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RESEARCH PAPER

Vitamin D status affects proteomic profile of HDL-associated proteins and inflammatory mediators in dyslipidemia

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Abstract

Vitamin D deficiency and dyslipidemia have substantial implications for human health globally. Vitamin D is essential for bone metabolism and immune modulation, and its insufficiency is linked to various chronic inflammatory conditions. Dyslipidemia, characterized by low levels of high-density lipoprotein (HDL) and elevated levels of low-density lipoprotein (LDL) and triglycerides, is also prevalent. Previous research has shown a connection between vitamin D deficiency and low HDL, but the precise mechanism by which vitamin D influences HDL production and its anti-inflammatory properties remains unclear. This study aimed to investigate the proteomic profiles of individuals with and without vitamin D deficiency and dyslipidemia, specifically focusing on the effects of vitamin D on HDL production, its anti-inflammatory potential, and the molecular pathways associated with vitamin D deficiency and dyslipidemia, particularly inflammation and cancer pathways. By analyzing the proteomic profiles of 274 participants from the Qatar Biobank database, we identified 1301 proteins. Our findings indicated a decrease in HDL-associated apolipoproteins (ApoM and ApoD) in individuals with both dyslipidemia and vitamin D deficiency. Conversely, participants with these conditions exhibited increased expression of acute-phase proteins (SAA1 and SOD1), which are associated with inflammation. Pathway enrichment analysis revealed heightened inflammatory activity in individuals with vitamin D deficiency and dyslipidemia, with notable enrichments in pathways such as MAPK, JAK-STAT, Ras signaling, cytokine-cytokine receptor interaction, AGE-RAGE, ErbB signaling, and cancer pathways. Overall, cases of vitamin D deficiency showed enrichment in inflammation pathways, while individuals with both vitamin D deficiency and dyslipidemia demonstrated enhanced activation of cancer and inflammation pathways.

Keywords: Proteomics; Vitamin D; HDL; Apolipoproteins; Inflammation; Dyslipidemia.

1. Introduction

Vitamin D deficiency is a global health concern, affecting populations worldwide [1]. In Qatar, more than half of the population (64%) has serum 25-Hydroxy Vitamin D (25(OH)D) concentrations below 12 ng/mL, which is the cutoff value for vitamin D deficiency [2]. Recent studies have linked vitamin D deficiency to various health problems, including conditions associated with high inflammatory status such as metabolic syndrome, dyslipidemia, chronic cardiovascular diseases, obesity, and autoimmune diseases [3].

The production of vitamin D begins with the synthesis of its precursors in the skin through exposure to UVB sunlight. These precursors are then converted into 25-hydroxyvitamin D (25(OH)D) metabolites by the vitamin D 25-hydroxylase enzyme. The hormonally active form of vitamin D, known as 1,25 dihydroxyvitamin D3 (1,25(OH)2D3), is primarily produced in the kidneys through the action of the 25-hydroxyvitamin D-1α-hydroxylase enzyme. However, other tissues besides the kidneys, such as the skin, immune cells, intestinal epithelium, parathyroid gland, prostate, and breast, also produce the active form of vitamin D, 1,25(OH)2D3 [4]. The active form of vitamin D, 1,25(OH)2D3, binds to the vitamin D receptor (VDR), initiating a cascade of signaling pathways that result in the upregulation or downregulation of various gene targets. Consequently, disturbances in vitamin D homeostatic levels can lead to dysregulation in the expression of downstream targets. Vitamin D deficiency occurs when the serum concentration of 25(OH)D falls below 12 ng/mL, often due to inadequate exposure to sunlight or poor dietary habits [5].

Some cases of vitamin D deficiency have a genetic predisposition, such as familial hypertension resulting from polymorphisms in the VDR gene, specifically the rs3847987 variant. Patients with this genetic condition experience hypertension, diabetes, insulin resistance, and vitamin D deficiency [6–8]. VDR polymorphisms have also been associated with an increased risk of developing metabolic syndrome, particularly the VDR ApaI variant linked to

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elevated levels of triglycerides in the blood. Other variants, such as BsmI and TaqI, have been associated with lower levels of HDL-cholesterol [9]. These findings suggest a potential role for vitamin D and VDR in regulating lipidomics and lipoproteins in the bloodstream.

Vitamin D is derived from cholesterol, serving as its backbone molecule [10]. Lipoproteins, on the other hand, play a vital role in organizing the trafficking and distribution of cholesterol. High-density lipoprotein (HDL), a member of the lipoprotein family, plays a crucial role in dyslipidemia and coronary heart disease (CVD) [11]. HDL functions by removing excess cholesterol from peripheral tissues and the bloodstream, transporting it to the liver for recycling. This process, known as reversed cholesterol transfer (RCT), affects multiple tissues, particularly the cardiovascular system [12]. Additionally, HDL acts as a carrier for various immune modulators and antimicrobial peptides, including cathelicidin or LL-37. The gene encoding LL-37 contains a vitamin D response element (VDRE) and is considered one of the target genes of vitamin D [13].

Both the production and function of vitamin D and HDL are influenced by several proinflammatory cytokines. For example, TNF-α attenuates HDL biogenesis by reducing the expression of the cellular cholesterol transporter ABCA1. Conversely, suppressing TNF-α using anti-TNF-α agents has been shown to increase HDL cholesterol levels [14,15]. Similarly, low vitamin D levels have been associated with an overexpression of proinflammatory cytokines such as TNF-α and IL-8 [16,17]. However, studies have not yielded conclusive results regarding the ability of vitamin D supplementation to reduce TNF-α levels [18,19]. Interestingly, the combination of anti-TNF-α therapy and vitamin D supplementation has shown promise in improving the clinical manifestations of inflammatory diseases such as Crohn's disease [20].

Vitamin D has also been found to affect the proteomic profile and expression levels of various proteins. In *in vivo* studies, the administration of 1,25-dihydroxyvitamin D3 resulted in modifications to the proteomic profile in mouse brains affected by multiple sclerosis. These modifications included proteins involved in myelin repair and calcium binding activity, such as calretinin, S10A5, and secretagogin, as well as proteins linked to mitochondrial function, such as NADH-ubiquinone oxidoreductase chain 3 and acyl-coenzyme A synthetase [21]. Furthermore, these proteomic profile modifications have the potential to predict the risk of cardiovascular disorders. For example, modifications in HDL proteins, including paraoxonase/arylesterase 1 (PON1), paraoxonase/arylesterase 3 (PON3), LCAT, and apolipoprotein A-IV, enable the prediction of cardiovascular disorder risk in patients with chronic kidney disease [22,23].

A study by Al-Daghri et al. utilized MALDI profiling to investigate proteomic profile alterations during vitamin D deficiency in obese participants. The study reported changes in apolipoprotein CIII, apolipoprotein B100, alpha-1-antichymotrypsin, and complement C3 in participants with high BMI compared to those with low BMI. MALDI mass spectrometry was employed to analyze the changes in Apo A1, Apo B100, and lipoprotein lipase. However, the results were inconclusive, although increased expression levels were reported in obese subjects using classical immunoblotting methods [24].

Vitamin D deficiency is associated with a high inflammatory status and disturbances in lipid profiles. Serum vitamin D concentration is inversely associated with the levels of tumor necrosis factor receptor 2 (TNFR-2) and C-reactive protein (CRP), while anti-inflammatory cytokines such as IL-10 and HDL are suppressed during vitamin D deficiency [25]. A clinical study by Jin et al. demonstrated that an increase in serum 25(OH)D levels in pregnant women significantly impacted lipid profiles by decreasing total cholesterol, triglyceride, LDL-c, and HDL-c levels. Additionally, CRP levels were reduced due to improved lipid metabolism [26]. Another study by Johny et al. revealed that vitamin D supplementation as supportive therapy in patients with type 2 diabetes mellitus reduced the risk of developing cardiovascular complications. This supplementation led to decreased serum levels of inflammatory markers such as IL-18, TNF-α, IFN-γ, CXCL-10, CXCL-12, CCL-2, CCL-5, CCL-11, and PF-4 [27].

