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Original Article

Association between vitamin D levels with IL-6 and IL-10 in umbilical cord blood of infants

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Abstract

A worldwide issue, vitamin D deficiency affects pregnant mothers and babies everywhere, including Indonesia. It involves the adaptive immune system by controlling the production of pro- and anti-inflammatory cytokines and the balance between humoral (Th2) and cell-mediated (Th1) immunity. The aim of this study was to investigate the relationship between vitamin D and the cytokines IL-6 and IL-10 in infants. It also examined the relationship between ferritin and IL-6/IL-10 in newborns. The study collected 114 umbilical cord blood samples from term-born mothers without clinical symptoms. IL-6 and IL-10 were among the cytokine profiles measured by the enzyme-linked immunosorbent assay (ELISA). SPSS was used for statistical analysis, and an in-silico investigation was carried out to examine the molecular relationships between vitamin D and IL-6/IL-10. Using the 20 ng/mL as the cut-off for vitamin D insufficiency suggested the insignificant association of vitamin D with IL-6 (p=0.42), IL-10 (p=0.76), and ferritin (p=0.47). When the umbilical cord vitamin D level was categorized into four quartiles, the association with the highest statistical significance (quartile 4 versus quartile 2) was observed for IL-6 (p<0.001), IL-10 (p<0.001), and ferritin (p<0.001). However, the linear regression did not suggest the significant correlations of vitamin D with IL-6 (p=0.40) and IL-10 (p=0.45). A significant correlation based on the linear regression was found between ferritin and IL-10 (p=0.03). Molecular docking studies demonstrated binding affinities of -8.04 kcal/mol for IL-6-vitamin D and -8.53 kcal/mol for IL-10-vitamin D complexes, with stable root mean square deviation throughout the simulations. This study contributes valuable insights into the clinical and computational analysis of the relationship of vitamin D with IL-6 or IL-10.

Keywords: Ferritin, IL-6, IL-10, umbilical cord, vitamin D

Introduction

A widespread vitamin D deficiency has been observed across different regions such as South America, Africa, India, the Middle East, and Australia. In the United States (US), serum 25(OH)D3 levels below 20 ng/mL were found in 48% of preadolescent white women and more than 50% of Hispanic adolescents, spanned from 2001 to 2004 [1]. In the same period, serum 25(OH)D3 levels of less than 15 ng/mL were reported in 42% of African American women in the US aged 15 and 49 years [1]. Research conducted across multiple South Asian and Southeast Asian

Received: May 23, 2024 - Accepted: October 3, 2024 - Published online: October 20, 2024

nations between 2005 and 2009 has demonstrated a high prevalence of vitamin D insufficiency and deficiency in this region [2]. Furthermore, an epidemic of vitamin D (25(OH)D3) deficiency was reported in Asia, with a prevalence ranging from 27% to 60%, depending on the season [3]. Additionally, vitamin D intake has been linked to the increased wound-healing process of diabetic foot ulcer patients [4]. Vitamin D has been found to have a significant role in determining bone mineral density in menopausal women [5]. Low vitamin D status (25(OH)D<50 nmol/L) is associated with an increased risk of iron deficiency and low ferritin levels. 25(OH)D concentrations interacted with inflammation, so in children with inflammation, higher 25(OH)D concentrations are predicted to lower ferritin concentrations than those without inflammation. In overall meta-analyses, we found limited evidence of increased risk of iron deficiency anemia and anemia in children with low vitamin D status [6].

Despite the multiple health benefits of vitamin D, pregnant women have been reported to be at risk of vitamin D deficiency [7,8]. The condition is particularly caused by the transfer of vitamin D from the mother to the fetus during the pregnancy. Indeed, this mechanistic pathway also applies to other micronutrient deficiencies [7,8], potentially impacting the child's growth and development [9]. According to recent epidemiological research, vitamin D deficiency has been linked to a higher frequency of respiratory tract infections [10].

Despite being a tropical nation with abundant sunshine, vitamin D deficiency remains a persistent issue in Indonesia [5,9]. Research conducted from 2010 to 2011 reported that 45.1% of Indonesian children aged between 2 and 12.9 years had vitamin D insufficiency [11]. Another study conducted from December 2015 to December 2017 in Yogyakarta, Indonesia, showed that approximately 54% of newborns had vitamin D levels below 30 mmol/L [12]. Vitamin D deficiency in newborns could pose a risk to their impaired innate and adaptive immune systems [13]. In an in vitro study, vitamin D has been associated with the alteration of several cytokines, such as interferon γ, IL-4, IL-5, IL-6, IL-10, and IL-13 [14]. In the case of an innate immune response, increased levels of pro-inflammatory cytokines like IL-1 and IL-6 and decreased levels of anti-inflammatory cytokines like interleukins IL-4 and IL-10 have been linked to adequate 25(OH)D3 levels [10]. Moreover, it is also evident that ferritin and vitamin D status interact during pregnancy, with low ferritin levels linked to a 6.59-fold increased risk of vitamin D deficiency in expectant mothers [16]. It is, therefore, crucial to comprehend the epidemiology and risk factors for vitamin D deficiency during early life [17,18]. The aim of this study was to preliminarily investigate the correlations between the levels of vitamin D, IL-6, and IL-10 in neonatal umbilical cord blood and its molecular interaction through molecular docking and molecular dynamics. Furthermore, this study also explores the correlation between vitamin D and ferritin to get a more holistic analysis of the interactions between vitamin D and key components of the immune and inflammatory response, providing valuable insights into their combined roles in health and disease.

