Impaired gallbladder motility and delayed orocecal transit contribute to pigment gallstone and biliary sludge formation in β-thalassemia major adults

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Abstract
AIM: Gallbladder and gastrointestinal motility defects exist in gallstone patients and to a lesser extent in pigment gallstone patients. To investigate the role of gallbladder and gastrointestinal motility disorders in pigment gallstone formation in β-thalassemia major.

METHODS: Twenty-three patients with β-thalassemia major (16 females; age range 18-37 years) and 70 controls (47 females, age range 18-40 years) were studied for gallbladder and gastric emptying (functional ultrasonography), orocecal transit (OCTT, H2-breath test), autonomic dysfunction (sweat-spot, cardiorespiratory reflex tests), bowel habits, gastrointestinal symptoms and quality of life (all with questionnaires). Gallbladder content (ultrasonography) was examined before and during 8-12 mo follow-up.

RESULTS: Gallstones and/or biliary sludge were found in 13 (56%) patients. β-thalassemia major patients had increased fasting (38.0±4.6 mL vs 20.3±0.7 mL, P = 0.0001) and residual (7.9±1.3 mL vs 5.1±0.3 mL, P = 0.002) volume, and slightly slower emptying (24.9±1.7 min vs 20.1±0.7 min, P = 0.04) of the gallbladder, together with longer OCTT (132.2±7.8 min vs 99.7±2.3 min, P = 0.00003) than controls. No differences in gastric emptying and bowel habits were found. Also, patients had higher dyspepsia (score: 6.7±1.2 vs 4.9±0.2, P = 0.027), greater appetite (P = 0.000004) and lower health perception (P = 0.00002) than controls. Autonomic dysfunction was diagnosed in 52% of patients (positive tests: 76.2% and 66.7% for parasympathetic and sympathetic involvement, respectively). Patients developing sludge during follow-up (38%, 2 with prior stones) had increased fasting and residual gallbladder volume.

CONCLUSION: Adult β-thalassemia major patients have gallbladder dysmotility associated with delayed small intestinal transit and autonomic dysfunction. These abnormalities apparently contribute together with haemolytic hyperbilirubinemia to the pathogenesis of pigment gallstones/sludge in β-thalassemia major.

INTRODUCTION
Patients with cholesterol gallstones have impaired gallbladder emptying[1] and may show dyspeptic symptoms with functional defects of both upper and lower gastrointestinal tract[2-4]. Recently, we reported that gallbladder emptying was also defective in patients with black pigment stones and such defect was less severe than in patients with cholesterol stones[5].

β-thalassemia is one of the most widespread single-gene disorders with 3-6% of the world’s population carrying the gene. The disease represents a major public health problem in the Mediterranean area, the Middle East, the India subcontinent and the Far East[6]. Increased production of bilirubin from chronic hemolysis is a prerequisite for formation of pigment gallstones[6,7], and black pigment gallstones often accompany thalassemia major[8]. However, despite similar biochemical and clinical features, many β-thalassemia major patients with marked hemolysis did not develop gallstones. One might hypothesize that gallbladder stasis and functional gastrointestinal disorders could contribute to gallstone pathogenesis in β-thalassemia major. In the present study, we investigated for the first time the role of gallbladder and gastrointestinal motility in adult β-thalassemia major patients in relation to gallstone/sludge formation. Autonomic neuropathy and gastrointestinal symptoms were also evaluated.

