Different tissue sensitivity to iron induced toxicity defines the spectrum of complications in Romanian β-thalassemia major patients

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BACKGROUND

Treatment of patients with β-thalassemia major has improved significantly over the past 40 years, expanding their life expectancy. However Romanian thalassemics gained widespread access to deferoxamine chelation therapy only in the last decade, which exposed them to an increased risk of significant iron overload complications, from obligatory lifelong transfusions of packed red blood cells. Transfusional therapy raised also the prevalence of bloodborne infections in this group. We tried to characterize these complications in the probably largest cohort of Romanian β-thalassemia major patients assembled to date, to identify ways of improving their management, through better allocation of resources. ❑

MATERIALS AND METHODS

We followed prospectively 90 patients, receiving standard treatment at the National Institute of Transfusional Hematology between January, 1999 and August, 2008. Regular transfusions were offered for a recommended pretransfusional hemoglobin level of 9 g/dl and subcutaneous deferoxamine was indicated to all patients for a target serum ferritin level of 1000 ng/ml. Serum ferritin was monitored every four to six months. All patients were serologically tested for the most frequent transfusion transmitted infections (HCV, HBV, HIV 1

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and 2, and HTLV). Since 2003 the entire cohort was systematically assessed for endocrinopathies in the Endocrinology Ward of Elias Emergency University Hospital. Subjects with symptoms of heart disease or electrocardiogram changes were offered a cardiologist consultation. Liver tests were performed every three months, with abdominal ultrasonographical evaluation at least once during the follow up period.

Interactions between gender, birth cohort and ferritin levels and their effect on complications development were defined.

Data were analyzed using SPSS 10.0 statistical software for Windows. Associations between categorical variables were tested by the Fisher exact test. Differences of medians for continuous measures were tested by exact Wilcoxon-Mann-Whitney rank sum tests. Significance was accepted for an alpha of .05 for 2-tailed tests. ❑

RESULTS

The study included 90 patients with β-thalassemia major; mean age at the end of the follow-up period was 23 years (range, 11-40 years) and mean age at first transfusion was 16 months (range, 2-72 months). Males and females were equally represented, their distribution being similar in the different birth cohorts.

All but one patient who initiated transfusions before 1996 tested positive for anti-HCV (81 out of 90 patients representing 90%), out of which 45 had detectable HCV-RNA (55.55%). Cured or chronic HBV infection prevalence was 5%. HIV infection was absent in all patients, whereas HTLV infection was documented in 3 (FIGURE 1).

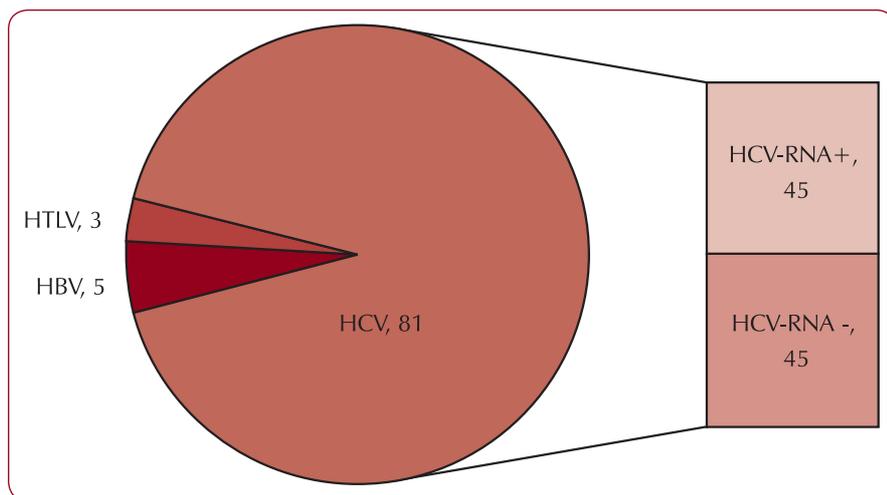FIGURE 2 illustrates the most frequently encountered complications.
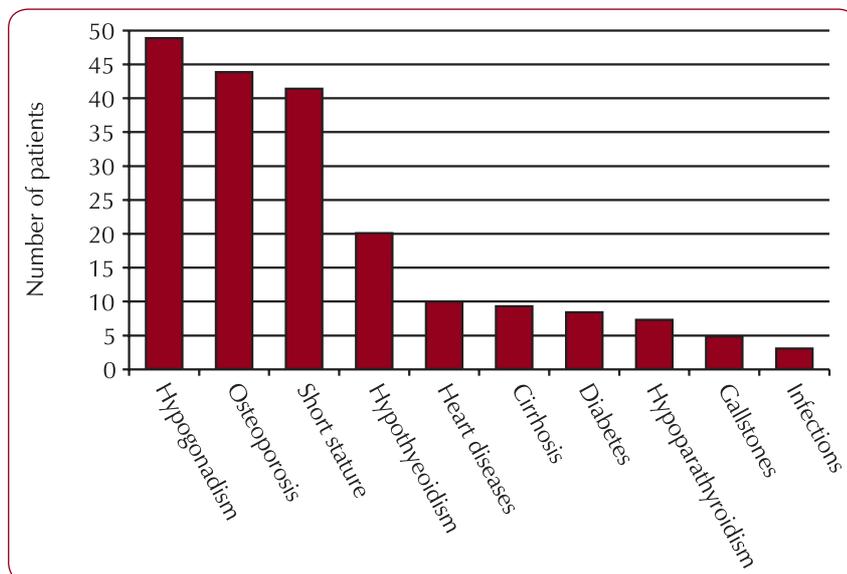

