The frequency of synovitis and calcium pyrophosphate deposition with ultrasound is increased in transfusion-dependent beta-thalassaemia patients: effect of iron accumulation?

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Abstract

Objective

To determine the frequency of synovitis and calcium pyrophosphate deposition (CPDD) with ultrasound (US) in the wrists of transfusion dependant (TD) beta-thalassaemia patients and to investigate the associated factors with these pathologies.

Methods

Eighty-seven beta-thalassaemia patients (46 thalassaemia major and 41 thalassaemia minor patients) were grouped into two as TD and transfusion non-dependent (TND)-thalassaemia patients. Under bilateral wrist US the presence of synovial hypertrophy (SH), power Doppler signal (PD) combined synovitis (SH+PD), tenosynovitis, and triangular fibrocartilage complex (TFC)-cartilage calcification (CC) were examined. SH, PD, and combined synovitis in the US were classified as Grade-0 (no), Grade-1 (minimal), Grade-2 (moderate), and Grade-3 (severe).

Results

The incidence of moderate/severe SH, PD, and combined synovitis with US was 34.8%, 17.4%, and 34.8% in TD-thalassaemia patients, respectively, but none in TND patients ($p<0.001$, $p=0.006$, $p<0.001$). The frequency of TFC-CC with US was 32.6% in TD and 2.4% in TND-thalassaemia patients ($p<0.001$). Ferritin level was positively correlated with SH ($r=0.414$, $p<0.001$), PD ($r=0.279$, $p=0.009$) and combined synovitis scores ($r=0.402$, $p<0.001$). Ferritin level (OR:1.001, CI:1.000-1.002) and the presence of TFC-CC (OR:25.048, CI:5.187-120.951) were determined as to be associated with moderate/severe combined synovitis.

Conclusion

The presence of synovitis and TFC-CC with the US is common in patients with beta-thalassaemia who have had recurrent blood transfusions. Iron overload in beta-thalassaemia patients may cause CPDD and synovial inflammation.

Key words

cartilage calcification, calcium pyrophosphate deposition, ferritin, ultrasound, synovitis, thalassaemia minor, thalassaemia major

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Introduction

Beta-thalassaemias (β-thalassaemia) are a heterogeneous group of autosomal recessive diseases characterised by hypochromic microcytic anaemia developing due to the damaged synthesis of one or more haemoglobin (Hb) chains (1, 2). In β-thalassaemia major (β-TM), the severe form of the disease, the excessive iron accumulation due to the need for frequent and regular transfusion leads to the structural and functional deterioration of the liver, heart, gonads, skin, and joints, and the development of such clinical manifestations as cardiomegaly, growth-development retardation, and arthropathy (3). A wide variety of musculoskeletal (MSC) involvements can be seen in patients with β-TM (4). Among such involvements are osteoporosis, bone fractures, epiphyseal deformities that may result from the enlargement of bone marrows, crystal arthropathies, and septic arthritis developing due to recurrent transfusions (5). Inflammatory arthritis is also another MSC involvement that may accompany β-TM. In the light of the data based on the limited number of studies so far, the frequency of rheumatoid arthritis has been detected between 4.4–6.4% in β-thalassaemia patients, more prevalent than that in the general population (6-9). However, there is insufficient information about the precise frequency of synovitis and the factors affecting it in patients with β-thalassaemia.

Conventional radiography (CR) is frequently used for imaging the existence of various joint pathologies in patients with β-thalassaemia (10, 11). Although used for imaging joint space narrowing, sclerosis, and calcium pyrophosphate deposition (CPPD), the CR is not sensitive for imaging soft tissue pathologies (12). The methods used for imaging, especially soft tissue pathologies in the MSC system are ultrasonography (US) and magnetic resonance imaging (MRI). As a safe and non-invasive imaging method widely used in MSC diseases, the US can distinguish between synovitis, tenosynovitis, bursitis, and other soft tissue lesions that can mimic clinical synovitis (13). Also, US is superior to CR in the identification of crystal arthropathies, and its superiority over MRI is that the US allows the clinicians to dynamically evaluate the detected pathological findings from different directions (14-17). Most studies also suggest that wrist US could be considered a relevant tool for the diagnosis of CPPD, with higher sensitivity and specificity than CR (18-20).

In the present study, we aimed to determine the frequency of synovitis, tenosynovitis, and CPPD with the US in β-thalassaemia patients, investigate the associated factors with these US findings and compare those patients who are transfusion-dependent and not.