MATERIALS AND METHODS
Subjects
β-thalassemia major patients (n = 23) Age 26±1 years (mean±SE, range 18-37 years), body mass index (BMI) of 21.4±0.6 kg/m². Sixteen were women. Patients attended regular review from October 2001 until June 2003 at the Referral Center of the University Hospital of Bari. All the patients were homozygous for a mutation β° or β° or double heterozygous for a mutation β° or β°. The genetic characteristics of the patients seen in Bari have been previously reported[9]. As β-thalassemia major requires regular blood transfusions and chelating therapy to alleviate the harmful accumulation of iron, all patients were on a program consisting of one or two monthly blood transfusion of packed red blood cells and desferrioxamine given as 40-60 mg/(kg·d) subcutaneously overnight using syringe pumps. The program...
lasted for 5 nights per week. Mean length of desferrioxamine treatment was 19.2±0.6 years. All patients had various stages of liver involvement, as confirmed by ultrasonography (e.g., liver steatosis, hyperechoic parenchyma) and/or liver biopsy (ranging from active/chronic siderotic and fibrotic hepatitis to definite liver cirrhosis, n = 1). The analysis of organ involvement confirmed that splenomegaly, hypothyroidism, and heart disease were the most frequent conditions with a prevalence of 39%, 35% and 18%, respectively. Hypogonadism was present in 35% of the patients. Type 1 insulin-dependent diabetes and a positive oral glucose tolerance test were present in 9% and 22% of the patients, respectively.

**Healthy subjects (n = 70)** Age 28±1 years (range 18-40 years), BMI 22.0±0.3 kg/m². Forty-seven were women. They were recruited from local staff members, students, and family practices. None of the healthy subjects complained of gastrointestinal symptoms or had previous gastrointestinal diseases or surgery. All had a negative abdominal ultrasound.

No significant difference existed in age, BMI and gender distribution between the patients and the normal controls. As expected[9], patients had shorter stature than their matched controls (males: 168±2 cm vs 178±7 cm, P = 0.0006 and females: 158±0.01 cm vs 167±6 cm, P = 0.00005). All subjects gave their informed consent and the study was approved by the University of Bari Human Subjects Committee.

**Study design**

After the outpatient clinic evaluation consisting of history, physical examination, and serum analyses, subjects were scheduled for the motility studies and tests for autonomic neuropathy. A clinical and ultrasonographic follow-up was planned within the next 8-12 mo. Ultrasonography was chosen because it was a non-invasive and validated technique allowing to study both gallbladder[1,10] and gastric[11-16] emptying, simultaneously[17]. We also developed a novel, one-day test for studying the upper gastrointestinal motility by simultaneous assessment of gallbladder, stomach, and small bowel transit[18].

**Gallbladder and gastric emptying**

The gallbladder was studied for content, wall, shape, and motility with standardized methodology[17]. Gallstones were diagnosed by the presence of mobile high-level echoes with acoustic shadows in the gallbladder, and sludge by the presence of mildly echogenic intraluminal sediment in the absence of acoustic shadows[19]. All gallstone patients had a small gallstone burden (i.e., less or equal to 20% of fasting gallbladder volume[20] and a thin gallbladder wall (i.e., less than 3 mm in the fasting state) delimiting a regularly pear-shaped organ[17]. Gallbladder motility was assessed by monitoring gallbladder volumes before and at 5-15 min intervals over 2 h after ingestion of a liquid test meal.

Gastric emptying was assessed by monitoring antral areas at the same time points as for the gallbladder[11,13]. The equipment consisted of an Esaote AUC50 equipped with a 3.5 MHz convex probe. The test meal consisted of 200 mL liquid formula containing fat 11.6 g (35%), protein 12 g (16%) and carbohydrate 36.8 g (49%) with a total of 300 kcal, 1 270 kJ, 445 mOsm/L (Nutridrink®, Nutricia S.p.A., Lainate Milano, Italy).

**Orocecal transit time**

Orocecal transit time was measured at the time when ultrasonographic studies were performed by the hydrogen breath test with a portable, previously validated device (EC60-Gastrolyzer, Bedfont, USA)[18,21]. The substrate consisted of 10 g lactulose (Duphalac Dry®, Solvay Pharma, Belgium) which was added directly to the standard liquid test meal. The accuracy of the detector was±2 p.p.m. A rise of 10 p.p.m. above baseline on two consecutive measurements was considered as orocecal transit time and expressed in minute[22].