FIGURE 1. Blood born infections

FIGURE 2. β-thalassemia major complications

10 individuals were considered to have advanced liver disease based on clinical, biochemical and ultrasonographic criteria. They were significantly older then the others (28.7 versus 22.56 years; p=0.002), none younger than 25. Seven out of 10 were males, though this was not statistically significant. Only one patient had serum ferritin under 2000 ng/ml (probably because of better compliance throughout the study period), while the rest presented considerable higher ferritin levels compared to other thalassemics (4094.5 compared to 2705 ng/ml; p=0.021). All cirrhotic patients had positive HCV serology, 9 out of 10 having also detectable HCV-RNA (p=0.019), with higher viraemia levels than the rest of chronic HCV infected individuals (p=0.049). Infection duration (calculated as the difference between the year of virological testing and that of the first transfusion), was considerably longer in cirrhotic than in other viraemic patients (24.44 versus 18.61; p=0.015).

Twelve thalassemics presented signs or symptoms of heart disease (13.33%). Nine were symptomatic, with heart failure in 5 and various arrhythmias in 4, including supraventricular and ventricular ectopic beats, supraventricular tachycardia through reentry, atrial fibrillation and sustained ventricular tachycardia. These patients were generally older than those without cardiac complications (27.33 compared to 22.62 years; p=0.033), only one being under 20 years. Mean serum ferritin was this time comparable to the rest of the cohort. Five cardiac patients associated advanced liver disease, the relationship between the two complications being more than obvious (p=0.003).

Nine patients had anomalies of carbohydrates metabolism, out of which 2 demonstrated impaired glucose tolerance, while 7 were diagnosed with diabetes mellitus. Their mean age was higher (28.44 versus 22.67 years; p=0.033), whereas serum ferritin was comparable to the rest of the patients. A single diabetic was younger than 25.

Hypothyroidism was present in 21 patients (23.33%), much more evenly distributed between birth cohorts than the previous described complications.

Seven individuals were diagnosed with hypoparathyroidism, all of them older than 25 (mean age 28.86 years compared to 22.77 years for those without this complication; p=0.007). Their mean serum ferritin was near-ly double when compared to others (5258.85 versus 2623.2 ng/ml; p<0.0001). Females were significantly older than males (33 versus 25.75 years; p=0.032), the last ones presenting in return much higher mean ferritin levels (7207 compared to 2661.26 ng/ml; p=0.014).

Hypogonadotropic hypogonadism was one of the most frequent complications, diagnosed in no less than 50 patients. In 12 of them gonadic insufficiency become manifest after sexual maturation (mostly so in females, overrepresented in this group in a 3 to 1 ratio), 17 presented delayed puberty (both sexes being equally represented), while 21 presented stopped puberty (dominated by males in a 5:2 ratio). After exclusion of younger than 14 patients, hypogonadic thalassemics proved to be older than others (26 versus 19.73 years; p<0.0001).

Short stature was seen in 44 patients (48.9%). Even if mean age was not significantly different from the rest of the group, cases distribution between birth cohorts was not uniform, this complication proving to be rare before 14 or after 30 years of age, at least as far as females were concerned (p=0.014). Mean ferritin values were similar to patients with normal development.

