three different genotypes.[6,7]

In the present study, eleven of the 30 children (36.7%) with gallstones and six of the 40 healthy children (15%) had the $(TA)_7/TA)_7$ genotype suggesting, for the first time, that GS promoter genotype might be a risk factor for the development of gallstones in children, in the absence of any other predisposing factors.

Previous studies have demonstrated an increased risk for developing gallstones in patients with co-inheritance of GS and hereditary spherocytosis (HS) or homozygous β-thalassemia.[5,8] More recently, Galanello *et al.* reported that the presence of GS genotype is associated with a statistically increased prevalence of cholelithiasis in both thalassemia major and intermedia patients.[8] Passon *et al.* also report that children with sickle cell anemia and cholelithiasis have a higher frequency of the abnormal $(TA)_7$ UGT1A promoter allele.[9]

All the above evidence and the results of the present study should alert pediatricians to investigate the GS promoter genotype in children with cholelithiasis even in the absence of other risk factor.

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Immunologic reconstitution in long-term survivors of thalassemia major after hematopoietic stem cell transplantation

We studied the immune function of 33 long-term survivors of thalassemia after hematopoietic stem cell transplantation. Lymphocyte subsets, lymphoproliferative response and immunoglobulin were normal but the level of natural killer cells was low. Five and seven patients had suboptimal antibody response at 4 week after pneumococcal and hepatitis B vaccine, respectively, but this response returned to normal by 6 months.

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Immune recovery after allogeneic haematopoietic stem cell transplantation (HSCT) is affected by many factors such as age and graft-versus-host disease (GVHD).[1] Transfusion-dependent thalassemia major (TM) patients have altered immune function,[2] and this study was aimed at evaluating the immune recovery of long-term survivors of TM after HSCT. Thirty-three TM patients who had survived more than 2 years after HSCT were studied. The donors were HLA-identical siblings and the conditioning was busulphan and cyclophosphamide. Antithymocyte globulin (ATG) was given at a daily dose of 10 mg/kg from day −5 to day +5 in 20 patients, 11 patients received 30 mg/kg/day for 3 days from day −4, and two did not receive ATG. GVHD prophylaxis was cyclosporin A and short course methotrexate.[3] The patients were re-immunized at one year with diphtheria, pertussis and tetanus vaccine. Three doses of hepatitis B vaccine were to be given if the antibody against hepatitis B (anti-HBs) was below the protective level (<10 IU/L).

At the study baseline, lymphocyte subsets were assessed by flow cytometry and were compared with those of an age- and sex-matched reference.[4] Lymphoproliferative response (LPR) was studied for mitogens and specific recall antigens (herpes simplex virus, cytomegalovirus, varicella-zoster virus). Serum immunoglobulins and anti-tetanus antibody were measured. Thirty patients were given a booster intramuscular injection of hepatitis B vaccine (Engerix-B), 33 patients received one dose of polyvalent pneumococcal vaccines (Pneumovax-23). Anti-HBs and anti-pneumococcal bodies were measured at baseline, and four weeks and six months following vaccination. An antibody titer more than double the baseline level was considered to represent an optimal response.

At the time of the study, the median age of the patients was 15.8 years (5.8 to 26.9 years) and their median follow-up from HSCT was 68 months (24 to 106 months). None of the patients was receiving desferrioxamine and their mean serum ferritin was 1383±1157 pmol/L. Acute GVHD grade I or II had occurred in 19 patients but all responded to a short course of steroids. None of them developed chronic GVHD and immunosuppressive treatment had been stopped more than one year prior to

Table 1. Lymphocyte subsets after HSCT (absolute count in $\times 10^9$/L).

	Mean number + SD	*percent of patients >75% of reference*	*percent of patients < 25% of reference*
T cells (CD3)			
Percentage	6.37±7.57	9%	45%
Absolute count	1.53±0.43	27%	12%
CD4 cells			
Percentage	31.16±5.09	18%	33%
Absolute count	0.75±0.23	12%	27%
CD8 cells			
Percentage	26.33±6.07	29%	40%
Absolute count	0.64±0.35	18%	18%
CD4/8 Ratio	1.23±0.36	27%	30%
B cells (CD19)			
Percentage	21.67±6.13	63%	0%
Absolute count	0.53±0.25	57%	9%
NK cells (CD16/56)			
Percentage	11.97±6.12	6%	48%
Absolute count	0.29±0.19	6%	42%

Table 2 . Antibody response of HSCT patients after vaccination.

