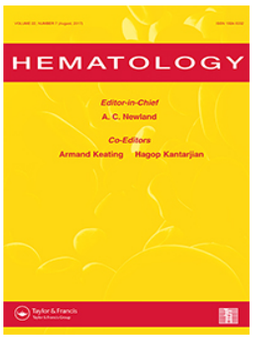

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Protector effect of α-thalassaemia on cholecystitis and cholecystectomy in sickle cell disease

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ABSTRACT

Objectives: Cholecystitis is one of the complications of symptomatic cholelithiasis responsible for high levels of morbidity of sickle cell disease (SCD) patients. Here, we investigated the possible protective role of single gene deletions of α-thalassaemia in the occurrence of cholelithiasis and cholecystitis in SCD patients, as well as the cholecystectomy requirements.
Methods: The α-globin genotype was determined in 83 SCD patients using the multiplex-polymerase chain reaction and compared with clinical events.
Results: Overall, in 23% of patients, $-\alpha^{3.7}$ deletion was found. α-Thalassaemia concomitant to SCD was an independent protective factor to cholecystitis (OR = 0.07; 95% CI: 0.01–0.66; $p = 0.020$) and cholecystectomy requirement (OR = 0.14; 95% CI: 0.03–0.60; $p = 0.008$). The risk of cholelithiasis was not affected by the α-thalassaemia concomitance.
Conclusions: To the best our knowledge, our study is the first to show the protective effect of α-thalassaemia on cholecystitis and cholecystectomy requirements in SCD, which may be due to an improved splenic function.

KEYWORDS
α-Thalassaemia; sickle cell disease; cholelithiasis; cholecystitis; cholecystectomy; multiplex-PCR

Introduction

The chronic hyperbilirubinaemia, due to haemolysis in sickle cell disease (SCD), frequently leads to the formation of pigment gallstones (cholelithiasis) [1,2]. Its onset can be as early as at around 5 years old, but its prevalence increases progressively with age reaching 50% around 22 years old [3]. Cholecystitis is responsible for high levels of morbidity in SCD patients, and elective cholecystectomy is, therefore, the treatment approach recommended to prevent acute complications as biliary tract obstruction, infection and emergency surgery [3,4].

Co-inherited α-thalassaemia (α-thal) occurs in approximately 30% of SCD patients , more commonly due to the $-\alpha^{3.7}$ deletion of single gene, and in African descendants. Studies have shown that these individuals have a lesser degree of haemolysis by decreasing Hb S intracellular concentration and Hb S polymerization [5–7].

Nevertheless, the role of α-thalassaemia in bilirubin levels and cholelithiasis is controversial in SCD [8–13]. Furthermore, there is a lack of information about complications from cholelithiasis in SCD patients with coinheritance of α-thalassaemia. In order to clarify these points, we investigated the possible protective role of $-\alpha^{3.7}$ and $-\alpha^{4.2}$ single gene deletions of α-thal in the occurrence of cholelithiasis and cholecystitis in SCD patients, as well as the cholecystectomy requirements.

Methods

Patients and samples

From October 2011 to December 2013, 102 SCD outpatients (47% male and 53% female) of Haematology Service at HUPE/UERJ – median age of 21 years old (ranging from 5 to 79) – were enrolled in the study, after informed consent, according to the ethical approval granted by the Research Ethics Committee (CAAE no.1543.0.000.228–1/HUPE3016/2011). High-performance liquid chromatography (HPLC, Variant I, CA, USA) was used to confirm haemoglobin (Hb) phenotype, Hb F and Hb A2 measures. After that, 19 patients were excluded due to their phenotypes – Hb SC ($n = 14/19$), Hb SD ($n = 3/19$) and Hb $S\beta^+$ ($n = 2/19$). Thus, multiplex polymerase chain reaction (multiplex-PCR) for α-thal deletions was performed with 83 patients (Hb SS or Hb $S\beta^0$), being classified according

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to α-thal genotype: (1) positive α-thal – one or two deletions and (2) negative α-thal – without deletions.

