH)2D and 25(OH)D. We performed a meta-analysis based on the correlation results of 1,25(OH)2D and 25(OH)D in different clinical conditions. We included a total of 63 studies and our laboratory's results in the meta-analysis. The studies were categorized into high-quality methods group (HQMG), medium-quality methods group (MQMG), and low-quality methods group (LQMG) based on the 25(OH)D and 1,25(OH)2D measurement.

Results: In the healthy, renal disease, and other disease groups, the highest correlation values were observed in the studies categorized as HQMG, with values of 0.35 (95 % CI; 0.23–0.48), 0.36 (95 % CI; 0.26–0.42), and 0.36 (95 % CI; 0.22–0.48), respectively. Significant statistical heterogeneity was observed in the healthy, renal disease, and other disease groups, with I2 values of 92.4 , 82.7, and 90.7 %, respectively ($p<0.001$). Both Funnel plots and the results of Egger's and Begg's tests indicated no statistically significant bias across all studies.

Conclusions: A significantly low correlation was found between 25(OH)D and 1,25(OH)2D. However, higher correlations were found in the studies categorized as HQMG. Various factors, including methodological inadequacies and disparities, might contribute to this. In the future, with more accurate and reproducible measurements of 1,25(OH)2D, a clearer understanding of vitamin D metabolism will be achieved.

Keywords: 1,25-dihydroxyvitamin D; 25-hydroxyvitamin D; analytical chemistry methods; correlation study; healthy subjects; renal disease

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Introduction

Vitamin D deficiency (VDD) is the most common worldwide, and more than 1 billion people are known to have a deficiency [1]. Although VDD was known long ago, rickets and osteomalacia were first distinctly described in 1645 [2]. The experimental animal studies have clarified its synthesis, mechanism, and treatments for its deficiencies [3–6]. It has been stated that there has been a considerable increase in the number of individuals affected by VDD in recent years, especially common in the elderly and those who stay indoors for longer periods, people with pigmented skin, pregnant women, vegans, and children of developmental age.

The regulation of vitamin D metabolism

Vitamin D metabolism and regulation are very complex (Figure 1). Vitamin D3 is produced in the skin by ultraviolet-B (290–315 nm) irradiation of 7-dehydrocholesterol (7-DHC). Irradiation of 7-DHC produces pre-D3 (which later becomes vitamin D3), lumisterol, and tachysterol [7]. Melanin in the skin absorbs UV radiation, potentially reducing the skin's ability to produce vitamin D from sunlight. This could be a key factor contributing to lower 25-hydroxyvitamin D (25(OH)D) levels (which is a well-established indicator of vitamin D levels) in Black and Hispanic individuals living in regions with less direct sunlight [8]. The 25(OH)D levels can exhibit significant seasonal fluctuations, with elevated concentrations in the summer and decreased levels in the winter.

Vitamin D is initially metabolized to 25(OH)D, predominantly in the liver, and then further converted to 1,25-dihydroxyvitamin D (1,25(OH)2D), mainly in the kidney. This final form, 1,25(OH)2D, is the primary active form responsible for most of vitamin D's effects in the body [9].

The 25-hydroxylase-CYP2R1 is highly controlled by a variety of diseases (obesity, diabetes mellitus, starvation, infection, inflammation, cancer, etc.), and although many regulatory factors have now been identified, significant gaps remain. Genetic silencing mutations in CYP2R1 can cause rickets, osteomalacia, or other clinical conditions. Serum 25(OH)D may not reflect only the vitamin D produced by diet and skin. In particular, hepatic 25(OH)D synthesis has a complex regulation involving many possible hormones and factors.

The enzyme 1,25-dihydroxylase CYP27B1 is mainly present in the renal proximal tubule, and its production is influenced by changes in parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), 1,25(OH)2D, calcium, and phosphate levels [10]. A specific region in the enhancer region of renal CYP27B1 is responsible for responding to PTH, FGF23, and 1,25(OH)2D regulation [11]. However, this region is not open to such regulation in non-kidney tissues like skin, immune cells, and adipose tissue. In non-renal tissues, a different enhancer region of CYP27B1 is regulated by various factors such as interferon-γ (IFN-γ), cytokines like tumor necrosis factor-α (TNF-α), or leptin [12, 13]. These feedback loops play a vital role in regulating 1,25(OH)2D production in the kidney, which differs from CYP27B1 in other cell types, including distal renal tubular cells that are minimally affected by PTH [14]. CYP24A1 regulates 1,25(OH)2D levels in other tissues and is stimulated by TNF-α and IFN-γ. However, in some conditions like sarcoidosis, where macrophages produce excess 1,25(OH)2D without proper CYP24A1 regulation, it can lead to hypercalcemia and hypercalciuria [15–17].

25(OH)D and 1,25(OH)2D are carried in the bloodstream by a protein called vitamin D-binding protein (DBP). This

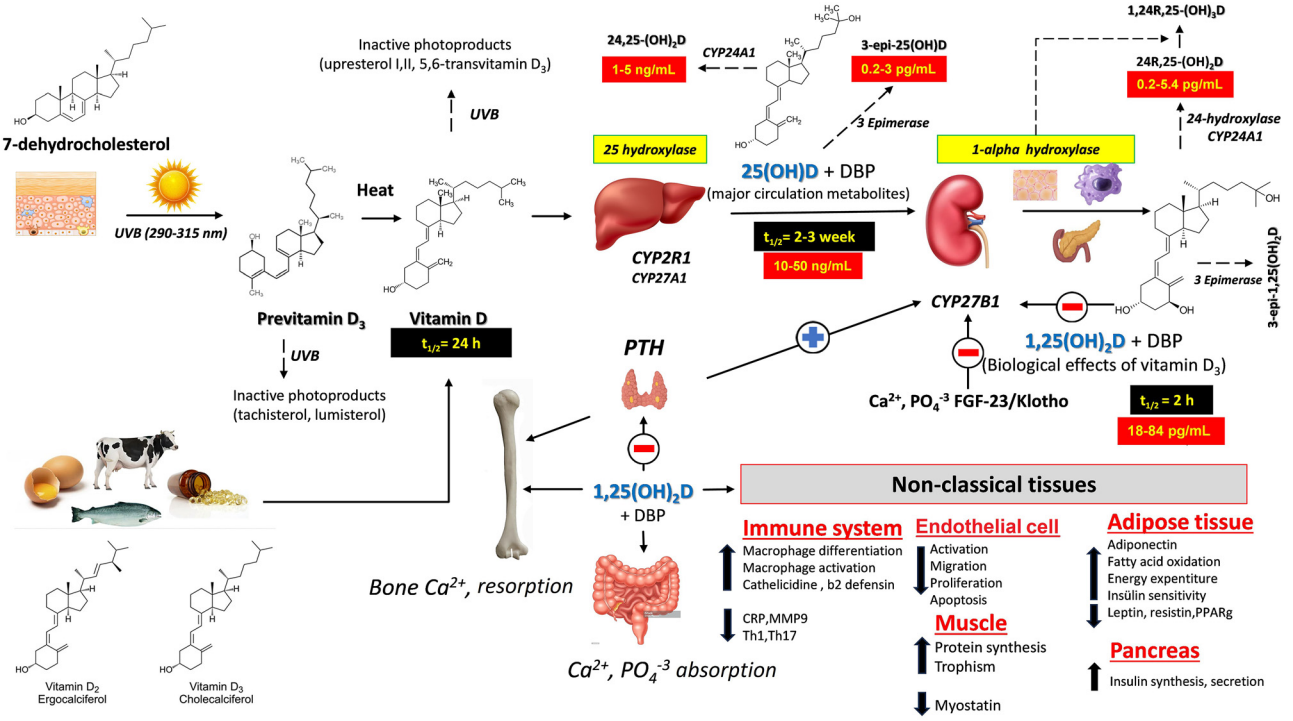

Figure 1: Vitamin D metabolism and regulation.

protein safeguards them from degradation and allows for their storage. About 85–90 % of the 25(OH)D/1,25(OH)2D pool is bound to DBP in the serum. The remaining 10–15 % is loosely attached to serum albumin and lipoproteins. Only a tiny fraction (less than 0.1 % of 25(OH)D and about 0.4 % of 1,25(OH)2D) is freely available [9]. Total 25(OH)D or 1,25(OH)2D includes three fractions: DBP-bound, weakly bound to albumin (also called bioavailable, as they can easily dissociate from albumin), and free compounds. Changes in DBP levels due to genetic factors, hormonal status, or liver and kidney conditions can impact the accuracy of 25(OH)D levels as a marker of vitamin D status, especially in pregnancy, estrogen-containing contraceptives, and liver or kidney diseases. Through the measurement of total 25(OH)D, DBP, and albumin by the principles of protein-ligand binding kinetics, it is possible to calculate both the 25(OH)D/1,25(OH)2D-DBP and 25(OH)D/1,25(OH)2D-albumin affinities [9, 18]. However, these are used limitedly due to their impracticality and complexity.