Pneumococcal vaccine (N=33)	*Antibody titer (IU/L) or fold increase*
Baseline Ab: mean ± SD (Median; range)	77±177 (35; 2-1038)
Ab at 4 weeks; mean ± SD (median; range)	638±622 (478; 26-3116)
Fold increase: mean ± SD (median; range)	21±24 (11; 0.6-90)
Ab at 6 months; mean ± SD (median; range)	4548±46386 (31407; 1817-232845)
Fold increase: mean ± SD (median; range)	1535±1771 (830; 620-6522)

Hepatitis B vaccine (N=30)	*Antibody titer (IU/L) or fold increase*
Baseline Ab: mean ± SD (Median; range)	623±1431 (88; 2-7460)
Ab at 4 weeks; mean ± SD (median; range)	57094±105673 (4222; 0-484000)
Fold increase: mean ± SD (median; range)	239±370 (47; 0-1516)
Ab at 6 months; mean ± SD (median; range)	24498±69413 (1771; 5-371245)
Fold increase: mean ± SD (median; range)	79±134 (12; 1-517)

the study.

The lymphocyte subsets did not differ significantly from normal except the natural killer cells: 48% and 42% of patients had, respectively, a percentage and number of NK cells below the lower quartile of normal (Table 1). Overall the LPR response to mitogens and specific stimuli showed a pattern similar to normal. None of the patients had decreased immunoglobulin levels. Four patients (12%) had anti-tetanus antibody levels below the protective titer (0.5 IU/L). At baseline, 9 patients (29%) had anti-HBs below the protective titer despite 3 having been reimmunized with hepatitis B vaccine after HSCT. At week 4, 7 patients (23%) and five patients (15.4%) had suboptimal antibody response to hepatitis B and pneumococcal vaccine, respectively. At 6 months they had all achieved optimal response but 2 still had anti-HBs <10 IU/mL (8.5 and 9.6 IU/mL) (Table 2). In the subgroup analysis, there was no difference in the immunological parameters between the two groups receiving the two different modes of ATG.

Immune reconstitution is important for long-term survival after HSCT and fatal opportunistic infections may occur.[5] Knowledge of immune recovery after HSCT is important when considering the use of prophylactic antibiotics and counseling patients on risk of infection. Chronic GVHD may affect immune recovery. None of the patients in our study had chronic GVHD and all had normal recovery of Ig G, M and A. However 12% could not produce anti-tetanus antibody to a protective level despite revaccination. A previous study of TM patients after bone marrow transplant (BMT) reported 86-100% antibody response to tetanus vaccine.[6] Another study showed a good response after hepatitis B vaccine in TM patients who had undegone HSCT.[7] In our study 23% and 15% of patients did not have optimal response after hepatitis B booster vaccine and pneumococcal vaccine at week 4. These suboptimal responders subsequently showed an adequate response at 6 months. The post-HSCT TM patients appeared to have a delayed response to vaccination. There were 2 patients with anti-HBs titers less than protective after booster vaccination; the clinical significance of this is unclear. We found rather low levels of NK cell more than 2 years after HSCT. Our previous study showed that iron overload might lead to low levels of NK cells.[2] The common occurrence of mixed chimerism in TM patients after BMT may be another contributory factor.[8] The residual host cells might produce NK cells less efficiently. The clinical significance of the lower levels of NK cells needs further study.

In conclusion, TM patients after HSCT had slower antibody responses to vaccination and lower NK cell numbers, such patients deserve serial monitoring of immune function.

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α-globin gene deletion and point mutation analysis among Iranian patients with microcytic hypochromic anemia

We tested 67 Iranian individuals, presenting with low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels, normal hemoglobin electrophoresis and iron status, for the presence of twelve common α-thalassemia gene deletions and point mutations. Five different mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{MED}$, $-(\alpha)^{20.5}$, Hb Constant Spring) were identified in a total of 43 cases.