Methods

Study design

This is a cross-sectional study conducted in the departments of rheumatology, paediatric haematology, and haematology of Bursa Training and Research Hospital, Bursa, Turkiye. After informing about the study design, written consent was acquired from each patient, and approval was also obtained from the local ethics committee for clinical research (ECCR) of the institution (registration number and date: 2011 ECCR-25-2021/07/25).

Inclusion and exclusion criteria

Patients between 8-60 years of age diagnosed with β-thalassaemia minor (HbA_2 ≥3.5%, mean corpuscular volume (MCV) <80 fL, and HgF between 2–10%) and β-TM (HbA_2 <4% and HbF >50% and Hg <7 g/dL) were included under the criteria of The Hemoglobinopathy Diagnostic Guide, 2015 (21). β-thalassaemia patients with more than 24 transfusions per annum constituted the participants in the study and they were defined as transfusion-dependant (TD) β-thalassaemia patients. β-thalassaemia patients who did not receive a transfusion were defined as non-transfusion dependent (TND) β-thalassaemia patients.

Patients with the diagnosis of any concomitant rheumatological disease, having wrist traumas or joint injections within the last 30 days, and those receiving immunosuppressive drugs for any reason, including steroids were excluded.

Patients

Forty-nine patients followed up with the diagnosis of β-TM in the departments of paediatric haematology and haematology, and 42 followed up with the diagnosis of β-thalassaemia minor were evaluated in terms of the study criteria. All β-TM patients evaluated in terms of study criteria were TD, and all thalassaemia minor patients evaluated in terms of study criteria were also TND. One TND-patient and three TD-patients diagnosed with any rheumatic disease were not included in the study. While two of the TD-patients had rheumatoid arthritis, merely one was observed to have connective tissue disease. However, one of the TND-patients was diagnosed with ankylosing spondylitis and detected to receive tumour necrosis factor inhibitors. Thus, a total of 87 thalassaemia patients, 46 with TD and 41 with TND, were included in the study.

Clinical evaluation

The demographic data, medical history, and medications were recorded. The number, frequency, and duration of the transfusions, chelation treatment, and comorbidities related to thalassaemia, including hepatomegaly, cardiomegaly, splenomegaly, diabetes mellitus, and hypogonadism were also investigated. Organomegaly was evaluated through the clinical examination and the abdominal US. The presence of cardiomegaly was determined based on the findings of electrocardiography, echocardiography, and cardiac MRI previously performed during the follow-ups in the outpatient clinics and existing in patients' records. The existence of hypogonadism and growth-developmental retardation was also obtained from patients' records.

Laboratory practice

The morning fasting plasma samples were acquired from all patients for the tests of haemogram, erythrocyte sedimentation rate (ESR) by the Westergren method, C-reactive protein (CRP) by the nephelometry, and such biochemical and hormonal parameters as iron, iron-binding capacity, and ferritin.

Ultrasound practice

Bilateral wrist US of all patients was performed by an experienced rheumatologist (KA). The physician performing the US was blinded to the clinical features of patients. The US examination of the wrist was conducted in two different sections (transverse and vertical) from the dorsal and volar directions with the use of the GE Logiq p6 device having a 9-15 MHz multi-frequency linear probe (GE Healthcare, Waukesha, Washington, USA). The frequency of the power Doppler (PD) pulse repetition was set to 700 Hz, and the Doppler color gain adjustment was decreased until the artifacts under the bone cortex disappeared.

On the joint US, the terms synovial hypertrophy (SH), PD, and tenosynovitis were defined using the US definitions of Outcome Measures in Rheumatology Clinical Trials (OMERACT) (22, 23). Combined synovitis was defined using the 2017 definition of the European League Against Rheumatisms (EULAR)-OMERACT (24).

SH was scored as; Grade-0 (no SH), Grade-1 (hypoechoic SH extending to the level of the horizontal line connecting the bone surfaces), Grade-2 (hypoechoic SH with a flat/convex upper surface extending beyond the joint line), and Grade-3 (hypoechoic SH with a flat/convex upper surface extending beyond the joint line). In grading SH, whether SH was accompanied by effusion was not taken into account. PD was scored as; Grade-0 (no/minimal vascularity), Grade-1 (up to three single Doppler spots/up to one confluent spot and two single spots/up to two confluent spots), Grade-2 (>Grade-1, but <50% of grayscale background), and Grade-3 (>50% of grayscale background). Even so, combined score (SH+PD) was defined as Grade-0 (SH and PD=Grade-0), Grade-1 (SH=Grade-1 and PD ≤Grade-1), Grade-2 (SH=Grade-2 and PD ≤Grade-2), or (SH=Grade-1 and PD ≤Grade-2), and Grade-3 (SH=Grade-3 and PD≤Grade-3) or (SH=Grade-1 or 2 and PD=Grade-3) (19). SH, PD, and combined synovitis in the US were classified as Grade-0 (no), Grade-1 (minimal), Grade-2 (moderate), and Grade-3 (severe) for all.