A similarly frequent complication was osteoporosis, diagnosed in 45 beta-thalassemia major patients (50%), which were a decade older than the others (28.13 versus 18.36 years; p<0.0001). Osteoporosis was never present before 14 years of age, was occasionally observed before 25, being almost universal after this age. Mean ferritin levels thalassemics with osteoporosis tended to exceed those of other patients (3218 compared to 2438.46; p= 0.052).

Another characteristic complication was biliary lithiasis, diagnosed by ultrasound in 6 patients, out of which 5 were women. Four needed cholecystectomy during the study period for acute cholecystitis. Thalassemics with biliary lithiasis were older (28.17 versus 22.89 years; p=0.045) and had a higher mean ferritin compared to others (4371.72 versus 2718 ng/ml; p=0.039).

Four patients developed severe infectious complications, such as pleurisy, pneumonia, bone abscess and pulmonary tuberculosis. Their mean ferritin was not different from the rest of the cohort, but mean pretransfusional hemoglobin levels proved to be obviously lesser (7.22 compared to 8 g/dl; p=0.031).

A 20 year old woman suffered a cardioembolic ischemic stroke during an episode of paroxysmal atrial fibrillation. Surprisingly, even if her mean ferritin was 6838.79 ng/ml, she did not associate any other iron related complication besides her heart condition.

Considering liver, heart and endocrine iron related complications, together with short stature and osteoporosis, only 16 of our patients were free of complications (FIGURE 3). Two thirds presented at least one but no more than three complications, while a quarter had at least four organ involvements. At univariate analysis age was the stronger predictor of the number of complications detected in a single patient (p<0.0001), along with high mean ferritin level (p=0.009). All thalassemics without complications were younger than 25, half of them being under 14 years old. After 25 years

of age the majority of our β-thalassemia major patients accumulated between three and six complications with the notable exception of those older than 35, where a relatively high proportion of single or double complication was noticed (FIGURE 4).

Cirrhosis was never diagnosed as an isolated manifestation (FIGURE 5), was seldom present in double or triple association (3 cases, all between 25 and 29 years old), being on the contrary more frequently observed in patients with at least four complications (7 cases; p=0.008). Advanced liver disease associated significantly with heart disease (p<0.0001), as well as with osteoporosis (p=0.007). Half of cirrhotic patients presented cardiac failure or rhythm disturbances.

In only one case heart disease was diagnosed as a single complication (FIGURE 5), in most patients being combined with at least other three iron-overload related injuries (10 out of 12 cases; p<0.0001). Letting aside the already mentioned correlation with advanced liver disease, cardiac pathology associated closely with hypogonadotropic hypogonadism (p=0.038) and again, with osteoporosis (p=0.013).

Glucidic metabolism abnormalities were frequently noticed in individuals with 5 or 6 complications (p<0.0001; FIGURE 5). Gonadic insufficiency was always present in cases with altered glucose tolerance (p=0.004). A positive correlation was established also with hypoparathyroidism (p=0.002).
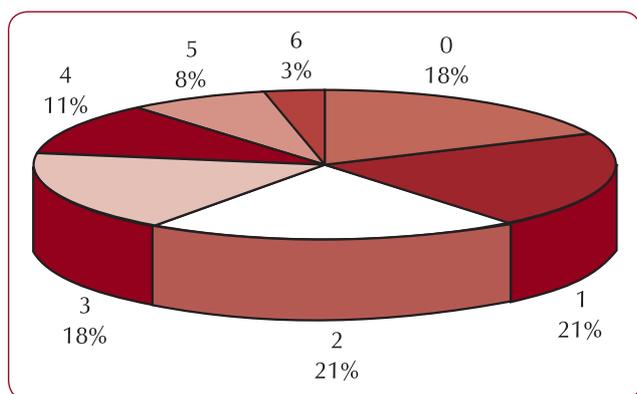