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α-thalassemia (α-thal) is one of the most common hemoglobin disorders in the world. It is characterized by the absence or reduced synthesis of α-globin chains. Unlike β-thalassemia (β-thal) mutations, which are mostly point mutations, the majority of α-thal mutations are deletions of one or both α-globin genes located on chromosome 16. The two most common single gene deletions are $-\alpha^{3.7}$ and $-\alpha^{4.2}$, while the Southeast Asian ($--^{SEA}$), the Mediterranean ($--^{MED}$), the Filipino ($--^{FIL}$) and the 20.5 kb ($-(\alpha)^{20.5}$) type are common α-thal double gene deletions. In addition, a small number of α-globin point mutations are known. Mutations are classified into α^+- and α^0-thalassemia to indicate, respectively, the reduced or absent output of α-globin chains from an affected chromosome. The clinical phenotype varies according to the number of affected genes.

Table 1. α-thal genotypes and associated hematologic values of 67 thalassemia patients.

Subject	*Mean Hb (g/dL)*	*Mean HbA_2 (%)*	*Mean MCV(fL)*	*Mutation*
23	14.1±1.0	2.4±0.6	73.9±6.4	$-\alpha^{3.7}/\alpha\alpha$
9	13.2±1.4	2.6±0.7	69.3±3.1	$-\alpha^{3.7}/-\alpha^{3.7}$
3	13.2±1.6	3.1±1.4	68.3±4.8	$--^{MED}/\alpha\alpha$
2	11.7±0.6	3.7±0.3	70.8±6.7	$-\alpha^{3.7}/-\alpha^{4.2}$
1	10.7	3.5	59.2	$-\alpha^{3.7}/--^{MED}$
1	13.6	3.8	66.0	$-\alpha^{4.2}/--^{MED}$
1	12.0	3.4	77.4	$-\alpha^{4.2}/\alpha\alpha$
1	11.4	2.4	68.3	$-(\alpha)^{20.5}/\alpha\alpha$
1	not available	3.4	76.5	$\alpha^{CS}\alpha/\alpha^{CS}\alpha$
1	14.8	3.5	78.4	$\alpha^{CS}\alpha/\alpha\alpha$
24	13.0±2.3	3.4±0.6	72.7±6.8	Normal

Carriers of three intact α-globin genes ($-\alpha/\alpha\alpha$) usually present with little, if any, detectable red blood cell abnormalities or globin chain imbalance, while double gene deletions ($--/\alpha\alpha$ or $-\alpha/-\alpha$) cause mild microcytic, hypochromic anemia with normal HbA_2 levels. Carriers with only one functional α-globin gene ($--/-\alpha$) present with hemoglobin (Hb) H disease, which is characterized by marked imbalance in globin chain synthesis ratios. Absence of all four α-globin genes (Hb Bart's hydrops fetalis syndrome) is incompatible with life.[1-3]

In an attempt to establish α-thal molecular diagnosis in Iran, a series of 67 individuals from different ethnic origins, randomly selected from a pool of patients presenting with low MCV (mean: 72.2±6.3 fL), low MCH (23.5±2.7 pg), normal or slightly reduced Hb (13.4±1.7 g/dL) levels, normal HbA_2 (3.0±0.8%), and negative results in β-thal genotyping (β-Globin StripAssay, Viennalab, Austria) were tested for the presence of α-thal mutations. Serum iron and ferritin levels, as well as total iron binding capacity were analyzed and confirmed to be in the normal range for all subjects in order to rule out iron deficiency. After comprehensive genetic counseling, blood was drawn from each individual, and genomic DNA was extracted according to established protocols.[4]

A polymerase chain reaction (PCR) method described by Baysal and Huisman[5] was used to detect the two α-thal single gene deletions $-\alpha^{3.7}$ and $-\alpha^{4.2}$. The Southeast Asian ($--^{SEA}$), Mediterranean ($--^{MED}$), Thai ($--^{THAI}$), Filipino ($--^{FIL}$) and 20.5 kb ($-(\alpha)^{20.5}$) double gene deletion variants, as well as point mutations Hb Constant Spring (cd 142: TAA-CAA), Hb Quong Sze (cd 125: CTG-CCG), Hb Pakse (cd 142: TAA-TAT), Hb Adana (cd 59: GGC-GAC) and cd 30 delGAG were investigated by a combined strategy based on multiplex PCR and reverse dot-blot analysis.[6,7] Southern-blotting using a ζ probe on DNA digested with BglII and Asp718[8] was performed to confirm the $-\alpha^{3.7}$,