Previous studies have reported that both vitamin D deficiency and dyslipidemia can modify the proteomic presentation to some extent. However, the effect of combined vitamin D deficiency and dyslipidemia on inflammatory markers and apolipoproteins remains unclear. This study aimed to investigate the proteomic profiles of individuals with and without vitamin D deficiency and dyslipidemia, focusing on the impact of vitamin D on HDL biogenesis, the anti-inflammatory potential of vitamin D, and the molecular pathways associated with vitamin D deficiency and dyslipidemia, particularly inflammation and cancer pathways.

2. Material and methods

2.1. Study design

This cohort is retrospective cross-sectional. Data from 274 participants was obtained from QBB database (https://www.qatarbiobank.org.qa/). Participants were selected randomly from a larger cohort of 1820 subjects. This study was performed in line with the World Medical Association Declaration of Helsinki–Ethical Principles for medical research involving human subjects. The Institutional Research Board of Qatar University QU-IRB form (1366-E/20), QBB-IRB form (EX-2020-QBB-RES-ACC-0237-0124), approved all protocols. All participants consented to the use of their samples for research. The cohort was divided into 4 groups according to vitamin D status (sufficient and deficient) and the presence or absence of Dyslipidemia. Serum 25 dihydroxy vitamin D (25(OH)D) concentrations were considered deficient $<$12 ng/mL and a sufficient $\geq$ 20 ng/mL according to Institute of Medicine recommendation [28]. Dyslipidemia status was determined according to the following criteria: high total cholesterol ($>$6.2 mmol/L), high LDL-c ($>$4.1 mmol/L), and high TG ($>$2.3 mmol/L) [29]. Accordingly, the four groups were: vitamin D sufficient and normolipidemic (Group 1, n=64); vitamin D sufficient and dyslipidemia (Group 2, n=26); vitamin D deficient and dyslipidemia (Group 3, n=85); vitamin D deficient and normolipidemic (Group 4, n=99).

The inclusion criteria were adults devoid of comorbidities except for dyslipidemia and vitamin D deficiency. The exclusion criteria included using vitamin D supplements, pregnancy, and those with chronic diseases such as diabetes, high blood pressure, asthma, hay fever, blood clot, heart attack, angina, stroke, emphysema/chronic bronchitis, hyperthyroidism hyperparathyroidism, Cushing syndrome, and cancer.

2.2. Measurements

QBB team collected venous blood samples from participants after their consent and sent to Hamad Medical Corporation Laboratories (College of American Pathologist Accredited Laboratory) to be analyzed. Serum 25(OH)D concentration was analyzed using electrochemiluminescence immunoassay (LIAISON 25-hydroxyvitamin D Total Assay, DiaSorin Inc., Stillwater, MN, USA) and both vitamin D2 and vitamin D3 fractions were measured. Lipid profiles, Alanine aminotransferase ALT and AST, Total Bilirubin, Total Protein, Albumin, Alkaline Phosphatase, Creatinine, Urea, Uric Acid and Sex Hormone binding Globulin were measured using automatic Biochemistry Analyzers at Qatar Biobank and Hamad General Hospital as

previously described [30,31]. Proteomic analysis has been done by QBB using SOMAscan aptamer-based affinity proteomics platform (SomaLogic, Boulder, CO, USA) more details and protocols mentioned in Thareja et al. study [32]. Briefly, EDTA-plasma was incubated with bead-coupled epitope-specific aptamers (SOMAmers). The bead-bound protein was then biotinylated, and the biotinylated target protein-fluorescently labeled SOMAmer complex was photocleaved and recaptured onto streptavidin beads. SOMAmers were then eluted and quantified by hybridization of SOMAmer-complementary oligonucleotides to custom arrays. The raw intensities obtained were processed using various standards as references, including hybridization normalization, median signal normalization, and signal calibration to control for plate-to-plate differences. Data for 1,305 aptamers were obtained. No samples or data points were excluded. Quality control was performed by measuring two QC samples in duplicate. The average coefficient of variation (CV) was 0.073 for both QC samples based on 51 and 54 replicates, respectively. Ninety-five percent of aptamers had a CV less than 0.172 or 0.176 and 5% had a CV less than 0.046 or 0.041. In other words, half of the proteins tested had CVs below 0.073 and most (95%) had CVs below 0.176 [32].

2.3. Analysis

Baseline characteristics were reported as means (SD) for normally distributed values and as median (interquartile) for the non-normally distributed variables. The linearity was assessed for ApoM, ApA-1, ApoD, and vitamin D levels. One-way ANOVA was used to detect the significant difference of mean for 122 proteins in the 4 groups. Whereas the T-test was used to address the differences in the significance of apolipoproteins (ApoA-1, ApoB, ApoD, ApoE, ApoM, and ApoL-1) means between the groups (Group 1 *vs.* Group 2), (Group 1 *vs.* Group 3), (Group 1 *vs.* Group 4), (Group 2 *vs.* Group 3), (Group 2 *vs.* Group 4), and (Group 3 *vs.* Group 4). The gender difference in protein level was reported for ApoM, ApoA-1, and lipocalin 2. All statistical tests were 2-tailed tests and the *P* value was considered significant if <.05. SPSS and GraphPad Prism 5 were used for data analysis.

2.4. Expression enrichment analysis and co-expression analysis

The total number of analysed protiens was 1,301 proteins for 274 participants. Gender and BMI were considered as confounders. To calculate the protein expression, we used the ProTExA workflow which facilitates differential expression analysis and co-expression network analysis as well as pathway analysis [33]. For each of the six comparison groups, we start the analysis by performing statistical analysis and filtering to highlight the genes of the differentially expressed proteins. For this purpose, we used the "default pre-processing scheme" option, which deletes rows that include NA and empty values. For entries that include protein names separated by semi-colon, the script keeps only the first name. For duplicate proteins, the script keeps one row per protein, that contains the mean value of all the duplicated rows per sample. This is followed by the normalization process where the pipeline uses the LIMMA statistical analysis package [34] that requires the dataset to be normalized and transformed to log 2 scale. For this purpose, we use the quantile algorithm as our normalization method of choice and log 2 transformation. The next step involved performing enrichment analysis to identify top-scored pathways, where we used *P* value <=.05 as our threshold but without implementing any filtering for the fold change threshold. This will give us a list of high-quality differentially expressed proteins, which we then use to perform further downstream analysis. The first such analysis involves enrichment analysis. We decided to do three types of enrichment analysis: (1) functional enrichment analysis using InterPro (InterPro Domain 2019) (2) pathway enrichment analysis using KEEG human pathway 2019, and (3) phenotype enrichment analysis using Human Phenotype Ontology. For all types of enrichment analysis, we use *P* value <=.05 for selecting a significant enriched result. Other than enrichment analysis, we also investigate the co-expression of the differentially expressed proteins. We used the Maximum Relevance Minimum Redundancy Network method (MRNET) algorithm to infer the network [35].

We performed a linear regression analysis between Vitamin D level and 4 Apolipoproteins (ApoM, ApoA-1, ApoB, and ApoD) by employing linear modeling procedures within the R statistical software environment. These analyses were carried out using the entirety of our cohort. Furthermore, we extended our analysis to encompass subgroup-level examinations. This approach allowed us to explore potential variations in the relationships between vitamin D levels and the APO proteins across distinct subgroups within our dataset.

3. Result

3.1. Cohort baseline characteristics

The cohort for this study consisted of a total of 277 participants, with three participants being excluded due to missing data. The participants were categorized into four groups based on their vitamin D status and serum lipid profile. Group 1 comprised 64 participants who were vitamin D sufficient (greater than 20 ng/mL) and had normolipidemia. Group 2 included 26 participants who were vitamin D sufficient but had dyslipidemia. Group 3 consisted of 85 participants who were vitamin D deficient (less than 12 ng/mL) and had dyslipidemia. Group 4 comprised 99 participants who exhibited vitamin D deficiency with normolipidemia. Group 1 served as the control group and reference point for the other groups. Baseline characteristics of the participants, such as gender, BMI, WBC, Monocyte, C-Peptide, and lipid profile components (total cholesterol, HDL, LDL, and TG), were reported in our previous study on metabolomics profiling for the same cohort [36]. In this paper, we focused on investigating liver and kidney function tests, as these systems have a significant impact on lipidomic profile components and vitamin D metabolism.