The clinical decision limits for vitamin D

Determining the clinical decision limits of 25(OH)D is complicated. In 1997, the Food and Nutrition Board of the Institute of Medicine identified serum 25(OH)D as a good marker for evaluating vitamin D status [19]. However, there was not enough data at that time to fully understand its normal range in the body. Estimated values were insufficient, and it has since become evident that vitamin D deficiency is more prevalent than previously thought. It is affected by various factors such as seasons, diet, medications, and inaccurate measurement methods. Studies have investigated the relationship between serum PTH levels and 25(OH)D levels. It is known that PTH levels increase at low levels of 25(OH)D values but decrease at 75–110 nmol/L levels [20–22]. In this context, high PTH levels suggest the body is adapting to lower calcium intake. However, whether this adaptation indicates better health is still uncertain [23]. To date, no definitive functional change in 25(OH)D levels is known at the point where PTH stabilizes at the lower end of the healthy reference value (or clinical decision threshold level) of 25(OH)D. Vitamin D significantly increases circulating 1,25(OH)2D concentrations, but in vitamin D users, this increase is suppressed by calcium co-administration.

Measuring 25(OH)D levels is crucial in assessing human vitamin D status. Cut-off values for various status categories are established based on correlations between circulating 25(OH)D concentrations and physiological/clinical changes in the body [24–26]:

- Increased risk of deficiency status (<30 nmol/L, <12 ng/mL)
- Increased risk of inadequacy (<40 nmol/L, <16 ng/mL)
- Adequacy (>50 nmol/L, >20 ng/mL)
- Increased risk of excess (or potentially harmful effects) (>125 nmol/L, >50 ng/mL).

The relationship between 25(OH)D and 1,25(OH)2D

Circulating 1,25(OH)2D level is generally not a good indicator of vitamin D status. 1,25(OH)2D has a short half-life; PTH, Ca, and PO4 tightly regulate serum levels and do not decrease until severe vitamin D deficiency. Additionally, the limitations of commercial measurement kits for 1,25(OH)2D have necessitated the development of new methods and the use of alternative reference ranges [27, 28].

The relationship between 25(OH)D and 1,25(OH)2D is multifaceted and complex. In this study, we planned to conduct a meta-analysis and systematic review to elucidate the relationship between 25(OH)D and 1,25(OH)2D, aiming to gain a better understanding of vitamin D metabolism. This correlation was designed considering two different scenarios. In the first scenario, the measurement methods for 25(OH)D and 1,25(OH)2D, along with the analytical limitations associated with these methods, were considered. In the second scenario, various clinical conditions, such as in healthy individuals, kidney diseases (where 1,25(OH)2D synthesis takes place), and systemic diseases, were individually evaluated by considering their specific effects on vitamin D metabolism.

Materials and methods

This meta-analysis was conducted following the guidelines recommended by the PRISMA statement [29].

The strategy of publication search

In this meta-analysis study, comprehensive search strategies were created to identify publications. Articles published between 2005 and 2023 without language restrictions are in MEDLINE (via PubMed), Embase, and Web of Science. Using the keywords "(25-hydroxy D OR 25-hydroxycholecalciferol OR 25OHD OR 25-OH vitamin D OR calcidiol) AND (1,25-dihydroxy vitamin D OR 1,25-dihydroxycholecalciferol OR 1,25-dihydroxy vitamin D OR calcitriol)) NOT (animal)) NOT (review)) NOT (case report)) AND (correlation))."

The selection and extraction criteria of publications

Four independent reviewers, who were blinded to the study details, read the titles and abstracts of all reports found through electronic searches (MAS, FDA, NYS, DY). For studies that seemed to fulfill the

inclusion criteria, or when the title and abstract provided insufficient data for a definitive decision, the complete report was acquired. The reliability between reviewers was assessed using Cohen's kappa test, setting an acceptable threshold at 0.74. Discussions among the reviewers resolved disagreements about whether to include or exclude certain studies. The relationship between 1,25(OH)2D and 25(OH)D levels was analyzed using various clinical conditions and analytical techniques.

For this meta-analysis, studies were included if they reported correlation test results (such as Pearson correlation without or with log transformation, Spearman correlation, or regression analysis) conducted in serum or plasma, were published in English, had accessible full texts, and were conducted on human subjects. Studies were excluded if they lacked correlation values but provided interpretations, were animal studies, or involved other biological samples (such as cord blood, cerebrospinal fluid, etc.).

The classification of analytical methods

High-quality methods group (HQMG): Automated and traceable 25(OH)D and 1,25(OH)2D measurements, commercial liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) measurement (ImmuTube® LC-MS/MS assay), in-house LC-MS/MS measurement, and automated repeatable immunoassay (like LIASON) methods were used and given analytical performance for both tests (limit of detection, limit of quantification, repeatability, linearity, etc.).

Medium-quality methods group (MQMG): This group contains non-automated 25(OH)D and 1,25(OH)2D radioimmunoassay (RIA), enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), and limited analytical performance information.

Low-quality methods group (LQMG): The methods have no or insufficient information regarding 25(OH)D and 1,25(OH)2D measurements.

The classification of clinical conditions

The clinical conditions were evaluated in three groups: healthy, kidney diseases, and other illnesses.

The results of 1,25(OH)2D and 25(OH)D in our laboratory

In addition to the studies, the total of 25.457 results of 5424 patients who applied for various reasons between 2015 and 2023 and had measurements of 1,25(OH)2D and 25(OH)D in the Acıbadem Labmed Laboratory have also been included. Patients have been presented in three groups, like the other groups, based on their ICD codes, diagnoses, clinical information, and other laboratory test results.

Presentation of data

Data extraction was achieved by the four authors of the meta-analysis (MAS, DY, NSY, FDA). Detailed data from 63 articles, along with our laboratory results, are included in this meta-analysis. The following information was extracted from the studies: the year of the research, place where it was conducted, study design, sample size, gender, age, diseases, analytical measurement procedures, sample size, gender, correlation results between 25(OH)2D and 25(OH)D, and analytical quality considerations. Data were compiled into evidence tables, and a descriptive summary was formulated to assess the volume of data, various study characteristics, and outcomes (Table 1).

For measurements of 25(OH)D and 1,25(OH)2D in this study, information about the manufacturers, methods, sample types, analytical performances, and interferences belonging to the most commonly preferred brands are provided in Supplemental Table 1 (for 25(OH)D) and Supplemental Table 2 (for 1,25(OH)2D).

Statistical analysis

Meta-analysis was achieved on the correlation between 25(OH)D and 1,25(OH)2D. These analyses were done using Stata MP17 4.6.241 (Stata Corp LLC, Texas, USA). Random effects meta-analyses were performed using the DerSimonian–Laird method. The $\chi 2$ and I2 statistics were used to assess the statistical heterogeneity among the included studies. Forest plots were drawn to describe the weighted correlation with 95 % confidence intervals (CI). In addition, the Funnel plot and Egger and Beggs tests were applied to explore the sources of bias.

Results

The flowchart of the study, according to the PRISMA statement, is presented in Figure 2. Initially, the search strategy retrieved 1388 references. After screening the titles and abstracts, 1325 articles were excluded due to unrelated topics. The entire texts of the remaining 264 articles were assessed, and 63 studies were included in the meta-analysis. In this meta-analysis, correlation analysis results of a total of 25.147 people, consisting of 63 studies and our laboratory data, were evaluated. The meta-analysis outcomes are represented according to clinical conditions in Figures 3–5.

Accordingly, in the healthy group, a total of 24 studies were evaluated. Among these, ten were classified as HQMG, eleven as MQMG, and three as LQMG. The correlation values were calculated as 0.35 (95 % CI, 0.23–0.48) with 91.2 % of heterogeneity (I2) for HQMG, 0.21 (95 % CI, 0.10–0.31) with 91.3 % for MQMG, and as 0.22 (95 % CI, 0.03–0.42) with 38.0 % for LQMG. The correlation value was determined in the total healthy group as 0.26 (95 % CI, 0.18–0.34) with 92.4 % of I2. It was observed that the correlation value was the highest in HQMG, followed by MQMG, and lastly, LQMG. Significant heterogeneity was detected in all groups except for the LQMG group and in the overall evaluation of the study.