Methods

Study design

This study employed a cross-sectional design by collecting samples at a single point in time. Cases of vitamin D deficiency and normal vitamin D levels in mothers were matched based on the frequency of umbilical cord sampling in newborns. The inclusion criteria were full-term newborns with a birth weight of ≥2.5 kg. Newborns with congenital anomalies or those suffering from acute infections at birth were excluded from the study. Despite their apparent health, all patients underwent a clinical evaluation for acute infection or congenital anomaly status. Signed informed consent was collected from the mother via a questionnaire prior to the study. As a complementary analysis, we performed an in silico analysis to observe the binding of vitamin D molecules to IL-6 or IL-10. The study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia, under Registration No. 571/UN4.6.4.5.3L/PP36/2023.

Umbilical cord blood collection

Umbilical cord blood samples (5 mL each) from 114 newborns were collected from various maternity hospitals in Makassar, Indonesia. The sample was then stored in a vacutainer tube and

left to clot for 30 minutes at room temperature. Following a 10-minute centrifugation at 3,000×g, the serum was separated, transferred into sterile tubes, and kept frozen until further analysis.

Measurement of vitamin D and cytokines

Quantification of serum 25(OH)D3 was carried out using a commercial radioimmunoassay kit (IDS Inc., Draper, UT, USA) that measures 25(OH)D in the range of 5–300 nmol/L with corresponding intra- and inter-assay coefficients of variation of 3.43% and 5.44%. The standard for determining normal 25(OH)D concentrations is commonly set at 20 ng/mL, which is often used as the cut-off for vitamin D insufficiency. However, for vitamin D deficiency, the cut-off is typically ≤12 ng/mL [19]. The range for serum ferritin detected was around 0.01–0.1 ng by ELISA [20]. Serum levels of ferritin, IL-6, and IL-10 were determined using ELISA with standard commercial kits, and the protocols followed those provided by the manufacturers.

Statistical methods

The SPSS 27 program was used to analyze the data and perform statistical analyses (IBM Corporation, Armonk, NY, USA). Continuous variables were compared using the Mann-Whitney U test and Spearman's test as a hypothesis test to ascertain the relationship between the serum levels of vitamin D (25(OH)D) and IL-6 or IL-10. The value was interpreted as significant at $p<0.05$ and very significant at $p<0.01$.

In silico study

System preparation and homology modeling

To gain more information about the molecular interactions between vitamin D with IL-6 and IL-10, an in silico analysis was performed. The AVOGADRO software was used to prepare the three-dimensional structures of vitamin D3 (25(OH)D3), acting as the ligand in this study. The structures were downloaded from the PubChem database at https://pubchem.ncbi.nlm.nih.gov/ (PubChem ID: 5283731). The IL-6 protein was modeled using its homologue (PDB ID: 1ALU, 1.90 Å) and sequence from Uniprot (ID: P05231). The sequences were aligned with the template on Jalview ver. 2.11.2.7 and MODELLER ver. 10.9 programs to generate the 3D protein molecules. Consequently, the model was assessed using PyMoL Software ver 2.3.4 for its root main square deviation (RMSD) and was further verified using ProssA-web Z score (https://prosa.services.came.sbg.ac.at), ERRAT, 3D-Verify, and PROCHECK computations through Ramachandran Plots (https://saves.mbi.ucla.edu). As for the IL-10 and ferritin, the protein structure was downloaded from the Protein Data Bank (PDB ID: 1ILK and 3AJO, respectively). The protein was prepared by removing unnecessary molecules and water, adding charged ions, and performing structural minimization using UCSF Chimera (Version 1.16). Thereafter, the steepest descent minimization was carried out on IL-6, IL-10 and ferritin using UCSF Chimera

Molecular docking study

AutoDock Tools (ADT) 1.5.7 was used for molecular docking analyses. Together with the ligand vitamin D3 (25(OH)D), the protein structures of IL-6, IL-10, and ferritin were also converted to a .pdbqt structure. Additions of nonpolar hydrogen atoms and assignment Kollman and Gasteiger charges were also carried out on the AutoDock Tools. Throughout the docking process, all torsional angles were allowed to rotate. CASTp, the Computed Atlas of Surface Topography of Proteins, was used to predict the binding sites of IL-6 and IL-10, particularly (http://sts.bioe.uic.edu/castp). AutoGrid was used to produce grid maps for the proteins. The gird box coordinates for IL-6 and IL-10 were centered at 12.6×62.5×1.78 Å and 7.3×54.25×30.3 Å, respectively. Meanwhile, the coordinates of ferritin were centered at 42.03, -9.98, and 29.21 Å, and the grid box was set at 52×56×54 Å. The orientation of the docked compound within the binding site, hydrogen bond interactions, and the binding energy score were used to evaluate each protein-ligand complex.