**Autonomic neuropathy**

Symptoms and signs of autonomic dysfunction were evaluated by taking the clinical history according to Rangari et al.[23]. The questionnaire was scored as “normal”, “mild” and “severe” for the presence of orthostatic hypotension, gastric symptoms, bowel disorders, sweating disorders, bladder dysfunction and in males, impotence. A combination of tests including the Sweat-spot-test (SST)[22,24,25] and cardiorespiratory reflex tests[26], were employed as sensitive methods to assess the presence of autonomic neuropathy[27]. The SST was used to investigate the involvement of cholinergic sympathetic fibers by analyzing sweat abnormalities on the dorsum of the foot. An example of positive and negative test is shown in Figure 1.

A portable device was used to measure the “beat-to-beat” modifications of the R-R interval using skin electrodes connected to Cardionomic® (Lifescan, Italy). Lying-to-standing and standing-to-lying tests were used for sympathetic involvement. Valsalva maneuver, deep-breathing, cough test, and postural hypotension test were used for parasympathetic involvement.

**Figure 1** Sweat spot test (SST) for assessment of sympathetic autonomic nervous system[22,24,25]. The skin is coated with iodine and a fine emulsion of starch in arachis oil. Sweat is stimulated by intra-dermal injection of 0.1 mL acetylcholine (the red dot indicates the point of injection). Denervated glands do not respond to the acetylcholine injection. A colorimetric reaction between starch and iodine is triggered by the sweat from stimulated glands, so that each pore appears as a small black dot after 2-5 min. A digital photo is taken and transferred to a magnifying software to measure the number and distribution of dots appearing in a standard squared grid of 529 mm² divided into 64 squared subareas. A normal SST implied a score 12 dots/subarea and/or <8% of abnormal subareas (each square of the grid having less than 6 dots) according to Ryder[24] and to our group[25]. Only patients with both indices (SST score and % abnormal subareas) outside normal limits were considered to have a positive test. A: Normal and even distribution of sweating glands seen as black dots; B: Defective response in a β-thalassemia major patient with sympathetic autonomic neuropathy.
involvement. The results of each test were scored as normal, borderline or abnormal, according to age-controlled values. The overall results for autonomic neuropathy were therefore normal (all tests normal), early involvement (one abnormal test or two borderline abnormal), definite involvement (two or more abnormal tests).[23]

**Questionnaires**

On the same day of the motility studies, subjects were asked to complete the following questionnaires under the supervision of one operator.

**Bristol stool scale form[28]** This is a semi-quantitative score to assess the quality of bowel movements. The weekly frequency of evacuations over a time span of one mo was also assessed.

**Dyspepsia score[29]** This is a semi-quantitative score from four symptoms such as epigastric pain, burning, belching/burping, postprandial fullness. Maximum score was equal to 48 with the upper normal limit equal to 8 and estimated from the mean±2SD of healthy control values.

**Visual analogue scales** (VAS) of upper gastrointestinal perception[28,30]. These are self-assessed 100 mm horizontal lines of upper gastrointestinal perception monitoring appetite, satiety, nausea, abdominal fullness and upper abdominal (epigastric) pain or discomfort. Scores in mm were obtained at baseline (i.e. time 0) and at 15, 30, 45, 60, 90 and 120 min postprandially.

**Rome criteria for biliary pain[31]** Patients with gallstones or sludge were considered “symptomatic” with a history of one or more episodes of colicky pain during the last 12 mo.