The clinical data were retrospectively collected from patient records, following these criteria to define the events. (1) *cholelithiasis*: The presence of pigmented gallstones with diagnosis was confirmed by ultrasonography (USG) ($n = 77/83$) and/or by cholecystectomy ($n = 38/83$). Additionally, the presence of recurrent acute abdominal pain associated with cholelithiasis was recorded as symptomatic cholelithiasis (26/38). (2) *Cholecystitis*: Any episode of right upper quadrant abdominal pain with fever or positive Murphy's sign [14] ($n = 21/38$) needed hospitalization. (3) *Acute splenic sequestration (ASS)*: Any record of ASS and/or splenectomy. (4) *Hydroxyurea (HU)*: the use of drug therapy is for a minimum period of 12 months ($n = 61/83$ patients). Other clinical events are defined in the Supplementary methods**.**

The samples were collected during outpatient clinic attendance over the study, without previous blood transfusion in the last 3 months (Table 2 and Supplementary Tables 1 and 2). Owing to the large variation of the reference, value of median corpuscular volume (MCV) stated to patient age, we standardized a value of MCV for all patients in relation to the minimum reference value for adults (80 fl). The formula (standard MCV = (MCV observed/MCV minimum expected for the age) × 80) was used to calculate the value of MCV standardization by age. This strategy allowed a homogeneous analysis in different groups of age studied. The MCV minimum expected values for the age were previously described in Vranken et al. [15].

Molecular study

Genomic DNA was extracted from peripheral blood samples. A single tube multiplex-PCR technique was used to determine the common α-thal single gene deletions $-\alpha^{3,7}$ and $-\alpha^{4,2}$ and did not delete α_2 gene, according to Chong et al. [16]. The PCR procedure and primers used in this study are described in the Supplementary methods and Table 1.

Statistical analysis

It was performed using the statistical software package SPSS 21.0 (Chicago, IL, USA). Univariate analysis was assessed using the Mann–Whitney *U*-test and X^2 test for continuous and categorical variables, respectively. The p value < 0.05 was considered significant. Variables with p-value < 0.20 in the univariate analysis were considered to enter in multivariate analysis, performed with three logistic regression analyses considering cholelithiasis, cholecystitis and cholecystectomy as outcomes. Collinearity was evaluated by the Spearman correlation coefficient. The sample (83 patients with phenotypes SS/$S\beta^0$) was used to build the parsimonious model by the backward stepwise method, by the likelihood ratio to select the models in each step.

Results

Analysis of laboratory and clinical parameters according to different α-genotypes and age in SS/$S\beta^0$ patients

Overall, $-\alpha^{3.7}$ deletion was found in 23% ($n = 19/83$) of SS/$S\beta^0$ patients, and $-\alpha^{4.2}$ deletion was not detected. The red blood cell (RBC), Hb and Hb A2 levels were significantly higher in the positive α-thal group than in the negative α-thal group. In contrast, the median corpuscular haemoglobin (MCH), reticulocytes count and indirect bilirubin (BI) levels were significantly lower ($p < 0.05$) in the positive α-thal group when compared with the negative α-thal group (Table 2).

Concerning clinical events, the frequency of cholelithiasis was 61.3% ($n = 47/77$) with 68.4% (26/38) of patients having significant symptoms and 55.2% ($n = 21/38$) progressing to cholecystitis. The frequency of cholecystitis was significantly lower in the positive α-thal group (12.5%, 1/8) than in the negative α-thal group (66.7%, 20/30), with OR = 0.07 [95% CI: 0.01–0.66; $p = 0.01$]. Similarly, cholecystectomy was less frequent in the group with α-thal (15.8%, 3/19) than the other (54.7%, 35/64), with OR = 0.15 (95% CI: 0.04–0.58; $p = 0.006$) (Table 2). In the descriptive analysis, however, the frequency of cholelithiasis (OR = 0.47; 95% CI: 0.16–1.35) was not different between the groups. In addition, this event was significantly more prevalent in patients who use HU (87%, $n = 41/47$, $p = 0.003$). See Table 2 for the descriptive statistics.