FIGURE 3. Number of iron related complications per patient
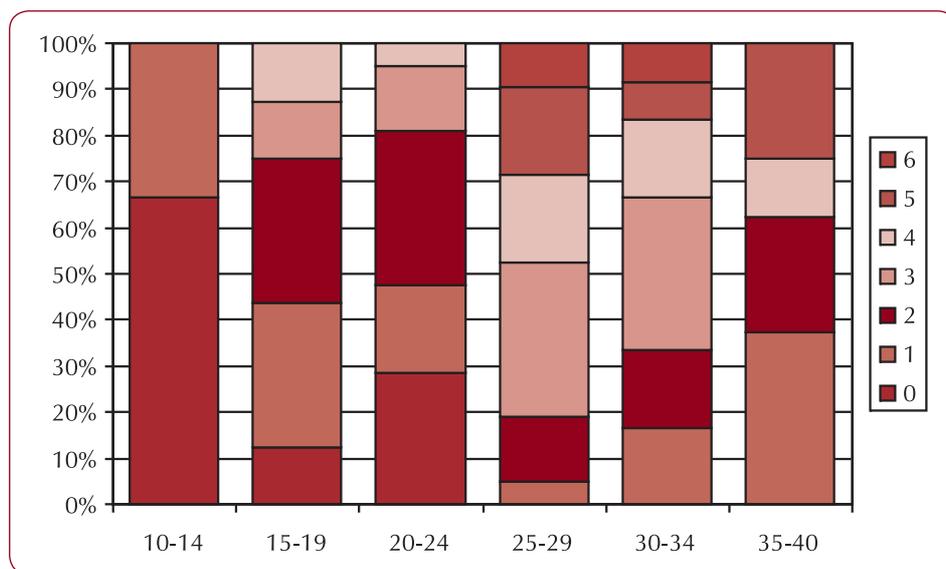

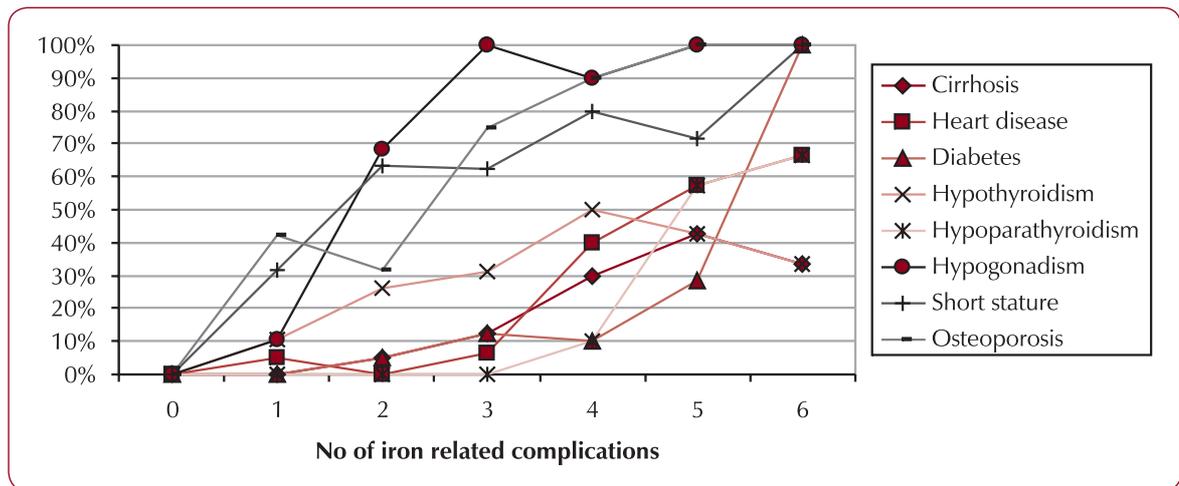**FIGURE 4.** Number of complications in different birth cohorts

FIGURE 5. Distribution of specific complications

Hypothyroidism was an isolated complication in two thalassemics (FIGURE 5), both younger than 20, but in the majority of cases it was combined with one to three other organ dysfunction (15 out of 21 patients; p=0.045), this tendency being more obvious in the 20 to 24 years age group (p=0.025). However it is the only complication that, although relatively common, didn't correlate with any other iron related disease.

Thalassemics with hypoparathyroidism associated at least 4 other complications each (p<0.0001; FIGURE 5). Without exceptions they were diagnosed with hypogonadotropic hypogonadism (p=0.013) and osteoporosis (p=0.005). In all but one of these patients short stature was also evident (p=0.043). Hypoparathyroidism correlated also, as already mentioned, with glucidic metabolism disturbances.

Even if hypogonadotropic hypogonadism was by far the most frequent complication, present in over half of our cohort patients, it was seldom isolated, being associated in all but one cases with at least three other complications (p<0.0001; FIGURE 5). Along with heart disease, glucose intolerance and hypoparathyroidism, gonadic insufficiency correlated also with short stature (p<0.0001) and osteoporosis (p=0.001).

In spite of being a relatively common isolated complication, the occurrence of short stature increased progressively with the number of synchronous complications, affecting all three thalassemics with six iron related diseases (p<0.0001; FIGURE 5). As already mentioned it correlated with hypogonadotropic hypogonadism and hypoparathyroidism.