**Health survey SF-36[32,33,34]** This is a short form of questionnaires assessing health-related quality of life (HRQOL). The SF36 includes 36 items which measure eight multi-item variables: general health, physical function, role physical, role emotional, social function, mental health, body pain, and vitality. Healthy subjects in this setting had a HRQOL profile remarkably similar to that derived from subjects across different cultures in USA and UK.[34]

**Statistical analysis**

Results are expressed as mean±SE. Demographic data, as well as baseline characteristics were checked for normal distribution (Kolmogorov-Smirnov goodness of fit test). Statistical significance in contingency tables was evaluated using χ²-test or Fisher’s exact test when appropriate. Comparison of continuous variables among groups was performed with unpaired Student’s t-test or Mann-Whitney rank sum test when appropriate. Comparison between multiple groups was assessed using one-way analysis of variance (ANOVA) or the Kruskall–Wallis non parametric ANOVA on ranks. Post-hoc multiple pair wise comparisons were calculated with the Fisher’s LSD test. Linear regression analysis was performed by the method of least square and Pearson’s coefficient. A multivariate analysis was constructed to study the effect on appetite of motility variables. The variables showing significant correlations in univariate analysis were included in a stepwise multiple regression analysis. All statistical calculations were performed with the NCSS2004 software (Kaysville, UT, USA). Statistical tests were conducted as a two-sided alpha level of 0.05.[35,36]

**RESULTS**

Gallbladder sludge and stones were found in 13 (56%) patients, all asymptomatic for previous episodes of biliary pain. Sludge was detected in 6 (26%) patients, while gallstones were observed in 7 (30%) patients, solitary and small in size (5-9 mm). The other 10 patients (44%) were gallstone/sludge-free.

**Routine biochemical analysis**

There was no difference in laboratory biochemistry among the three subgroups of thalassemia patients, according to gallbladder content (Table 1). There was a statistically significant relationship between levels of ferritin and AST and ALT in the serum (0.73<r<0.77; P>0.00002, P<0.00007).

**Table 1** Laboratory biochemistry in β-thalassemia major patients with gallstones, biliary sludge and gallstone-free

|                      | Gallstones | Sludge | Gallstone/ | P     |
|----------------------|------------|--------|------------|-------|
| No. of subjects      | 7          | 6      | 10         |       |
| Total bilirubin (mg/dL) | 2.3±0.4   | 1.4±0.4 | 1.8±0.3    | NS    |
| AST (U/L)            | 50.9±11.5 | 32.8±12.5 | 44.9±9.7 | NS    |
| ALT (U/L)            | 76.7±17.7 | 42.7±19.1 | 59.8±14.8 | NS    |
| Ferritin (mg/dL)     | 1 922±445 | 1 165±481 | 1 900±372 | NS    |
| Hb (g/dL)            | 9.3±0.1   | 9.5±0.2 | 9.5±0.1    | NS    |
| Iron (mg/dL)         | 186.8±16.8 | 189.6±8.4 | 156.5±16.8 | NS    |
| Total proteins (g/dL)| 7.8±0.2   | 7.9±0.2 | 7.4±0.2    | NS    |
| GGT (U/L)            | 34.4±5.6  | 18.3±6.0 | 19.4±4.7  | NS    |
| ALK Phosphatase (U/L)| 1.15±0.2  | 0.98±0.2 | 0.9±0.2    | NS    |
| LDH (U/L)            | 331.3±46.7 | 293.7±56.7 | 310.7±28.4 | NS    |
| Cholesterol (mg/dL)  | 98.3±10.2 | 129.4±12.1 | 96.9±9.0  | NS    |
| Tryglicerides (mg/dL)| 114.9±23.5 | 71.6±27.9 | 84.4±22.0  | NS    |

Data are expressed as mean±SE; NS: Not significant; AST: Aspartate transaminase; ALT: Alanine transaminase; GGT: Gamma-glutamyl transeptidase; LDH: Lactate dehydrogenase.

**Ultrasonographic studies**

The study of gallbladder emptying showed that β-thalassemia major patients had increased fasting gallbladder volume (38.0±4.8 mL vs 20.3±0.7 mL, P = 0.0001), and residual volume (7.9±1.3 mL vs 5.1±0.3 mL, P = 0.002), decreased percent residual volume (20.3±1.5% vs 24.6±1.0%, P = 0.03) and slightly slower emptying (24.9±1.7 min vs 20.1±0.7 min, P = 0.04) than controls, as also suggested by the analysis of the gallbladder emptying curves (Figure 2).

