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Assessment of Vitamin D3 and Iron Status and their correlation among Libyan Children at a Tertiary Care Centre in East Libya

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Abstract:

- ***Background***:

Recent studies have suggested a relationship between serum vitamin D (25-hydroxyvitamin) and iron. Vitamin D regulates iron through hepcidin, an iron regulatory hormone; on the other hand, iron controls vitamin D metabolism. Nevertheless, vitamin D and iron deficiency are highly prevalent worldwide. We have no data on their interactions among our Libyan children.

- ***Aim***:

To assess the correlation (association)between 25-hydroxyvitamin D (25(OH)D) and iron status and evaluate the frequency(prevalence) of iron deficiency anemia (IDA) and vitamin D deficiency (VDD) in our study group. Method: An observational descriptive study of 94 Libyan children (1-14 years) diagnosed with iron deficiency (low serum ferritin). The following data were collected from a medical record: age, sex, and laboratory data, including Hemoglobin (Hb), serum ferritin, and vitamin D levels. All our cases were classified according to hemoglobin level in each specific age group into iron deficiency (ID) and iron deficiency anemia (IDA). The study population will then be categorized into vitamin D deficiency (VDD), vitamin insufficiency (VDI), and vitamin D sufficiency (VDS) based on the cutoff vitamin D values. Statistical analysis: Data entered in SPSS 23rd version. Descriptive statistics were used for numerical variables. Spearman's rho correlation and the Kruskal-Wallis test were used to estimate the correlation between vitamin D3 with serum ferritin levels and between vitamin D level and iron deficiency anemia of study participants. Results: Of the total 94 studied iron-deficient children, 50% of the study group were males, and 53% of the study children were aged 1-5 years. The mean age was 6.45 years with a standard deviation of 4.2. The mean serum ferritin is 7.55 and the mean vitamin D is 21.78. Sixty-seven percent (67%) of them exhibited iron deficiency anemia (IDA), Additionally, 53% and 24% of the subjects had vitamin D deficiency and vitamin D insufficiency, respectively. However, approximately 51% of the children had concurrent low serum ferritin and vitamin D deficiency. The Spearman's rho correlation showed a statistically significant correlation between Vitamin D3 and ferritin (p-value of 0.002). In addition, the nonparametric Kruskal-Wallis test also reveals a significant association between them (p = 0.039).

- ***Conclusion***:

The result of our study showed a high prevalence (51%) of concurrent low ferritin and vitamin D status. In addition to a strongly positive significant correlation between vitamin D and ferritin According to WHO-definitions of iron deficiency anemia (IDA). IDA in our study children is considered a severe public health problem in Libyan children in addition to it is association with low vitamin D status. So we need further studies to explain the causal and risk factors as well as their relationship mechanism.

Key words: *Correlation Statistics, Serum Ferritin, Vitamin D3, Hemoglobin, Children.*

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I. INTRODUCTION

Recently low vitamin D level is linked to anemia especially IDA, as demonstrated in various observational studies[1,2]. While iron deficiency anemia is the most prevalent health concern globally, vitamin D deficiency and iron deficiency have become among the most widespread and preventable nutritional deficiencies. [3,4] These deficiencies often appear together as concurrent health concerns and have a notably detrimental effect on community health, particularly among children, especially in developing nations. About 52% of children in Africa experience iron deficiency, while roughly 23% suffer from vitamin D deficiency[7,8]

In young, growing children, iron deficiency and iron deficiency anemia are linked to several serious health issues, including a weakened immune system that increases susceptibility to infections, abnormal motor and neurocognitive development during infancy, heightened risk of prematurity, poor academic performance, stunted growth, and in cases of severe anemia, elevated maternal and infant mortality[9]. What is particularly noteworthy about iron deficiency anemia is that it is easily preventable, even in highly disadvantaged social groups. Nevertheless, specific issues resulting from iron deficiency can be reversed and corrected with iron supplements; on the other hand, severe cases of iron deficiency anemia may result in permanent complications if treatment is postponed or if damage has already occurred.