All extensor and flexor tendons were also evaluated in both wrists to evaluate the presence of tenosynovitis. Tenosynovitis was defined as the presence of hypoechoic or anechoic fluid or tissue associated with intratendinous changes that can be seen in two different planes, surround the tendon, and generally reveal a Doppler signal, and the presence of tenosynovitis was scored as yes/present or no/absent (22).

The triangular fibrocartilage complex (TFC) of both hands was evaluated to investigate the presence of fibrocartilage calcification attributable to haemochromatosis-associated CPPD. The term 'cartilage calcification (CC)' was used in our study and scored as yes/present or no/absent (25).

Statistical analysis

All statistical analyses were performed through the statistical analysis package program of the Statistical Package for the Social Sciences for Windows, version 21.0 (IBM Corp., Armonk, New York, USA). The descriptive statistics of the features obtained were calculated as mean, standard deviation (SD), and frequencies (n, %), depending on the type of features. The distribution was examined using the Shapiro-Wilks test. While the independent samples *t*-test was used to compare two independent groups in terms of the features with normal distribution, the Mann-Whitney U-test was utilised for the numerical variables showing no normal distribution. The relationships between the categorical features were examined using the Pearson Chi-Square test or the Fisher-Freeman-Halton test by considering the expected values of the frequencies in the Tables. Besides, the correlations were analysed with the non-parametric Spearman correlation analysis. The univariate and multivariate evaluations were also conducted with the binary logistic regression model, and a *p*-value of <0.05 was accepted as statistically significant.

Results

Clinical data and laboratory findings of participants

The clinical characteristics and laboratory findings of patients are presented in Tables I and II. Frequencies of cardiomegaly (23.9%), splenomegaly

Table I. Demographic data, clinical background and comorbidities of thalassaemia patients.

	All patients (n=87)	TD thalassaemia (n=46)	TND thalassaemia (n=41)	*p*-value**
Age, mean±SD	24.8 ± 13.1	19.5 ± 9.2	30.1 ± 14.6	**<0.001**ᵗ
Age at diagnosis, mean±SD	13.6 ± 14.1	5.1 ± 9.2	23.3 ± 12.2	**<0.001**ᵗ
Gender, F, n (%)*	47 (54.0)	22 (47.8)	25 (61.0)	0.282
Thalassaemia minor		0 (0)	41 (100)	
Thalassaemia major		46 (100)	0 (0)	
Tx time (years), mean±SD	14.5 ± 7.0	14.5 ± 7.0	-	NA
Number of Tx in the last year, mean±SD	28.3 ± 6.4	28.3 ± 6.4	-	NA
Chelation therapy, n (%)*	36 (41.4)	36 (78.3)	-	NA
Clinical background, n (%)*				
Myalgia	27 (31.0)	15 (32.6)	12 (29.3)	0.818
Arthralgia	34 (39.1)	17 (37.0)	17 (41.5)	0.826
Arthritis	6 (6.9)	4 (8.7)	2 (4.9)	0.680
Back pain	40 (46.0)	22 (47.8)	18 (43.9)	0.830
Hip pain	7 (8.0)	3 (6.5)	4 (9.8)	0.702
Gout	2 (2.3)	1 (2.2)	1 (2.4)	0.934
Skeletal changes	4 (4.6)	1 (2.4)	3 (6.5)	0.619
Fractures	7 (8.0)	5 (10.9)	2 (4.9)	0.439
Comorbidities, n (%)*				
DM	8 (9.3)	7 (15.6)	1 (2.4)	0.060
Hearing loss	6 (6.9)	5 (10.9)	1 (2.4)	0.207
Cardiomegaly	11 (12.6)	11 (23.9)	0 (0)	**0.001**
Hepatomegaly	30 (34.5)	29 (63)	1 (2.4)	**<0.001**
Splenomegaly	25 (28.7)	25 (54.3)	0 (0)	**<0.001**
Hypogonadism	12 (13.8)	12 (26.1)	0 (0)	**<0.001**
Splenectomy	16 (18.4)	15 (32.6)	1 (2.4)	**<0.001**

ᵗ Independent samples t-test, *Pearson Chi-Square test, ***p*-values are the comparisons of TD and TND patients.
DM: diabetes mellitus; F: female; NA: non-applicable; SD: standard deviation; TD: transfusion dependent; TND: transfusion non-dependent; Tx: transfusion. Skeletal changes (spinal fractures, spinal deformity, intervertebral disc changes).