Although all subgroups compared to controls had a significantly greater fasting gallbladder volume, only patients with biliary sludge and normal gallbladder had increased residual volume and delayed emptying. Indeed, relative gallbladder contraction (percent residual volume) was increased in patients with gallstones. β-thalassemia patients and controls showed comparable fasting and postprandial indices of gastric emptying (Table 2).

**Orocecal transit time**

Mean OCTT was longer in β-thalassemia patients than in controls (132.2±7.8 min vs 99.7±2.3 min, P = 0.00003) (Figure 3). Seven patients (30.4%) but none of controls had OCTT above the upper normal value of 140 min (i.e. mean±2SD) derived from the control group (P = 0.000026, χ²-test). Similar results were found among different subgroups (Table 2).

**Autonomic neuropathy**

Twenty-one patients agreed to undergo the tests. Symptoms and signs of autonomic dysfunction were found in 11 patients (52%), which were mild in all but 2 cases (asymptomatic). Early or definitive parasympathetic and sympathetic involvement was present in 76.2% and 66.7% of cases, respectively. Early involvement was more frequent in the cases of parasympathetic AN system than those of sympathetic AN system (81.3% vs 28.6%, P = 0.00037). Overall, there were 3 (14%), 5 (24%), and 13 (62%) patients with normal tests, early involvement (mainly parasympathetic), and definitive involvement (both parasympathetic and sympathetic), respectively. All 5 patients with type I insulin-dependent diabetes or impaired oral glucose tolerance test and all 7 patients with thyroid involvement had
Orocecal transit time (OCTT) by lactulose H2-breath test in β-thalassemia major patients and controls. Patients had significantly longer OCTT than controls. Data are expressed as individual points and means (bars).

Questionnaires
Overall, bowel habits were comparable in controls and patients (overall: 3.5±0.01 vs 3.6±0.2, respectively), while the score for dyspepsia was slightly higher (and statistically significant) in patients than in controls (6.7±1.2 vs 6.1±0.9, P=0.0001) (Table 2).

The results of VAS for satiety and appetite (as postprandial OCR) showed that the two feeling feelings were strongly and negatively correlated in both patients (r=-0.87, P<0.0001 n=3) and compared to controls (r=-0.03, P=0.0001, n=70). Profiles for both appetite and satiety, however, were different in patients and controls. Fasting and postprandial appetite scores were invariably greater in patients than in controls. In particular, VAS for appetite was increased at baseline and showed a rapid decrease followed by a rapid and marked postprandial increase, compared to controls (Figure 4). Whereas univariate analysis suggested an inverse relation between appetite and OCTT, gallbladder contraction (as residual volume in percent) and gallbladder half emptying time, multiple regression analysis identified gallbladder half-emptying time as the only predictor (P<0.002) of time-related changes of appetite perception in patients. The relationship is shown in Figure 5.

There was no difference in nausea, fullness and abdominal pain between patients and controls either at baseline or postprandially (data not shown).
The follow up was completed after 8-12 mo in 16 out of 23 (69.6%) major patients. All other domains were similar between the two groups.

**Follow-up**

The follow up was completed after 8-12 mo in 16 out of 23 (69.6%) patients (2 patients were lost at follow-up because one died and the other moved to a different city). All patients remained asymptomatic for biliary symptoms. Gallbladder ultrasound was utilized to identify three subgroups of patients according to ultrasonographic appearance of the gallbladder during 8-12 mo follow-up.

**Quality of life**

Patients showed a significant deterioration in general health and a lower score than controls (51.7±4 vs 73.1±2, P = 0.00002, ANOVA). All other domains were similar between the two groups.

