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Vitamin D Level in Pediatric Autoimmune Hepatitis: Relation to Features and Response to Therapy

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Abstract

Background: Vitamin D has diverse immunomodulatory, anti-inflammatory, antioxidant, and anti-fibrotic actions, that have warranted its consideration as acritical pathogenic factor in the occurrence and severity ofdiverse immune-mediated diseases, including autoimmunehepatitis.

Aim of Study: Primary aim: To assess serum levels of 25(OH)D in children and adolescents with AIH, Secondary aim: To evaluate the association between serum 25(OH)D levels and clinical, histological, biochemical features of AIH, Tertiary aim: To evaluate the association between serum 25(OH)D levels and response to immunosuppressive therapy.

Patients and Methods: This controlled cross sectional study was performed on a total of 30 children and adolescences diagnosed with autoimmune hepatitis following-up in Pediatric Hepatology Clinic, Faculty of Medicine, Ain Shams University. They were 13 males and 17 females, their age ranged from 5-17 years old and 15 age and sex matched children as control group starting from October 2021 till July 2022.

Results: This study study results revealed that there were no statistically significant differences between vitamin D level and sex, treatment, symptoms (right hypochondrial pain and abdominal enlargement), other autoimmune diseases of thyroid and DM and interface hepatitis.

Conclusion: As evident from the current study, severe vitamin D deficiency occurs in patients with AIH. Interface hepatitis was found in 93.3%, bridging fibrosis in 86.7% and lymphocytic infiltration in 80% in case group of autoimmune hepatitis.

Abdominal enlargement is the most common symptom in liver disease (46.7%) followed by right hypochondrial pain (26.7%).

Key Words: *Pediatric autoimmune hepatitis – Relation to features – Response to therapy.*

Introduction

AIH is a generally unresolving inflammation of the liver. Its pathogenesis postulates that environmental triggers, a failure of immune tolerance mechanisms, and a genetic predisposition collaborate to induce a T cell-mediated immune attack upon liver antigens, leading to a progressive necroinflammatory and fibrotic process in the liver **[1,2]**. With progress made in diagnostic techniques, the detection rate of autoimmune hepatitis has remarkably increased, attracting more attention from clinicians and patients **[3]**.

Vitamin D has received special attention, among the environmental triggers of autoimmunity as it has an immunomodulatory role and prevents inflammatory process by suppressing T- and B-cell auto-aggression. Lower vitamin D levels are frequently encountered in several autoimmune diseases, including multiple sclerosis, systemic lupus erythematous, inflammatory bowel disease and rheumatoid arthritis **[4]**.

Low 25(OH)D levels have been correlated with increased histological damage in patients with chronic hepatitis C virus infection and in patients with non-alcoholic fatty liver disease **[5,6]** higher viral loads in patients with hepatitis B virus infection **[7]**, and severe histological features and poor response to therapy in AIH in adults **[8]** but the relationship between severity of AIH and vitamin D status in paediatrics and adolescents warrants investigation.

Aim of the work:

Primary aim: To assess serum levels of 25(OH)D in children and adolescents with AIH.

Secondary aim: To evaluate the association between serum 25(OH)D levels and clinical, histological, biochemical features of AIH.

Tertiary aim: To evaluate the association between serum 25(OH)D levels and response to immunosuppressive therapy.

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Patients and Methods

This controlled cross sectional study was performed on a total of 30 children and adolescences diagnosed with autoimmune hepatitis following-up in Pediatric Hepatology Clinic, Faculty of Medicine, Ain Shams University. They were 13 males and 17 females, their age ranged from 5-17 years old and 15 age and sex matched children as control group starting from October 2021 till July 2022.

Theinclusion criteria were: Patients confirmed to have autoimmune hepatitis by serologic, immunologic, and histopathologic findings, Children and adolescents of both sexes (age >18 years) while the exclusion criteria were: Patients receiving vitamin D therapy prior to the study.

All patients were subjected to history: Age at study, age at disease onset, sex, symptoms at presentation, hepatic symptoms as abdominal distension, jaundice, change in colour of urine and stool and itchinghistory suggestive of hepatic decompensation as bleeding tendency, oedema, and abdominal distension history suggestive of other autoimmune diseases e.g. thyroid disease, Diabetes mellitus.