Osteoporosis was the most common single complication (FIGURE 5), but the majority of cases were documented in thalassemics with at least three iron related organ dysfunctions (p< 0.0001). A close association was demonstrated with advanced liver disease, heart disease, hypoparathyroidism, and hypogonadotropic hypogonadism.

Infectious complications developed in patients with at least two other secondary hemosiderosis determinations (p=0.01). ❑

DISCUSSIONS

In our study we have tried to define the current status of complications among 90 β-thalassemia major patients, focusing on transfusion-transmitted infections and transfusional iron overload. Even if monocentric, it offers the clear advantage of a high number of patients uniformly treated and followed up in a specialized center. Moreover our cohort represents a significant proportion of Romanian thalassemics, being highly representative, at least for the southern part of the country, where the disease is anyhow more prevalent.

Like in other high prevalence areas (1), HCV infection is almost universal in those transfused before 1996, after that infection risk decreasing sharply, probably by efficient screening of transfused blood. On the contrary HBV and HTLV infection is uncommon in our cohort, while HIV infection was never found. Spontaneous HCV clearance is very high, but is nevertheless concordant with other published data and meets the virological pattern already described in infancy acquired infection (2-4).

Both detectable HCV-RNA and severe iron overload, as reflected by high mean serum ferritin, are independent predictors of advanced liver disease (5). Given the relatively severe iron overload in our cohort, cirrhosis prevalence is not very high, but we have to take into account that this was only a clinical diagnosis. Cirrhotics present characteristically longer duration of infection, higher viraemia, persistently elevated aminotransferases and a supra-unitary AST/ALT ratio.

Iron induced cardiopathy prevalence is unexpectedly low (2), probably because of high cardiac mortality observed in Romanian beta thalassemic major patients before the follow up period of our study, which reached a peak in the second decade of life. Heart disease is common after 20 years of age, preceding advanced liver disease by at least five years. Along with lower mean ferritin levels, found in four patients to be even less than 2000 ng/ml, this suggests a higher susceptibility of the myocardium to the toxic effects of iron (6). In the presence of heart disease, the relative risk of having cirrhosis, calculated by Cox regression, was 5.34.

The 10% prevalence of carbohydrate metabolism abnormalities in our thalassemics is comparable with that reported elsewhere (2). Age was the only predictive factor of this complication, all but one patient being over 25 years old. Over this age cases are evenly distributed, which contrasts with studies that report decreasing diabetes frequencies in successive thalassemics cohorts born after 1970 (6). A logical explanation comes from the lack of adequate β-thalassemia major management before 1999. Our data suggest that females are more susceptible to develop glucidic metabolism anomalies than males.

Hypothyroidism is twice as frequent as diabetes in our cohort, similar to other data (7). Age groups distribution is in this case uniform. Some authors report declining prevalence of this complication in successive birth cohorts, but again our data come from a cohort with correspondingly high mean ferritin, suggesting that thyroid is very sensitive to any significant, even if short lived, increase in iron overload (8). The thyroid susceptibility to iron induced toxicity can explain also the lack of correlation between hypothyroidism and any other complication.

As in other studies hypoparathyroidism is an infrequent anomaly (8) which relates not only to age, all cases being diagnosed after 25 years of age, but also to high mean ferritin level. It seams to develop after prolonged exposure to excessive iron overload, parathyroid tissue being therefore more resistant to iron related injury.

Over half of our patients have been diagnosed with hypogonadotropic hypogonadism, which therefore represents the most frequent endocrine complication in this group, which again corresponds to other reports (7,8). In spite of being more common after 25 years of age, gonadic insufficiency has a relatively normal distribution throughout the cohort, which implies that the pituitary gland is vulnerable not only to iron excess level but also to the duration of its persistence. It appears therefore that iron induces progressive alteration in hypophyseal tissue. Females prevail in the group of gonadic failure installed after complete sexual maturation, while males were in the majority in puberty stopped in evolution category. Thus hypophysis appears to be more resistant to iron toxicity in women.

Short stature is another common complication (8), affecting nearly a half of all subjects. It was more often observed in males, yet the difference was not statistically significant. Even if multifactorial nature, which explains the distribution of cases throughout all the age groups, short stature relates more closely to hypogonadotropic hypogonadism. So development of gonadic insufficiency after complete sexual maturation contributes probably to the relatively low prevalence of short stature in female subjects.