**Table 3** Prior gallbladder motility indices in β-thalassemia major patients according to ultrasonographic appearance of the gallbladder during 8-12 mo follow-up

|                      | Still anechoic | Developed sludge | Prior sludge/ gallstones |
|----------------------|---------------|------------------|--------------------------|
| No. of subjects      | 5             | 4                | 7                        |
| Fasting volume (ml)  | 29.2±7.3      | 60±17*           | 29.2±4.2                 |
| (ml), basal          |               |                  |                          |
| Residual volume (ml) | 5.5±1.4       | 14.7±4.6*        | 4.9±0.9                  |
| (ml), basal          |               |                  |                          |
| Fasting volume       | 30.9±6.5      | 72.8±20.1*       | 36.7±5.1                 |
| at follow-up (ml)    |               |                  |                          |

Data are expressed as mean±SE; *P<0.017 vs still anechoic group; †P<0.05 vs prior sludge/gallstones group (ANOVA and Fisher’s LSD multiple comparison test).

Variations in individual fasting gallbladder volumes for each subgroup are depicted in Figure 6. Major changes were evident in patients developing biliary sludge (panel A), also when gallbladder volume was expressed as percent increase (panel B). Interestingly, maximum percent increase in fasting gallbladder volume was observed in 2 patients (89% and 130%) who developed sludge in a gallbladder which previously contained only stones and showed normal fasting volume (i.e. <cut-off value of 32 mL). These two patients had clinical evidence of diabetes mellitus (and autonomic neuropathy) and hypothyroidism.

Furthermore, patients who developed sludge during follow-up had also significant changes in postprandial gallbladder volume (Table 3), with a trend towards decreased gallbladder contraction (residual volume: 25.6±4.7% vs 19.5±2.8% vs 17.9±3.0%), and longer transit time (OCTT: 150±15.8 vs 108±9.7 vs 126±12.9 min) when compared with the other two subgroups.

**Figure 4** Time-course of visual analogue scale (VAS) for appetite and satiety in β-thalassemia major patients and controls. Data are mean±SE. On the X-axis time “0” is before ingestion of test meal. Asterisks indicate significant differences of controls vs patients (0.001<P<0.001, at various time-points and overall ANOVA).

**Figure 5** Correlation between appetite sensation (area under curve during 120 min of visual analogues scale) and speed of gallbladder emptying (half-emptying time) in β-thalassemia major patients.

**Figure 6** Variations in individual fasting gallbladder volumes for each subgroup in β-thalassemia major patients. A: Major changes in patients who developed biliary sludge: asterisks indicate significant differences, compared to the other two groups of patients (*P<0.05 vs prior sludge/gallstones group and †P<0.017 vs still anechoic group). B: Percent increased fasting gallbladder volume in the three groups of patients. The last bar represents those gallbladders with stones that developed additional sludge (n = 2).
DISCUSSION

The present study examined the role of gallbladder and gastrointestinal motility in a well-characterized group of adult β-thalassemia major patients in their 3rd decade of life, in relation with the presence of gallstones/biliary sludge. The results suggested the coexistence of impaired gallbladder motility, delayed small intestinal transit, and high prevalence of autonomic neuropathy in β-thalassemia major patients. These abnormalities might contribute, together with haemolytic hyperbilirubinemia to the pathogenesis of gallstones/sludge in β-thalassemia major.

As for other hemolytic disorders including sickle hemoglobinopathy[37], both hyperbilirubinemia and bilirubin overload/precipitation in bile were definite predisposing factors for pigment gallstone in β-thalassemia major patients during chronic hemolysis[6]. Indeed, in this study gallstones and/or biliary sludge were found in 56% of β-thalassemia major patients at entry, increasing to over 80% during follow-up (8-12 mo). A previous study[7] in young patients reported a prevalence of 11.8% and 29.4% for gallstones and sludge, respectively. However, the mean age of β-thalassemia major patients in the present series was 26 years, while it was 12 years in the above mentioned study.