Molecular dynamics study

The complex structures obtained from the previous docking simulation were analyzed through molecular dynamics simulations to examine the effects of their binding on conformational

flexibility and structural stability. This set of simulations was run with the GROMACS 2023 software. The ligand topology was created using SwissParam (https://www.swissparam.ch), whereas the protein topologies were produced using the CHARMM36 force field. Each protein-ligand complex was encased in a 1.0 nm dodecahedron box and solvated with water molecules of the SPC216 type using periodic boundary conditions. In order to achieve system neutrality, one chloride ion (Cl^-) was added to IL-6-vitamin D3 (25(OH)D3), two chloride ions (Cl^-), and five Sodium (Na^+) were added to ferritin-vitamin D3 (25(OH)D3). Position constraints were placed on both complexes, and the steepest descent algorithm was used to minimize energy over a 1,000-step process. The NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensembles were subjected to a 100 picosecond-long two-phase equilibrium step. A 50-nanosecond production molecular dynamics simulation run was performed with the pressure set to 1 bar and the temperature kept at 300°K. Root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) were among the metrics used to analyze the simulation trajectory data was used to visualize the simulation results, while Xmgrace was used to represent the data graphically.

Results

Correlation between vitamin D and IL-6, IL-10, and ferritin

In this study, the median for each group was determined for the sufficient group (vitamin D>20 ng/mL, n=19), with a median of 5.80 ng/mL, and insufficient group (vitamin D<20 ng/mL, n=95), with a median of 7.00 ng/mL. Results from the Mann-Whitney test for the difference of IL-6, IL-10, and ferritin between the vitamin D sufficiency and deficiency groups are presented in **Table 1**. The vitamin D status was not associated with IL-6 (p=0.41), IL-10 (p=0.76), and ferritin (p=0.47). Subsequently, this study conducted further tests by dividing the subjects into four groups based on quartiles to examine the differences in IL-6, IL-10, and ferritin levels across the vitamin D quartiles (**Table 2**). In quartile 1, which had the lowest vitamin D levels, the median IL-6 was recorded at 11.0655 pg/mL with a range of 0.66–1,495.45 pg/mL, showing the highest value compared to other quartiles. IL-10 in this quartile also had the highest median of 273.8 pg/mL, with a range of 69.87–295.48 pg/mL. Meanwhile, the median ferritin in quartile 2 was the highest, reaching 13,653.22 ng/mL with a range of 2,460.63–17,038.78 ng/mL. The Kruskal-Wallis test showed results for IL-6 (p=0.82), IL-10 (p=0.95), and ferritin (p=0.01), where ferritin levels were significant (p<0.05), indicating that there was a significant difference between the medians of the groups tested (**Table 2**).

Table 1. Comparison between IL-6, IL-10, and ferritin with insufficient and normal levels of vitamin D

Markers	Median (minimum-maximum)		*p*-value
	Sufficient vitamin D (n=19)	Insufficient vitamin D (n=95)	
IL-6 (pg/mL)	5.80 (1.61–119.50)	7.00 (0.19–2,005.28)	0.42
IL-10 (pg/mL)	268.92 (112.14–293.58)	270.82 (69.87–1,525.19)	0.76
Ferritin (ng/mL)	13,450.27 (7,095.04–17,337.52)	13,088.79 (421.91–22,119.75)	0.47

Table 2. Comparisons of IL-6, IL-10, and ferritin levels according to the quartiles of umbilical cord vitamin D level

Vitamin D level (ng/mL) (quartile)	IL-6 (pg/mL)	*p*-value	IL-10 (pg/mL)	*p*-value	Ferritin (ng/mL)	*p*-value
<11.89 (Q1)	11.07 (0.66–1,495.45)	<0.001	273.80 (69.87–295.48)	0.017	11,876.60 (421.91–22,119.75)	0.103
11.89–14.30 Q2)	8.92 (0.19–2,005.09)	<0.001	268.44 (199.30–1,525.19)	<0.001	13,653.22 (2,460.63–17,038.78)	<0.001
14.30–18.11 (Q3)	4.79 (0.74–1,481.25)	<0.001	270.90 (148.65–300.4)	0.002	12,340.04 (7,568.93–15,346.20)	0.476
>18.11 (Q4)	5.80 (1.02–585.27)	Ref	268.92 (112.14–293.58)	Ref	13,450.26 (7,095.04–17,691.41)	Ref

To get more information about the relationship between levels of vitamin D, IL-6, IL-10, and ferritin, this present study utilized the Spearman test, where the results showed that there was no significant relationship between vitamin D levels and IL-6 (ρ=-0.079, p=0.402) and IL-10 (ρ=-0.072, p=0.449) (**Table 3**). The correlation between vitamin D and these two biomarkers was weak and insignificant. On the other hand, the analysis between ferritin and IL-6 shows a correlation coefficient (ρ) of 0.104 with a p-value of 0.104. However, there was a significant correlation between ferritin and IL-10 (ρ=-0.202, p=0.031) (**Table 3**).