The unique aspect of vitamin D as a nutrient is that it is synthesized in the skin as a prohormone and subsequently activated in the liver and kidneys. It is an essential nutrient for most body organ and system especially that supports the preservation of strong bones, and it is necessary for the development of neuronal axons and brain cells. Vitamin D deficiency results in rickets, particularly in children aged 3–18 months 10.

In addition to rickets in children, vitamin D deficiency (VDD) has been related to a higher risk of chronic health disorders such as autoimmune disorders, infectious diseases such as tuberculosis, acquired immune deficiency syndrome (AIDS), cardiovascular conditions, and cancer [11].

Vitamin D deficiency is frequently observed in individuals suffering from iron deficiency anemia. Numerous studies have indicated a relationship between vitamin D deficiency and iron deficiency anemia. Recent findings reveal an interaction between vitamin D levels and iron status, as identified in research involving children from five African countries[12].

On another note, vitamin D plays specific biological roles involving iron metabolism, which may clarify the relationship between vitamin D and iron metabolism identified in laboratory and animal studies. Elevated levels of vitamin D may enhance iron status by reduction of hepcidin, the key iron regulating hormone, through the binding of the 1,25-dihydroxy vitamin D (1,25(OH)2D)–vitamin D receptor complex to the vitamin D response element (VDRE) on the hepcidin gene (HAMP), thereby inhibiting its transcription [13], As well as vitamin D had increase iron availability by down regulatory or an inhibitory effect on proinflammatory cytokines (IL6 and IL1B)[14] which increases the sensitivity to erythropoietin promotes erythropiosis[15,16].

In contrast, a deficiency of iron may contribute to vitamin D deficiency by inhibiting the function of heme-containing enzymes responsible for activating vitamin D, such as 1-hydroxylase and 25-hydroxylase, as demonstrated in rodent studies[17], or by elevating levels of fibroblast growth factor 23 (FGF23) [18], which would decrease the activity of 1-hydroxylase, resulting in lower concentrations of 1,25(OH)2D[19].In this study, we aim to assess the correlation between serum ferritin levels and vitamin D levels in children who have iron deficiency, while also examining the prevalence of iron deficiency anemia and vitamin D deficiency in our study group. We expected these findings would encourage pediatricians and healthcare providers to screen for these dual deficiencies and prevent this emerging health issue and there disaster sequeles.

II. METHODOLOGY

- *Study Design and Setting*

This observational descriptive study was conducted from August 2019 to December 2024 at the hematology clinic of the pediatric department at Benghazi Medical Center (BMC), Benghazi, Libya.

- *Study Population and Sample Size*

A total of 94 Libyan children who have latent iron deficiency (ID), aged 1 to 14 years, were examined at the hematology clinic and enrolled in the study according to the following selection criteria:

- *Inclusion Criteria:*

Children aged 1-14 years with iron deficiency who were referred to the pediatric hematology outpatient clinic between January 2019 and August 2024 were included in this study.

- *Exclusion Criteria:*

Any patient who has a condition that may have a potential effect on iron status biomarker(ferritin), including Patients with chronic disease, infection, inflammatory diseases, unexplained hematological parameters, missing data, and children receiving supplements, were excluded from the study. Sick febrile illness (increased CRP) and children with malnutrition.

Any children who do not have the inclusion criteria, like age >14 years, and do not have an ID.

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➢ *Definitions and Cut-Offs*

All our study group has an iron deficiency status, which is defined using WHO guidelines as plasma ferritin <12µg/L or < 30µg/L in the presence of inflammation in children <5 years old, and as <15µg/L or <70µg/L in the presence of inflammation in children ≥5 years old [20]

Measurement of hemoglobin level is a vital physiological parameter that helps diagnose the extent and severity of anemia. We use the criteria for determining the presence of anemia, as recommended by the World Health Organization, based on hemoglobin cut-off values for age and sex [21]. So, we divided our study group into two groups: iron deficiency (ID) and iron deficiency anemia (IDA). WHO's definition of anemia, according to age, is:

Children, 6–23 months <10.5 g/dl.