In the case of renal diseases, a total of 19 studies were assessed. Among these, nine were categorized as HQMG, seven as MQMG, and three as LQMG. The correlation values were calculated as 0.34 (95 % CI, 0.26–0.42) with 78.6 % of I2 for HQMG, 0.28 (95 % CI, 0.17–0.38) with 75.0 % for MQMG, and 0.27 (95 % CI, 0.25–0.37) with 92.6 % for LQMG. In the overall analysis, encompassing both healthy and renal disease groups, the correlation value was determined to be 0.31

Table 1: Detailed data from 63 articles and our laboratory results are included in this meta-analysis. These data consist of the year of the research, place where it was conducted, study design, sample size, gender, age, diseases, analytical measurement procedures, sample size, gender, correlation results between 25(OH)2D and 25(OH)D, and analytical quality performances.

No.	Study	Study type	Country	Population	n	Age group	Female/male	25 (OH)D measurement	25 (OH)D brand/procedure	1,25 (OH)2D measurement type	1,25 (OH)2D brand/procedure	Analytical performance	Correlation, r	p-Value	Analytical quality
1	Salle, 1983 [30]	Non-RCT	France	Premature infants supplemented with vitamin D	61	Children		Radioligand assay	Sigma Chemicals, MO, USA	Specific receptor assay	In-house/no data	CVinterassay CVintraassay Analytical sensitivity	0.79 (LR)	<0.001	LQMG
2	Shany, 1984 [31]	Cross-sectional	Israel	Women in normal labor-serum	20	Adult	20/0	Chromatographic competitive protein binding assays	In-house	RIA	In-house/no data	No data	0.22 (LR)	>0.05	LQMG
3	Mosekilde, 1989 [32]	Case-control	Denmark	Primary hyperparathyroidism and controls	75	Adult	PTH 24/10-H 28/12	RIA	In house	RIA	In-house	CVinterassay CVintraassay Analytical sensitivity Cross reaction	0.39 (MR)	<0.05	LQMG
4	Bettica, 1999 [33]	Cross-sectional	Italy	Postmenopausal women	570	Adult	23/0	RIA	After extraction (Nichols Institute Diagnostics, CA)	RIA	Nichols Institute Diagnostics, CA	CVinterassay CVintraassay	0.49 (P)	<0.03	MQMG
5	Panidis, 2005 [34]	Case-control	Greece	PCOS and healthy control	228	Adult	228/0	RIA	BioSource Europe, Nivelles, Belgium	RIA	BioSource Europe, Nivelles, Belgium	No data	0.204 (P)	0.013	MQMG
6	Malavolta, 2005 [35]	Cross sectional	Italy	Postmenopausal women	156	Adult	156/0	RIA	Nichols Institute Diagnostics, Paris, France	RIA	DiaSorin, Stillwater, MN, USA	IVD No data	0.177 (P)	0.027	MQMG
7	Li, 2007 [36]	RCT	United States	Prostate cancer and control group	480	Adult	0/480	RIA	No data	RIA	No data	CVintraassay	0.17 (S)	<0.001	LQMG
8	London, 2007 [37]	Cross-sectional	France	ESRD	40	Adult		CLIA	LAISON, DiaSorin, MN, USA	RIA	DiaSorin, MN, USA	No data	0.365 (P)	<0.05	HQMG
9	Moosgaard, 2007 [38]	Cross-sectional	Denmark	Primary hyperparathyroidism	252	Adult	215/37	RIA	DiaSorin, MN, USA	RIA	Nichols Institute, California, USA	IVD CVinterassay CVintraassay Cross reaction	0.15 (LR)	<0.05	MQMG
10	Jean, 2008 [39]	Non-RCT	France	CKD stage 5	43	Adult	17/26	CLIA	LAISON, DiaSorin, MN, USA	RIA	DiaSorin, MN, USA	CVinterassay Analytical sensitivity	0.283 (LR)	0.02	HQMG
11	Matias, 2008 [40]	Cross-sectional	Portugal	ESRD	223	Adult	107/116	RIA	IDS Ltd, Boldon, UK	RIA	IDS Ltd, Boldon, UK	IVD CVinterassay CVintraassay	0.25 (S)	<0.001	MQMG
12	Need, 2008 [41]	Cross-sectional	Australia	Osteoporosis	319	Adult	8/311	Competitive protein binding assay	In house-No data	HPLC and RIA	In house-no data	CVinterassay Analytical sensitivity	0.115 (no data)	<0.05	LQMG
13	Shroff, 2008 [42]	Cross-sectional	UK	CKD and health control	101	Adult	Patients; 24/37, controls; 18/22	EIA	IDS Ltd, Boldon, UK	RIA	DiaSorin, MN, USA	No data	0.11 (no data)	0.4	MQMG
14	Inoue, 2008 [43]	Cross-sectional	Japan	Postmenopausal primary hyperparathyroidism	30	Adult	30/0	Competitive protein binding assay	In house	Radioreceptor assay	In house	No data	−0.401 (P)	0.0462	LQMG
15	Zittermann, 2009 [44]	Prospective cohort	Germany	End-stage heart failure and health control	510	Adult	98/190 213/69	RIA	DiaSorin. MN, USA	EIA	IDS Ltd, Boldon, UK	CVinterassay CVintraassay Analytical sensitivity Cross reaction	0.218 (P)	<0.001	MQMG
16	Marcen, 2009 [45]	Cross-sectional	Spain	Renal transplant (12. months)	509	Adult	214/295	EIA	IDS, Boldon, UK	RIA	Biosource Europe, Nivelles, Belgium	IVD No data	0.138 (Log, P)	0.008	MQMG
17	Boudville, 2010 [46]	Cross-sectional	Australia	CKD (stage 5) with pre-dialysis	25	Adult	5/20	RIA	DiaSorin, MN, USA	RIA after extraction	DiaSorin, MN, USA	IVD CVinterassay	0.54 (S)	0.005	MQMG

Table 1: (continued)

No.	Study	Study type	Country	Population	n	Age group	Female/male	25 (OH)D measurement	25 (OH)D brand/procedure	1,25 (OH)2D measurement type	1,25 (OH)2D brand/procedure	Analytical performance	Correlation, r	p-Value	Analytical quality
18	Nguyen, 2010 [47]	Cross-sectional	France	Idiopathic hypercalcemia and hypercalciuria	20	Adult	45,150	Chromatographic competitive protein binding assays	In house-no data	Chromatographic competitive protein binding assays and HPLC	In house No data	No data	0.434 (LR)	0.0383	LQMG
19	Christensen, 2010 [48]	Cross-sectional	Norway	Healthy subjects	3484	Adult	1551/1933	RIA	IDS, Boldon, UK	RIA	IDS, Boldon, UK	IVD CVinterassay Analytical sensitivity Cross reaction	0.14 (P)	<0.001	MQMG
20	Zhou, 2010 [49]	Cross-sectional	United States	Osteoarthritis	27	Adult	13/14	RIA	DiaSorin, MN, USA	RIA	DiaSorin, MN, USA	CVinterassay Analytical sensitivity	0.016 (S)	0.94	MQMG
21	Thrailkill, 2011 [50]	Prospective cohort	United States	Healthy subjects Type 1 DM without proteinuria Type 1 DM with proteinuria	55 99 16	Adult Adult Adult	31/24 53/46 8/8.	Competitive immunoluminometry	No data, ARUP Laboratories	RIA	No data, ARUP Laboratories	No data	0.451 (S) 0.19 (S) 0.096 (S)	<0.001 0.062 0.744	MQMG MQMG MQMG
22	Walker, 2011 [51]	Case-control	United States	Healthy subjects	44	Adult	35/9	RIA	In-house	RIA	In-house	CVinterassay CVintraassay Analytical sensitivity Cross reaction	0.02 (no data)	0.89	LQMG
23	Stein, 2012 [52]	Cross-sectional	United States	Children with CKD (1–5)	100	Adult	40/60	RIA	DiaSorin, MN, USA	Column chromatography, RIA, and kinetic methods	Laboratory Corporation of America	CVinterassay CVintraassay	0.38 (P)	<0.001	MQMG
24	Moen, 2012 [53]	Case-control	Norway	Multiple sclerosis Healthy subjects	99 159	Adult Adult	71/28 117/42	RIA	DiaSorin, MN, USA	RIA	DiaSorin, MN, USA	CVinterassay CVintraassay	0.342 (no data) 0.255 (no data)	0.001 0.001	MQMG MQMG
25	Jovanovich, 2012 [54]	RCT		CKD is not yet on dialysis and ESRD	1497	Adult	White 17/856 Black 12/612	CLIA	LAISON, DiaSorin, MN, USA	RIA	DiaSorin, MN, USA	CVinterassay CVintraassay Analytical sensitivity	0.33 (S)	<0.001	HQMG
26	Kox, 2012 [55]	Non-RCT	The Netherlands	Young, healthy, non-smoking male	112	Adult	0/112	ECLIA	Roche Diagnostics, Burgess Hill, UK	RIA	IDS Ltd, Boldon, UK	No data	0.23 (S)	0.02	HQMG
27	Carpenter, 2012 [56]	Cross-sectional	Connecticut, US	Healthy infants and toddlers	715	Adult	25(OH)D group; 401/352, 1,25(OH)2D group; 379/355	RIA	DiaSorin, MN, USA	RIA	DiaSorin, MN, USA	IVD CVinterassay CVintraassay	0.15 (P)	<0.001	MQMG
28	Denburg, 2013 [57]	Prospective cohort	United States	Children with CKD (stage 2–5)	171	Adult	70/101	RIA	DiaSorin, MN, USA	RIA	DiaSorin, MN, USA	CVintraassay	0.47 (S)	<0.001	MQMG
29	Muindi, 2013 [58]	Non-RCT	United States	Colorectal cancer	313	Adult	147/116	LC-MS/MS	TSQ Quantum ULTRA Mass Spectrometer	RIA	DiaSorin, MN, USA	IDMS IVD Analytical sensitivity	0.308 (S)	<0.05	HQMG
30	Viapiana, 2013 [59]	Cross-sectional	Italy	Postmenopausal women affected by primary hyperparathyroidism and healthy postmenopausal women	63	Adult	63/0	EIA	IDS Ltd, Boldon, UK	EIA	IDS Ltd, Boldon, UK	CVinterassay CVintraassay Analytical sensitivity	−0.46 (MR)	<0.01	MQMG
31	Camargo, 2014 [60]	Cross-sectional	Sao Paulo, Brazil	Postmenopausal women	50	Adult	50/0	CLIA	LAISON, DiaSorin.Inc, Stillwater, MN	RIA	IDS Ltd, Boldon, UK	CVinterassay CVintraassay	0.584 (P)	<0.01	HQMG
32	Swanson, 2014 [61]	Cross-sectional	United States	Osteoporotic fractures in men	679	Adult	0/679	LC-MS/MS	In-house	LC-MS/MS	In-house	CVinterassay CVintraassay Analytical sensitivity	0.5 (S)	<0.001	HQMG