Table 3. Linear regression of umbilical cord vitamin D with IL-6 and IL-10

Correlation	ρ	p-value
Vitamin D and IL-6	-0.079	0.40
Vitamin D and IL-10	-0.072	0.45
Ferritin and IL-6	0.104	0.10
Ferritin D and IL-10	-0.202	0.03*

Analyzed using Spearman correlation test
*Statistically significant at p<0.05

In silico interactions

Herein, the IL-6 protein molecule was prepared through homology modeling, where structure, template, and sequence alignment are presented in **Figure 1.** The model construction had 86.98% sequence identity similarity and an RMSD of 0.418 Å as compared to the template. The ProSA-web Z-score was found to be -6.69. The ERRAT results indicated that all models met the standard score for acceptance as high-quality models, with the model protein scores surpassing 95.75%.

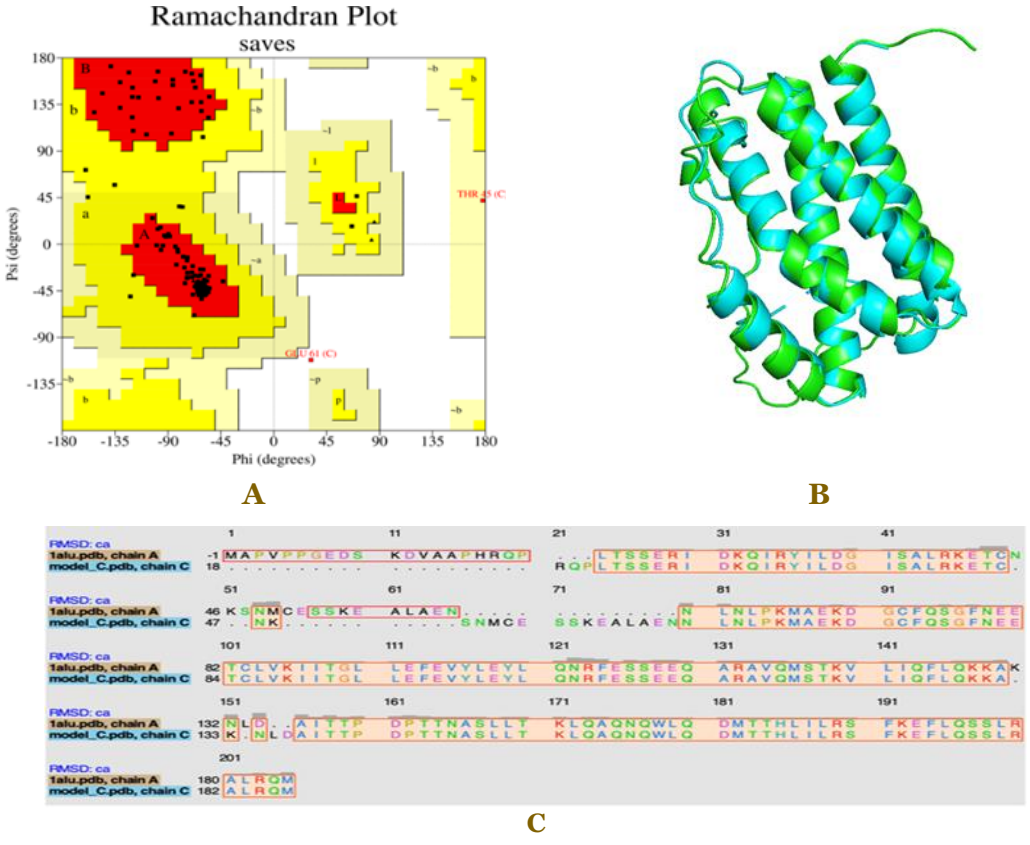

Figure 1. Ramachandran plot of the modeled protein IL-6 (residues in most favoured regions (A,B,L), residues in additional allowed regions (a,b,l,p), residues in generously allowed regions (-a,-b,-l,-p), and residues in disallowed regions) (A). Superimposition of the template IL-6 (blue) (PDB ID: 1ALU) and the modeled protein (green) (B). The sequence alignment between the template and the modeled protein (C).

Analysis of the PROCHECK program revealed that the overall G-factor for the stereochemical quality of the model was -0.03. This G-factor value was plotted using the

Ramachandran plot, where the values corresponded to four primary categories of different residual regions. The plot suggested that 93.1% of the model's residues were in the "allowed" region. The additional "allowed" region contained 5.7% of residues. Notably, the "generously allowed" and "disallowed" regions collectively accounted for 0.6% of the total residues, with specific residues such as Thr45 and Glu61 predominantly contributing to these regions. This minimal percentage of residues in the disallowed region indicated that only a small portion of the protein structure should be of concern.

The protein structure of IL-6 has 169 residues consisting of 6 α-helices and 7 loops. The binding pocket in IL-6 was positioned between the sixth α-helix and the second loop. The binding pocket in IL-6 was positioned between the sixth α-helix and the second loop. Visual representations of the docking analysis of this structure with the vitamin D ligand are presented in **Figure 2**. The interaction involved Leu59, Glu61, Leu66, Met151, Leu167, Arg170, and Glu174 with a binding affinity of -8.04 kcal/mol. This complex had two hydrogen bonds, involving Glu61 and Glu174.

As for the IL-10, its structure has 151 residues that form seven α-helices and eight loops. The binding pocket was situated at the convergence of loops 1, 2, 3, and 4. The interaction with vitamin D involved Leu17, Phe21, Val24, Phe28, Phe47, Leu56, Met59, Ile60, Tyr63, Val67, Met68, Ala71, His81, Val82, Leu85, Gly86 and Leu89 (**Figure 3**). The total binding affinity in this interaction is -8.53 kcal/mol. The complex was formed through alkyl and pi-alky interactions via Leu26, Phe30, Val33, Phe37, Ile60, Tyr72, Met77, Ala80, Val91, Leu94, and Leu98 (**Figure 3**).

