GENETIC AND HEMATOLOGICAL PROFILES OF B-THALASSEMIAS IN EASTERN ALGERIA

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Résumé

Le présent travail est une étude rétrospective transversale portant sur des cas de β- thalassémies suivis au sein du service de Pédiatrie de l'Hôpital Militaire Régional Universitaire de Constantine (HMRUC), sur une période de 3 mois. Notre objectif est d'étudier à travers ces patients, d'une part, les aspects épidémiologiques et para-cliniques de la β-thalassémie, et d'autre part, une étude génétique en recherchant par RFLP-PCR d'éventuelles associations entre les polymorphismes T3801C du gène *CYP1A1* et C677T du gène de la *MTHFR* et la β-thalassémie. Nous avons colligé durant cette période 36 cas de β-thalassémies âgés entre 3 mois et 10ans avec une moyenne de 1.9 ans et un sexe ratio (M/F) de 2.25. Les parents sont consanguins dans 55,56% des cas. L'hémogramme a été marqué par une pseudo-polyglobulie et une anémie microcytaire hypochrome chez les porteurs de trait thalassémiques et une diminution du nombre de globules rouges et une anémie microcytaire sévère chez les patients atteints de formes sévères. L'électrophorèse de l'hémoglobine a objectivé une β-thalassémie hétérozygote dans 11 cas (30.55%) et une β-thalassémie homozygote dans 25 cas (69.44%). L'analyse statistique des résultats préliminaires des génotypage moléculaires, représentée par l'Odds ratio et la *p-value*, indique l'absence d'association entre les polymorphismes, C677T de la *MTHFR* et T3801C du *CYP1A1* et la β-thalassémie. Cependant, la taille de l'échantillon ne permet pas d'infirmer ou de confirmer avec certitude la présence ou l'absence de cette association.

Mots clés : *β-thalassémie, anémie, polymorphisme, MTHFR, CYP1A1, RFLP-PCR.*

Abstract

The present work is a retrospective cross-sectional study of cases of β-thalassemia in the Pediatric Department of the Constantine Regional Military Hospital (HMRUC) over a period of 3 months. Our objective is to study through these patients, on the one hand, the epidemiological and para-clinical aspects of β-thalassemia, and on the other hand, a genetic study by searching by RFLP-PCR for possible associations between T3801C polymorphisms of the gene *CYP1A1* and C677T of the *MTHFR* gene and β-thalassemia. We collected during this period 36 cases of β-thalassemias aged between 3 months and 10 years with an average of 1.9 years and a sex ratio (M/F) of 2.25. The parents are consanguineous in 55,56% of the cases. The blood count was marked by pseudo-polycythemia and hypochromic microcytic anemia in thalassemic trait carriers and a decrease in red blood cell count and severe microcytic anemia in patients with severe forms. Hemoglobin electrophoresis revealed heterozygous β-thalassemia in 11 cases (30.55%) and homozygous β-thalassemia in 25 cases (69.44%). Statistical analysis of the preliminary results of molecular genotyping, represented by Odds ratio and the p-value, indicates the absence of association between the polymorphisms, C677T of *MTHFR* and T3801C of *CYP1A1* and β-thalassemia. However, the size of the sample does not make it possible to invalidate or to confirm with certainty the presence or absence of this association.

Keywords: *β-thalassemia, anemia, polymorphism, MTHFR, CYP1A1, RFLP-PCR.*

ملخص

هذا العمل هو دراسة استعادية مستعرضة حالات β–الثلاسيميا المتبعة في قسم طب الأطفال بالمستشفى الجامعي الجهوي العسكري بقسنطينة, على مدى 3 أشهر. هدفنا هو دراسة من خلال هؤلاء المرضى, من جهة, الجوانب الوبائية و السريرية من β-الثلاسيميا, ومن جهة أخرى, دراسة وراثية من خلال البحث بتقنية RFLP-PCR عن الارتباطات المحتملة بين تعدد الأشكال T3801C من الجين CYP1A1 و C677T من الجين MTHFR و β–ا لثلاسيميا. جمعنا خلال هذه الفترة 36 حالة من β– الثلاسيميا تتراوح أعمارهم بين 3 أشهر و 10 سنوات بمتوسط 1.9 سنة ونسبة الجنس (عدد الذكور / عدد الاناث) 2.25. نجد زواج القرابة بين الوالدان في 55.56 ٪ من الحالات. تقنية تعداد الدم تميزت بفقر الدم مصحوب بنقص الانصباغ و صغر حجم الكريات الحمراء عند متخالفة التلاسيميا, و فقر دم حاد مصحوب بصغر حجم الكريات الحمراء و نقص شديد في عدد الكريات الحمراء عند المرضى الذين يعانون من أشكال حادة .الفصل الكهربائي للهيموغلوبين تمكن من تشخيص 11 حالة (30.55 %) لمتخالف β– الثلاسيميا و 25 حالة (69.44 %) متماثلة β–الثلاسيميا. التحليل الاحصائي للنتائج الأولية لتحديد النمط الوراثي الجيني الجزيئي الذي قدمته نسبة الاحتمالات و ذات القيمة ص, تدل على عدم وجود علاقة بين الأشكال CYP1A1 T3801C و C677T MTHFR و β–الثلاسيميا.و مع ذلك ، فإن حجم العينة لا يسمح بدحض أو تأكيد بيقين وجود أو عدم وجود هذه العلاقة.