Children 24–59 months <11.0 g/dl.

Children, 5–11 years <11.5 g/dl.

Children, 12–14 years, boys and nonpregnant girls <12.0 g/dl.

- *Definitions:*

Patients were also categorized based on Vitamin D3 levels cutoffs of vitamin D values as follows [22], vitamin D status was defined using 25(OH)D cutoffs of vitamin D values higher than 30 ng/dl are considered normal, between 30 and 20 ng/dl are insufficient, and less than 20 means vitamin D deficiency.

✓ Vit D3 Deficiency (VDD) = Vit D3 (less than 20 ng/ml).
✓ Vit D3 Insufficiency (VDI) =Vit D3 (20-30 ng/ml).
✓ Vit D3 Sufficiency (VDS) =Vit D3 (>30 ng/ml).

➢ *Data Collection:*

Data was collected from the study group's medical records, including demographic data, age, gender, and laboratory data, including the hemoglobin (Hb), serum ferritin (S. ferritin), and serum vitamin D (vit. D) levels.

➢ *Data Analysis:*

The collected data was managed and analyzed by using Statistical Package for Social Sciences (SPSS) version 23[23], and the demographic features of the participants in the study were displayed through tables and charts using descriptive statistics. Appropriate statistical tests were employed to attain a statistical power of 80 % for the study while maintaining a type I error of 5%.

➢ *Ethical Consideration:*

Ethical approval was obtained from the hospital authority to access the patients, and verbal consent was secured from the patients' parents.

III. RESULTS

This observational descriptive study was conducted to investigate the prevalence of VDD in Libyan children who have iron deficiency status whether anemic or, not as well as demonstrate the prevalence of iron deficiency anemia in addition to proving if there is any correlation between Vitamin D level and serum ferritin level among 94 Libyan children aged (1-14 years) who attended a pediatric hematology clinic at Benghazi Medical Center (BMC), the study found that.

➢ *Regarding The Sociodemographic Characteristics of Study Participants*

- *Age Characteristics of Study Participants*

Regarding age characteristics of study participants,53.19% of the participants were aged 1-5 years, while 24.47% were in the 11-14 age group and 22.34% in the 6-10 age group; as illustrated in **Figure 1.**

- *Gender Distribution of Study Participants Across Age Groups.*

The study also found that 50% of the participants were males**, i.e** equal age distribution as shown in **Figure 2**.

- *Gender Distribution of Study Participants Across Age Groups.*

Figure 3 depicts the gender distribution of study participants across age groups. Most males were in the 1-5 age group, followed by the 11-14 age group at 62% and 22%, respectively. Meanwhile, 44.6% of females were also in the 1-5 age group

Descriptive statistics for hemoglobin, serum iron, and vitamin D levels among study participants are presented in Table 1. The descriptive statistics for age, with Mean plus standard deviation, were 6.37 +4.2 years. The minimum age was 1 year to a maximum of 14 years. The mean plus a standard deviation of vitamin D3 was 21.78 + 14.2 ng/ml, with a minimum of 3.1 ng/ml and a maximum of 57.4 ng/ml. The mean plus a standard deviation of serum ferritin was (7.55+ 2.6 µg/L), with a minimum of 1.06 µg/L to a maximum of 14.9 µg/L and the Mean plus a standard deviation of hemoglobin (HB) was (Mean 7.55+ 2.6 g/dl) with a minimum of 5.2 g/ dl to a maximum of 13.8g/dl as recorded in our study population.