Risk evaluation for the outcomes: cholelithiasis, cholecystitis and cholecystectomy

In univariate analysis, we observed that cholelithiasis was associated with Hb levels, packed cell volume (PCV), MCV and HU. On the other hand, the parameters associated with cholecystitis and cholecystectomy were slightly different. These are gender, α-genotype and MCV for cholecystitis and age, gender, α-genotype, Hb, PCV and MCV for cholecystectomy (Table 3).

After performing a collinearity analysis, the parameters Hb, PCV, total bilirubin (BT) and BI, Hb F and MCV were considered redundant and were not included in multivariate analysis. For the logistic regression multivariate modelling, the parameters (gender, HU usage, age and α-genotype) were all initially included in the full controlled model for the evaluation of three outcomes (cholelithiasis, cholecystitis and cholecystectomy). The logistic regression parsimonious model maintained the use of HU, female

Table 1. Sequence, location Genbank® of oligonucleotide and size of the fragments generated in multiplex-PCR for -$\alpha^{3.7}$ and -$\alpha^{4.2}$ deletions.

Primers	5′ → 3′ Sequence	Localization: Genbank®	Size of the fragment
α2/3.7-F	CCCCTCGCCAAGTCCACCC	HUMHBA4:5676 → 5694	Junction of fragment
3.7/20.5-R	AAAGCACTCTAGGGTCCAGCG	HUMHBA4:11514 → 11494	-$\alpha^{3.7}$ (2022/2029 bp)
α2-R	AGACCAGGAAGGGCCGGTG	HUMHBA4:7475 → 7457	gene α_2 (1800 bp)
α2/3.7-F	Above	Above	
4.2-F	GGTTTACCCATGTGGTGCCTC	HUMHBA4:3064 → 3084	Junction of fragment
4.2-R	CCCGTTGGATCTTCTCATTTCCC	HUMHBA4:8942 → 8920	-$\alpha^{4.2}$ (1628 bp)

F= forward primer; R= reverse primer

gender and older age as independent risks for cholelithiasis. The α-genotype (presence of -$\alpha^{3,7}$ deletion) was the only significant variable, being an independent protective factor to cholecystitis. Furthermore, we observed that the use of HU and female gender were risk factors, while the presence of -$\alpha^{3,7}$ deletion was a protective factor for cholecystectomy (Table 4).

Discussion

Several research groups have reported controversial results about the protective effect of α-thal on clinical outcomes in SCD patients [6,17,18]. Such controversies allow approach to new studies in this field. The Brazilian population has a high degree of miscegenation of African inheritance, in consequence a high prevalence of -$\alpha^{3.7}$ deletion [19–22] that permitted us to analyse its impact on concomitant SCD.

In the present study, about 23% of patients with SS/$S\beta^0$ phenotypes were concomitant with α-thal (-$\alpha^{3.7}$ deletion), as expected from the population studied [19,21,23,24]. Concerning haematologic parameters, our data indicated that α-thal concomitance with SCD promoted an increase in the RBC, Hb and HbA2 values, and a decrease in the MCH, percentage of reticulocytes and BI-levels in SS/$S\beta^0$ patients analysed. These results were concordant with previous studies [5,6,24] and could be due to a reduction in the haemolytic rate, reflecting that all parameters are associated with Hb metabolic pathway.

Our data showed that the frequency of cholelithiasis was not affected by -$\alpha^{3.7}$ deletion. In line with these results, two studies found a similar prevalence of cholelithiasis between SCD patients with and without -$\alpha^{3.7}$ deletion [10,12]. In contrast, Vasavda et al. [11] observed a reduced risk of cholelithiasis in the positive α-thal patients. Interestingly, Chaar et al. [10] and Vasavda et al. [11] showed similar effects of the α-thal concomitant with SCD on lower BI-levels, but it was not reflected in the reduction of cholelithiasis in one of them.