(54.3%), hepatomegaly (63%), and hypogonadism (26.1%) were significantly detected to be higher in TD-thalassaemia patients than in TND-thalassaemia patients. Iron and ferritin values were detected to be significantly higher in those with TD-thalassaemia patients ($p<0.001$, $p<0.001$, respectively).

Ultrasound findings

The US findings of thalassaemia patients are shown in Table III, and US images of selected patients are shown in Figure 1.

- Evaluation of SH

The frequencies of SH in the right and left wrists of TD-thalassaemia patients were found significantly higher than those of TND-thalassaemia patients ($p=0.002$, $p=0.016$, $p=0.002$, respectively), and the percentage of SH in the right hand was higher in both groups. The frequencies of minimal SH were detected as 17.4% in TD and 17.1% in TND-thalassaemia patients. While the rates of moderate/severe SH were detected as 30.4% and 4.3% in TD, no patients with moderate/severe SH were found among TND-thalassaemia patients. While the rate of the moderate/severe SH in any wrist was 34.8% in TD-thalassaemia patients, no or minimal SH was observed in any patient in TND-thalassaemia patients' ($p<0.001$).

- Evaluation of PD

Although the presence of PD in the right hand was found not to be different between the groups, the number of patients revealing PD in the left hand was significantly higher in TD-thalassaemia patients' group ($p=0.014$). The rates of minimal PD were detected as 15.2% and 14.6% in TD and TND-thalassaemia patients', respectively. However, moderate/severe PD was found 15.2% and 2.2% in TD-thalassaemia patients respectively, no patients with moderate/severe PD signals were determined among TND-thalassaemia patients. While the rate of clinically moderate/severe PD in any wrist was 17.4% in TD-thalassaemia patients, no/minimal PD signal was observed in any of the TND-thalassaemia patients ($p=0.006$).

- Evaluation of combined scores

The rates of minimal combined synovitis were 17.4% in TD and 19.5% in TND-thalassaemia patients. Among TD-thalassaemia patients, however, the rates of moderate/severe combined synovitis were found as 28.3% and 6.5%. No patients with moderate/severe combined synovitis were encountered among TND-thalassaemia patients.

- Evaluation of tenosynovitis

The presence of tenosynovitis in any wrist was found as 39.1% (n=18) in TD and 14.6% (n=6) in TND-thalassaemia patients, and the difference was significantly higher in TD patients ($p=0.016$). While there was no difference in terms of tenosynovitis rates in the right hand between the two groups, the number of those with tenosynovitis was detected to be significantly higher in the left hand in TD-thalassaemia patients ($p=0.008$).

- Evaluation of cartilage calcification

The rate of TFC-CC in any hand of all patients was found as 18.4%, and the presence of TFC-CC in any hand was also found to be 32.6% in TD and 2.4% in TND-thalassaemia patients ($p<0.001$).

- Evaluation of factors associated with ultrasound findings

No correlation was observed between such US findings as SH, PD, combined synovitis score and TFC-CC, and age, transfusion time, and the number of transfusions per annum. However, a positive correlation was seen between the level of ferritin, and SH (r=0.414, $p<0.001$), PD (r=0.279, $p=0.009$) and combined synovitis scores (r=0.402, $p<0.001$).

- Comparison of no/mild and moderate/severe ultrasonographic synovitis

All patients with moderate/severe synovitis were TD-thalassaemia patients. Among patients with moderate/severe

Table II. Comparisons of laboratory findings of transfusion-dependent and non-dependent thalassaemia patients.

	TD thalassaemia (n=46)	TND thalassaemia (n=41)	*p*-value*
Laboratory, mean±SD			
Whole blood count			
WBC, 10^3 cells/ μL	14 ± 19.3	7.6 ± 2.2	0.286
Neu, 10^3 cells/ μL	7.8 ± 10	4.7 ± 2	0.647
Lymp,10^3 cells/ μL	3.1 ± 1.7	2.1 ± 0.5	**0.011**
Hg, g/dL	9.4 ± 1.2	11.6 ± 1.8	**<0.001**
Hct, %	29.3 ± 3.3	36.1 ± 5.1	**<0.001**
MCV, fL	77.1 ± 7.9	65.1 ± 8.5	**<0.001**
PLT, 10^3 cells/ μL	416.1 ± 225.2	268.4 ± 98.7	**0.001**
Biochemical and hormonal parameters			
BUN, mg/dL	12.9 ± 4.1	10.5 ± 2.9	**0.006**
Cr, mg/dL	0.5 ± 0.2	0.6 ± 0.2	**<0.001**
AST, mg/dL	26.3 ± 10.2	16.7 ± 4.9	**<0.001**
ALT, mg/dL	26.2 ± 20.3	17.6 ± 11.5	**0.043**
Uric acid, mg/dL	4.2 ± 1.8	4.1 ± 1.3	0.858
Ca, mg/dL	9.5 ± 0.5	9.3 ± 0.4	**0.003**
P, mg/dL	4.1 ± 0.6	3.5 ± 0.9	**<0.001**
Vit D, ng/mL	19.3 ± 10.4	15.9 ± 11.4	0.053
PTH, pg/mL	32.5 ± 21.9	35.3 ± 16.1	0.071
Iron parameters			
Iron, ng/mL	218.1 ± 67.4	90.2 ± 40.3	**<0.001**
IBC, ug/dL	218.4 ± 198.9	260.8 ± 111.3	0.070
Ferritin, ng/mL	1711.1 ± 1043	63 ± 56.9	**<0.001**
Acute phase reactants			
ESR, mm/h	8.9 ± 11.3	10.6 ± 12.2	0.304
CRP, mg/L	3.7 ± 1.4	3.8 ± 2.6	0.588