Liver function tests were compared among the four groups, and ALT values were found to be within the normal range for all participants (Table 1). However, a significant variation in the median values was observed, particularly in Group 4, where participants had vitamin D deficiency and normolipidemia, and the median value was slightly on the lower borderline (9.5 U/L). Moreover, the dyslipidemic groups (Group 2 and Group 3) showed a slight increase in median values compared to the control Group 1 (vitamin D sufficient with normolipidemia). No significant difference was observed among the other liver biomarkers, including AST, total bilirubin, total protein, albumin, and alkaline phosphatase. On the other hand, the kidney function biomarker creatinine showed a slightly higher level in both Group 2 and Group 3 (the dyslipidemic groups) compared to the other normolipidemic groups. The highest creatinine level was observed in Group 3, where participants had vitamin D deficiency and dyslipidemia. Furthermore, uric acid levels were highest in the groups with dyslipidemia, specifically in Group 2 participants (338.2 μmol/L) who were vitamin D sufficient with dyslipidemia, and in Group 3 participants (302.5 μmol/L) who were vitamin D deficient with dyslipidemia. In contrast, the lowest value of uric acid was seen in participants from Group 4 who had vitamin D deficiency with normolipidemia. The liver-produced protein Sex Hormone Binding Globulin, which binds to three steroid hormones (estrogen, dihydrotestosterone [DHT], and testosterone),

Table 1
Base line characteristics of participants in this study

	VitD sufficient, No dyslipidemia (G1)	VitD sufficient, Dyslipidemia (G2)	VitD deficient, Dyslipidemia (G3)	VitD deficient, No dyslipidemia (G4)	
Number of subjects	*n*=64	*n*=26	*n*=85	*n*=99	
	Mean (SD) (IQR)	Mean (SD) (IQR)	Mean (SD) (IQR)	Mean (SD) (IQR)	*P* value
25(OH) D (ng/mL)	24.41(7.4)	25.91(7.0)	10.04 (1.9)	9.77 (1.8)	<.001
Age	38.67(11.0)	47.93 (23.3)	39.09 (17.3)	28.39 (15.0)	<.001
Liver function test					
ALT(GPT) (U/L)	19.64 (12.09) (24.75-12.00)	20*(11.73) (30.00-14.75)	31.22 (23.05) (36.00-18.00)	22.35 (36.78) (21.00-10.00)	.044
AST(GOT) (U/L)	16 (12.09) (20.00-14.00)	20 (5.08) (24.50-16.00)	21.34 (9.8) (22.50-16.50)	19.64 (20.30) (20.00-14.00)	.389
Total Bilirubin (μmol/L)	7.2 (4.5) (8.9-4.4)	9.5 (13.12) (8.9-5.35)	8.8 (9) (9.38-5.2)	9.64 (8.11) (11.3-5.1)	.302
Total Protein (g/L)	73.38 (3) (75-71)	73.5* (6.47) (77-70)	73.13 (4.79) (76-70)	73.04 (4.88) (76-71)	.935
Albumin (g/L)	45.56(2.26) (47-44)	45* (3.87) (48-43)	45.66 (2.84) (47.5-44)	46 (4.23) (47-44)	.856
Alkaline Phosphatase (U/L)	65 (15.38) (75-54)	67* (24.28) (79.75-56.25)	72.05 (20.44) (81-57.5)	69.71 (19.65) (81-55)	.947
Kidney function test					
Creatinine (μmol/L)	68.5 (14.2) (78.75-56.25)	73.0* (19.53) (86.75-64)	74.9 (16.43) (83-67)	64.60 (14.71) (77-53)	<.001
Urea (mmol/L)	4.8 (1.3) (5.7-3.8)	4.9* (9.55) (5.75-4.15)	5.4 (7.8) (5.15-3.65)	4.68 (6.2) (4.7-3.2)	.493
Bicarbonate (mmol/L)	26.1 (2.04) (27-25)	26.0* (5.01) (29-25)	25.7 (3) (27-24.5)	26 (2.8) (27-24)	.646
Uric Acid (μmol/L)	292.5(67.92) (332.5-237.25)	317.0* (80.06) (364.2-274.2)	340.5(77.64) (396.5-274.5)	288.58 (87.16) (367-227)	<.001
Sex Hormone binding Globulin (nmol/L)	47*[35.16] (61-34.50)	37* (49.70) (57-32.90)	29.2* (30.80) (41.58-20.6)	36.8* (46.53) (58-23.28)	.092

P value was considered significant if value <.05.
VitD, vitamin D metabolite 25(OH)D3.
* Data are represented as mean (SD) or Median [Interquartile] for skewed data.

did not show any statistically significant differences among the four groups (Table 1).

3.2. Impact of vitamin D deficiency on HDL-associated protein expression

To gain insight into the interplay between vitamin D deficiency and lipid profile homeostasis, we conducted a proteomics profiling analysis to investigate the expression of HDL-associated proteins. Specifically, we focused on apolipoproteins and examined how their concentrations varied based on vitamin D and lipid profile status (Table 2). Our findings reveal noteworthy associations between vitamin D deficiency, dyslipidemia, and the expression of these proteins (Fig. 1). In Group 4, which comprised individuals with vitamin D deficiency and normolipidemia, we observed the lowest expression values for ApoA-1. However, the combination of dyslipidemia and vitamin D deficiency led to an increase in ApoA-1 levels (Fig. 1A). As anticipated, ApoB exhibited a significant increase in dyslipidemia, given its predominant association with LDL. Interestingly, the presence of vitamin D deficiency alongside dyslipidemia resulted in a slight reduction in ApoB compared to dyslipidemia with vitamin D sufficiency, indicating that vitamin D deficiency modifies the effect of dyslipidemia on ApoB levels (Fig. 1B). ApoD showed a reduction specifically in the presence of combined dyslipidemia and vitamin D deficiency (Fig. 1C). On the other hand, ApoE displayed increased expression in individuals with dyslipidemia, and the combination of dyslipidemia and vitamin D deficiency had a substantial effect on further elevating ApoE levels (Fig. 1D). Dyslipidemia alone led to a decrease in ApoM expression, and when combined with vitamin D deficiency, this reduction in ApoM levels was even more pronounced (Fig.1E). This finding provides robust evidence supporting the notion that vitamin D status influences HDL levels, as ApoM plays a vital role in HDL biogenesis. Furthermore, vitamin D deficiency resulted in increased levels of ApoL1, although its impact on dyslipidemia was limited (Fig. 1F). In summary, our analysis demonstrates the impact of vitamin D deficiency on the expression of HDL-associated proteins.