The use of HU and older age were predictive of a higher risk of cholelithiasis, probably because the use of HU reflects the clinical severity of the disease as this is the primary indication for its use [25,26]. Older patients also had higher risk for gallstone formation in SCD as described in the previous studies [1,3].

Conversely, -$\alpha^{3.7}$ deletion was a protective factor on the occurrence of cholecystitis in SS/$S\beta^0$ patients. Interestingly, it does not seem to be mediated by the haemolytic rate, because BI and Lactate dehydrogenase (LDH) were not significantly increased in patients with cholecystitis compared to others. One possible explanation for the protective effect of α-thal in the

Table 2. Laboratory parameters and clinical events by α-globin gene status.

Parameters data	Negative α-thal	Positive α-thal	*p* value	OR (CI 95%)
Patients % (*n*/*n*)	77.1 (64/83)	22.9 (19/83)		
Age in years (median range)	21 (5–61)	22 (5–56)	NS	–
Gender, M/F	31/33	9/10	NS	–
RBC ($\times 10^6$ ml^{-1})	**2.3 (1.3–4.3)**	**2.6 (2.3–5.0)**	**0.001**	–
Hb (g dl^{-1})	**7.7 (4.8–11.4)**	**8.3 (6.3–11.3)**	**0.04**	–
PCV (%)	22 (14–33)	24 (18–33)	NS	–
MCV (fl)	95 (64–131)	89 (66–111)	NS	–
MCH (pg)	**33 (19–44)**	**30 (22–38)**	**0.02**	–
Hb F (%)	8.0 (1.1–27.5)	10.1 (1.6–27.6)	NS	–
Hb A_2 (%)	**3.8 (3.1–7.4)**	**4.6 (3.6–5.0)**	**0.001**	–
Reticulocytes count (%)	**5.0 (1.0–14.9)**	**2.5 (0.2–10.8)**	**0.03**	–
BT (mg dl^{-1})	2.9 (0.8–10.9)	1.8 (0.5–5.7)	NS	–
BI (mg dl^{-1})	**2.3 (0.4–10.4)**	**1.2 (0.3–5.3)**	**0.03**	–
LDH (U/L)	1006 (352–2956)	807 (234–1513)	NS	–
Cholelithiasis[a] % (*n*/*n*)	65.5 (38/58)	47.4 (9/19)	NS	0.47 (0.16–1.35)
Cholecystitis[a] % (*n*/*n*)	**66.7 (20/30)**	**12.5 (1/8)**	**0.01**	**0.07 (0.01–0.66)**
Cholecystectomy % (*n*/*n*)	**54.7 (35/64)**	**15.8 (3/19)**	**0.006**	**0.15 (0.04–0.58)**
HU % (*n*/*n*)	76.6 (49/64)	63.2 (12/19)	NS	0.52 (0.17–1.57)
ASS % (*n*/*n*)	21.9 (14/64)	5.3 (1/19)	NS	1.19 (0.24–1.61)
Splenectomy % (*n*/*n*)	17.2 (11/64)	5.3 (1/19)	NS	0.26 (0.03–2.22)

[a]Diagnostic cholelithiasis by USG was available in 77/83 patients. Clinical events cholelithiasis and cholecystitis were observed in 47/77 and 21/38 patients, respectively; *p* value obtained by the Mann–Whitney *U*-test; significant *p* value < 0.05 (in bold); NS = *p* value not significant.

CI (95%), confidence interval 95%; OR, odds ratio; RBC, red cell blood; Hb, haemoglobin; PCV, packed cell volume; MCV, median cell volume; MCH, median cell haemoglobin; ASS, acute splenic sequestration.

Table 3. Univariate analysis of laboratorial parameters with a significant difference between patients with the presence or absence of α-thal deletion.