The prevalence of iron deficiency anemia, iron deficiency (low ferritin level), and various vitamin D level statuses among study participants

In the present study, all participants, 94(100%), were iron deficient according to the WHO definition, while 67% and 33% were IDA and ID, respectively once hemoglobin for age was included. Additionally, 77% of the Children had vitamin deficiency (VDD),22(23.4%) had vitamin D insufficiency (VDI), and only 21(22.3%) had vitamin D sufficiency, as shown in **Table 2.**

The association between iron deficiency anemia, iron deficiency, vitamin D level, and age of study participants

Table 3) shows a high prevalence of iron deficiency anemia (67%) and iron deficiency (33%) in our study participants, and higher rates of iron deficiency anemia (52%)

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and iron deficiency (48%) in children aged 1-5 years. While vitamin D deficiency was reported in 36%, and vitamin D insufficiency (23%), and higher rates of VDD (46%) and VDI (59%) in children 1-5 years old

The association between Vitamin D3 and serum ferritin levels in the study participants

Table 4) illustrates the association between serum vitamin D and serum ferritin levels among participants, revealing that 51% had both iron and vitamin D deficiencies, with a p-value of 0.608.

Spearman's rho correlation analysis showed a significant relationship between serum ferritin and vitamin D levels, with a p-value of 0.002, as indicated in **Table 5**.

Table 6) illustrates that the nonparametric Kruskal-Wallis test was applied to assess the association between vitamin D level and ferritin level among study participants; there was a significant association between them (0.039), so in this case, the null hypothesis is rejected.

The association between Vitamin D level and hemoglobin among study participants:

A nonparametric Kruskal-Wallis test was applied to assess the association between vitamin D level and hemoglobin level among study participants, There was no significant association between them; in this case, the null hypothesis couldn't be rejected, as shown in **Table 7.**