الكلمات المفتاحية: *β- الثلاسيميا, فقر الدم, تعدد الأشكال, MTHFR, CYP1A1, RFLP-PCR.*

Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin (Hb) resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. They include three main forms: Thalassemia Major, Thalassemia Intermedia and Thalassemia Minor also called "heterozygous beta-thalassemia" (Galanello and Origa 2010). Of recessive autosomic transmission, β-thalassemia presents public health problems seen its frequency and its difficulties of treatment. Not assumption of responsibility, it involves the death of the patients in childhood. Whereas it is asymptomatic in a heterozygous state, it is translated in a homozygous state by a more or less severe anemia and a martial overload. The latter being due not only to the multiple transfusions of globular concentrates necessary to ensure the good ponderal development stature children but also to the physiopathology of the disease (Lahlou 2016).

More than 300 point mutations, and rarely deletions, affecting the expression of the β-globin gene have been reported. Those constitute the heterogeneous group of thalassemias, but this diversity explains only very partially heterogeneity of the clinical presentation. Molecular diagnosis currently plays an important role in diagnosis, genetic counseling and prenatal diagnosis, but it requires a precise phenotypic analysis always as a preliminary (Couque and *al.* 2016).

Our work includes a cross-sectional study and a molecular analytical study. It has as principal objectives:

- Characterization of familial, hematological, biological and biochemical criteria of patients with β-thalassemia collected in pediatric HMRUC.
- Research by RFLP-PCR of possible associations between the polymorphisms T3801C of *CYP1A1* and C677T of *MTHFR* and β-thalassemia.

MATERIALS AND METHODS

Patients

Recruitment : Our study related to 36 children reached of β-thalassemia coming from various areas of the Algerian East, diagnosed and treated in the pediatry of HMRUC over a period of 3 months (from March to May 2017). The genetic study was carried out at the laboratory of biology and molecular genetics of the university hospital center Ibn Badis Constantine 3 (DNA extraction) and the laboratory of molecular biology – Faculty of Sciences of Nature and Life-Constantine 1 (PCR/Digestion).

Criteria of inclusion / exclusion

- All the children reached of a β-thalassemia and whose diagnosis was confirmed by an electrophoresis of Hb were included. The children whose diagnosis is evoked but not confirmed by the electrophoresis of Hb as well as those reached of an association β-thalassemia / sickle cell anemia were excluded from recruitment.

Methods

We undertook a cross-sectional study of the familial, hematological, biological and biochemical criteria of patients with β-thalassemia, as well as an analytical study of the genotypic and allelic profiles of 12 patients by RFLP-PCR of two polymorphisms (T3801C of the *CYP1A1* gene and C677T of the *MTHFR* gene).

Blood sampling

The blood sample was taken for each patient from the venous blood at the elbow crease under sterile conditions. The blood is collected in vacutainer tubes containing EDTA anticoagulant (in a quantity of 5 ml). Blood collection took place within a one-month transfusion time interval. All samples were stored at + 4 ° C for a maximum of one week.

Hemogram

Hemogram is the first examination giving useful information to suspect a hemoglobin abnormality. It is carried out at a distance from any transfusion. The parameters included in the blood count are: Red blood cell (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin content (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Lahlou 2016).

Blood smear

The blood smear used to detect morphological abnormalities (size, form, color, inclusions) of the RBCs. It consists of the spreading of a drop of blood on a glass slide, colored by the May Grünwald Giemsa (MGG) and read under an optical microscope (Picaut 2006).