Clinical examination which included: Thorough clinical examination with stress on: Weight in kg, height in cm and plotting them on the age and sex standard percentile according to Egyptian growth charts [9]. Body mass index (BMI): Was calculated as weight in (kg) / height in (m^2), the obtained number was plotted on appropriate CDC gender - specific BMI-for-age growth chart to determine BMI percentile and Z score [10]. Abdominal assessment of the patients for hepatomegaly ± splenomegaly and ascites Signs of liver cell failure or hepatic decompensation as jaundice, oedema.

Laboratory studies of AIH patients: Complete blood picture,erythrocyte sedimentation rate, C reactive protein, serum alanine transaminase, aspartate transaminase levels, Serum total proteins and albumin level, Total bilirubin, direct bilirubin. Serum calcium, phosphoru, alkaline phosphatase and gamma GT levels. Prothrombin time, INR. Serum 25-hydroxy vitamin D levels:

Quantitative determination of total 25-hydroxy vitamin D by chemiluminescence competitive assay on Cobas e411 analyzer using Elecsys Vitamin D (Roche Diagnostics), Serum protein electrophoresis.

Antinuclear antibody (ANA) with titre: Smooth muscle antibody (SMA) with titre. Liver-kidney microsomal antibody (LKM-1) with titre.

Controls: Serum 25-hydroxy vitamin D levels as before.

Imaging technique for patients: Abdominal ultrasound with portal vein Doppler, commenting on size of liver, echo texture, focal lesions, size of spleen, portal vein diameter, direction of blood flow and resistance to portal venous flow.

Histopathological assessment of liver biopsy of AIH patients at time of diagnosis: Fibrosis was graded using the METAVIR score on a five (0-4)-point scale. Briefly, F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = few septa; F3 = numerous septa without cirrhosis; F4 = cirrhosis. Presence of lymphocytic piecemeal necrosis was graded as mild (necrosis around 1-2 portal tracts), moderate (necrosis at the periphery of about half of the portal tracts) and severe (necrosis surrounding more than half of the circumference of almost all portal tracts). The typical and compatible histology for AIH was defined according to the International Autoimmune Hepatitis Group (IAIHG) criteria.

Study procedures: Patients with confirmed diagnosis of Autoimmune hepatitis which included 30 patients collectively. All patients were subjected to the above-mentioned history, examination, and investigations. Control cases were subjected to measuring 25(OH)D.

Statistical analysis:

The collected data was coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013 and Microsoft Office Excel 2007. Descriptive statistics were done for quantitative data as minimum & maximum of the range as well as mean ±SD (standard deviation) for quantitative normally distributed data, while it was done for qualitative data as number and percentage. Inferential analyses were done for quantitative variables using Shapiro-Wilk test for normality testing, independent *t*-test in cases of two independent groups with normally distributed data. In qualitative data, inferential analyses for independent variables were done using Chi square test for differences between proportions and Fisher's Exact test for variables with small expected numbers. The level of significance was taken at *p*-value <0.050 is significant, otherwise is non-significant.

Results

Table (1) show that there was no statistically significant difference between control and cases group regarding demographic data.

Table (2) shows there was no statistically significant differences between both groups regarding Weight, Height, BMI of the studied patients.

Table (3) show there were no statistically significance differences between both groups regarding the level of 25 hydroxy vitamin D of the studied patients (p=0.739). Table (3) show that 93.3% of AIH patients had deficient levels of 25 hydroxy vitamin D.

Table (4) shows that the median age of diagnosis was 9 years and the median duration of disease was 2 years. The treatment included azathioprine in 13.3%, prednisolone in 6.7% and prednisolone-azathioprine combination in 80%. 11 of patients showed relapses during their illness and the remaining (4 patient) showed smooth response to treatment.

Table (1): Comparison between control and cases group regarding demographic data.

	Control group No.=15	Cases group No.= 15	Test value	p-value	Sig.
Sex:					
Female	6 (40.0%)	11 (73.3%)	3.394*	0.065	NS
Male	9 (60.0%)	4 (26.7%)			
Age (years):					
Mean ± SD	9.53±3.16	11.93±3.56	–1.954•	0.061	NS
Range	4-14	5-17			

p-value >0.05: Non significant. *: Chi-square test.
p-value <0.05: Significant. •: Independent t-test.
p-value <0.01: Highly significant.

Table (2): Comparison between both control and cases groups regarding Weight, Height, BMI and their Z scores.