*Mann-Whitney U-test; SD: standard deviation; TD: transfusion dependent; TND: transfusion non-dependent; WBC: white blood cell; Neu: neutrophil; Lymp: lymphocyte; Hb: haemoglobin; Hct: haematocrit; MCV: mean corpuscular volume; PLT: platelet; BUN: blood urea nitrogen; Cr: creatinine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Ca: calcium; P: phosphorus; Vit D: vitamin D; PTH: parathormone; IBC: iron binding capacity; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Table III. Ultrasonography findings of patients with β-thalassaemia and comparison of findings between transfusion-dependent and non-transfusion-dependent patients.

	All patients (n=87)	TD thalassaemia (n=46)	TND thalassaemia (n=41)	*p*-value***
SH, (n,%)				
Right wrist	30 (34.5)	23 (50.0)	7 (17.1)	**0.002***
Left wrist	24 (27.6)	18 (39.1)	6 (14.6)	**0.016***
SH with moderate/severe score PD, (n,%)	16 (18.4)	16 (34.8)	0 (0)	**<0.001***
Right wrist PD	17 (19.5)	11 (23.9)	6 (14.6)	0.296*
Left wrist PD	16 (18.4)	13 (28.3)	3 (7.3)	**0.014***
PD with moderate/severe score	8 (9.2)	8 (17.4)	0 (0)	**0.006***
Combined score (SH+PD), (n,%)				
0 (no)	55 (63.2)	22 (47.8)	33 (80.5)	**<0.001****
1 (minimal)	16 (18.4)	8 (17.4)	8 (19.5)	
2 (moderate)	13 (14.9)	13 (28.3)	0 (0)	
3 (severe)	3 (3.4)	3 (6.5)	0 (0)	
Effusion, (n,%)				
Right wrist	7 (8.0)	6 (13)	1 (2.4)	0.114*
Left wrist	5 (5.7)	5 (10.9)	0 (0)	0.057*
Tenosynovitis, (n,%)				
Right wrist	20 (23.0)	14 (30.4)	6 (14.6)	0.125*
Left wrist	14 (16.1)	12 (30.4)	2 (4.9)	**0.008***
Hand TFC CC, (n,%)	16 (18.4)	15 (32.6)	1 (2.4)	**<0.001***

*Pearson Chi-Square test, **Fisher-Freeman-Halton exact test, [m]Mann-Whitney U-test, ****p*-values are the comparisons of TD and TND patients.
TD: transfusion dependent; TND: transfusion non-dependent; SH: synovial hypertrophy; PD: power Doppler; TFC: triangular fibrocartilage complex; CC: cartilage calcification.

synovitis, the number of those with hepatomegaly, splenomegaly, and the level of ferritin were significantly higher (p=0.018, p=0.002, p<0.001). Although present in 68.8% of patients with moderate/severe synovitis, TFC-CC existed in 7% of those with no/mild synovitis. Even so, TFC-CC was significantly higher in patients with moderate/severe synovitis (p<0.001). Comparison of demographic data and laboratory and clinical findings between patients with no/mild and moderate/severe combined synovitis are given in Table IV.

- Evaluation of factors associated with moderate/severe ultrasonographic synovitis

Ferritin level, hepatomegaly, splenomegaly, and presence of TFC-CC were found as predictive factors affecting moderate/severe ultrasonographic synovitis (p<0.001, p=0.013, p=0.002, p<0.001, respectively). Even so, when evaluated with the multivariate analysis, ferritin and TFC-CC were found as independently associated factors (p<0.001, p<0.001). Logistic regression analysis results investigating the associated factors of the presence of moderate/severe combined synovitis in patients with β-thalassaemia are presented in Table V.