Table 1: (continued)

No.	Study	Study type	Country	Population	n	Age group	Female/male	25 (OH)D measurement	25 (OH)D brand/procedure	1,25 (OH)2D measurement type	1,25 (OH)2D brand/procedure	Analytical performance	Correlation, r	p-Value	Analytical quality
33	Kamphuis, 2014 [62]	Retrospective cohort	Netherlands	Sarcoidosis patients	172	Adult	174/127	RIA	IDS Ltd, Boldon, UK	RIA	IDS Ltd, Boldon, UK	No data	0.36 (P)	<0.001	MQMG
34	Pasquali, 2015 [63]	Case-control	Italy	Renal transplant	135	Adult	79/56	RIA	DiaSorin, MN, USA	RIA	IDS Ltd, Boldon, UK	IVD	0.45 (S)	<0.001	HQMG
				Haemodialysis	76	Adult	43/34					CVinterassay	0.51 (S)	<0.001	HQMG
				CKD stage 2–5	111	Adult	72/39					CVintraassay	0.51 (S)	<0.001	HQMG
				Healthy subjects	290	Adult	146/141						0.25 (S)	0.003	HQMG
				Primary hyperparathyroidism	20	Adult	9/11						0.51 (S)	0.042	HQMG
35	Kondo, 2016 [64]	Cross-sectional	Japan	Diabetic nephropathy (low risk)	151	Adult	43/108	CLIA	Abbott Laboratories, Chicago, USA	RIA	DiaSorin, Saluggia, Italy	Automated	0.31 (P)	>0.05	HQMG
				Diabetic nephropathy (moderate risk)	102	Adult	37/65					IVD	0.06 (P)	>0.05	HQMG
				Diabetic nephropathy (high risk)	113	Adult	40/73					No data	0.44 (P)	<0.05	HQMG
				Diabetic nephropathy (Vert high risk)	78	Adult	33/45						0.1 (P)	<0.05	HQMG
36	Zhang, 2016 [65]	Prospective cohort	United States	HIV-infected man	640	Adult	0/640	Immunoaffinity LC-MS/MS	In-house	Immunoaffinity LC-MS/MS	In-house	IDMS	0.32 (no data)	0.001	HQMG
				Healthy adults, HIV-uninfected men	99	Adult	0/99					CV			
												Analytical sensitivity	0.09 (no data)	0.623	HQMG
37	Souberbielle, 2016 [66]	Cross sectional	France	Healthy subjects	892	Adult	429/463	CLIA	LIASON XL, DiaSorin, MN, USA	CLIA	LIASON XL, DiaSorin, MN, USA	CVinterassay CVintraassay Analytical sensitivity	0.21	0.001	HQMG
38	Ter Horst, 2016 [67]	Prospective cohort	The Netherlands	Morbidly obese women	37	Adult	37/0	Isotope dilution LC-MS/MS	In-house	Isotope dilution LC-MS/MS	In-house	No data	0.2 (P)	0.25	HQMG
39	Doyon, 2016 [68]	Cross-sectional	12 European countries	Children with CKD (stage 3–5)	500	Children	179/321	LC-MS/MS	In-house	LC-MS/MS	In-house	CVinterassay CVintraassay Analytical sensitivity	0.56 (LR)	<0.001	HQMG
40	Valcour, 2016 [69]	Cross-sectional	United States	Newborn infants	78	Children		No data	No data	CLIA	LAISON XL, DiaSorin, MN, USA	CVinterassay CVintraassay Analytical sensitivity Cross reaction	0.15 (P)	0.18	MQMG
41	Ghazi, 2016 [70]	RCT	Iran	Children	210	Children	105/105	EIA	IDS Ltd, Boldon, UK	ELISA	Cusabio Biotech Co.	CVinterassay CVintraassay Analytical sensitivity	−0.111 (S)	0.126	MQMG
42	Bima, 2017 [71]	Cross-sectional	Australia	Healthy subjects	322	Adult	145/177	Solid-phase extraction and LC-MS/MS	Qu Lab, University, Buffalo, New York	Solid-phase extraction LC-MS/MS	Qu Lab, University, Buffalo, New York	IDMS Accuracy CVinterassay CVintraassay Analytical sensitivity	0.53 (Log, P)	<0.0001	HQMG
43	Marques Vidigal, 2017 [72]	Case-control	Brazil	Healthy subjects	321	Adult	%50.8 male	HPLC	No data	HPLC	No data	No data	0.35 (P)	<0.001	MQMG
				Colorectal cancer	152	Adult	%53.3 male						0.09 (P)	>0.05	MQMG
44	Yadav, 2017 [73]	Cross-sectional	India	Nephrotic syndrome and healthy controls	141	Adult	42/59 15/25	EIA	IDS Ltd, Boldon, UK	EIA	IDS Ltd, Boldon, UK	Accuracy CVinterassay CVintraassay Analytical sensitivity	0.321 (S)	0.001	HQMG

Table 1: (continued)