Electrophoresis

The electrophoresis separates hemoglobins as a function of their charge difference in an electric field. The alkaline pH electrophoresis method was undertaken in our study. At pH 8.6, the negatively charged Hb molecule migrates to the (+) anode, and the hemoglobins that have a positive charge increase migrate more slowly (Couque and De Montalembert 2013).

Questionnaire

A collection of information was carried out on level of the pediatric service ; starting from the files of the patients, supplemented by ourpersonal investigat ions. Oral consent for the inclusion of this study was obtained from parents or legal tutors. The confidentiality of the data was respected throughout our study.

Family trees

A family from Azzaba (Skikda) was chosen for the establishment of the representative family tree, as it contains all the studied cases.

Extraction of DNA

The extraction of the DNA was carried out from the blood leukocytes of each individual. During this study, the NaCl extraction method was undertaken. The extraction of the leukocyte DNA is summarized in 3 steps:

- Preparation of leukocytes;
- Extraction of the DNA proper;
- Solubilization.

Genotyping of polymorphism T3801C of the *CYP1A1*

Genotyping of the 3801T> C allelic variant of the *CYP1A1* gene was performed by RFLP-PCR using the MspI restriction enzyme. The digestion profile was obtained by several successive steps:

- PCR followed by an electrophoresis of the products on agarose gel.
- Digestion of the PCR product with the restriction enzyme MspI.
- Separation of digestion products by electrophoretic migration on agarose gel.
- Visualization of digestion products by trans-illumination under UV.

The PCR

- Dilution of DNA

To proceed to the PCR, the highly concentrated DNAs must be diluted (10 µl of DNA in 30 µl of distilled water).

- Preparation of the reaction medium

The reagents used in this PCR step must first be diluted according to the following formula: C1 x V1 = C2 x V2
Where:

C1: Initial concentration of each reagent.

V1: Initial volume required for dilution (unknown).

C2: Final concentration.

V2: Final volume.

Once the initial volume (V1) is known, the volume of distilled water required for dilution of each reagent is calculated as follows:

V water distilled = V2 - V1

The composition of the reaction mixture is mentioned in Table 1.

Table 1: Components of the PCR reaction mixture.

Reagents	Volumes (µL)
dNTP 0.2mM	4.8
Buffer 10x	3
ADN of 20ng/µL at 50ng/µL	3
Oligo F **GGCTGAGCAATCTGACCCTA** (100ng/µL)	3
Oligo R **TAGGAGTCTTGTCTCATGCCT** (100ng/µL)	3
$MgCl_2$ 1.5mM	0.9
Taq Polymérase 5 U/µL	0.24
Distille water	12.06

After preparing the mix, in a PCR tube, 27 µL of this mixture was added to 3 µL of DNA for each sample.

- Progress of the PCR

We have programmed the thermo cycler for 30 cycles. The conditions for the progress of the PCR amplification are shown in Table 2.

Table 2: Programming a PCR cycle

Steps	Temperature (°C)	Duration
Initial denaturation	94	4min
Denaturation	94	30sec
Hybridization	61	30sec
Elongation	72	30sec

- Electrophoresis of PCR products

Electrophoresis is needed to control the size of the amplified fragments by PCR and to detect any contamination of the DNA (with the negative control). In our study, we carried out this control in a horizontal tank on a 2% agarose gel (TBE 1X) in which were incorporated 10µl of ethidium bromide (BET). In each well of the gel and on the (-) cathode side, we deposited a mixture of 7 µl of the amplification product and 3 µl of the Bromo Phenol Blue (BBP) mobility marker, reserving 2 wells, one for the deposition of the size marker (100pb) and the second for the deposition of white (negative control). Then, the system is subjected to a migration under a current of 100 volts for 30 min. After migration, visualization of amplified products is performed under UV. the visualization of the amplified products is carried out under UV.

- Digestion of PCR products by the restriction endonuclease MspI

In our study, 20µl of the PCR product are mixed with 0.7µl of MspI restriction enzyme. The whole is then incubated overnight at 37 ° C. The T3801C mutation of the *CYP1A1* creates a recognition site for the restriction enzyme MspI. The cleavage action of this enzyme is detected by a variation in the number and the length of restriction fragments obtained after enzymatic digestion.

- Electrophoresis of digestion products

The DNA fragments digested with the restriction enzyme are separated by electrophoresis; the small size of these fragments required the preparation of a more resolutive agarose gel at 3%. In each well, +/- 20µl of the digested product and 3µl of BBP are deposited. The migration is carried out under a current of 100volts during 30min. The resulting fragments are then visualized under UV. The gel is then photographed.