The structure of ferritin consisted of six main α-helices and seven loops, whereas the binding pocket was located between the second and the fourth α-helix. The residues that were included at the binding site Leu26, Tyr29, Val33, Pro88, Asp89, Cys90, Trp93, Cys102, His105, Leu106, Asn109, and Ser113, whereas Tyr29 and Asp89 have formed hydrogen bond interactions with the ligand (**Figure 4**).

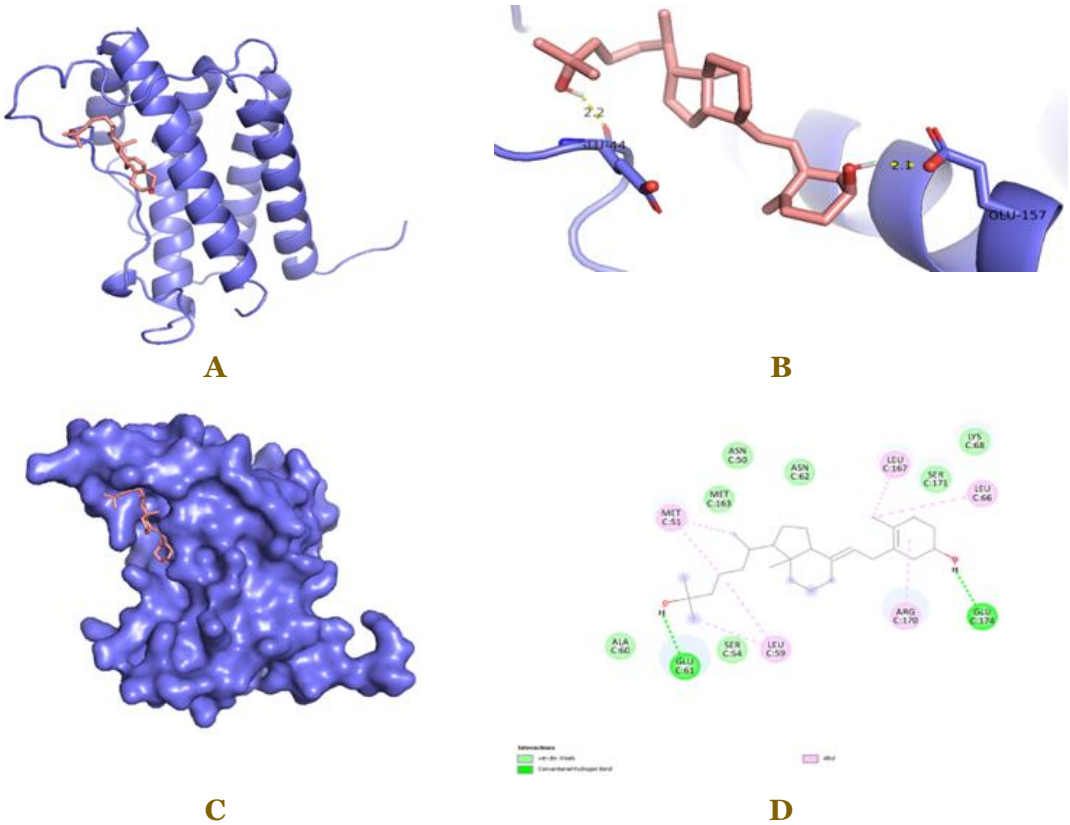

Figure 2. Three-dimensional representation of the IL-6-ligand complex (A), vitamin D representation within the binding pocket of IL-6 (B), the surface representation of the IL-6-vitamin D complex (C), and molecular interaction between vitamin D and the residue of IL-6 with binding affinity of -8.04 kcal/mol (D).