3.3. Gender effect on apolipoproteins concentrations

Since HDL levels are higher in females than males [37], we further investigated apolipoprotein concentrations to identify if there is a gender-based difference. Here we report that ApoA-1 has a significant gender-based difference among participants with vitamin D deficiency without dyslipidemia. Males expressed significantly higher levels and ApoA-1 compared to females (Fig. 2A). In contrast, ApoM was higher in females compared to males in the control group 1 which represents the normal physiological conditions, and in vitamin D deficient group 4 without dyslipidemia (Fig. 2B). On the other hand, combined vitamin D deficiency and dyslipidemia (group 3) seem to impact ApoM levels in men more than women although statistically not significant, but lower levels of ApoM are visible (Fig. 2B). ApoD has not had a significant variation in groups' levels although females have higher ApoD levels than males in all groups (Fig. 2C). ApoM and ApoD belong to the lipocalin family, therefore we investigated lipocalin 2 (LCN2) expression among the four groups in this study. Lipocalin 2 is a carrier protein and anti-inflammatory molecule that in-

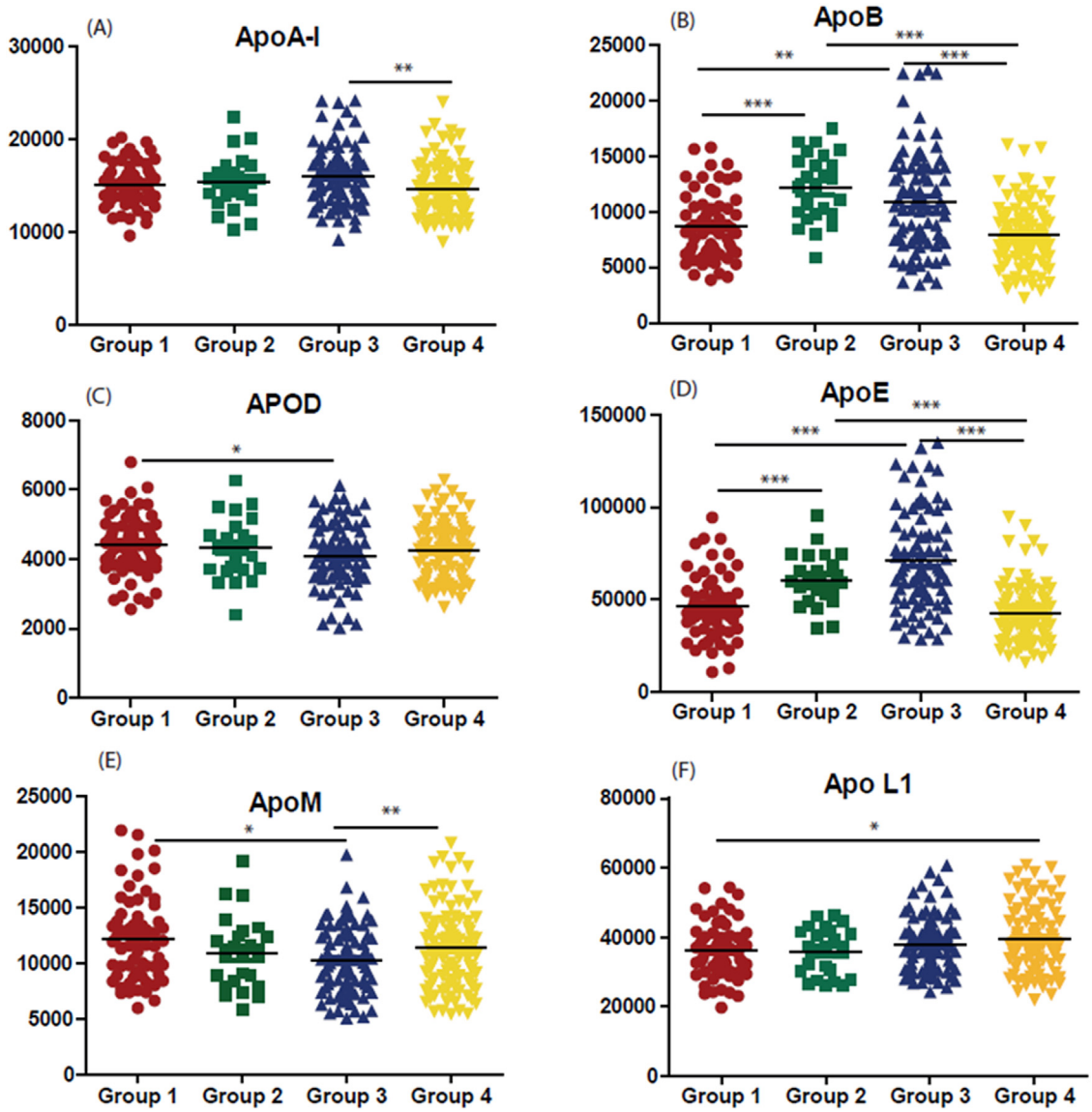

Fig. 1. Apolipoproteins concentrations are represented with their means among the four groups. Group 1 (n=64) represents participants who were vitamin D sufficient with normolipidemia (control group). Group 2 (n=26) participants who were vitamin D sufficient with dyslipidemia. Group 3 (n=85) represents participants who were vitamin D deficient with dyslipidemia. Group 4 (n=99) represents participants who exhibited vitamin D deficiency with normolipidemia. A: ApoA-1; B: ApoB; C: ApoD; D: ApoE; E: ApoM and F: ApoL1.* P<.05, **P<.01, ***P<.001.

creases during acute phase inflammation and was used as a control for gender differences. No significant difference was observed in lipocalin 2 expression between males and females. However, there was an obvious reduction in lipocalin 2 levels in Group 3 participants with combined vitamin D deficiency and dyslipidemia (Fig. 2D).

3.4. HDL-associated proteins expression profile during vitamin D deficiency and dyslipidemia

Table 2 shows the expression of various HDL-associated proteins among participants included in the four groups (group 1 (n=64) vitamin D sufficient participants with normolipidemia (control group); group 2 (n=26) vitamin D sufficient participants with dyslipidemia; group 3 (n=85) vitamin D deficient participants with dyslipidemia and group 4 (n=99) vitamin D deficient participants with normolipidemia). The expression of proteins such as ApoM, ApoA-1 and Serum amyloid A (SAA), which directly affects HDL biogenesis was significantly varied among the four groups of participants. ApoM protein level was significantly reduced among dyslipidemia groups 2 and 3 compared to healthy controls group 1. Whereas ApoA1 was slightly elevated in those groups as expected. Apo E and its isoforms ApoE2, Apo E3, and Apo E4 were tremendously elevated in group 3 (vitamin D deficient with dyslipidemia). SAA protein level reflects the disturbance in HDL level. In this study, we observed that SAA1 protein in particular has its lowest value in the dyslipidemic groups (groups 2 and 3) but it was enriched in group 4 suffering from vitamin D deficiency without dyslipidemia. The concentrations of cathepsin family components including (Cathepsin B, G, and S), which are also found associated with HDL particles, had insignificant fluctuation among the four groups. In contrast, Cathepsin V level was decreased in Group2 vitamin D sufficient with dyslipidemia, while its highest value was recorded in Group 4 (vitamin D deficiency with normolipidemic) (Table 2).

Table 2
HDL-associated proteins semi-quantitative abundance* among the four groups

	VitD sufficient, No dyslipidemia (G1)	VitD sufficient, Dyslipidemia (G2)	VitD deficient, Dyslipidemia (G3)	VitD deficient, No dyslipidemia (G4)	
Number of subjects	*n*=64	*n*=26	*n*=85	*n*=99	
Protein	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*P* value
ApoM	12166 (3800)	10960 (3184)	10286 (3009)	11474 (3786)	.01
ApoA-1	15091 (2283)	15418 (2766)	15974 (3239)	14609 (2843)	.01
ApoD	4423 (867)	4317 (853)	4154 (1002)	4299 (880)	.36
ApoB	8725 (2940)	13052 (5364)	10927 (4556)	8644 (7819)	.00
ApoE	46210 (17154)	60738 (13826)	72484 (28737)	42396 (15366)	.00
Apo E2	288792 (36396)	296457 (26392)	312578 (36880)	289620 (37046)	.00
Apo E3	24253 (53475)	278302 (31064)	307414 (69002)	230874 (50492)	.00
Apo E4	251335 (47162)	278980 (31551)	306950 (54539)	245443 (44302)	.00
ApoL-I	36136 (7813)	36758 (8073)	37997 (8078)	39817 (9604)	.051
SAA1	3490 (3952)	2644 (2181)	2722 (2926)	3574 (6756)	.572
Cathepsin B	1474 (336)	1445 (375)	1389 (261)	1369 (318)	.17
Cathepsin G	944 (412)	895 (241)	957 (394)	1088 (738)	.19
Cathepsin S	836 (183)	867 (186)	818 (164)	809 (153)	.41
Cathepsin V	2011 (817)	1816 (510)	1871 (500)	2351 (827)	.00

P-value was considered significant for value <.05.
VitD, vitamin D metabolite 25(OH)D3.
* HDL associated proteins concentrations in the four groups represented by Mean (SD).