Genotyping of polymorphism C677T of the *MTHFR*

To genotype the 677 C > T allelic variant of the MTHFR gene, we followed a similar protocol to the genotyping of the CYP1A1 T3801C polymorphism, with the exception of a few modifications:

-The sequences of the primers of the MTHFR gene used:

oligo F: TGAAGGAGAAGGTGTCTGCGGGA
oligo R: AGGACGGTGCGGTGAGAGTG

- The thermo cycler was programmed for 40 cycles.
-The hybridization temperature of the PCR is 69 ° C.
-The digestion of the PCR product was carried out with the restriction enzyme HinfI.

Statistical analysis

In this work, we performed a statistical association study between β-thalassemia and polymorphisms of *CYP1A1* T3801C on one side and the polymorphism of *MTHFR* C677T on the other hand. The statistical study is based on OR and *p-value* in order to determine whether there is a significant association between the studied polymorphisms and β-thalassemia (Table 3). The calculations were done using the EPI-info 5.01b software. For the calculation of the OR, we have established a contingency table. It is presented as a 2 × 2 cross-tab. The sick / non-sick status of study subjects is presented in columns and the exposed / unexposed on a line. The IC is 95% (or 0.95).

Table 3: Contingency table.

	Patients	Controls	Total
Sick	a	b	a + b
Non sick	c	d	c + d
Total	a + c	b + d	a + b+ c+ d

The OR is calculated as follows: OR = a * b / c * d
OR = 1: no association between exposure and disease.
OR <1: negative association.
OR> 1: positive association.

For the *value p*, the critical threshold a *priori* is 0.05 (since the IC for the OR is 95%). If the *p value* calculated *a posteriori* is lower than this threshold, the difference between the parameters is declared statistically significant.

RESULTS

Epidemiological characteristics

The patients in our series are distributed in 25 boys and 11 girls. We emphasize a male predominance with a sex ratio (M / F) of 2.27. Patient age at diagnosis ranged from 3 months to 10 years, with an average of 1.9 years. 13 patients from a consanguineous marriage 1st degree, 2 patients from a consanguineous marriage 2nd degree and 3rd degree 5 patients, while 16 patients are from a non-consanguineous marriage, as presented in Table 4 .

Table 4: General Characteristics of Patients

Characteristics	Effective	Percentage
Age of diagnosis (years)		
]0-4[	32	88.89%
]4-8[	3	8.33%
]8-12[	1	2.78%
Sex		
Male	25	69.44%
Female	11	30.56%
Consanguineous marriage		
1st degree	13	36.11%
2nd degree	2	5.56%
3rd degree	5	13.89%
Non-consanguineous marriage	16	44.44%

Hematological and biochemical profiles

Hemoglobin electrophoresis

Electrophoresis was performed in all patients at least once. She objectified a -β thalassemia heterozygous in 11 patients (30.55% of cases), and β-thalassemia homozygous in 25 patients (69.44% of cases). The results of electrophoresis of 11 heterozygous patients revealed an increased HbA2 varies between 3.7 and 6.8% with an average of 5.6%. The results of patients with severe forms of β-thalassemia (β-thalassemia major and β-thalassemia

intermediate) revealed an increase in HbF which varies between 6.4 and 98.6%. Electrophoretic profiles are shown in Figure 1.

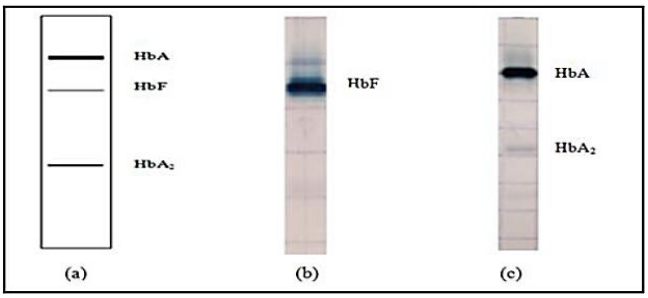

Figure 1: Electrophoretic profiles of individuals a)healthy b)homozygous c)heterozygous

Hemogram

Table 5 reports the mean values of hematology parameters heterozygous β-thalassemia and that of β-thalassemia homozygous such that, the mean of hemoglobin is 10.12 g/dl with a standard deviation of 1.30 in heterozygotes, and of 7.03 g/dl with a standard deviation of 1.28 in homozygotes.