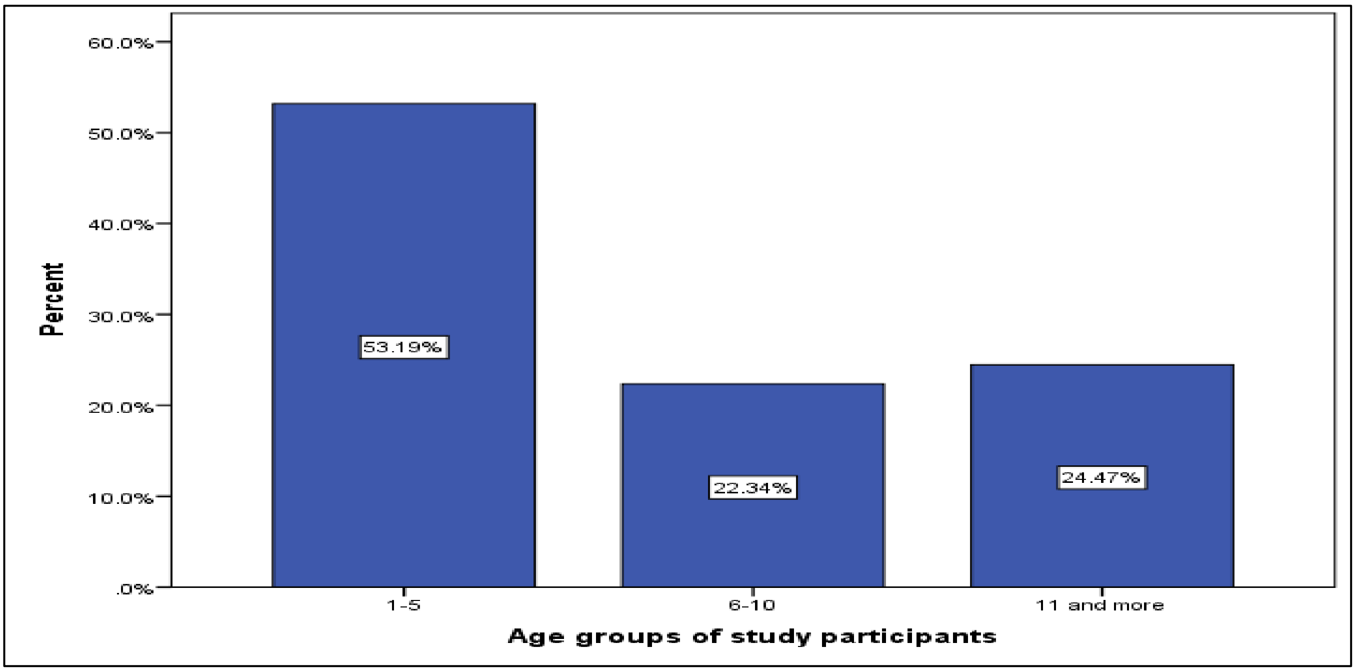

Fig 1 The Distribution of Study Participants According to Age

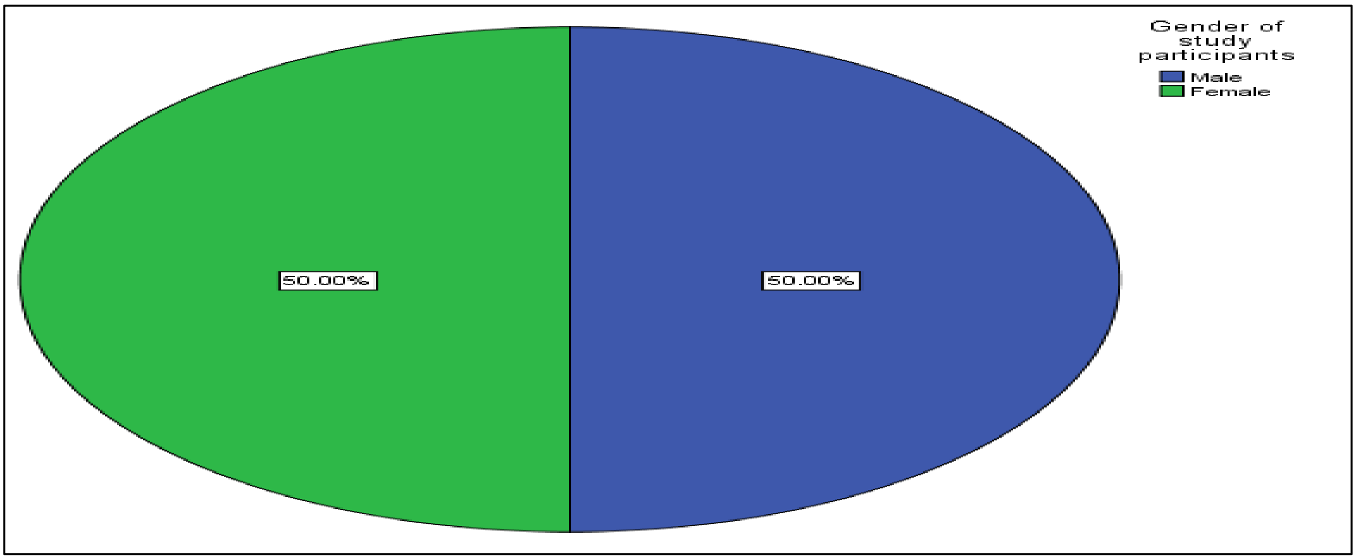

Fig 2 The Distribution of Study Participants According to Gender

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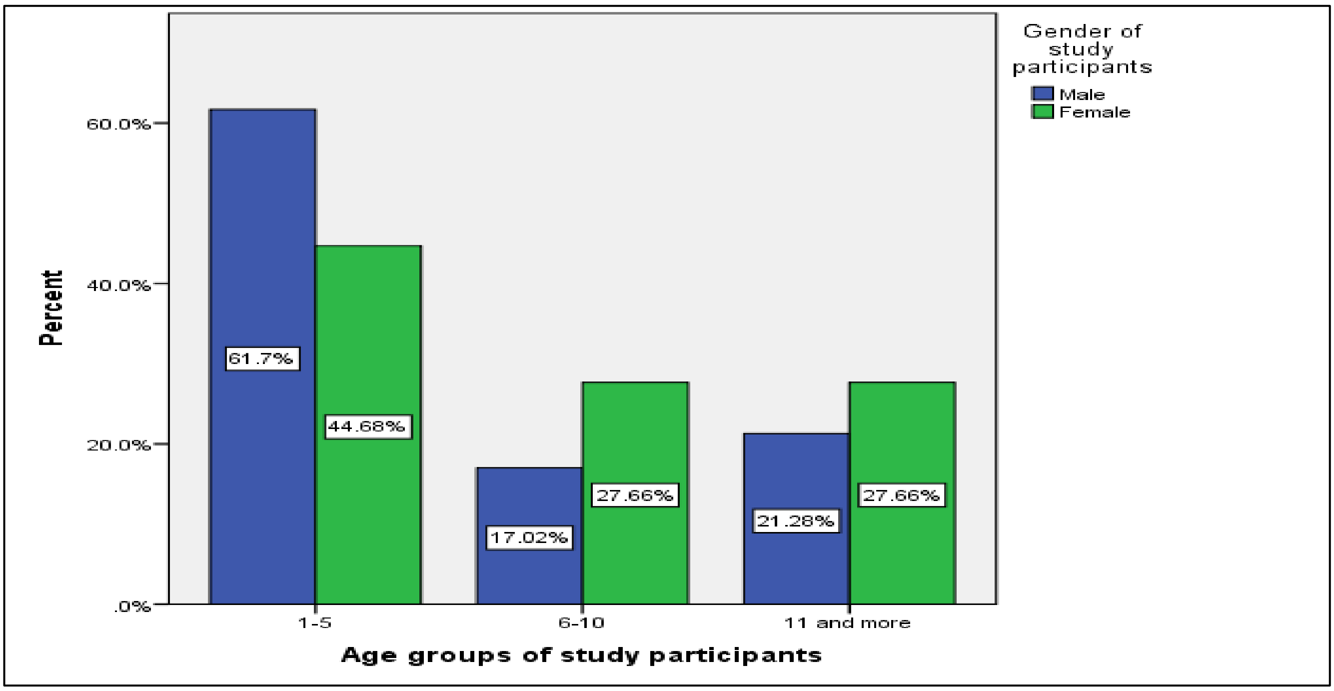

Fig 3 Distribution of Study Participants According to Gender in Each Age Group

Table 1 Descriptive Statistics of Hemoglobin, Serum Ferritin, and Vitamin D Levels of the Study Group:

Descriptive statistics	Age of study group/yrs	HB level gm/dl	Serum ferritin µg/L	Vitamin D level ng/ml
Mean	6.372	10.233	7.5519	21.7833
Median	5.000	10.550	7.4500	18
Standard deviation	4.2	1.6	2.6	14.2
Minimum	1	5.2	1.06	3.1
Maximum	14	13.8	14.9	57.4

Hemoglobin (HB) (g/dl) = Gram per deciliter, Years(yrs), Nanogram per milliliter (ng/ml), microgram per liter (µg/L)

Table 2 The frequency of iron deficiency anemia, iron deficiency, and vitamin D levels among the 94 study group.

Variable		Frequency	Percent (%)
IDA		63	67%
ID		31	33%
Vitamin D level	**VDD**	51	54.3
	VDI	22	23.4
	VDS	21	22.3
Total		94	100%

Vitamin D deficiency (VDD), Vitamin D insufficiency (VDI), Vitamin D sufficiency = Normal level of Vitamin D(VDS), Iron deficiency anemia (IDA), Iron deficiency (ID).

Table 3 The Association between Iron Deficiency Anemia, Iron Deficiency, Vitamin D Level, and Age of the Study Group.

Variable		Age groups of study participants (Year)			Chi-square (X^2)/ Fisher's Exact Test	Level of significance
		1-5	**6-10**	**11 -14**		
IDA		35	12	16	1.195	0.550
ID		15	9	7		
Vitamin D level among study participants	**VDD**	24	12	3.199	3.199	0.534
	VDI	13	6	3		
	VDS	13	3	5		

Table 4 The Relationship between Iron Deficiency Anemia, Iron Deficiency, and Vitamin D Level Among Study Participants.