	Control group No.=15	Cases group No.=15	Test value	p-value	Sig.
Weight (kg):					
Mean ± SD	26.27±8.26	32.33± 11.22	–1.686•	0.103	NS
Range	13-40	16-52			
Z-score:					
Weight:					
Median (IQR)	-0.32 (-0.92-0.46)	0.27 (-0.72-1.25)	–1.392‡	0.164	NS
Range	-1.6-1.05	-1.31-2.23			
Height (cm):					
Mean ± SD	117.93± 15.32	120.00±20.39	–0.314•	0.756	NS
Range	93-142	92-162			
Z-score:					
Height:					
Median (IQR)	0.28 (-0.96-0.62)	-0.22 (-0.84-0.85)	–0.083‡	0.934	NS
Range	-1.46-1.3	-1.52-2.42			
BMI (kg/m^2):					
Mean ± SD	19.22±5.94	21.78±2.80	–1.512•	0.142	NS
Range	8-33.6	17.6-26.9			
Z-score:					
BMI:					
Median (IQR)	-0.11 (-1.05-0.63)	0.11 (-0.21-0.74)	–1.683‡	0.092	NS
Range	-2.63-2.76	-0.61-1.35			

p-value >0.05: Non significant. •: Independent t-test.
p-value <0.05: Significant. ‡: Mann Whitney test.
p-value <0.01: Highly significant.

Table (3): Comparison between control and cases group regarding the level of Vitamin D among the studied subjects.

25 hydroxy vitamin D (ng/ml)	Control group No.=15	Cases group No.=15	Test value	*p*-value	Sig.
Median (IQR)	14 (10-18)	12 (10-17)	–0.333	0.739	NS
Range	5-29	5-21			
Deficient	13 (86.7%)	14 (93.3%)			
Insufficient	2 (13.3%)	1 (6.7%)			

p-value >0.05: Non significant.
p-value <0.05: Significant.
p-value <0.01: Highly significant:
Mann-Whitney test.

- 25 hydroxy vitamin D (ng/ml): Normal range: 30-100, insuffieciency: 10-29, marked deficiency: <10, toxic values: >100

Table (4): Timing of diagnosis, treatment regimens and number of relapses among the autoimmune hepatitis group.

Timing		No.=15
Age of diagnosis (years)	Median (IQR)	9 (7-11)
	Range	4-13
Duration of disease (years)	Median (IQR)	2 (1-7)
	Range	1/12-8
No. of relapses	Median (IQR)	2 (1-3)
No. of relapsers=11	Range	0-6
No. of non relapsers=4		
Treatment at time of the study	Azathioprine	2 (13.3%)
	Prednisolone	1 (6.7%)
	Prednisolone-Azathioprine	12 (80.0%)
Dose of steroid (mg/kg/day)	Median (IQR)	0.4 (0.2-0.7)
	Range	0.1-2
Dose of azathioprine (mg/kg/day)	Median (IQR)	1 (0.7-1)
	Range	0.6-1.4
Duration of treatment (months)	Median (IQR)	24 (12-84)
	Range	3-98

Table (5) show that abdominal enlargement was the most common symptom in AIH patients (46.7%) followed by right hypochondrial pain (26.7%). Only one case presented with oedema and one presented with hepatic encephalopathy. None of our patients presented with bleeding tendency.

Table (6) show that history of other autoimmune diseases (thyroid disease and diabetes mellitus) among the autoimmune hepatitis group was found in 13.3% of cases.

Table (7) shows that 8 (53%) of patients with AIH had anemia, 10 (67%) of them had thrombocytopenia and none of them showed any leucopenia. As regards, parameters of synthetic function of liver, 9 (60%) showed hypoalbuminemia and 11 showed prolonged INR.

Table (8) show that Pelvi-abdominal ultrasound revealed hepatomegaly in 66.7%, splenomegaly in 6.7%, hepatosplenomegaly in 6.7% and parenchymatous liver disease in 20% of them.

Table (9) shows that ANA, ASMA and LKM-1 were found in 20%, 53.3% and 20% of cases with autoimmune hepatitis, respectively, Serum protein electrophoresis showed Polyclonal hypergammaglubinemia in all cases of autoimmune hepatitis.

Table (5): Symptoms of liver disease among the autoimmune hepatitis group.