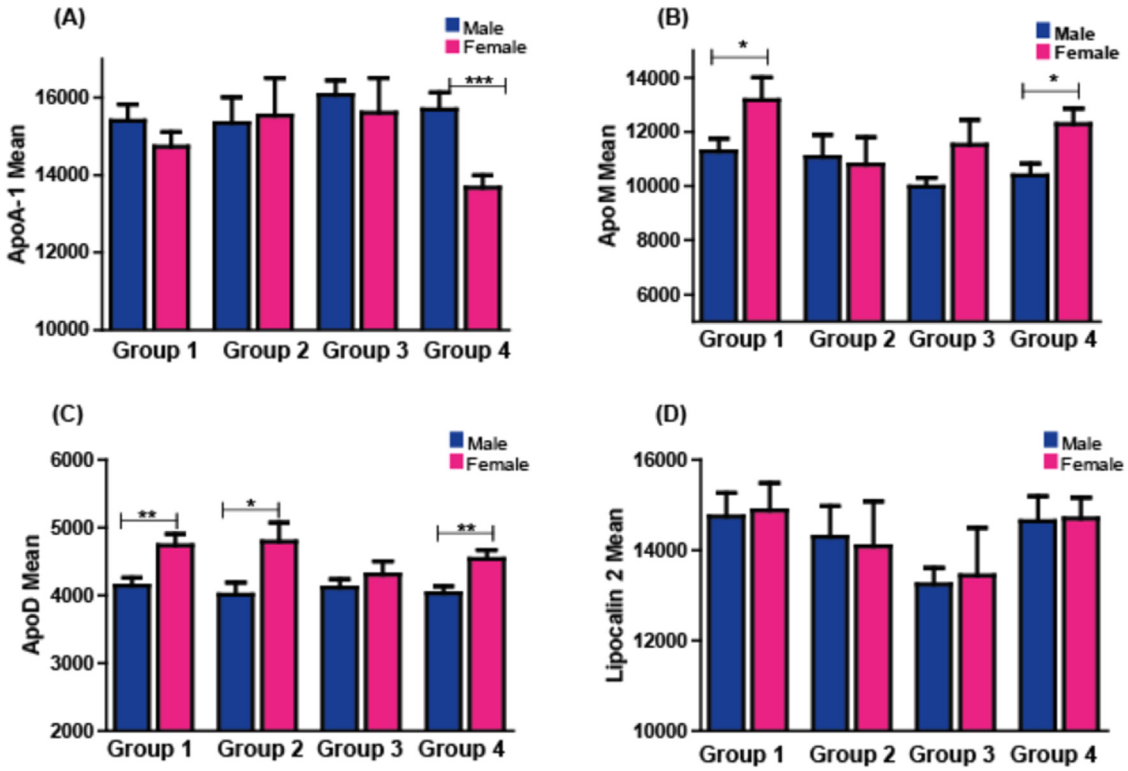

Fig. 2. The difference of means between males and females in four groups of Apolipoproteins (ApoA-1, ApoM, ApoD) and Lipocalin 2. Group 1 (*n*=64) represents participants who were vitamin D sufficient with normolipidemia (control group). Group 2 (*n*=26) participants who were vitamin D sufficient with dyslipidemia. Group 3 (*n*=85) represents participants who were vitamin D deficient with dyslipidemia. Group 4 (*n*=99) represents participants who exhibited vitamin D deficiency with normolipidemia. A: ApoA-1; B: ApoM; C: ApoD ; D: Lipocalin 2 $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$.

3.5. Semi-quantitative expression of cytokines and inflammatory mediators during vitamin D deficiency and dyslipidemia

Subclinical inflammation is a common feature during vitamin D deficiency and dyslipidemia. In order to understand how vitamin D deficiency affects HDL anti-inflammatory potential, we examined the expression of cytokines and inflammatory mediators among the participants in this study. Supplementary Table S1 compares the expression level of various proinflammatory cytokines and other inflammatory mediators among the four groups as explained above. Here, we selected few commonly reported pro-inflammatory cytokines however, no significant changes were observed for the majority of these cytokines levels among the four groups except for CXCL8 and IL-18Ra Supplementary Table S1.

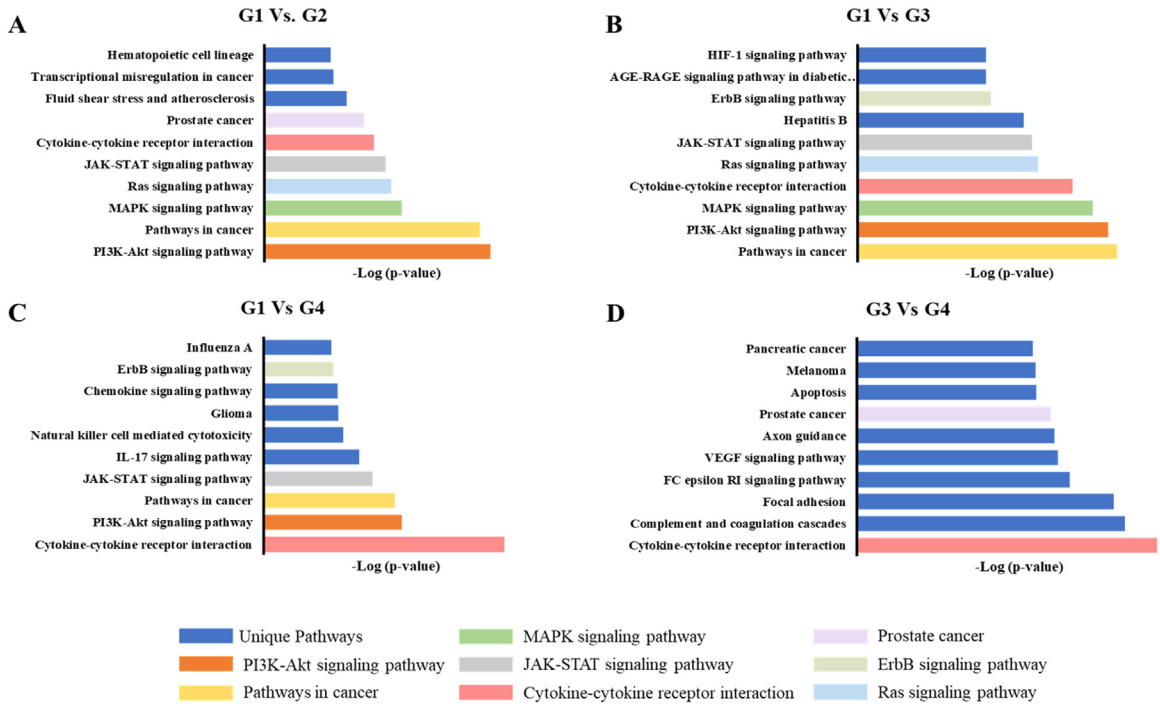

Fig. 3. Proteomics pathways enrichment during vitamin D deficiency and dyslipidemia. Bar graphs illustrates a comparison between the different groups in the level of statistical significance represented by -log of *P* value of enriched pathways. Group 1 (n=64) represents participants who were vitamin D sufficient with normolipidemia (control group). Group 2 (n=26) participants who were vitamin D sufficient with dyslipidemia. Group 3 (n=85) represents participants who were vitamin D deficient with dyslipidemia. Group 4 (n=99) represents participants who exhibited vitamin D deficiency with normolipidemia. A: The top 10 pathways with the highest level of statistical significance for Group 1 *vs.* Group 2 (G1 *vs.* G2). B: Group 1 versus Group 3 (G1 *vs.* G3). C: Group 1 *vs.* Group 4 (G1 *vs.* G2). D: Group 3 *vs.* Group 4 (G3 *vs.* G4). P<.05 considered significant.