- Laboratory findings of patients with and without cartilage calcification

Given the laboratory data of the patients with and without TFC-CC, the levels of serum ESR (p=0.895), CRP (p=0.374), uric acid (p=0.848), calcium (p=0.192), phosphorus (p=0.092), vitamin D (p=0.734), and parathormone (p=0.295) were not different between both groups.

Discussion

In this study, the presence of moderate/severe synovitis and TFC-CC in the US were significantly higher in the wrists of TD-thalassaemia patients compared to TND-thalassaemia patients. TFC-CC and ferritin levels were found to be associated factors with synovitis. It could be concluded that transfusion-related iron load in thalassaemia patients may affect the MSC system as well as

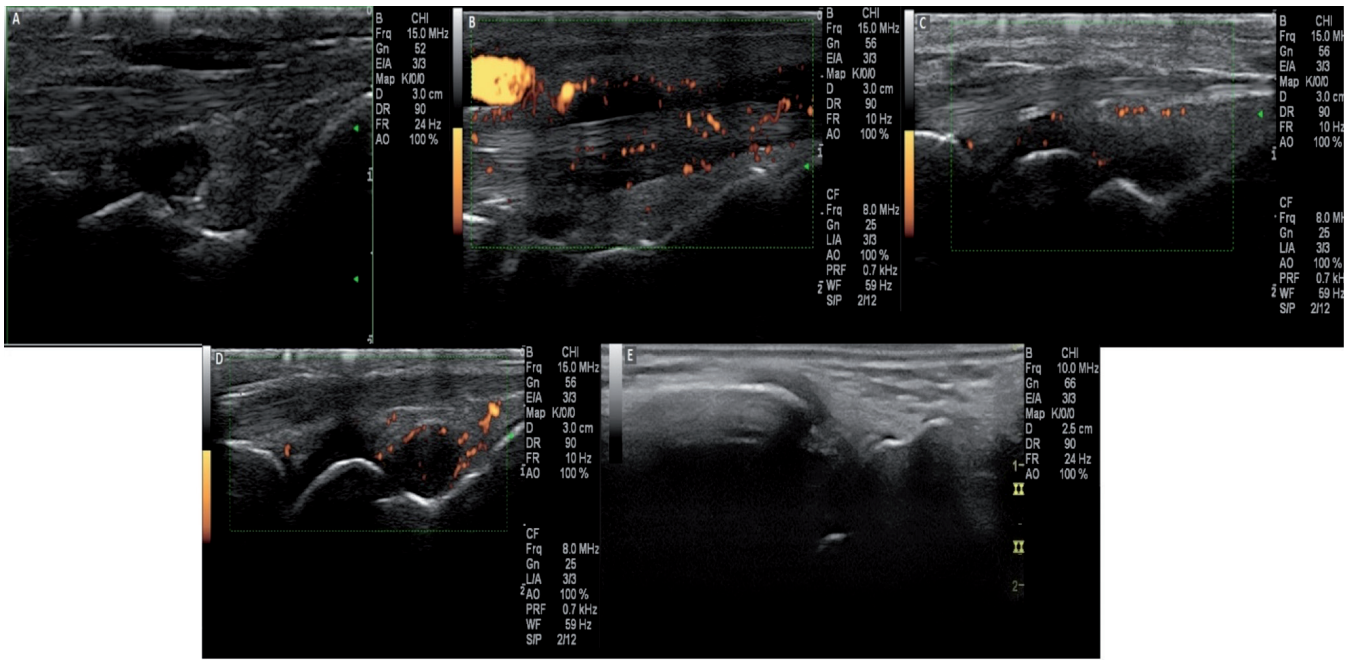

Fig. 1. US images of thalassaemia patients.
A: US image of a patient with synovial hypertrophy; **B**: US image of a patient with tenosynovitis; **C**: US image of a patient with Grade 2 synovitis; **D**: US image of a patient with Grade 3 synovitis; **E**: US image of a patient with a TCC cartilage calcification.

Table IV. Comparison of demographic data, laboratory and clinical findings between patients no/minimal and moderate/severe ultrasonographic synovitis.