Table 5: Mean values of hematological parameters of patients.

Parameters	heterozygous β-thalassemia	homozygous β-thalassemia
RBC(x10^6/µL)	5.48 ± 1.07	3.34 ± 0.96
Hb(g/dl)	10.12 ± 1.30	7.03 ± 1.28
MCV (fL)	62.04 ± 7.40	74.33 ± 6.44
MCHC (g/dl)	19.34 ± 4.14	25 ± 3.77

Blood smear

The blood smear of a homozygous patient before transfusion compared to a smear of a healthy person (Figure 2) revealed:

- hypochromia with anisocytosis poikilocytosis-schizocytosis.
- The presence of target cells.

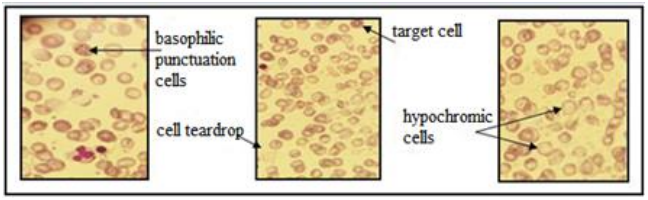

Figure 2: Blood smear cells of a homozygous β-thalassemic (Gx100)

Family tree

Figure 3 represents the family tree of the representative family.

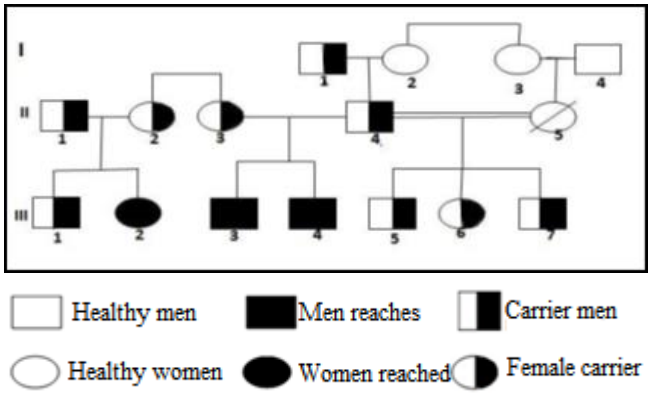

Figure 3: Family tree of the representative family

Genetic profile

Results of electrophoresis of PCR

- Electrophoretic profiles of polymorphisms C677T of the *MTHFR* gene and T3801C of the *CYP1A1* gene

The electrophoretic profiles of polymorphisms of the C677T of the *MTHFR* gene and T3801C of the *CYP1A1* gene are shown in Figure 4.

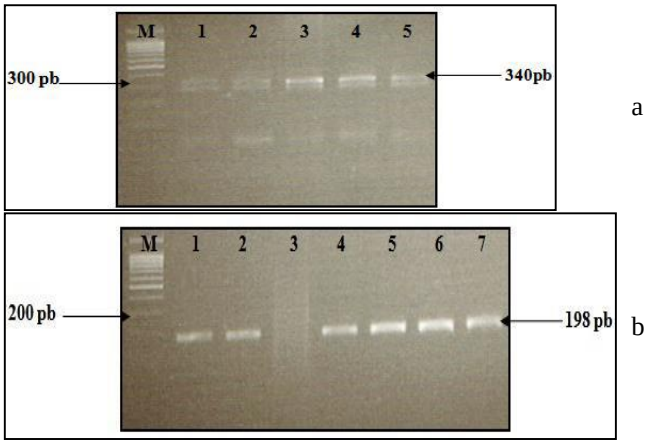

Figure 4: PCR Control
a) T3801C Polymorphism of CYP1A1m1. M: size marker; 1-5: patients; **b)** C677T polymorphism of MTHFR. M: size marker; 1-7: patients

Case-control analytical study of genotypic and allelic profiles

- Case-control analytical study of genotypic and allelic profiles of T3801C polymorphism of the *CYP1A* gene

Our molecular analysis aims to investigate a possible association between the T3801C allelic variant of the *CYP1A1* gene and β-thalassemia. Digestion of the amplification product of the *CYP1A1* gene by the restriction enzyme *MspI* revealed 3 fragments. The first one appears on the electrophoretic profile in the form

of a single band corresponding to the normal homozygous type TT (a band of 340pb), the second in the form of two bands (one of 200pb and the other of 140pb) corresponding to the mutated homozygous type CC, the third in the form of 3 bands corresponding to the heterozygous TC type (bands 340pb, 200pb and 140pb) (Figure 5).