The inflammatory chemokine CXCL8 (IL8) concertation was significantly upregulated in the dyslipidemic group (Group 2). A similar increase was also seen in the antimicrobial peptide LEAP-1 or hepcidin, the major iron-regulating hormone. Both dyslipidemic groups (group2 and group3) had an elevated level of LEAP-1. Whereas the normolipidemic groups (Group1 and 4) has diminished level of hepcidin / LEAP-1, particularly Group 4 (vitamin D deficiency with normolipidemia). The antimicrobial protein Lipocalin 2 was observed to be elevated in the control group, while the combination of vitamin D deficiency and dyslipidemia (Group 3) lead to a decrease in Lipocalin 2. Lipopolysaccharide Binding Protein (LBP), which plays a role in inflammation and endotoxin detoxification was also reduced in participants with vitamin D deficiency and dyslipidemia Supplementary Table S1.

3.6. Holistic proteomic profile analysis of the participants with or without vitamin D deficiency and dyslipidemia

Holistic proteomic profile analysis was conducted to investigate the global impact of vitamin D deficiency and dyslipidemia on the serum proteomics profiles of participants. Table 3 provides a comprehensive list of the top 20 most enriched proteins in each group. To illustrate the effect of dyslipidemia on the proteomic profile, a comparison was made between Group 1 (vitamin D sufficient with normolipidemia) and Group 2 (vitamin D sufficient with dyslipidemia), as depicted in Figure 3A. The data revealed significant enrichment of apolipoproteins, namely ApoE and ApoB, as well as the LDL receptor protein LRP1B. Additionally, several proteins involved in the immune system, such as pro-inflammatory cytokines IL7 and neutrophil AZU1, were significantly enriched. The PI3K-Akt signaling pathway emerged as the most significant pathway, followed by pathways in cancer, MAPK signaling, Ras signaling, JAK-STAT signaling, cytokine-cytokine receptor interaction, prostate cancer, fluid shear stress and atherosclerosis, transcriptional misregulation in cancer, and hematopoietic cell lineage.

To investigate the effect of combined vitamin D deficiency and dyslipidemia, a comparison was made between Group 1 (control group) and Group 3 (vitamin D deficiency with dyslipidemia). In this comparison (Fig. 3B), ApoE and LDL receptor protein LRP1B were elevated. Furthermore, proteins involved in inflammation, such as phosphatidylethanolamine-binding protein PEBP1 and antioxidant SOD1, showed significant upregulation. Conversely, TNF-receptor superfamily member TNFRSF17, immunoglobulin member LSAMP, and appetite stimulator GHRL were downregulated. The enriched pathways included inflammation and cancer pathways, such as pathways in cancer, PI3K-Akt signaling, MAPK signaling, cytokine-cytokine receptor interaction, Ras signaling, JAK-STAT signaling, Hepatitis B, ErbB signaling, AGE-RAGE signaling pathway in diabetic complications, and HIF-1 signaling pathway.

The proteomic profile modifications induced by vitamin D deficiency were examined by comparing Group 1 (control) with Group 4 (vitamin D deficiency with normolipidemia) in Figure 3C. Proteins involved in calcium or bone metabolism, such as calcium-binding protein ANXA2, CAMK2D calcium/calmodulin-dependent kinase, and calcium and hydroxyapatite binding protein IBSP, were increased in vitamin-deficient participants. Insulin-like growth factor binding protein IGFBP3 also exhibited increased expression. Conversely, FAS protein, TNF-receptor superfamily member, and interleukin-5-specific subunit IL5RA were downregulated. The enriched pathways primarily related to inflammation, including cytokine-cytokine receptor interaction, IL-17 signaling pathway, Natural killer cell-mediated cytotoxicity, Chemokine signaling pathway, and Influenza A, indicating a high sub-inflammatory status. In addition to the PI3K-Akt signaling pathway, pathways in

Table 3
The most significant proteins expression levels between different groups

G1-G2				G1-G3			
Protein	*P* value	FC	logFC	Protein	*P* value	FC	logFC
APOE	5.60E-03	1.11	0.16	APOE	4.64E-09	1.21	0.27
CRYZL1	1.63E-05	1.32	0.4	CRYZL1	2.71E-09	1.38	0.47
CA2	4.42E-03	0.67	-0.57	GDI2	4.80E-06	1.29	0.37
LAMA1	4.75E-03	1.17	0.23	LAMA1	5.44E-08	1.25	0.32
LRP1B	1.23E-05	1.24	0.31	LRP1B	1.02E-08	1.23	0.3
APOB	2.15E-06	1.49	0.57	GHRL	2.71E-06	0.77	-0.39
ADAMTS13	5.45E-03	0.86	-0.22	HTRA2	1.77E-06	1.18	0.23
AZU1	1.70E-03	1.15	0.2	LSAMP	1.18E-07	0.83	-0.27
CD70	3.62E-03	0.86	-0.22	METAP2	4.03E-06	1.37	0.45
CDH12	4.19E-03	1.36	0.44	NEGR1	1.33E-06	0.87	-0.2
EIF5	1.28E-04	1.31	0.39	PEBP1	4.07E-06	1.29	0.36
FGF10	2.43E-03	1.16	0.21	PPA1	4.56E-06	1.29	0.37
FGFR2	2.66E-03	0.76	-0.4	PRKCI	4.28E-06	1.38	0.46
HDAC8	4.06E-03	1.12	0.16	PSMA6	4.37E-06	1.27	0.34
HDGFL2	5.56E-03	1.08	0.12	SHC1	3.31E-06	1.42	0.51
IL7	4.62E-03	1.27	0.35	SKP1	4.57E-06	1.23	0.3
MAP2K4	6.93E-04	0.82	-0.28	SMAD3	5.22E-07	1.43	0.51
S100A4	5.03E-03	1.2	0.27	SOD1	2.75E-07	1.21	0.28
SERPINC1	3.74E-03	0.92	-0.12	TNFRSF17	1.93E-06	0.83	-0.27
TGM3	8.53E-04	1.62	0.7	VTA1	2.37E-06	1.58	0.66

G1-G4				G3-G4			
Protein	P value	FC	logFC	Protein	P value	FC	logFC
CGA	8.85E-04	0.66	-0.6	APOE	1.29E+01	0.81	-0.3
ANXA2	1.76E-03	1.15	0.2	CRYZL1	1.72E+01	0.66	-0.61
CA2	1.27E-03	0.77	-0.38	UNC5D	8.24E-07	1.25	0.32
CAMK2D	2.27E-03	1.4	0.49	LAMA1	7.49E+00	0.8	-0.33
LRP1B	1.46E-03	0.9	-0.15	LRP1B	2.15E+01	0.73	-0.45
IBSP	3.69E-05	1.29	0.36	IBSP	7.91E+00	1.36	0.44
OMD	3.74E-06	1.33	0.41	OMD	8.76E+00	1.37	0.45
SOST	8.92E-12	0.66	-0.59	SOST	9.66E+00	0.73	-0.45
FAS	1.38E-03	0.85	-0.24	A2M	2.81E-07	1.28	0.36
IGFBP3	1.67E-03	1.13	0.17	ACY1	5.01E-07	0.64	-0.65
IL5RA	5.77E-04	0.82	-0.29	ADIPOQ	8.47E+00	1.38	0.46
MYBPC1	1.03E-03	0.74	-0.43	ESM1	8.87E+00	1.31	0.39
NAGK	7.03E-05	1.18	0.24	GAS1	3.67E-07	1.12	0.16
NRG4	3.62E-04	0.83	-0.26	GHR	6.09E-07	0.79	-0.34
PGK1	2.55E-03	1.23	0.3	MMP3	9.24E+00	0.73	-0.45
PRSS1	6.86E-04	0.86	-0.22	NRP1	3.06E-07	1.16	0.21
PTH	1.26E-03	1.22	0.28	PIANP	1.23E-07	1.24	0.31
SERPINA1	8.67E-04	1.16	0.21	PLAT	1.75E-07	0.76	-0.4
SERPING1	9.71E-04	0.88	-0.18	SELL	8.35E-08	1.16	0.21
VEGFD	2.67E-03	0.91	-0.14	TFPI	2.46E-07	0.84	-0.25

The highest 20 expressed proteins are selected based on their statistical significance, $P<.05$ considerd significant.
Values highlited in green represent the log fold change of downnregulated proteins.

cancer, JAK-STAT signaling pathway, ErbB signaling pathway, and Glioma were also significant.