Symptoms of Liver disease	No.=15
Right hypochondrial pain:	
No	11 (73.3%)
Yes	4 (26.7%)
Abdominal Enlargement:	
No	8 (53.3%)
Yes	7 (46.7%)
Jaundice:	
No	13 (86.7%)
Yes	2 (13.3%)
Pale stool:	
No	13 (86.7%)
Yes	2 (13.3%)
Dark urine:	
No	12 (80.0%)
Yes	3 (20.0%)
Oedema:	
No	14 (93.3%)
Yes	1 (6.7%)
Bleeding tendency:	
No	15 (100.0%)
Yes	0 (0.0%)
Hepatic encephalopathy:	
No	14 (93.3%)
Yes	1 (6.7%)
Itching:	
No	13 (86.7%)
Yes	2 (13.3%)

Table (6): Other autoimmune diseases among the autoimmune hepatitis group.

History of other autoimmune diseases	No.=15
Thyroid disease:	
No	13 (86.7%)
Yes	2 (13.3%)
Diabetes mellitus:	
No	13 (86.7%)
Yes	2 (13.3%)

Table (7): Laboratory investigations among the autoimmune hepatitis group.

Laboratory data		No.=15
Hemoglobin (g/dl)	Mean±SD	9.65± 1.22
	Range	7.4-12.1
Anemia	Yes	8 (53%)
White Blood Cells (/mm^3)	Mean±SD	6.73± 1.10
	Range	5-9
Leucopenia	Yes	0 (0%)
Platelets (/mm^3)	Mean±SD	177.53±51.90
	Range	103-283
Thrombocytopenia	Yes	10 (67%)
Alanine Transaminase	Median (IQR)	61 (33-81)
(U/L)	Range	21-102
Aspartate Transaminase	Median (IQR)	82 (54-104)
(U/L)	Range	38-142
Albumin (g/dL)	Mean±SD	2.83±0.25
	Range	2.3-3.2
Hypoalbuminemia	Yes	9 (60%)
Total protein (g/dL)	Mean±SD	4.07±0.57
	Range	3-5
Total Bilirubin (mg/dL)	Mean±SD	4.11± 1.74
	Range	1.8-7
Direct Bilirubin (mg/dL)	Mean±SD	2.29±0.97
	Range	1.1-4.2
Prothrombin Time	Mean±SD	23.00±6.00
(seconds)	Range	14-36
International Normalized	Mean±SD	1.44±0.27
Ratio	Range	1-2
Prolonged INR	Yes	11 (73.3%)
Alkaline phosphatase	Mean±SD	247.13± 112.66
(U/L)	Range	133-586
Gamma-glutamyl	Mean±SD	159.93±72.78
Transferase (U/L)	Range	83-390
Calcium (mg/dL)	Mean±SD	7.99± 1.56
	Range	6.3-10.2
Phosphorus (mg/dL)	Mean±SD	3.91±0.61
	Range	2.6-5
C-reactive protein	Median (IQR)	7 (4-9)
(mg/dL)	Range	2-12
Erythrocyte Sedimentation	Median (IQR)	18 (10-22)
Rate (mm/h)	Range	5-50

Table (8): Pelvi-abdominal ultrasound (PAUS) among the autoimmune hepatitis group.

Other Investigatory data	No.=15
PA US:	
Hepatomegaly	10 (66.7%)
Hepatosplenomegaly	1 (6.7%)
Splenomegaly	1 (6.7%)
Parenchymatous liver disease	3 (20.0%)

Table (9): Autoimmune markers and serum protein electrophoresis among the autoimmune hepatitis group.

Autoimmune hepatitis markers	No.=15
Anti-nuclear antibody (ANA):	
Negative	12 (80.0%)
Positive	3 (20.0%)
Anti-smooth muscle antibody (ASMA):	
Negative	7 (46.7%)
Positive	8 (53.3%)
Liver kidney microsomal -1 antibody (LKM-1):	
Negative	12 (80.0%)
Positive	3 (20.0%)
Serum Protein electrophoresis Polyclonal hypergammaglubinemia	15 (100.0%)

Table (10) shows that patients with autoimmune hepatitis can present with variable degrees of activity and fibrosis.

Table (11) show that interface hepatitis was found in 93.3%, bridging fibrosis in 86.7%, lymphocytic infiltration in 80% and piecemeal necrosis in 33.3% of autoimmune hepatitis patients.

Tables (12) showed that 25 hydroxy vitamin D level was negatively correlated with patient age at time of study, at time of diagnosis, weight and height z scores, dose of steroid and treatment at time of the study. 25 hydroxy vitamin D showed a positive correlation with BMI Z score, duration of the disease, treatment durations and dose of azathioprine at time of study, but all these correlations were statistically insignificant.