Several pathways might be involved in the pathogenesis of sludge and pigment stones in β-thalassemia. Glucuronidation of excess bilirubin was a predisposing factor in chronic haemolitic diseases[68]. After injection of desferoxamine, both fecal and urinary excretion were observed and there was formation of iron-desferoxamine complex[69] which might potentially interfere with the gallbladder microenvironment, already enriched in calcium bilirubinate. Moreover, increased secretion of glycoproteins by epithelial cells was seen after iron-induced stimulation in the guinea pig gallbladder[49], and this might also take place in β-thalassemia major patients who developed sludge. However, since not all β-thalassemia patients with haemolytic hyperbilirubinemia had gallstones, other pathogenetic mechanisms underlying gallstone formation in β-thalassemia major may play a role.

The present study for the first time suggested that bile stasis and intestinal dysmotility might be key factors in the pathogenesis of pigment gallstones in β-thalassemia major. Pigment stones were common in patients with cirrhosis[41,42], chronic hemolysis[73,43,44], ileal Crohn’s disease[45], and conditions associated with impaired gallbladder kinetics[37,44,46-48]. A recent study by our group found a certain degree of impaired motorfunction of the gallbladder also in non-hemolytic pigment stone patients[51]. Compared with controls, there was about 100% increase of fasting gallbladder volume across 3 β-thalassemia major subgroups: i.e. patients with stones, sludge and stone-sludge free. Increased fasting gallbladder volume seems to be common in hemolytic conditions leading to pigment stones. Everson et al. found a volume of about 27 mL in adolescents and young adults with sickle hemoglobinopathy[71]. Nevertheless, we found that adult “symptomatic” pigment (non-hemolytic) stone patients had a fasting volume comparable to controls[2]. In general, fasting gallbladder volume increased with body size[49] and obesity[49,50]. In this respect, the finding of increased fasting gallbladder volume was even more striking if one considered that β-thalassemia major patients had reduced growth with short stature, compared with matched controls[9]. The increment in fasting gallbladder volume in β-thalassemia major, therefore, represents a true phenomenon and seems to point to defective interprandial (fasting) gallbladder motility. It has been found that fluctuations in fasting gallbladder volume (leading to 20-30% contraction) are normally synchronized with the intestinal migrating motor complex (MMC) and release of the intestinal hormone motilin[31]. Patients with cholesterol gallstones had frequently increased fasting gallbladder volume[41] with abnormal MMC and motilin release pattern. Interdigestive gallbladder emptying was reduced, contributing to gallstone formation[52,53].