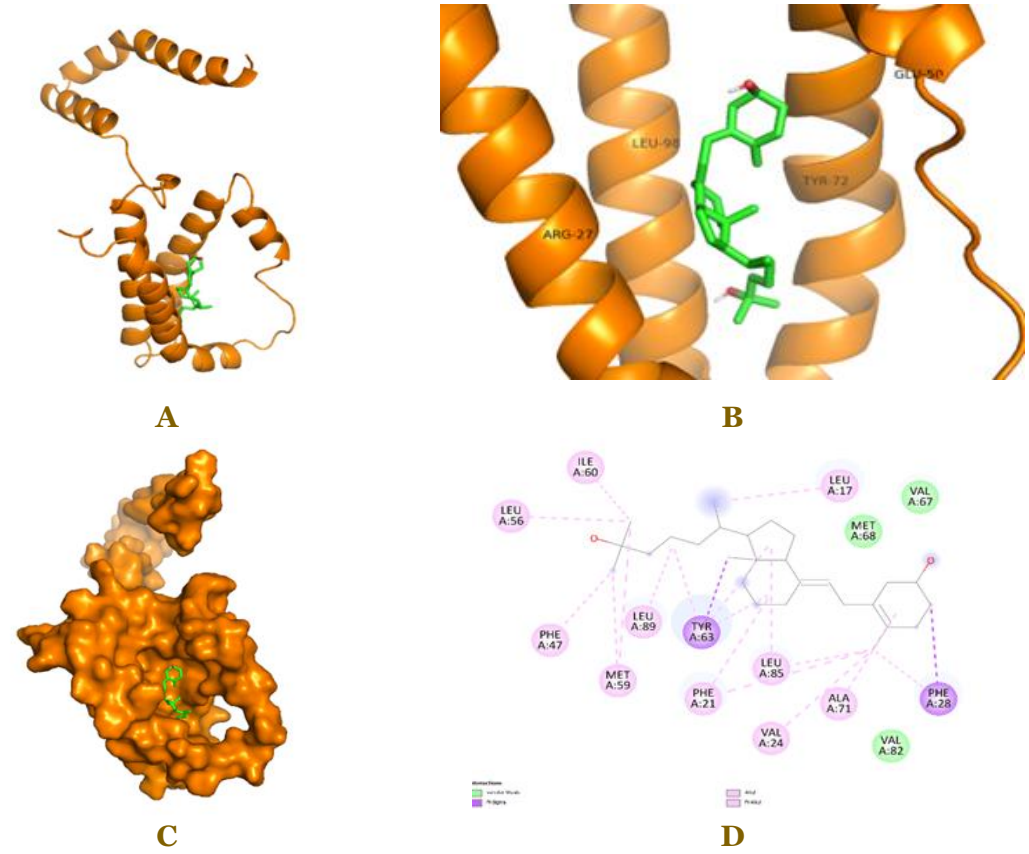

Figure 3. Three-dimensional representation of the IL-10-vitamin D complex (A), vitamin D representation within the binding pocket of IL-10 (B), the surface representation of the IL-10-vitamin D complex (C), and molecular interaction between vitamin D and the residue of IL-10 with binding affinity of -8.53 kcal/mol (D).

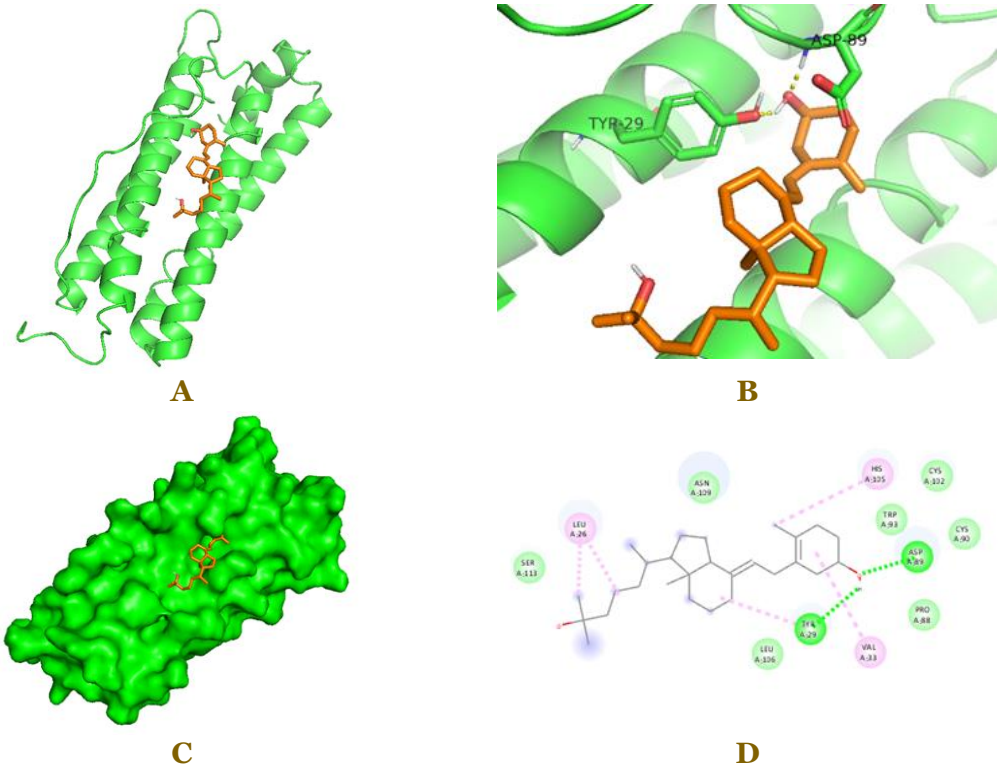

Figure 4. Three-dimensional representation of the ferritin-vitamin D complex (A), vitamin D representation within the binding pocket of ferritin (B), the surface representation of the ferritin-vitamin D complex (C), and molecular interaction between vitamin D and the residue of ferritin with binding affinity of -6.68 kcal/mol (D).