No.	Study	Study type	Country	Population	n	Age group	Female/male	25 (OH)D measurement	25 (OH)D brand/procedure	1,25 (OH)2D measurement type	1,25 (OH)2D brand/procedure	Analytical performance	Correlation, r	p-Value	Analytical quality
45	Pauwels, 2017 [74]	Case-control	Belgium	CKD and healthy	121	Adult	Healthy (7/13) Patients (51/50)	RIA	DiaSorin, MN, USA	LC-MS/MS	In-house	IDMS Accuracy CVinterassay Analytical sensitivity	0.24 (P)	0.09	HQMG
46	Chung, 2017 [75]	Cross-sectional	Korea	Isolated haematuria, proteinuria, or renal dysfunction of unexplained cause.	199	Adult	0/94	No data	No data	No data	No data	No data	0.179 (LR)	0.02	LQMG
47	Best, 2018 [76]	Prospective cohort	United States	Pregnant women	58	Adult	58/0	LC-MS/MS	In-house	LC-MS/MS	In-house	CVinterassay CVintraassay	0.14 (P)	>0.05	HQMG
48	Havens, 2018 [77]	Cross-sectional	United States	Youth without HIV, Group 2	209	Adult	33/176	No data	No data	No data	No data	No data	0.319 (S)	<0.0001	LQMG
				Youth without HIV (before of prophylaxis), Group 1	99	Adult	0/99						0.189 (S)	>0.05	LQMG
				Youth without HIV (12 weeks of prophylaxis), Group 2	77	Adult	0/77						0.041 (S)	>0.05	LQMG
49	Ouma, 2018 [78]	Case-control	Japan	Alzheimer's disease	108	Adult	69/39	RIA	DiaSorin, MN, USA	RIA	IDS Ltd, Boldon, UK	No data	0.301 (P)	0.372	MQMG
				Mild cognitive impairment	61	Adult	31/30						0.254 (P)	0.05	MQMG
				Healthy subjects	61	Adult	28/31						0.162 (P)	0.221	MQMG
50	Baumann, 2018 [79]	Cross-sectional	Switzerland	Early breast cancer	310	Adult	310/0	HPLC	Chromsystems, Gräflingen, Germany	RIA	IDS Ltd, Boldon, UK	No data	0.21 (S)	<0.001	HQMG
51	Zittermann, 2018 [80]	RCT	Germany	Heart failure	165	Adult	Vitamin D group 64 (male) Placebo group 71 (male)	CLIA	DiaSorin, MN, USA	CLIA	LAISON, DiaSorin. MN, USA	No data	0.205 (S)	<0.01	HQMG
52	Evenepoel, 2019 [81]	Prospective cohort	Belgium	Renal transplant	518	Adult	202/316	RIA	No data	RIA	No data	No data	0.49 (S)	<0.0001	LQMG
53	Albahlol, 2020 [82]	Cross-sectional	Saudi Arabia	Pregnant women (preeclampsia, GDM, undisturbed ectopic pregnancy abortion, premature rupture of membranes) and control	322	Adult	322/0	ELISA	Sunlong Biotech Co. Ltd., Zhejiang, China	ELISA	Sunlong Biotech Co. Ltd., Zhejiang, China	No data	0.157 (S)	<0.05	LQMG
54	Martin, 2020 [83]	Cross-sectional	United States	Early onset Preeclampsia-EOP -S	7	Adult	7/0	EIA	IDS Ltd, Scottsdale, AZ	EIA	IDS Ltd, Scottsdale, AZ	IVD	0.542 (LR)	0.03	HQMG
				Early onset controls- EOC -S	5	Adult	5/0					CVinterassay CVintraassay Analytical sensitivity Cross reaction	0.324 (LR)	0.221	HQMG
55	Harmon, 2020 [84]	Prospective cohort	Western New York	Healthy subjects	86	Adult	86/0	ELISA	BioVendor R&D, Asheville, NC	EIA	IDS Ltd, Scottsdale, AZ	CVinterassay	0.41 (P)	<0.001	MQMG
				Late onset Controls-LOC-S	9	Adult	9/0					CVintraassay	0.133 (P)	0.61	MQMG
				Late onset Controls- LOC -S	10	Adult	10/0						−0.11 (P)	0.673	MQMG
56	Ismail, 2021 [85]	Case-control	Saudi Arabia	Acute coronary syndrome and controls	123	Adult	ACS 57/16 Control 38/12	UPLC-MS	In house	UPLC-MS	In house	No data	0.88 (P)	<0.001	HQMG
57	Meza-Meza, 2021 [86]	Cross-sectional	United States	SLE with renal disease	88	Adult	0/88	ELISA	Cusabio, China	ELISA	Eagle Biosciences, USA	Analytical sensitivity	−0.26 (S)	0.001	LQMG
58	Tsuprykov, 2021 [87]	Prospective cohort	Germany	Pregnant healthy women	427	Adult	427/0	CMIA	Architect i2000 (Abbott Laboratories, Wiesbaden, Germany)	CLIA	IDS GmbH, Frankfurt am main, Germany	Cross reaction	0.572 (S)	<0.001	HQMG
59	Ogura, 2021 [88]	Case-control	Japan	Parkinson's disease	27	Adult	10/9	RIA	DiaSorin, MN, USA	RIA	IDS Ltd, Boldon, UK	No data	0.423 (S)	0.028	MQMG
				Multiple system atrophy	19	Adult	10/9						0.356 (S)	0.039	MQMG
				Healthy subjects	61	Adult	28/33						−0.234 (S)	0.069	MQMG
60	Saghir Afifeh, 2021 [89]	Prospective cohort	Italy	Acute coronary syndrome	228	Adult	%76.6 (male) %69.2 (male)	CLIA	LAISON XL, DiaSorin, MN, USA	CLIA	LAISON, DiaSorin, MN, USA	No data	0.175 (LR)	0.035	HQMG

Table 1: (continued)

No.	Study	Study type	Country	Population	n	Age group	Female/male	25 (OH)D measurement	25 (OH)D brand/procedure	1,25 (OH)2D measurement type	1,25 (OH)2D brand/procedure	Analytical performance	Correlation, r	p-Value	Analytical quality
61	Lotfollahi, 2021 [90]	Cross-sectional	Iran	ESRD	156	Adult	52/104	HPLC	Chrom Abzar Parse Co, Iran	EIA	ZellBio GmbH, Germany	Analytical sensitivity	0.12 (P)	>0.05	LQMG
62	Meng, 2021 [91]	Case-control	United States	Primary hyperparathyroidism and healthy	60	Adult	60/0	RIA	No data	No data	No data	No data	0.11 (LR)	>0.05	LQMG
63	Bacanakgil, 2022 [92]	Prospective cohort	Istanbul, Turkey	Infertile women (before with vitamin D replacement)	62	Adult	62/0	ECLIA	Roche Cobas 6000, Germany	ELISA	No data	No data	0.164 (P)	0.202	MQMG
				Infertile women (after with vitamin D replacement)	62	Adult	62/0						0.508 (P)	<0.001	MQMG
64	Our results, 2023	Cross-sectional	Istanbul, Turkey	Healthy subjects	485	Adult	308/177	CLIA	Advia Centaur (Siemens Healthineers, USA)	EIA	IDS Ltd, Boldon, UK		0.5 (P)	<0.001	HQMG
				Other diseases	4544	Adult	250/142						0.26 (P)	<0.001	HQMG
				Renal diseases	395	Adult	3128/1416						0.26 (P)	<0.001	HQMG

UPLC-MS, ultra-performance liquid chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; RIA, radioimmunoassay; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence immunoassay; RCT, randomized controlled trial; MR, multiple regression; LR, linear regression; P, Pearson correlation; S, Spearman correlation; Log, logarithmic transformation; HQMG, high-quality methods group; MQMG, medium-quality methods group; LQMG, low-quality methods group; IVD, invitro diagnostic device; CVinterassay, interassay coefficient of variation; CVintraassay, intraassay coefficient of variation; IDMS, isotope dilution tandem mass spectrometry.

(95 % CI; 0.25–0.37) with 82.7 % of I2. It was observed that the correlation value was the highest in HQMG, followed by MQMG, and lastly, LQMG. Notably, significant heterogeneity was detected in all groups except for the LQMG group and in the comprehensive study assessment.

In the context of other diseases, 36 studies were examined. Among these, 12 were classified as HQMG, 13 as MQMG, and 11 as LQMG. The correlation values were calculated as 0.36 (95 % CI; 0.22–0.48) with 94.8 % of I2 for HQMG, as 0.19 (95 % CI; 0.09–0.30) with 71.6 % for MQMG, and as 0.16 (95 % CI; 0.01–0.32) with 89.6 % for LQMG. The correlation value was determined to be 0.25 (95 % CI; 0.17–0.32) with 90.7 % of I2 in the comprehensive analysis covering healthy and disease groups. It was observed that the correlation value was the highest in HQMG, followed by MQMG, and lastly, LQMG. Importantly, significant heterogeneity was detected in all groups.

As a result, the correlation values obtained from measurements conducted with HQMG are higher than those of MQMG and LQMG.

The results of the assessment for publication bias in the conducted study are presented in Figure 6. According to both the Funnel plots and the results of Egger and Begg's tests, it was determined that there was no statistically significant bias.

Discussion

The relationship between 25(OH)D and 1,25(OH)2D is quite complex. In addition to the different reasons mentioned above, it is especially related to the measurement of 1,25(OH)2D. The characteristics of 25(OH)D and 1,25(OH)2D methods are presented in Supplemental Tables 1 and 2.

Since 2010, the U.S. National Institutes of Health, Office of Dietary Supplements (NIH-ODS), through the Vitamin D Standardization Program (VDSP), has been working to standardize the measurement of serum total 25(OH)D, which is the primary indicator of vitamin D levels. Studies have shown that the results of assays used to determine serum total 25(OH)D, comprising both 25-hydroxyvitamin D2 [25(OH)D2] and 25-hydroxyvitamin D3 [25(OH)D3], may vary depending on the specific assay method employed [93–95].