25 hydroxy vitamin D showed a negative correlation with the number of relapses reported in our patients, yet this correlation was statistically not significant.

Table (10): Metavir score of liver biopsy at time of diagnosis among the autoimmune hepatitis group.

Liver biopsy at time of diagnosis Metavir score	Total number of patients No.=15
A0F2	1 (6.7%)
A1F1	2 (13.3%)
A1F2	3 (20.0%)
A1F3	1 (6.7%)
A2F2	2 (13.3%)
A2F3	2 (13.3%)
A3F2	1 (6.7%)
A3F3	3 (20.0%)

A = Activity. F = Fibrosis.

Table (11): Detailed findings of liver biopsy among the autoimmune hepatitis group.

Microscopic picture of liver biopsy	Total number of patients No.=15
Interface hepatitis:	
No	1 (7%)
Yes	14 (93.3%)
Bridging fibrosis:	
No	2 (13.3%)
Yes	13 (86.7%)
Lymphocytic infiltration:	
No	3 (20.0%)
Yes	12 (80.0%)
Piece meal necrosis:	
No	10 (66.7%)
Yes	5 (33.3%)

Table (12): Correlation between 25 hydroxy vitamin D level and demographic data, treatment doses, anthropometric measures z scores and number of relapses in autoimmune hepatitis patients

	25 hydroxy vitamin D	
	r	*p*-value
Age of patients at time of study	–0.032	0.911
Age of diagnosis	–0.489	0.065
Duration of disease	0.357	0.192
Dose of steroid	–0.541	0.056
Dose of azathioprine	0.036	0.902
Duration of treatment (months)	0.326	0.236
Z score: Weight	–0.161	0.568
Z score: Height	–0.119	0.672
Z score: BMI	0.008	0.977
Number of relapses	–0.085	0.762

p-value >0.05: Non significant. *p*-value <0.01: Highly significant.
p-value <0.05: Significant. Spearman correlation coefficient.

Table (13): Correlation between 25 hydroxy vitamin D level and sex, symptoms of liver disease and other autoimmune diseases in autoimmune hepatitis patients.

		Vitamin D		Test value	*p*-value	Sig.
		Median (IQR)	Range			
Sex	Females	12 (11-16)	5-18	0.262•	0.793	NS
	Males	13.5 (7.5-20)	7-21			
Right hypchondrial pain	No	12 (10-16)	5-19	0.589•	0.556	NS
	Yes	14.5 (9.5-19.5)	8-21			
Abdominal Enlargement	No	12 (10.5-17.5)	5-21	0.174•	0.862	NS
	Yes	14 (8-17)	7-18			
Jaundice	No	12 (11-17)	5-21	0.511•	0.609	NS
	Yes	11.5 (7-16)	7-16			
Pale stool	No	12 (11-17)	5-21	0.511•	0.609	NS
	Yes	11.5 (7-16)	7-16			
Dark urine	No	12 (10.5-15.5)	5-21	0.289•	0.772	NS
	Yes	16 (7-18)	7-18			
Odema	No	12 (10-16)	5-21	0.928•	0.353	NS
	Yes	17 (17-17)	17-17			
Hepatic encephalopathy	No	12 (10-16)	5-21	1.160•	0.246	NS
	Yes	18 (18-18)	18-18			
Itching	No	12 (11-16)	5-21	0.341•	0.733	NS
	Yes	12 (7-17)	7-17			
Thyroid disease	No	12 (11-16)	7-19	0.000•	1.000	NS
	Yes	13 (5-21)	5-21			
Diabetes mellitus	No	12 (10-16)	5-21	1.107•	0.268	NS
	Yes	16 (14-18)	14-18			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant.
*: Kruskall-Wallis test. •: Mann-Whitney test.

Table (13) showed positive correlation between 25 hydroxy vitamin D and sex of patients, all hepatic symptoms and other autoimmune disorders, but all these correlations were statistically insignificant.

Table (14) showed that 25 hydroxy vitamin D level was negatively correlated with hemoglobin level, total white blood cell count, alanine aminotransferase level, serum albumin and total protein, bilirubin levels, alkaline phosphatase and gamma glutamyltransferase, C-reactive protein and ESR. However, all correlations were statistically insignificant.