	No/minimal synovitis (n=71)	Moderate/severe synovitis (n=16)	*p*-value
Age, mean±SD	25.7 ± -13.6	19.1± 8.7	0.067[t]
Age at diagnosis, mean±SD	15.8 ± 14.5	3.87± 5.81	**0.002**[t]
Gender, n, (%), F	39 (54.9)	8 (50.0)	0.786*
Thalassaemia, n, (%)			
TD	30 (42.3)	16 (100)	**<0.001***
TND	41 (57.7)	0 (0)	
Tx, mean±SD			
Total Tx time, (years)	13.3 ± 7.4	14.9 ± 6.6	0.472[t]
Number of tx in the last year	23.1 ± 12.5	24.7 ± 9.9	0.658[t]
Laboratory, median (min-max)			
Ferritin, ng/mL	132 (4-4012)	1906 (589-4403)	**<0.001**[m]
ESR, mm/h	5 (1-66)	5 (2-38)	0.895[m]
CRP, mg/L	3.10 (1.7-17.2)	3.11 (1.8-4.65)	0.374[m]
Organomegaly, n (%)			
Cardiomegaly	7 (9.9)	4 (25.0)	0.113*
Hepatomegaly	20 (28.2)	10 (62.5)	**0.018***
Splenomegaly	15 (21.1)	10 (62.5)	**0.002***
TFC CC, n, (%)	5 (7.0)	11 (68.8)	**<0.001***

[t]Indepentdent sample t-test, [m]Mann-Whitney U test, *Pearson Chi-Square test.
F: female; TD: transfusion dependent; TND: transfusion non-dependent; Tx: transfusion; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TFC: triangular fibrocartilage complex; CC: cartilage calcification.

some other organs, leading to synovial inflammation and CPPD accumulation in the wrist. According to our knowledge, this is the first study showing that continuous repetitive blood transfusions may cause subclinical synovitis and CPPD accumulation in the US, in thalassaemia patients.

Although arthropathy is widespread in thalassaemia patients, its pathogenesis has yet to be fully understood. Thus, the most important theory concerning pathogenesis is that excessive iron load causes the arthropathy of haemochromatosis by accumulating in the synovium, and the synovial destruction occurs due to the production of free radicals during the iron exchange (26, 27). In hereditary haemochromatosis (HH) studies, the indication that iron causes arthropathy by accumulating in the synovium supports the theory. Secondary haemochromatosis performed due to frequent transfusions also develops in TD-thalassaemia patients, and the joint involvements due to iron accumulation are anticipated to be similar to HH. Since the most commonly involved joints in HH are the wrist and knee, the wrists were targeted to evaluate the thalassaemia arthropathy in the present study (3).

Systematic imaging studies investigating inflammatory arthritis and structural changes in thalassaemia patients are rarely performed, and the number of studies evaluating previously thalassaemia arthropathy through MRI and US is also quite limited. In a study investigating haemochromatosis arthropathy in TD-thalassaemia patients, fluid accumulation around the scaphoid bone and a decrease in the density of wrist bones were

Table V. Logistic regression analysis results investigating the predictors of the presence of clinically combined synovitis in β-thalassaemia patients.

	Univariate analysis 95% CI for OR				Multivariate analysis 95% CI for OR			
	OR	Lower	Upper	*p*-value	OR	Lower	Upper	*p*-value
Age	0.952	0.900	1.006	0.078				
Age at diagnosis	0.882	0.807	0.964	**0.006**				
Gender	1.219	0.411	3.610	0.721				
Total Tx time	1.033	0.947	1.127	0.464				
Number of Tx*	1.012	0.960	1.068	0.650				
Ferritin, ng/mL	1.001	1.000	1.002	**<0.001**	1.001	1.000	1.002	**<0.001**
Cardiomegaly	3.048	0.771	12.048	0.112				
Hepatomegaly	4.250	1.364	13.243	**0.013**				
Splenomegaly	6.222	1.948	19.878	**0.002**				
TFC CC	29.040	7.202	117.091	**<0.001**	25.048	5.187	120.951	**<0.001**

*Number of transfusions within the last year.
CI: confidence interval; OR: odds ratio; Tx: transfusion, TFC: triangular fibrocartilage complex; CC: cartilage calcification.

determined in 23.3% of the patients. They also found an association between the imaging findings, ferritin levels, and organomegaly (28). Likewise, moderate/severe synovitis findings were detected in the wrists of TD-thalassaemia patients receiving frequent transfusions and developing secondary haemochromatosis, and a significant correlation was also found between ferritin levels and synovitis in the present study.