	Cholelythiasis				Cholecystitis				Cholecystectomy			
		95% CI				95% CI				95% CI		
Variables	OR	Lower	Higher	*p* value	OR	Lower	Higher	*p* value	OR	Lower	Higher	*p* value
Age	1.032	0.993	1.072	0.108	1.019	0.972	1.068	0.430	1.023	0.989	1.058	0.182
Gender	3.529	1.345	9.259	0.010	0.379	0.098	1.461	0.159	2.885	1.176	7.075	0.021
Genotype α	0.474	0.166	1.355	0.163	0.071	0.008	0.663	0.020	0.155	0.041	0.586	0.006
HU	5.225	1.704	16.023	0.004	0.491	0.072	3.343	0.468	0.176	0.053	0.583	0.004
Hb (g dl^{-1})	0.680	0.475	0.972	0.034	0.849	0.509	1.415	0.530	0.646	0.457	0.915	0.014
PCV (%)	0.876	0.776	0.988	0.032	0.928	0.779	1.105	0.402	0.859	0.764	0.967	0.012
MCV (fl)	1.037	0.999	1.077	0.056	1.051	0.994	1.112	0.083	1.041	1.005	1.078	0.023
Hb F (%)	1.031	0.968	1.099	0.344	1.004	0.927	1.087	0.992	1.009	0.952	1.068	0.770
BT (mg dl^{-1})	1.061	0.829	1.359	0.636	1.279	0.861	1.899	0.223	1.143	0.900	1.450	0.273
BI (mg dl^{-1})	1.050	0.813	1.356	0.709	1.279	0.851	1.922	0.236	1.129	0.952	1.068	0.770

Significance *p* value < 0.20.
OR = odds ratio; CI (95%) = confidence interval 95%; RBC, red cell blood; Hb, haemoglobin; PCV, Packed cell volume; MCV, median cell volume; BT, bilirubin total; BI, bilirubin indirect.

risk of cholecystitis is the preservation of splenic function in patients with concomitant α-thal , once the presence of -$\alpha^{3.7}$ deletion can lead to a delay in polymerization of Hb S and reduce the risk of spleen infarction [27–29]. This hypothesis is in line with our findings about the relationship between decreased MCV, MCH and cholecystitis.

Furthermore, -$\alpha^{3.7}$ deletion was also protective to cholecystectomy in SS/Sβ^0 patients, which we attributed to the low frequency of cholecystitis in this group and undergoing surgical treatment.

To the best of our knowledge, the present study is the first to describe the protective effect of -$\alpha^{3,7}$ deletion on cholecystitis and cholecystectomy occurrence in SS/Sβ^0 patients. Furthermore, studies with a prospective cohort are necessary to evaluate other genetic modifiers (e.g. *UGT1A1* polymorphism) and/or concomitantly with α-thal in SCD patients. Finally, the incorporation of α-thal detection in the clinic routine appears to be important to SCD patient evaluation, especially in services that use cholecystectomy prophylactically, once such a procedure could be avoided in α-thal/SCD patients.

Geolocation information: Sickle cell disease in Brazilian patients.

Table 4. Multivariate analysis for risk of cholelithiasis cholecystitis, and cholecystectomy in patients SS/Sβ^0 ($n = 83$), using a logistic regression model.

	OR (Exp(*B*))	95% CI	*p* Value
Cholelithiasis			
HU	8.38	2.20–31.65	**0.002**
Gender (female)	5.56	1.74–17.77	**0.004**
Age	1.04	0.99–1.08	0.093
Cholecystitis			
Genotype α (deletion -$\alpha^{3.7)}$	0.07	0.01–0.66	**0.020**
Cholecystectomy			
HU	7.29	1.92–27.64	**0.003**
Genotype α (deletion $\alpha^{3.7}$)	0.14	0.03– 0.60	**0.008**
Gender (female)	4.42	1.51–12.86	**0.006**

Significance *p* value < 0.05. Intercept not shown.
OR, odds ratio; CI (95%), confidence interval 95%.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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