Although osteoporosis was until recently an underestimated iron overload complication, it is extremely frequent in our high mean serum ferritin cohort, which is in accordance to lately published data (7). Half of patients demonstrate significant bone density abnormalities, revealed by DEXA. Age and serum ferritin influence on osteoporosis suggest that it is the consequence of long periods of severe iron overload. Our data does not support deferoxamine implication in osteoporosis, thalassemics older than 25 suffering long periods of suboptimal treatment before 1999, characterized by a chronic shortage of deferoxamine.

Gallstones were frequent enough, as expected given the excess hem catabolites resulted from inefficient erythropoiesis or transfused blood. They were almost exclusively diagnosed

in females and were often complicated by acute cholecystitis. We can therefore argue that β-thalassemia major patients diagnosed ultrasonographically with biliary lithiasis should undergo prophylactic cholecystectomy with intraoperative hepatic biopsy, for staging of liver disease.

In contrast to blood borne agents, infectious complications are not very common in the present cohort, even if three quarters of our patients have been splenectomized at a median age of 8 years (range 1-28 years). Besides, the majority of severe infections, which represent in other series the second death cause among β-thalassemia major patients (6), are related to surgically placed central vascular access devices (CVAD's) used in other countries for intensive iron chelation (9). The case of pulmonary tuberculosis illustrates the supplementary risk that iron induced immunosuppression poses in a country where tuberculosis is endemic. Insufficient transfusional therapy has been identified as a possible risk factor for infectious complications in our cohort.

To complete the spectrum of complications frequently listed in the literature, in the present cohort there was also a cardioembolic stroke, diagnosed in a young female with severe iron overload, after an episode of paroxysmal atrial fibrillation. However the presence a procoagulant state, which has been already demonstrated in thalassemic patients (10), could not be excluded.

Taking into consideration all these iron related complications, with the exception of gallbladder and infections, it seams that every tissue has a particular sensibility to the toxic effect of the iron. The major influence exerted by age and serum ferritin level on progressive accumulation of an increasing number of complications supports this hypothesis. The high proportion of cases with only one or two complications after 35 years of age reflects the reduced survival of those who accumulate more than three complications.

Short stature is the first complication to become manifest, probably because of its multifactorial nature, being the direct consequence of the disease as much as the result of transfusional and chelator treatment. However the most important determinant of short stature after 14 years of age is hypogonadotropic hypogonadism.

Among the endocrine glands the most sensitive to iron toxicity seems to be the thyroid tissue, its dysfunction depending more on iron overload severity than on its duration. This peculiar behavior could explain also the lack of correlation of hypothyroidism with any other complication.

Gonadic insufficiency is the next iron related endocrine disorder that becomes manifest, its occurrence increasing with age, which suggests that the duration of iron overload exposure is of paramount importance in generating hypophyseal lesions. Osteoporosis has a similar behavior, belonging along with hypogonadotropic hypogonadism and short stature to the combination met in the majority of thalassemics with at least three complications.

Glucidic metabolism abnormalities and hypoparathyroidism are closely associated, but rarely appear before patients accumulate at least the other three endocrine complications already mentioned.

We could therefore establish the following order of increasing iron susceptibility of endocrine tissues: parathyroids < pancreas < hypophysis < thyroid.

The liver and the heart have an intermediary behavior, but their dysfunction becomes evident only in the presence of at least four associated complications, the other two being represented in general by hypogonadotropic hypogonadism and osteoporosis. ❏

CONCLUSION

1. In β-thalassemia patients, complications accumulate progressively, depending on the severity and duration of tissular exposure to excess iron, the order of injury being conditioned by the different susceptibility of tissues to iron related toxicity. Our data suggest the following scale of increasing sensibility: parathyroid, pancreas, liver, heart, hypophysis, and thyroid.

2. For cardiac and liver iron dependent disease the serum ferritin level which confers protection is well under the previously recommended value of 2500 ng/ml, our days the target of chelator therapy being 1000 ng/ml.

3. Short stature is the first and apparently the most sensitive indicator of inadequate treatment, calling out for a search of potentially correctable factors, such as compliance to transfusional or chelator treatment.

4. Endocrinological surveillance, besides identifying hemosiderosis induced endocrine dysfunction and offering substitutive treatment, could detect sooner patients with iron related toxicity, before progression to clinical relevant complications and death.

Thyroid and hypophysis malfunction should be probably searched for first.

5. Women seem to have increased susceptibility for developing glucidic metabolism disorders, while being more resistant to hypophyseal iron related toxicity. ❑

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