Ligand-protein complex stability

The molecular dynamic simulation results are presented in **Figure 5**. The apo form of IL-6 is shown to have steady equilibration after 30 ns, further showing an increase in equilibration until the end of the simulation. The apo form of the IL-10 simulation and the RMSD of the IL-10-vitamin D complex had a similar pattern that fluctuated under 20 ns before remaining stable until the end of the simulation. In the IL-6 complex and apo form, the residues ranging from 50 to 60 demonstrated the highest degree of fluctuation. However, the IL-6 apo form exhibited more significant fluctuation in specific residues when compared to the corresponding complex form, particularly residue number 150. In contrast, the RMSF values for the IL-10 complex and its apo formed show notable differences between the two forms. Specifically, the initial residue of the IL-10 complex had an RMSF value above 1 Å, while the IL-10 apo formed started with a value of 0.5 Å. Interestingly, both forms displayed similar patterns up to a certain point, with distinctions emerging after residue 140, where the RMSF values in the IL-10 complex form notably exceeded those in the IL-6 complex form. Additionally, likely in IL-6 and IL-10 patterns in RMSD, the stability of the ferritin complex showed its stability at around 15 ns. During the simulation, most of the residues of ferritin that fluctuated slightly were located on loops.

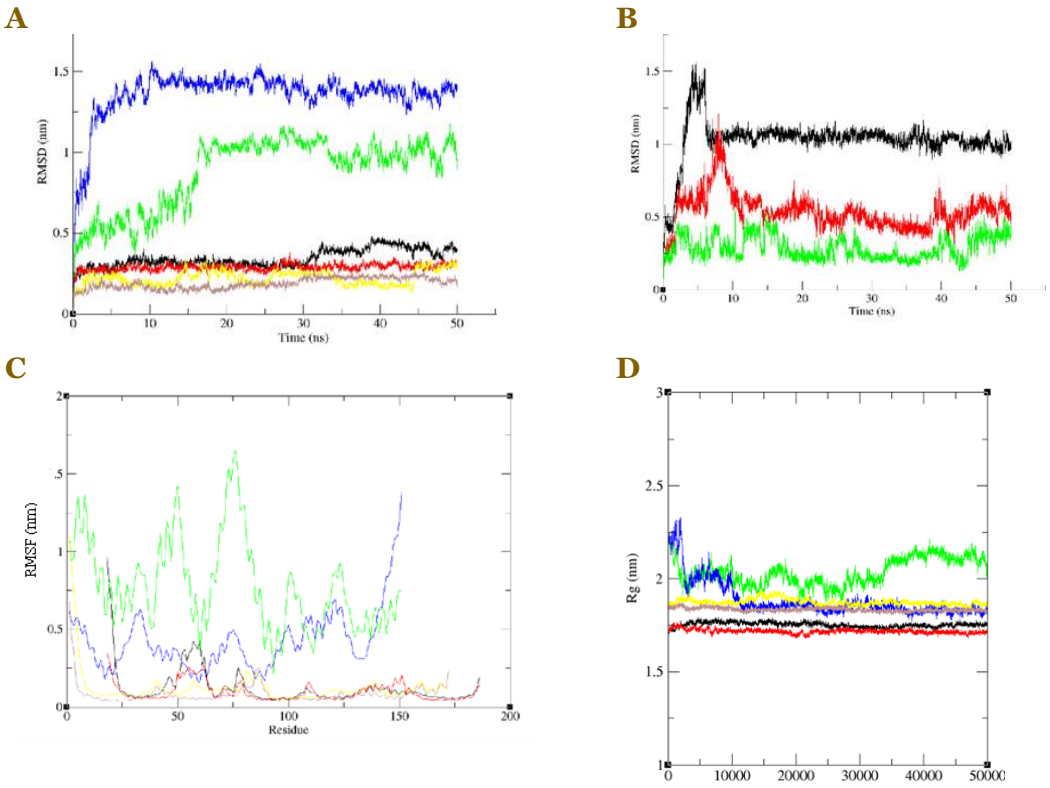

Figure 5. Root mean square deviation (RMSD) C-alpha of the complexes and apo forms of IL-6 and IL-10 (A), RMSD of the vitamin D (black: vitamin D-IL-6 complex, black: vitamin D-IL-10 complex, and green: vitamin D-ferritin complex) (B), root mean square fluctuation (RMSF) of the complexes and apo form of IL-6 and IL-10 (C), and radius of gyration (Rg) (red: IL-6-ligand complex, black: Apo IL-6, blue: IL-10-ligand complex, and green: Apo IL-10) (D). For RMSD C-alpha, RMSF, and Rg, they are categorized by the colors red: IL-6-vitamin D complex, black: Apo IL-6, blue: IL-10-vitamin D complex, green: Apo IL-10, yellow: ferritin-vitamin D complex, and brown: Apo ferritin.

The stability of a protein in a biological system can also be assessed through an analysis of its radius of gyration (Rg), which evaluates the structural compression changes during simulation time. In this context, the behavior of the IL-6 and ferritin complexes and their apo forms remained consistently stable throughout the simulation. In contrast, both the IL-10 complex and its apo form displayed fluctuations during the initial 10 ns of the simulation. However, after the first 10 ns, the Rg of the IL-10 complex exhibited an upward trend, surpassing 2 nm. At the same time, the apo form of IL-10 showed a declining pattern, falling below 2 nm before achieving stability for the remaining simulation period.