The VDSP is a cooperative venture involving the National Institutes of Health, National Institute of Standards and Technology (NIST), Office of Dietary Supplements (NIH-ODS) [96], Centers for Disease Control and Prevention (CDC), as well as the national survey laboratories in multiple countries, and vitamin D investigators worldwide [97].

The VDSP has enforced a reference measurement system that includes reference measurement procedures conducted at NIST and CDC, along with NIST Standard Reference Materials [98–102]. Additionally, it comprises the CDC

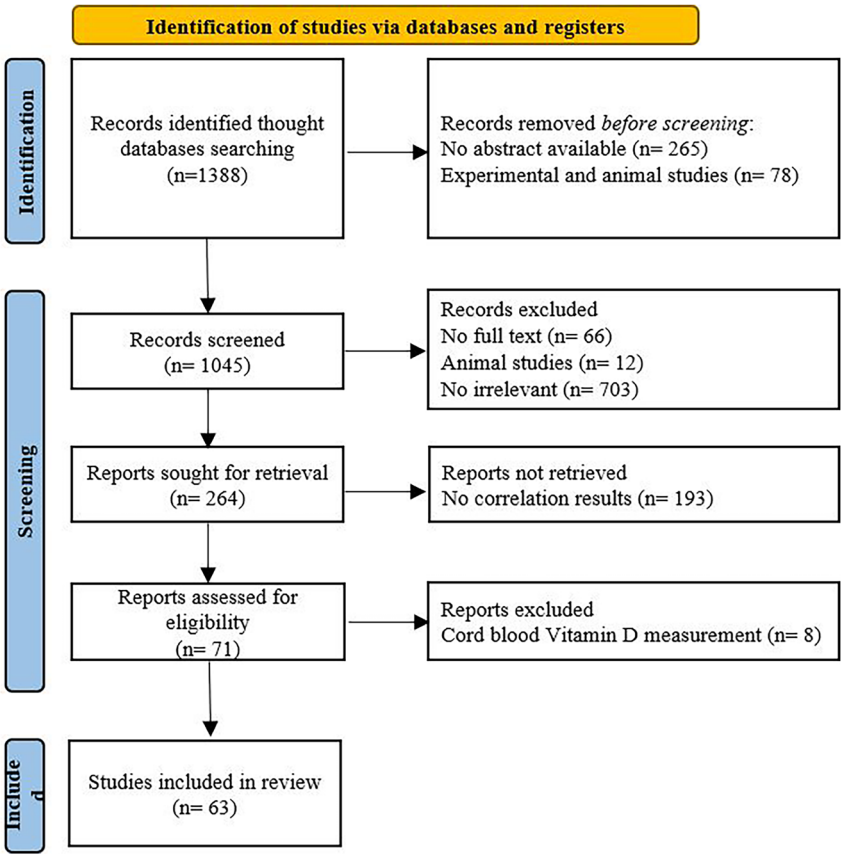

Figure 2: The flowchart of the study is based on the PRISMA* statement. *PRISMA (preferred reporting items for systematic reviews and meta-analyses). From: Page et al. [29].

Vitamin D Standardization Certification Program and partnerships with the College of American Pathologists and Vitamin D external quality assessment scheme [103–105]. The VDSP has set strict criteria for assay performance, ensuring that measurement variability and bias meet the standards of a coefficient of variation (CV) of ≤10 % and a mean bias of ≤ 5 % [106, 107].

Despite highly successful standardization efforts in 25(OH)D measurements, the issues still need to be solved. In the VDSP's Intra-laboratory Study for the Assessment study, 12 assays were compared, and 9 out of the 12 assays demonstrated a mean bias within ≤ 5 %. Samples with high levels of 25(OH)D2 were essential in evaluating the effectiveness of the immunoassays, highlighting possible differences in response or recovery between 25(OH)D2 and 25(OH)D3 in various assays [108].

Serious problems were also encountered in the LC-MS/MS method, which was presented as a better method. Only 53 % of the LC-MS/MS assays met the VDSP criterion of mean %bias ≤5 %. Four assays showed a mean %bias between 12 % and 21 % among those that did not. A regression study using the concentrations of four vitamin D metabolites in 50 single donor samples found that implementing several LC-MS/MS assays was affected by the presence of 3-epi-25(OH)D3 [109]. Significant correlation discrepancies and high bias values have also been reported for 25(OH)D measurements by immunoassay, chromatography, and mass spectrometry [110–112].

It has been observed that the analytical measurement difficulties are much greater in 1,25(OH)2D measurements compared to 25(OH)D. 1,25(OH)2D is a compound found in very low concentrations (pmol/L) in circulation and is highly lipophilic. Furthermore, the structurally similar metabolic precursor 25(OH)D circulates at nmol/L concentrations, making assay specificity an analytical concern. Significant advancements have been made in measuring 1,25(OH)2D. In 1974, a radioreceptor assay (RRA) was developed, utilizing the competitive binding of 1,25(OH)2D and a tritiated tracer to its nuclear receptor isolated from the calf thymus [113]. The first RIA that measured 1,25(OH)2 D was introduced in 1978 [114]. RIA for 1,25(OH)2 D using a radio iodinated (125 I) tracer was invented [115]. The assay involves acetonitrile extraction and purification of endogenous 1,25(OH)2 D by solid phase chromatography and quantification by RIA.

Kissmeyer and Sonne developed an LC-MS/MS method that quantified the ammonium adduct of 1,25-(OH)2D3 in rat and pig serum [116]. Later, methods utilizing 4dd-phenyl-1,2,4-triazoline-3,5-dione (PTAD) as a derivatizing reagent

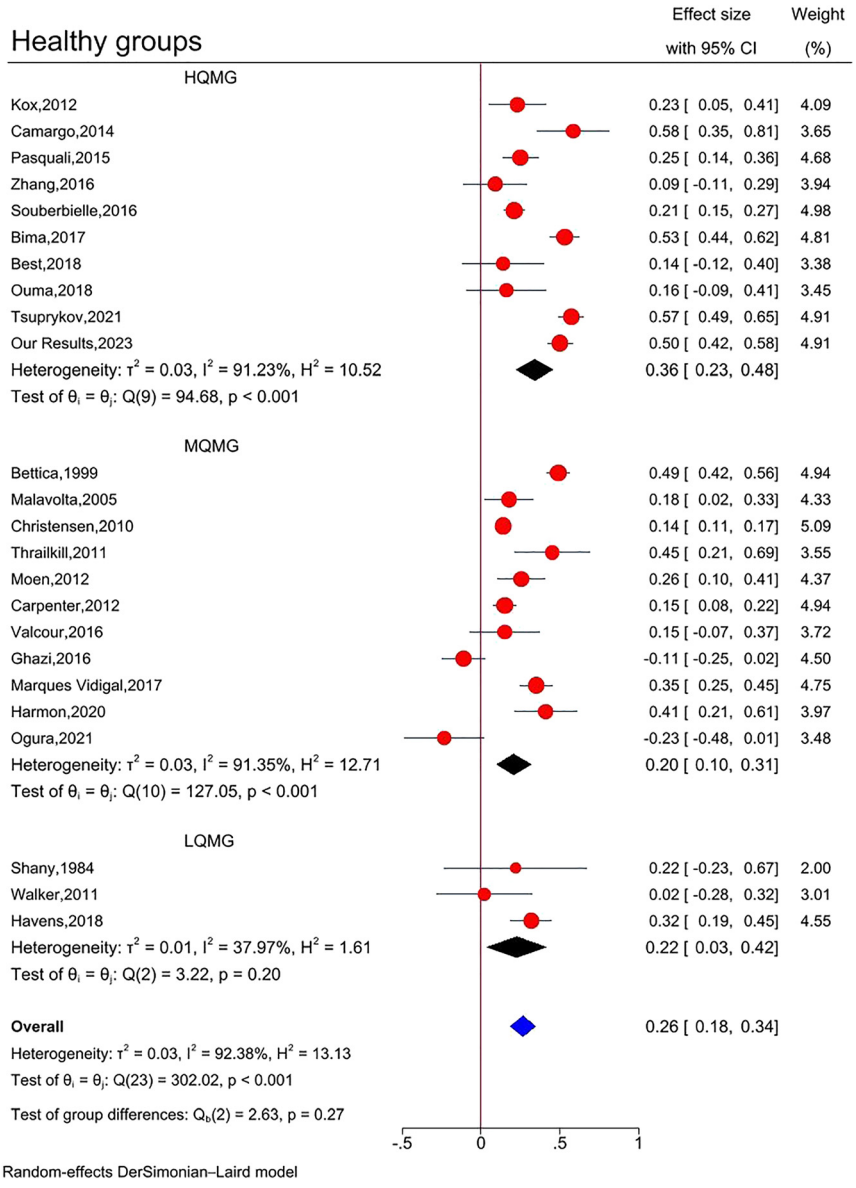

Figure 3: Forest plot depicting the correlation between 25(OH)D and 1,25(OH)2D in the healthy group.

were developed, further lowering the limit of quality (LOQ) values. In recent years, methods incorporating a single step of immunoaffinity extraction were developed, and assay kits were commercialized [117–124]. It has been specially done using Immunoaffinity extraction with ImmunoTubeÂ® 1,25(OH)2 Vitamin D LC-MS/MS Kit (Immunodiagnostic GmbH, Germany) or Immunodiagnostic Systems (IDS, UK) antibody. These commercially available kits can be applied to different brands of LC-MS/MS systems.