A similar derangement of fasting gallbladder motility might also develop in β-thalassemia major patients, and contribute to stasis and sludge/stone formation. A mechanical effect on fasting volume due to the physical presence of gallstones in the gallbladder lumen in β-thalassemia major was unlikely since fasting volume was larger in patients with sludge than in those with stones and also greater in patients without gallstone/sludge than in controls. Also, patients were all asymptomatic for biliary colicky pain (excluding an impacted stone within the cystic duct and/or acute cholecystitis). Our data indicate that postprandial gallbladder motility in β-thalassemia major patients is deranged. Not only fasting, but also residual volume was increased and emptying speed was somewhat slower (although still in the normal range) in sludge or gallstone/sludge-free patients, compared to both patients with gallstones and controls. The large residual gallbladder volume might be the consequence of a greater starting fasting volume[3,36,54,59]. By contrast, we found that “crude” contraction was preserved (i.e. similar or even smaller percent residual volume than in controls, Table 2). Thus, findings in the present study mimicked those seen in sickle hemoglobinopathy[37], with a smaller contraction index characteristic of β-thalassemia major patients (without mentioning gallbladder emptying speed). Differences in patients’ characteristics, age and study protocol might partly account for the discrepancies across studies. Why in our study those patients with gallstones had better gallbladder contraction than controls, remains to be elucidated. Prospective observations are needed to see if, at equilibrium (i.e. after stone have formed), the gallbladder adapts to a smaller fasting volume and therefore better contraction (smaller residual volume). According to Laplace’s law, in fact, the smaller the radius of a given shape (i.e. the gallbladder), the smaller the wall tension (from smooth muscle contraction) required to withstand a given internal fluid pressure[59]. It is highly unlikely that release of endogenous cholecystokinin was deranged in this series of pigment gallstone patients, as seen during total parenteral nutrition[60]. Whereas in cholesterol gallstone patients excess biliary cholesterol might alter cholecystokinin smooth muscle receptors[61,62], resulting in secondary impaired gallbladder motility. This possibility has been ruled out in β-thalassemia major patients. In fact, biliary cholesterol saturation did not increase in pigment stone patients and gallbladder smooth muscle contractility in vitro was normal[23]. We also found that OCCT was slightly delayed in β-thalassemia major patients, evidently across all subgroups. A clear explanation of the finding is not readily available, but a condition secondary to bacterial overgrowth or delayed colonic transit was highly unlikely, since there was no evidence of bacterial translocation with H₂-breath test and all patients had normal weekly bowel habits and a stool scale form. Small bowel dysmotility might accompany abnormal intestinal migrating motor complexes, as seen in a subgroup of patients with irritable bowel[64] and functional dyspepsia[65]. Also, intestinal mucosal changes have been described in β-thalassemia major patients[66] and might influence some motility patterns in the small bowel. Others have found that small bowel inflammation and/or malabsorption are not more frequent in β-thalassemia major patients[67]. Patients with ileal disease, bypass, or resection were at increased risk for developing pigment gallstones, so were patients with ileal Crohn’s disease. Increased bilirubin levels in bile of patients with Crohn disease were caused by lack of functional ileum[45]. One might speculate that delayed OCCT in β-thalassemia major patients might lead to increase of enterohepatic cycling of bilirubin[68], therefore predisposing to pigment gallstone formation.

Autonomic neuropathy might be associated with gastrointestinal dysmotility. As expected, all β-thalassemia major patients with diabetes or abnormal oral glucose tolerance test had impaired tests for autonomic neuropathy (i.e. 23% of all patients).
Surprisingly abnormal tests of sympathetic and parasympathetic system were found in 86% of the patients. Potential causes for autonomic neuropathy in β-thalassemia major patients were the coexistence of diabetes mellitus, neurotoxicity of desferroxamine ad iron overload (or both)\textsuperscript{69,70} (for example, visual and auditory neurotoxicity were found in 1 patient of this series). The presence of hemolysis might also contribute to neuropathy, as abnormal autonomic cardiovascular response was described in patients with sickle cell anemia\textsuperscript{71}. Last but not least, the presence of autonomic dysfunction was also a feature in patients with different types and stages of chronic liver disease,\textsuperscript{72} and this might also the case in chronic liver disease of β-thalassemia major patients. Further studies are needed to clarify if the presence of autonomic dysfunction is a useful tool for the prediction of prognosis and outcome of patients with β-thalassemia major.

Positive tests for autonomic neuropathy tended to relate to abnormal gallbladder and small bowel motility. Interestingly, we found delayed OCTT with increased bowel movements in chronic alcoholic patients during abstinence, was associated with dysfunction of autonomic nervous system. A form of subclinical autonomic neuropathy might predispose to a diffuse disorder of smooth muscle, suggesting the multi-organic involvement of the gastrointestinal tract. In the determination of altered OCTT a possible role for dysfunction of autonomic nervous system has been suggested in other series of patients\textsuperscript{73} and might deserve further investigations also in β-thalassemia major patients.

In conclusion, the present study shows for the first time that in β-thalassemia major patients in the 3rd decade of life, increased volume and deranged motility of the gallbladder are associated with delayed small intestinal transit and mild dyspeptic symptoms. Such functional abnormalities apparently contribute together with haemolytic hyperbilirubinemia to the pathogenesis of gallstones/slugde in β-thalassemia major.

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Edited by Wang XL. Proofread by Chen WW and Xu FM.