Discussion

Vitamin D, a fat-soluble vitamin, is produced in the skin in response to the sun's ultraviolet (UV) rays. The second source of it comes from diet, which includes supplements that contain ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3). Vitamin D status is assessed using serum 25(OH)D levels [18]. The active metabolite of vitamin D reduces the production and expression of numerous pro-inflammatory cytokines, thereby decreasing inflammation in the monocyte inflammatory profile. Specifically, calcitriol inhibits the production of type 1 pro-inflammatory cytokines such as IL-12, IFN-gamma, IL-6, IL-8, TNF-, and IL-9, which helps to reduce Th1 immune responses [19,20].

This study investigated the relationship between vitamin D levels and biomarkers IL-6, IL-10, and ferritin in newborns. Participants were divided into quartiles based on their vitamin D levels. The findings revealed consistent and statistically significant associations between vitamin D and both IL-6 and IL-10 across all quartiles, reinforcing the role of vitamin D in modulating inflammatory pathways. However, the associations between vitamin D and ferritin were not significant in the first and third quartiles (Q1 and Q3). While Q2 (11.89–14.30 ng/ml) correlated with ferritin levels ($p<0.001$), Q1 and Q3 showed insignificant correlations for ferritin levels. In Q4, with higher vitamin D levels (>18.11 ng/mL), IL-6 levels decreased, and IL-10 remained more stable compared to other quartiles, which served as a baseline.

Ferritin is a protein that stores excess iron in cells and releases it when there is an increase in demand [24]. However, serum ferritin can also be elevated due to factors such as inflammation, infection, and malignancy [24,25]. These unmeasured confounding variables may have an impact on the lack of significant relationships in Q1 and Q3. Specifically, elevated ferritin levels could result from iron deficiency, underlying inflammatory diseases, or other systemic variables that our analysis did not specifically control for. Ferritin levels vary, and there is no obvious correlation between these quartiles and vitamin D. This could be explained by the possibility that individuals in Q1 and Q3 had varying inflammatory conditions or different iron levels. Furthermore, the wide range of ferritin levels, particularly in the higher quartiles, introduces additional variability that may have affected the statistical power to detect significant associations. However, it is unclear whether serum ferritin reflects or causes inflammation or whether it is involved in an inflammatory cycle [26,27].

Regarding the in silico study, molecular docking and dynamics studies have determined the molecular interaction between IL-6/IL-10/ferritin-vitamin D3 (25(OH)D) complexes. The docking study demonstrated that the ligand (vitamin D3 (25(OH)D)) was precisely docked into its respective receptors (IL-6, IL-10 and ferritin), with conformations deeply seated within the binding site cleft, establishing favorable interactions with surrounding residues, such as hydrogen bonds and non-bonded interactions. Hydrogen bonds, a form of electrostatic interaction, are considered critical factors that stabilize the ionization state of both the protein and the ligand during molecular interactions [28]. Additionally, non-bonded interactions, like alkyl, pi-alkyl, and pi-sigma interactions, represent non-covalent bonds that significantly enhance the stability of the protein's conformation [29]. Furthermore, the molecular dynamics simulations revealed that both the complexes and apo forms maintained stable conformations, as observed through their C-alpha RMSD values and the ligand's behavior. These findings collectively illustrate how vitamin D3 (25(OH)D), as a ligand, can effectively interact with ferritin and cytokine proteins such as IL-6 and IL-10. This analysis underscores the importance of understanding these protein-ligand complexes' molecular interactions and dynamics, shedding light on their functional mechanisms.

Throughout this study, all these analyses have underscored the importance of understanding the correlation of vitamin D with IL-6, IL-10, and ferritin. This study has several limitations, including a small sample size and challenges in collecting samples from mothers needing follow-up treatment. Conducting research during the COVID-19 pandemic also posed logistical challenges that might have affected data collection and analysis. Additionally, while the in silico analysis provided insights into molecular interactions, it cannot fully replicate in vivo conditions, thus requiring further validation through laboratory experiments. These limitations suggested that future studies aim for larger sample sizes and more comprehensive data to strengthen the findings.

Conclusion

When the 20 ng/mL cut-off was used, infants with normal or low levels of vitamin D did not exhibit a trend of elevated umbilical cord IL-6, IL-10, or vitamin D. However, the associations between umbilical cord vitamin D levels with IL-6 and IL-10 were found to be significant when the cut-offs were based on the quartile. The molecular docking and dynamics analyses highlighted the stability of protein-ligand complexes and the successful ligand-receptor interactions, which enhanced our understanding of the complex interactions between vitamin D and cytokines. Further investigation should be carried out on a more homogenous population to minimize the confounding effects.

Ethics approval

The study protocol complies with the Declaration of Helsinki and has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia (No. 571/UN4.6.4.5.3L/PP36/2023, August 21, 2023). Each participant gave written informed consent and agreed to participate in the study.

Acknowledgments

The authors wish to express their gratitude to all of the study participants, the laboratory staff of Hasanuddin University Medical Research Center and StudiBio for their support, and Najdah Hidayah for her assistance in data analysis.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study was funded by the Ministry of Education, Culture, Research, and Technology (Kemendikbudristek) Indonesia under Indonesia Collaboration Research (RKI) Scheme C, project number 1825/UN4.22/PT.01.03/2023.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Massi MN, Fikri B, Putera AM, *et al*. Association between vitamin D levels with IL-6 and IL-10 in umbilical cord blood of infants. Narra J 2024; 4 (3): e889 - http://doi.org/10.52225/narra.v4i3.889.

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