In this study, our laboratory results are particularly crucial. According to the correlation results obtained with automated systems in a quite extensive patient group, 1,25(OH)2D and 25(OH)D measurements were found to be significantly higher in healthy individuals compared to the groups with renal and other diseases, with correlation coefficients of 0.50 (0.42–0.58), 0.26 (0.16–0.36), and 0.26 (0.23–0.29) respectively. A moderately significant correlation was observed in the healthy group. We believe that these results, obtained through the use of the same systems across all groups and with a sufficient amount of data, represent the best data currently available that demonstrates the current relationship.

Exacerbating the issue of low concentration is the poor ionization of the analyte, coupled with the potential complications arising from the derivatization required for sufficient analytical sensitivity. Additionally, poor sample preparation techniques can have a significant adverse impact on clinical performance in LC-MS/MS-based methods [125]. It is worth noting that LC-MS/MS is a complex and specialized technique that requires advanced equipment

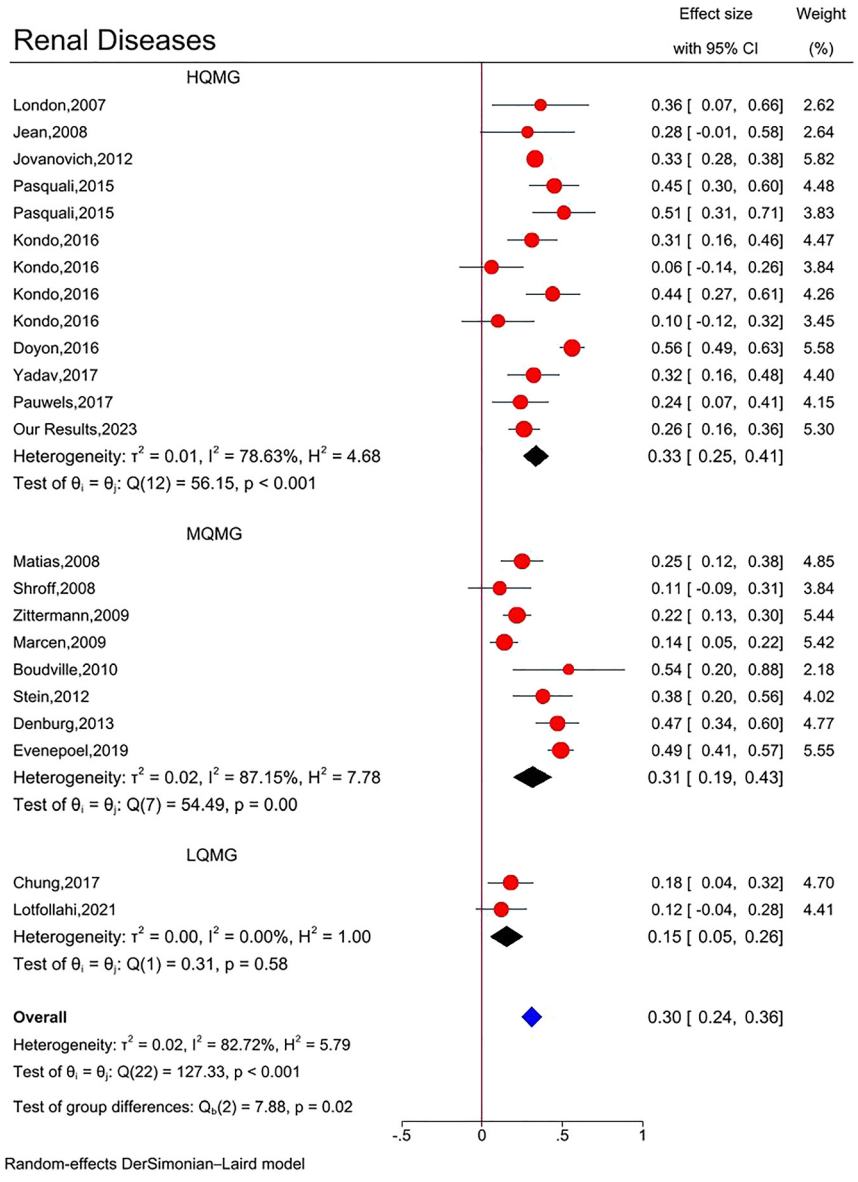

Figure 4: Forest graph of the correlation between 25(OH)D and 1,25(OH)2D in the renal diseases group.

and trained personnel, making it relatively expensive compared to some other testing methods. Despite these considerations, LC-MS/MS is recognized as the gold standard for accurate and reliable measurement of 1,25(OH)2D3 in biological samples.

The development of fully automated chemiluminescence immunoassays has indeed contributed to significant progress in accurately and efficiently measuring 1.25(OH)2D. Two notable products in this category are produced by IDS-iSYS, 1,25-dihydroxy vitamin D (Immunodiagnostic Systems, UK) and LIAISON® XL 1,25 dihydroxyvitamin D (DiaSorin Inc, USA). These assays represent a notable step forward in the accuracy and efficiency of measuring 1,25(OH)2D levels.

Under standardized conditions, a recently introduced automated immunoassay demonstrates strong agreement with measurements obtained using a liquid chromatography-tandem mass spectrometry reference method (LC-MS/MS) [126]. Nonetheless, recent findings from DEQAS reveal that coefficients of variation within specific tests and mean 1,25(OH)2D levels between different test procedures can exhibit fluctuations of over 20 % [127].

According to a study conducted by Zittermann and colleagues, the measurement of circulating 1,25(OH)2D was carried out using two different methods: an LC-MS/MS method provided by Immundiagnostik and an automated immunoassay test provided by DiaSorin. The study found a correlation (r=0.534) and an agreement (62 %) between the two methods and highlighted the need for additional standardization studies [128]. A recent meta-analysis has also revealed that the measurement procedure can significantly