Lastly, to examine the effect of dyslipidemia in vitamin D-deficient participants' proteomics profiles, a comparison was made between Group 3 (combined vitamin D deficiency and dyslipidemia) and Group 4 (vitamin D deficiency only) in Figure 3D. Interestingly, the level of ApoE was decreased in vitamin D deficiency without dyslipidemia. Calcium and bone mineralization proteins IBSP and OMD showed significant upregulation. Alpha-2-Macroglobulin A2M, Adiponectin protein ADIPOQ, and growth arrest GAS1 were also upregulated. Conversely, Growth Hormone Receptor GHR and matrix Metallopeptidase MMP3 were decreased. In this comparison, several pathways involved in inflammation, such as cytokine-cytokine receptor interaction, complement and coagulation cascades, and apoptosis cascades, were highly significant. Furthermore, pathways related to cancer, including prostate cancer, melanoma, and pancreatic cancer, were also prominent. Other pathways, such as focal adhesion, FC epsilon RI signaling pathway, VEGF signaling pathway, and axon guidance, were also significant. Overall, the holistic proteomic analysis revealed significant alterations in protein expression and associated pathways during vitamin D deficiency and dyslipidemia. These findings emphasized the physiological role of vitamin D in bone homeostasis, immune modulation, and anti-inflammatory effects.

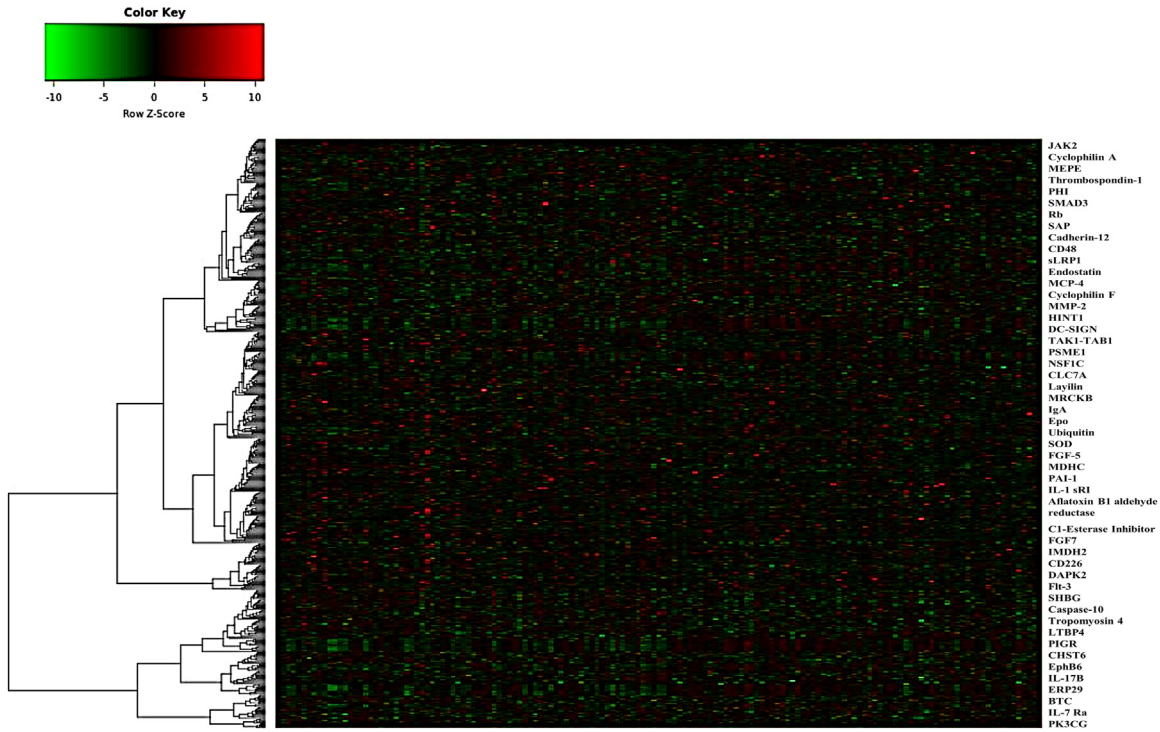

Fig. 4. Hierarchically clustered heatmap showing the expression of 50 most significant proteins between Group 1 (n=64) vitamin D sufficient with normolipidemia (control group), and Group 3 (n=85) of participants with combined vitamin D deficiency and dyslipidemia. Red and green represent expression abundance (green: low; red: high) as indicated in the legend.

3.7. Heatmap clustering of proteins expression differences observed in participants with or without vitamin D deficiency and dyslipidemia

The combination of vitamin D deficiency with dyslipidemia showed most effects on proteomics profiles. Here, we focused on the differences in protein expression between Group1 (the control group) and Group 3, which represents participants who were vitamin D deficient with dyslipidemia. The heatmap illustrates that many participants have a high level of the proteins involved in the inflammation process and the immune system were enhanced *i.e.,* IL-1sR, IgA, and the immunoglobulin CD226. The data indicate the elevation of the inflammatory biomarkers reflecting on the association between vitamin D status and dyslipidemia (Fig. 4).

The linear regression analysis between Vitamin D levels and Apolipoproteins (ApoM, ApoA-1, ApoB, and ApoD) didn't show significant associations. For instant, the coefficient estimate for ApoM is approximately 0.0002655. This estimate suggests that, on average, for each one-unit increase in ApoM, the predicted value of vitamin D increases by about 0.0002655 units. However the *P*-value associated with the coefficient for APOM is not significant (.0639). This suggests that there may be some evidence of a relationship between ApoM and vitamin D, but it is not statistically strong enough due to small number of samples. When cohort was stratified into 4 groups some linearity was observed. For example, the linear relationship between ApoM and vitamin D level showed positive and negative association in group 2 and group 3 respectivly (Supplementry Table S2 and Supplementary Fig. S1 and S2).

4. Discussion

This study aims to elucidate the molecular connection between vitamin D deficiency and dyslipidemia, specifically focusing on the association between vitamin D deficiency, low HDL levels, and reduced anti-inflammatory activity of HDL. Our findings demonstrate that concentrations of apolipoproteins vary according to vitamin D and lipid profile status.

Firstly, we observed a significant reduction in ApoA-1 expression, the major protein in HDL particles, during vitamin D deficiency, irrespective of the presence of dyslipidemia. Clinical studies have shown that vitamin D supplementation increases ApoA-1 serum levels [38]. ApoA-1 is integral to HDL formation and has been linked to the anti-inflammatory functions of HDL. Transgenic mice overexpressing ApoA-1 exhibited decreased expression of toll-like receptor 4 (TLR4) and improved survival following infection [39]. Our proteomic data suggest that the reduction in ApoA-1 expression during vitamin D deficiency may directly contribute to the diminished anti-inflammatory potential of HDL.

In contrast, ApoB and ApoE were elevated in dyslipidemia due to their association with LDL and chylomicrons, respectively [40]. Elevated levels of these lipoproteins are typically observed during dyslipidemia and are associated with an increased risk of cardiovascular disease [41]. The combination of dyslipidemia and vitamin D deficiency drastically reduces ApoM expression, which strongly supports the notion that vitamin D status influences HDL levels since ApoM is crucial for HDL biogenesis. However, limited studies have investigated the relationship between ApoM and vitamin D. ApoM has been found to induce the expression of the vitamin D receptor (VDR), and reduced ApoM levels correlate with decreased VDR expression [42,43]. ApoM is a lipocalin member that binds to HDL and carries sphingosine-1-phosphate (S1P). The ApoM/S1P complex plays a critical role in HDL's antiatherogenic and anti-inflammatory effects [44,45]. The complex suppresses the surface abundance of vascular adhesion molecules during inflammation [45]. The combined effect of low vitamin D concentrations and dyslipidemia leads to a substantial reduction in ApoM expression, exacerbating the disturbance in HDL level and function since

ApoM is essential for HDL biogenesis [46]. Moreover, HDL levels are known to be gender-dependent, and our study sheds light on how gender impacts the expression of certain apolipoproteins [37]. For instance, males expressed higher levels of ApoA-1 compared to females during vitamin D deficiency, while ApoM was higher in females under normal physiological conditions and during vitamin D deficiency.