Variable		IDA	ID	Total	Chi-square (X^2) / Fisher's Exact Test	Level of significance
Vitamin D level among study participants	VDD	37	14	51	1.580	0.454
	VDI	13	9	22		
	VDS	13	8	21		

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Vitamin D deficiency (VDD), Vitamin D insufficiency (VDI), Vitamin D sufficiency = Normal level of Vitamin D(VDS), Iron deficiency anemia (IDA), Iron deficiency (ID).

Table 5 Non-Parametric Correlation between Vitamin D Level and Serum Ferritin Level of Study Participants

		Serum ferritin level	
Spearman's correlation	**Vitamin D level**	**Correlation coefficient**	**0.312****
		P-value	**0.002**
		Total	**94**

**. Correlation is significant at the 0.01 level (2-tailed).

There was a significant positive correlation between vitamin D level and serum ferritin level, r_s (92) = 0.312, p-value = 0.002

Table 6 A Non-Parametric Test (Kruskal-Wallis Test) between Vitamin D Level and Ferritin Level of Study Participants.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Serum Ferritin level is the same across categories of Vitamin D level among study participants.	Independent-Samples Kruskal-Wallis Test	.039	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 8 A Non-Parametric Test (Kruskal-Wallis Test) between Vitamin D Level and Iron Deficiency Anemia of Study Participants:

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Vitamin D level the same across categories of Anemia among study participants.	Independent-Samples Kruskal-Wallis Test	.173	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

IV. DISCUSSION

Vitamin D and iron deficiencies are prevalent globally, and a potential connection between these two deficiencies has been proposed. The occurrence of vitamin D deficiency and iron deficiency as coexisting nutritional challenges has risen in recent years. Researchers worldwide are attempting to clarify their interaction. However, this area of research has not yet been explored in Libyan children. Therefore, we aim to focus our study on assessing the prevalence of iron deficiency anemia (IDA), low vitamin D status, and the interplay between vitamin D and low iron status in Libyan children.

We reveal a significant prevalence of low (suboptimal) vitamin D levels (77%) in our iron-deficient study children, which included 54.3% with vitamin D deficiency (VDD) and 23.4% with vitamin D insufficiency (VDI). A similar study conducted in East Africa[24] reported that 87% of their sample exhibited vitamin D deficiency or insufficiency, closely aligning with our findings. Conversely, our results diverge from a study conducted in South Africa[25], which indicated a lower prevalence rate (20.3%).

On the other hand, our study shows a high prevalence of iron deficiency anemia (IDA) (67%) and iron deficiency (ID) (33%), with higher rates of iron deficiency anemia (52%) and iron deficiency (48%) in children aged 1-5 years. Using the World Health Organization (WHO) criteria, if anemia prevalence in a country reaches approximately 40%, it is a significant community health problem[26], so the anemia in our study population is considered a severe public health problem, while Geltman PL[27] study found a prevalence of anemia in Tanzanian preschool children (84%) and school-aged children (55–67%), which represent an a very severe health problem. In Turkey, there estimation of iron deficiency (ID) and iron deficiency anemia (IDA) in children ranged between 21% and 35% across various studies[28], which is also considered a "moderate" public health issue in that population. In contrast to the prevalence of anemia to be just (1.1%) among young Australian children of mixed origin[29] which does not constitute a public health problem.

These findings in our community may be attributed to inadequate nutritional education, poor dietary habits, consumption of non-fortified iron foods, and a lack of adherence to international guidelines for screening,

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prevention, and treatment of iron deficiency. Additionally, the high rate of parasitic infections in our community may be a contributing factors. In African children, similar issues such us in addition to poverty and HIV infection, malaria, alongside other parasitic infections, serve as predisposing factors, whereas health services in Australia are generally optimal.