Table (15) showed no significant correlation between 25 hydroxy vitamin D and pelviabdominal ultrasound findings, autoimmune markers, serum protein electrophoresis, Metavir score and histopathological findings of liver biopsy.

Table (14): Correlation between 25 hydroxy vitamin D level and laboratory investigations in autoimmune hepatitis patients.

	25 hydroxy vitamin D	
	r	*p*-value
Hemoglobin (Hb)	–0.221	0.429
White blood cell (WBCs)	–0.081	0.773
Platelets (PLT)	0.041	0.884
Alanine transaminase (ALT)	–0.041	0.884
Aspartate transaminase (AST)	0.120	0.669
Albumin	–0.264	0.342
Total protein	–0.373	0.171
Total Bilirubin	–0.404	0.136
Direct Bilirubin	–0.353	0.196
Prothrombin time	0.264	0.342
INR	0.250	0.370
Alkaline phosphatase (ALK-P)	–0.021	0.942
Gama glutamyltransferase (GGT)	–0.014	0.960
Calcium	0.731**	0.002
Phosphorus	0.160	0.569
C- reactive protein (CRP)	–0.253	0.362
Erythrocyte sedimentation rate (ESR)	–0.161	0.567

p-value >0.05: Non significant. *p*-value <0.01: Highly significant
p-value <0.05: Significant. Spearman correlation coefficient.

Table (15): Correlation between 25 hydroxy vitamin D level and pelvi-abdominal ultrasound, autoimmune markers, serum protein electrophoresis, Metavir score and histopathological findings of liver biopsy in autoimmune hepatitis patients.

		Vitamin D		Test value	*p*-value	Sig.
		Median (IQR)	Range			
PAUS	Hepatomegaly	14 (11-17)	5-19	1.304*	0.728	NS
	Hepatosplenomegaly	11 (11-11)	11-11			
	Picture of parenchymtous liver disease	12 (7-21)	7-21			
	Splenomegaly	10 (10-10)	10-10			
ANA	Negative	12 (9.5-16)	5-21	0.434•	0.664	NS
	Positive	16 (10-17)	10-17			
ASMA	Negative	14 (10-17)	5-21	0.696•	0.486	NS
	Positive	11.5 (9.5-15)	7-19			
LKM-1	Negative	12 (10.5-17)	7-21	0.217•	0.828	NS
	Positive	14 (5-17)	5-17			
Serum protein electrophoresis	Normal	0	0	0.302	0.715	NS
	Polyclonal hypergammaglobulinemia	15				
Metavir score	A0F2	7 (7-7)	7-7	11.162*	0.132	NS
	A1F1	14 (12-16)	12-16			
	A1F2	8 (5-11)	5-11			
	A1F3	10 (10-10)	10-10			
	A2F2	18 (17-19)	17-19			
	A2F3	12.5 (11-14)	11-14			
	A3F2	18 (18-18)	18-18			
	A3F3	14 (12-21)	12-21			
Interface hepatitis	No	7 (7-7)	7-7	1.392•	0.164	NS
	Yes	13 (11-17)	5-21			
Bridging fibrosis	No	15 (11-19)	11-19	0.596•	0.551	NS
	Yes	12 (10-16)	5-21			
Lymphocytic infiltration	No	8 (7-12)	7-12	1.664•	0.096	NS
	Yes	14 (11-17.5)	5-21			
Piece meal necrosis	No	13 (11-16)	7-21	0.307•	0.759	NS
	Yes	12 (10-17)	5-18			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant.
*: Kruskall-Wallis test. •: Mann-Whitney test.

Discussion

Consequently, this study was conducted and aimed to assess serum levels of 25(OH)D in children and adolescents with AIH and to evaluate the association between serum 25(OH)D levels and clinical, histological, biochemical features and response to immunosuppressive therapy in patients with AIH.

This Cross sectional, case-control study was conducted at tertiary care hospital at Paediatric Hepatology Clinic, Ain Shams University from June 2021 till July 2022 and performed on a total of 30 children and adolescents with autoimmune hepatitis following-up in Paediatric Hepatology Clinic, Faculty of Medicine, Ain Shams University.

During this study, 43 patients were assessed for eligibility and 30 patients were included in the study (15 in each group). Of all eligible patients, 7 patients were excluded from the study based on the inclusion criteria and 5 parents or guardians of the patients refused to participate in the study.