Furthermore, the study revealed a reduction in ApoD levels during combined dyslipidemia and vitamin D deficiency. ApoD, a lipocalin that binds hydrophobic ligands, is found in body secretions and serum in larger amounts compared to other apolipoproteins. ApoD is reported to decrease immune responses during acute inflammation, inhibit T-cell infiltration into the central nervous system, reduce the production of pro-inflammatory cytokines, and downregulate the activity of phospholipase A2 (PLA2) [47,48]. ApoD is typically increased in the presence of vitamin D, and this increase is associated with an inhibitory effect on certain cancers, such as breast and prostate cancer cells [49,50]. The reduction in ApoD levels due to the lack of stimulation from vitamin D, combined with dyslipidemia, indicates a profound disturbance in ApoD expression and immunological function.

SAA1, an indirect indicator of HDL status, plays a role in HDL remodeling and function. During high inflammatory states like sepsis, SAA1 replaces ApoA-1 in HDL particles, leading to a reduction in the anti-inflammatory function [51,52]. Our findings demonstrate that SAA1 levels are highest during vitamin D deficiency, suggesting that SAA1 contributes to subclinical inflammation and dysfunction in HDL particles.

Iron homeostasis is known to be disrupted during inflammation, and altered iron homeostasis is observed in various chronic inflammatory diseases. Hepcidin or LEAP-1, the master iron-regulating protein produced by the liver, is part of the innate immune system's iron-hepcidin-ferroportin axis. This axis inhibits microbial growth during infection by depleting iron levels, sequestering iron in macrophages [53–56]. In our study, hepcidin (LEAP1) was found to be elevated in dyslipidemia but significantly reduced in normolipidemic participants with vitamin D deficiency.

The effect of dyslipidemia on the overall proteomic profile was observed to increase the expression of several proteins involved in the immune system, such as IL7 and the protein coding lysosomes in neutrophil AZU1, which possesses antimicrobial activity. Conversely, the TNFα ligand family member CD70 was decreased. CD70 is highly expressed in activated lymphocytes and macrophages [57]. It serves as a regulator of endothelial nitric oxide and reactive oxygen species. Knocking out the CD70 gene in endothelial cells impairs eNOS expression and function and increases intracellular hydrogen peroxide, thereby exacerbating atherosclerotic events [58]. Moreover, the most enriched pathways in dyslipidemia included inflammatory responses, apoptosis, the MAPK pathway, JAK-STAT signaling pathway, Ras signaling pathway, and cytokine-cytokine receptor interaction. These findings suggest the enrichment of inflammatory pathways during combined vitamin D deficiency and dyslipidemia.

The combination of vitamin D deficiency with dyslipidemia significantly enhanced the expression of inflammatory proteins, such as the phosphatidylethanolamine-binding protein PEBP1, and antioxidant enzymes like superoxide dismutase (SOD1), which detoxify free superoxide radicals [59]. Additionally, SOD1 has been found to suppress pro-inflammatory immune responses [60]. The elevation of SOD1 observed in our study could be a compensatory mechanism due to increased inflammatory cytokines. Conversely, the protein HTRA2, known to induce apoptosis, was elevated. On the other hand, members of the TNF-receptor superfamily, TNFRSF17, and immunoglobulin member LSAMP, were decreased. Additionally, pathways such as AGE-RAGE, which are disturbed in diabetes, and the ErbB signaling pathway were affected. These data further support the notion that the combination of vitamin D deficiency and dyslipidemia severely impacts the anti-inflammatory potential of HDL.

Moreover, our study reports that vitamin D deficiency enhances the expression of proteins related to calcium homeostasis and bone metabolism, reaffirming the importance of vitamin D sufficiency for bone health. Upregulated proteins included the calcium-binding protein ANXA2, CAMK2D calcium/calmodulin-dependent kinase, and calcium and hydroxyapatite binding protein IBSP, as well as the bone mineralization regulator OMD. Notably, the cell death and TNF-receptor FAS were downregulated. FAS downregulation is associated with high tumorigenicity and was observed to be decreased in many cancers *e.g.*, gastric cancer [61], prostate cancer [62], and bladder cancer [63].

In general, vitamin D status has a profound effect on HDL homeostasis and inflammation in the body. Previous studies by Yarparvar et al. have demonstrated a positive correlation between vitamin D levels and anti-inflammatory cytokines like IL-10, and a negative correlation with pro-inflammatory markers such as Tumor Necrosis Factor receptor 2 (TNFR-2) and high-sensitivity C-reactive protein (hsCRP), which supports our study findings. Furthermore, the study found that healthy adolescents with low serum vitamin D levels also have low HDL levels [25]. Another recent study by Sharif-Askari et al. [64] supports these findings, as they investigated the lipid profile in insulin-resistant individuals and revealed that low concentrations of serum 25(OH)D were accompanied by low levels of HDL-C and increased circulatory levels of cytokines IL-6 and IL-8. Our study confirms that the combination of vitamin D deficiency and dyslipidemia has a significant impact on the proteomic profile and is associated with an inflammatory state among participants.

Our study has a cross-sectional design, and therefore, it does not track participants longitudinally, which represents a limitation. As the proteomic profile can undergo dynamic changes, a longitudinal study that monitors the alterations in both vitamin D status and dyslipidemia would provide valuable insights and aid in the identification of biomarkers associated with the interplay between vitamin D deficiency and dyslipidemia. Dietary intake also influences vitamin d status and lipids levels which is missing in our data set. Additionally, our data was obtained from Qatar Biobank where the most common metabolite (25-Hydroxyviatmin D3) was measured as it has the longest half-life of 40 d which in turn reflects vitamin D status. In contrast, the hormonally active form 1,25-Dihydroxy vitamin D3 metabolite half-life is 4 h [1]. Therefore, investigating the effect of vitamin D metabolites on the proteomic profile of participants may shed new insight and warrant further investigation. Currently, it is not clear if vitamin D deficiency leads to proteomics and lipidomics alterations or vice versa, alterations in lipidomics and proteomics affecting vitamin D metabolism leading to vitamin d deficiency A future longitudinal study with greater sample size including insufficient participants assessing vitamin D metabolites in relation to proteomics and lipidomics profile is warranted.

5. Conclusion

The proteomic profiling conducted in this study unveiled significant changes in HDL-associated proteins. Notably, it revealed that inflammation pathways were enriched in individuals with vitamin D deficiency. Moreover, participants who had both vitamin D deficiency and dyslipidemia exhibited heightened activation of not only inflammation pathways but also cancer pathways. These findings highlight the distinct proteomic alterations associated with vitamin D deficiency and the effect of combining vitamin

D deficiency with dyslipidemia on inflammatory and cancer-related pathways.

Author contributions

Conceived the study: S.M.Z.; data collection: S.M.Z. and H.M.; data analysis: H.M., R. M.R. A.N.A. and S.M.Z. Supervision: S.M.Z. Writing manuscript draft: H.M., S.M.Z. Critically reviewing and finalizing manuscript: S.M.Z., H.M., R. M.R. and A.N.A. All authors have read and agreed to the published version of the manuscript.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Qatar Biobank, Doha, Qatar (QBB-RES-ACC-0237-0142) and confidentiality agreements were obtained before conducting this study.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

Restrictions apply to the availability of these data. Data was obtained from Qatar Biobank (https://www.qatarbiobank.org.qa. Accessed on 12, August 2022) under confidentiality agreement with Qatar University. Supplementary data are freely available.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2023.109472.

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