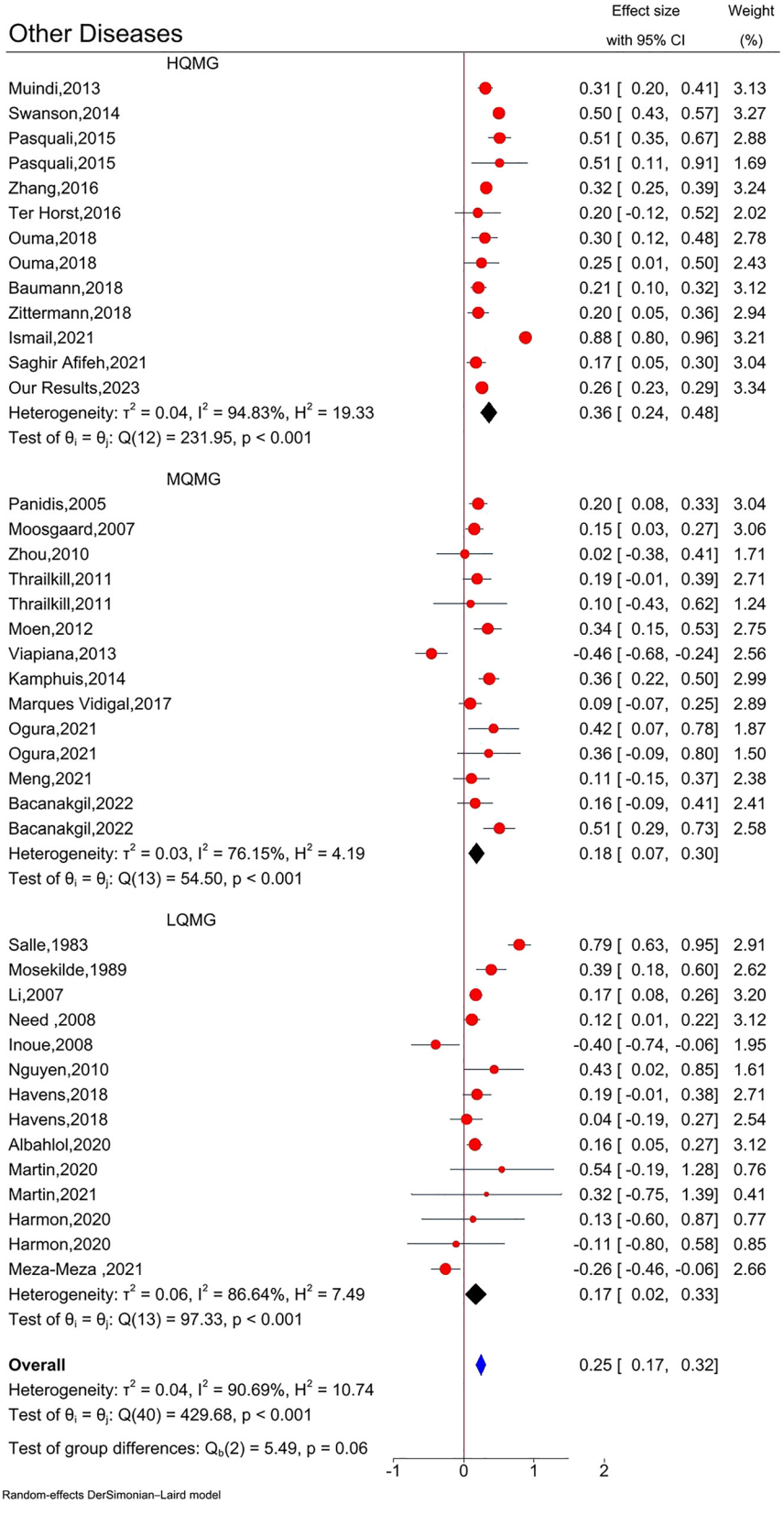

Figure 5: Forest graph of the correlation between 25(OH)D and 1,25(OH)2D in other disease groups.

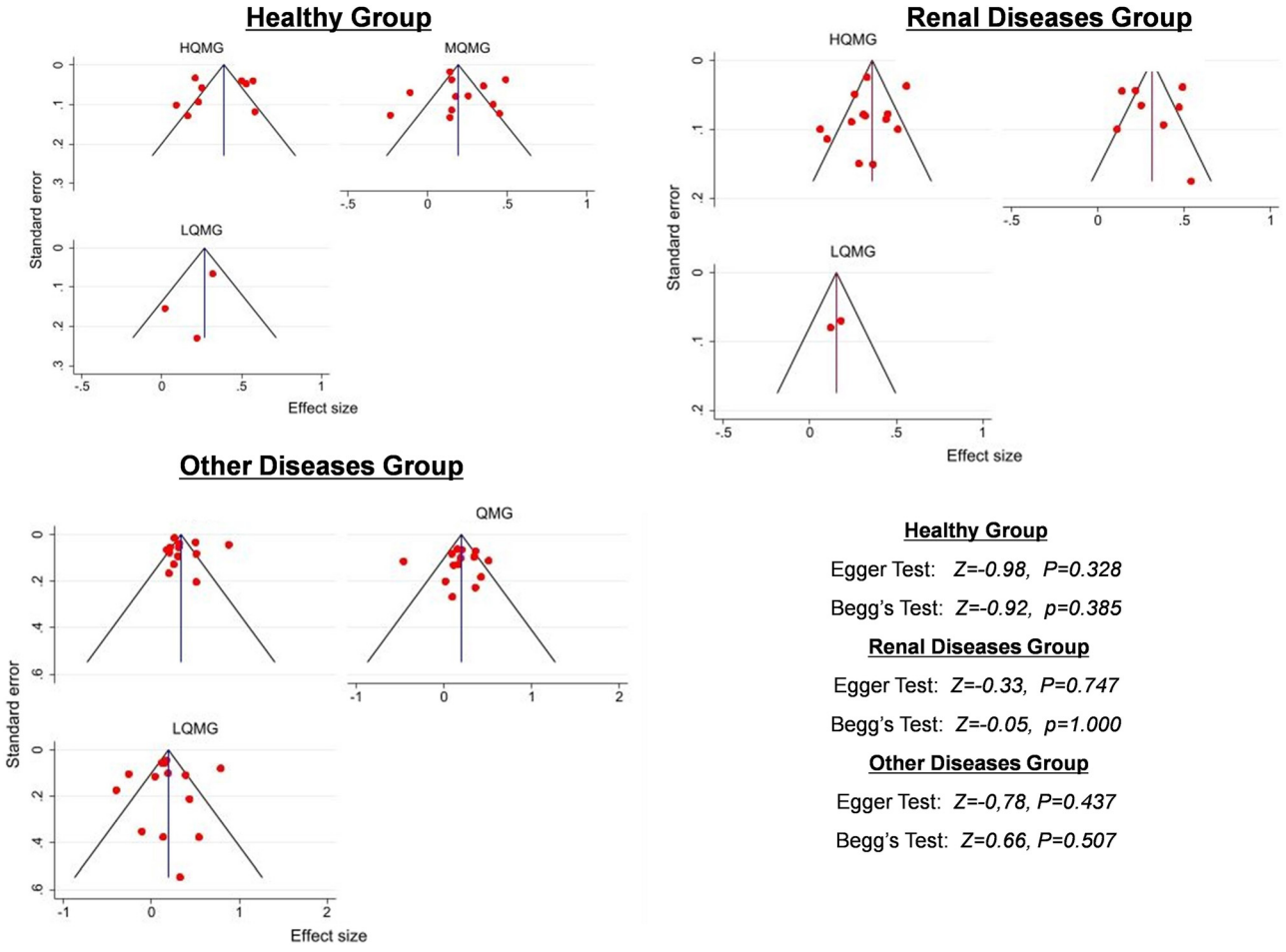

Figure 6: Funnel plots, Egger's and Begg's test results of all groups.

impact circulating 1,25(OH)2D levels. These differences in measurement make it challenging to compare results between labs and establish consistent reference values for circulating 1,25(OH)2D levels. Consequently, automation and standardization are crucial for improving the reliability of testing procedures [129]. It is worth noting that 80.8 % of the 1,25(OH)2D assays included in the meta-analysis were RIA and radioreceptor assays.

Upon closer examination, it is observed that there is a statistically insignificant or low correlation between 25(OH)D and 1,25(OH)2D in general. However, as can be seen in our results, the highest correlation in all three disease groups is found in HQMG. The highest correlations in the HQMG group are observed in all groups. The results in the renal diseases group are exciting. This may be due to this patient group taking Vitamin D supplements. However, the lower correlation in other diseases suggests that the relationship is highly complex and disrupted by different mechanisms in various diseases.

Even though the HQMG shows the highest correlation values in the healthy group, this is still a weak correlation. However, vastly different and heterogeneous results are present. The most significant factors here are methodological challenges, the short half-life of 1,25(OH)2D, and its complex regulations. While there is a direct enzymatic transformation of 25(OH)D into 1,25(OH)2D, a relationship between their serum levels may be noted. 1,25(OH)2D can directly suppress the production of 1α-hydroxylase and indirectly by reducing PTH levels and promoting FGF23 production. This feedback mechanism is crucial for preventing hypercalcemia. As a result, the level of 1,25(OH)2D is not influenced by the circulating amount of 25(OH)D [130, 131]. We anticipate that with improved methodologies, the correlation value could increase in the future.

This study has notable limitations. In the search that was carried out, the term 'correlation' was explicitly looked for in the title, keywords, and abstract. There might be studies

that do not mention the term “correlation” but discuss it within the text. While some studies may have used successful measurement procedures, they may not have been explicitly stated or may have been inadequately presented. Another significant limitation is that we did not consider age and gender differences in our analysis. We refrained from making distinctions based on age and gender as we believed it might reduce the number of studies in each group. In the studies included in this meta-analysis, different correlation analyses (Pearson correlation without or with log transformation, Spearman correlation, or regression analysis) were used. We included all of these correlation analyses in our study.

When all the results are evaluated, this study is the first meta-analysis conducted considering differences in methodological and health situations between 25(OH)D and 1,25(OH)2D. Both in the examination of Vitamin D metabolism and the relationship between 25(OH)D and 1,25(OH) 2D, differences in methodological and health situations are crucial and must be considered.

Research ethics: This study was approved by the Bakırçay University Local Ethics Board with 1303 of decide number on 08.11.2023.

Informed consent: Not applicable.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest.

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Data availability: Not applicable.

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