Ultimately, the analysis was based on the data of 30 children and adolescents with autoimmune hepatitis following-up in Paediatric Hepatology Clinic, Faculty of Medicine, Ain Shams University.

To the best of our knowledge, there is a paucity of studies in literature evaluating vitamin D in patients with autoimmune hepatitis, and that represents a strength point of our study.

As regards clinical features, the current study revealed that there was no statistically significant difference between control and cases group regarding demographic data (age, sex, weight, height and BMI) and symptoms of the studied subjects while there was statistically significant difference regarding Abdominal distension and Jaundice were found higher incidence in cases than control group (p-values = 0.065, 0.065, 0.103, 0.756, 0.142) respectively.

Our study results revealed that abdominal enlargement is the most common symptom in liver disease (46.7%) followed by right hypochondrial pain (26.7%) with 13.3% of patients had history of other autoimmune diseases of thyroid and diabetes mellitus.

As regards biochemical features, our study results revealed that there was statistically significant decrease in level of vitamin D and serum calcium in cases of autoimmune hepatitis (p-value=0.002) with no statistically significant association between vitamin D level and age, BMI, dose or duration of treatments (steroids, azathioprine), platelets, ALT, AST, Albumin, Bilirubin, INR, CRP and ESR.

EBADI, et al. **[11]** conducted a study that included two hundred and nine patients to determine the frequency of severe vitamin D deficiency in autoimmune hepatitis (AIH), assess its association with treatment non-response, and evaluate the relationship between vitamin D status and liver-related mortality and need for transplantation and reported that there were no difference was observed in other clinical characteristics, including age at diagnosis (37±17 vs 40±20, p=0.36), BMI (28±9 vs 30±8, p=0.17), serum level of ALT (900±778 vs 748±701, p=0.33), ALP (193±108 vs 207±124, p=0.59), albumin (30±7 vs 33±6, p=0.11), and treatment non-response (59% vs 49%, p=0.27).

EFE, et al. **[8]** conducted a cross-sectional study that enrolled 102 patients to evaluate the association of serum 25(OH)D levels with clinical, biochemical and histological features and response to therapy in AIH and revealed that Circulating levels of 25-hydroxyvitamin D are significantly lower (less than 30ng/mL) in 81% of patients with autoimmune hepatitis (compared to 44% of healthy individuals), and these patients have had a higher frequency of non-response to glucocorticoid therapy than patients without vitamin D deficiency (80% versus 43%, p=0.04).

In another studies, performed by **[5,6,11]** , low levels of 25(OH)D were also found to be associated with the severity of underlying autoimmune liver disease which is in harmony with our results.

MANNS, et al. **[4]** Reported that patients with low serum 25(OH)D levels were less likely to respond to immunosuppressive therapy. Especially, patients who had vitamin D deficiency (\10 lg/L) exhibited poorer response to therapy compared to those with levels higher than 10 lg/L. Importantly, all standard therapies of AIH include steroids and current guidelines recommend regular vitamin D supplementation in patients with AIH.

As regards histological features, on liver biopsy, our results revealed that interface hepatitis was found in 93.3%, bridging fibrosis in 86.7% and lymphocytic infiltration in 80% in case group of autoimmune hepatitis.

Our study results revealed that there were no statistically significant differences between vitamin D level and sex, treatment, symptoms (right hypochondrial pain and abdominal enlargement), other autoimmune diseases of thyroid and DM and interface hepatitis.

Interface hepatitis is the histological hallmark of AIH, consisting of infiltration of lymphocytes, plasma cells and macrophages in the liver parenchyma **[8]**. In agreement with our results, **[11]** revealed that cirrhosis at diagnosis (40% vs 22%, p=0.009) and liver-related events (60% vs 30%, p=0.001) occurred more frequently in patients with severe deficiency compared to their counterpart. The frequency of treatment non-response and liver-related events was higher in patients with mild to moderate deficiency compared to sufficient patients with no association between vitamin D status and outcomes in patients without severe deficiency.

The high frequency of vitamin D deficiency in chronic liver diseases of diverse etiology suggests that the near universal finding reflects impaired hepatic hydroxylation of vitamin D3. The observed improvement in circulating 25-hydroxyvitamin D levels during immunosuppressive therapy suggests that the deficiency is correctable by improved liver function **[11]**.