SH appearing hypoechogenic on the grey scale and PD revealing active inflammation/vascularisation in the synovial tissue are utilised in evaluating synovitis through US (29). Since minimal SH, PD and combined synovitis can often be seen in other pathologies or in people without significant joint complaints, patients with at least 'moderate' synovitis were considered significant in terms of the presence of ultrasonographic synovitis. (30, 31) In the present study, the rates of minimal SH and PD were found similar in both groups. However, although not observed in any of those with TND, the US scores of moderate/severe were found significantly higher in TD-patients. When TD and TND-patients were compared in terms of MSC symptoms, no difference was found between the groups. Most patients with moderate/severe ultrasonographic synovitis did not have arthritis attacks. Patients with moderate/severe synovitis in the US had not yet developed clinical signs of synovitis, and these were defined as patients with subclinical synovitis. The presence of SH in the right hand was higher in both groups, and the condition may be due to the dominant use of the right hand. Albeit the similar values in patients' right hands in both groups, the score of PD and presence of tenosynovitis were determined to be significantly higher in the left hands of TD-patients. The fact that both PD score and presence of tenosynovitis are higher in the non-dominant hand in TD-patients is significant, revealing that a true history of inflammation is more frequent in TD-patients.

CC developing due to the accumulation of CPPD in the articular and periarticular soft tissues is frequently encountered in diseases with iron accumulation. While seen as 10-15% in individuals between 65–75 years of age in the general population, the prevalence of CC was reported to be observed in 30–50% of HH patients, and the wrists and knees are the most commonly affected joints (29-31). In the present study, TFC-CC was found in 15 (32.6%) in TD-patients, as consistent with the findings reported in previous studies evaluating CC in HH patients (32-34). The diagnosis of CPPD is based on synovial fluid (SF) analyses that reveal typical crystals at polarised light microscopy, but sometimes SF is not available. US has been demonstrated to be a reliable and accurate tool for determining CPPD. Also, Filippou *et al*. showed that US revealed higher grade synovitis in patients affected by CPPD, and in their SF analyses had a higher number of pro-inflammatory cytokines. They emphasised that the number of CPPD crystals in the SF influences directly synovitis and can cause acute/chronic inflammatory arthritis attacks (35). In our study, none of the patients in whom CPPD was detected in the US have clinically acute/chronic arthritis, that's why none of the patients had SF analysis or CR. Increased ESR or CRP levels were also not observed in patients with neither CPPD nor moderate/severe synovitis. According to EULAR definitions, the patients in our study were considered 'asymptomatic CPPD' (25). The deposition of crystals could be asymptomatic as long as they do not pass in the synovial space. Once shedding occurs, inflammatory pathways are triggered and clinical symptoms of inflammation appear (35). This may be the reason why patients in our study were clinically asymptomatic.

CC is not always due to CPPD and may occur as an isolated finding, so it is debatable whether CC detected in the US is associated with CPPD or whether it is an isolated finding. It is well known that CC increases with age, when seen in younger patients, secondary causes should be considered such as predisposing metabolic diseases. In our study, CC has observed primarily in TD-patients and the average age of the patients was quite low even though most of them were children. The patients did not have a history of trauma, and the biggest common risk factor for those was frequent transfusions. Therefore, it seems reasonable to interpret these

lesions as transfusion-associated CPPD rather than isolated lesions.

The risk of developing both ultrasonographic synovitis and CC seem to be increasing with a high iron load in TD-patients. Also, one of the most related factors in moderate/severe synovitis was the presence of CC according to our study. In a study with untreated HH patients, CC prevalence was 30% and CC was correlated positively with ferritin level as in our study (36).

Ordinarily, TD-thalassaemia patients are diagnosed in early childhood due to their dependence on transfusion. TND-patients, on the other hand, are diagnosed at a later age, sometimes even by chance. Organomegaly is seen more in these patients than in TND-thalassaemia patients for the same reason. Although this is an expected difference between the groups, the significant difference is one of the limitations of the study. The most important limitation is that investigations through the US were performed by a single physician; if conducted by another physician, those investigations would have been a factor to strengthen the study findings. CR is the most adopted imaging technique in daily practice. Another important limitation is the lack of a reference imaging test for the identification of CPPD at the wrist level (*e.g.* CR or CT) and the fact that other joints in which US CPPD has frequently been detected in previous studies were not included in the current US protocol, such as the knee or the hip. It is a matter of curiosity whether thalassaemia patients with subclinical synovitis or CPPD accumulation will develop arthritis or inflammatory disease in the future. Anti-inflammatory drugs may be effective in such patients. Untreated CPPD cases in TD-thalassaemia patients may lead to severe attacks, chronic pain, or inflammation. Over time joints may degenerate and result in long-term disability, so, early diagnosis of CC may be important to prevent joint degeneration and may enable the development of treatments. Further studies are needed to explain these unanswered questions.

In conclusion, the iron load seems to be an important factor that affects the synovium in TD patients, as in all other organs in our study. Although having no clinical findings or high inflammation markers, significant synovitis and CPPD can be detected in the US in TD patients. The presence of CC may be a factor that triggers synovitis in thalassaemia patients.

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