Our research reveals that the occurrence of iron deficiency anemia (IDA) was recorded at 58.8%, 20.6%, and 20.6% across the vitamin D deficient (VDD), vitamin D insufficient (VDI), and vitamin D sufficient (VDS) groups, respectively. While there is a relationship between vitamin D and hemoglobin levels, it is not statistically significant. A nonparametric Kruskal-Wallis test showed no significant link between these variables (p-value = 0.173). Conversely, vitamin D does show a significant correlation with serum ferritin levels. In contrast, Sharma's 2015[30] study in North India reported a slightly higher incidence of IDA, at 66%, 49%, and 25% in the VDD, VDI, and VDS groups, respectively, which does not align with our results that indicate a positive correlation between vitamin D levels and hemoglobin levels, but not with ferritin or iron levels. While in a study by Lee et al, which included healthy children and adolescents, found an association between vitamin D deficiency and an elevated risk of anemia, particularly iron deficiency anemia[31]. Qader and Alkhateeb observed that children with iron deficiency have a higher likelihood of suffering from vitamin D deficiency, indicating that any child diagnosed with iron deficiency anemia should also be evaluated for vitamin D deficiency[32].

Our findings show a higher prevalence (frequency) of iron deficiency and/or anemia in patients deficient in 25(OH)D, and a higher occurrence of vitamin D deficiency and insufficiency among those with iron deficiency and iron deficiency anemia, supporting the claims made in the previously mentioned studies.

Recently, global researchers have been investigating the relationship between vitamin D and serum Ferritin levels[33]. In our study, 51% of participants exhibited both iron and vitamin D deficiencies, with a p-value of 0.608. The Spearman's rho correlation analysis indicated a meaningful positive correlation between serum ferritin and vitamin D levels, arriving at a p-value of 0.002, in addition to the nonparametric Kruskal-Wallis test also revealed and supporting this significant association between these factors (P 0.039); thus, the null hypothesis is rejected in this instance. Similarly, studies have indicated a strong positive linear correlation between vitamin D3 and serum ferritin in Pakistan (Khan H, Basharat M) [34]. Furthermore, Andıran and colleagues[35] also reported this link in populations from Korea and the United States. These studies' observation can be explained by other researcher including Pike JW et al. [36], who demonstrate the presence of vitamin D receptors in bone marrow progenitor cells, and Riccio E et al.[37], who show the beneficial effects of calcitriol on erythropoiesis. However, other researcher have argued that serum 25(OH)D exhibits a negative correlation with serum ferritin in Asian infants in the UK[38].

V. CONCLUSIONS

In summary, our findings reveal a significant prevalence of both IDA and vitamin D deficiency, with a substantial occurrence (51%) of simultaneous low ferritin and low vitamin D levels, indicating a strong connection between low vitamin D and low iron status regardless of anemia in Libyan children. This relationship suggests that both conditions should be recognized as major public health concerns. Therefore, further researches are necessary to clarify the causal factors and the mechanisms underlying the association.

- *Limitation of The Study:*

our research design as a descriptive retrospective observational study, with a limited sample number, includes a specific age group, and is only in one center in Libya, not include the causality and the risk factors for this health problem but should be used as the reference and the stimulus for the researcher who working in this field.

- *Limitations of The Study:*

This research is a descriptive retrospective observational study; in addition to it is designed with a limited sample size, focusing on a specific age group and conducted at a single center in Libya. It does not explore causality or risk factors related to this health issue, but should serve as a reference and motivation for researchers in this area.

- *Conflict of Interest:*

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENT

The authors express gratitude to all study participants' parents

CONSENT TO PARTICIPATE

Parents of all respondents who consented to participate in the study were required to sign the informed consent form.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author Haloom Abdelsalam Elhashmi is the corresponding author, designed the study, wrote the protocol, and wrote the first draft of the manuscript, manuscript conceptualization, writing, and manuscript submission and revision Asma Albarasi contributed to data collection and revising the research, Nadia AM Eldarogi performed the statistical analysis and revisions. Fatma Aldarat contributed to data collection. All the Authors managed the literature searches.

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