Furthermore, the association of poor outcomes with serum calcium level, Metavir score, and severe vitamin D deficiency suggests that the serum vitamin D level was a marker of severe liver disease rather than a major pathogenic factor. This consideration was supported by the demonstration that vitamin D deficiency did not correct after vitamin D supplementation and that the persistently deficient patients continued to have poor outcomes. The persistence of severe vitamin D deficiency despite supplementation may reflect failure of the liver disease to improve with glucocorticoid therapy, and this possibility was supported by a higher frequency of treatment non-response in these patients. Consequently, vitamin D deficiency was not only more common in patients with cirrhosis at presentation (40% vs 19%, p=0.007) but also a predictor of future cirrhosis **[11]**.

In concordance with our findings, **[8]** reported that clinical, laboratory and histological features of AIH patients according to 25(OH)D levels revealed that Severe interface hepatitis and advanced fibrosis scores were associated with low 25(OH)D levels, while no association was observed between 25(OH)D levels and age, sex, BMI, ALT or sampling time and fibrosis scores and severe interface hepatitis were independently associated with low 25(OH)D levels (p=0.014; and p=0.020, respectively) and vitamin D deficiency has been proposed as a prognostic biomarker that predicts response to therapy and histological features in AIH.

The strength points of this study are that it is prospective study design and having no patients lost to follow-up in three months. It is the first study in Ain Shams University Hospitals to evaluate the association between Vitamin D and autoimmune hepatitis as a biomarker for response to immunosuppressive therapy.

The limitations of the study are worthy of mention including relatively smaller sample size relative to the previous studies, not being a multicentric study and this represents a significant risk of publication bias. The main limitation of the study is that it cannot definitively distinguish whether vitamin D deficiency itself contributes to poor outcomes or whether it simply reflects more severe liver disease.

Conclusion:

As evident from the current study, severe vitamin D deficiency occurs in patients with AIH. Interface hepatitis was found in 93.3%, bridging fibrosis in 86.7% and lymphocytic infiltration in 80% in case group of autoimmune hepatitis.

Abdominal enlargement is the most common symptom in liver disease (46.7%) followed by right hypochondrial pain (26.7%).

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مستوى فيتامين د فى الدم فى الأطفال المصابين بالالتهاب الكبد المناعى الذاتى وعلاقته بخصائص الالتهاب المناعى ومدى الاستجابة للعلاج

يعد نقص فيتامين د شائعاً فى أمراض الكبد غير الصفراوية ويرتبط بشدة المرض. يعانى مرضى التهاب الكبد المناعى الذاتى من انخفاض مستويات فيتامين (د) مقارنة بمجموعة التحكم. أظهرت العديد من الدراسات تأثيراً كبيراً للكالسيتريول على فسيولوجيا خلايا الكبد.

تقييم مستويات المصل من 25 (OH) D فى الأطفال والمراهقين المصابين بالتهاب الكبد المناعى الذاتى ولتقييم الارتباط بين مستويات المصل 25 (OH) D والسمات السريرية والنسيجية والكيميائية الحيوية والاستجابة للعلاج المثبط للمناعة . فى مرضى التهاب الكبد المناعى الذاتى.

أجريت هذه الدراسة القطعية المستعرضة، وضبط الحالات فى مستشفى الرعاية الثالثية فى عيادة أمراض الكبد للأطفال، جامعة عين شمس من يونيو ٢٠٢١ حتى يوليو ٢٠٢٢ وأجريت على ما مجموعة ٣٠ طفلاً ومراهقاً مصابين بالتهاب الكبد المناعى الذاتى فى عيادة أمراض الكبد للأطفال ، كلية الطب، الطب جامعة عين شمس.

أظهرت نتائج دراستنا عدم وجود فروق ذات دلالة إحصائية بين مستوى فيتامين (د) والجنس والعلاج والأ عراض (ألم المهاد الأيمن وتضخم البطن) وأمراض المناعة الذاتية الأخرى للغدة الدرقية والتهاب الكبد الوبائى والتهاب الكبد السطحى.

خلصنا إلى أن نقص فيتامين (د) الحاد يحدث فى مرضى التهاب الكبد المناعى الذاتى. لوحظ التهاب الكبد فى ٩٣.٣٪ وسد التليف فى ٨٦.٧٪ وتسلل الخلايا الليمفاوية فى ٨٠٪ فى حالة مجموعة التهاب الكبد المناعى الذاتى. تضخم البطن هو أكثر الأعراض شيوعاً فى أمراض الكبد (٤٦.٧٪) يليه ألم المراق الأيمن (٢٦.٧٪).