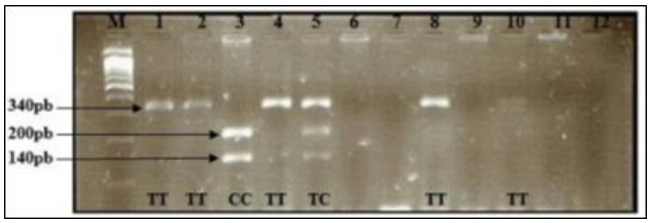

Figure 5: Agarose gel electrophoresis profile (3%) of the fragments after digestion with the *MspI* enzyme. M: size marker; 1-12: subjects.

Note that we did not obtain results for individuals 6, 7, 9, 11, 12. This could be due to manipulation error during extraction or contamination.

The genotype and allelic frequencies of the different forms were calculated for genotyped subjects (7 patients and 10 controls) (Table 6, Figure 6).

Table 6: Distribution of genotypic and allelic frequencies of the polymorphism T3801C of *CYP1A1*

		Patients		Controls		OR	*p value*
		n	%	n	%		
Genotypic frequencies	3801 TT	5	71 ,43	5	50	/	/
	3801 TC	1	14,28	5	50	0,20[0,01-3,32]	0,43
	3801 CC	1	14,29	0	0	undefined	0,92
Allelic frequencies	T	11	78,57	15	75	/	/
	C	3	21,43	5	25	0,82 [0,12-5,31]	0,86

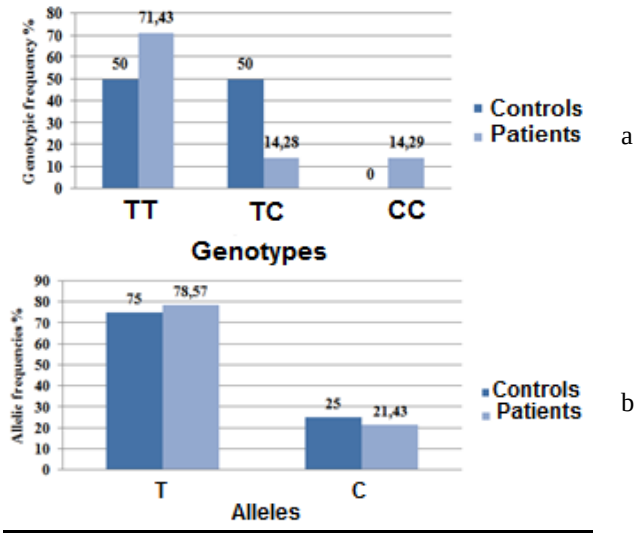

Figure 6: a- Genotypic frequencies of T3801C of *CYP1A1* in patients and controls. b- Allelic frequencies of T3801C of *CYP1A1* in patients and controls.

- Case-control analytical study of genotypic and allelic profiles of C677T polymorphism of *MTHFR* gene

Digestion of the amplification product of the *MTHFR* gene by the restriction enzyme *HinfI* reflected 2 fragments.

The first appears on the electrophoretic profile in the form of a single band (198pb) corresponding to the normal homozygous type CC, the second which is normally in the form of a single band (175pb) corresponding to the homozygous type mutated TT, did not found in our samples, the heterozygous CT type appears as two bands (198pb and 175pb) (Figure 7).

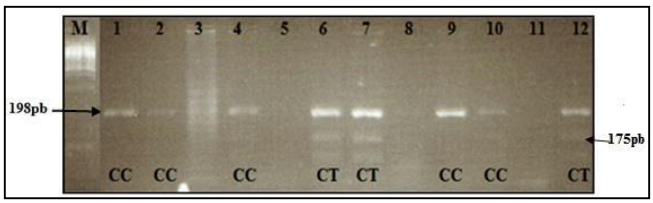

Figure 7: Profile of agarose gel electrophoresis (3%) of the fragments after digestion with the *HinfI* enzyme. M: size marker; 1-12: subjects.

Genotypic and allelic frequencies of the different forms were calculated for the genotyped patients (8 patients and 10 controls) (Table 7, Figure 8).

Table 7: Distribution of genotypic and allelic frequencies of the polymorphism C677T of *MTHFR*

		Patients		Controls		OR	*p value*
		n	%	n	%		
Genotypic frequencies	677CC	5	62 ,5	6	60	/	/
	677CT	3	37,5	2	20	1,80[0,14-25,91]	1
	677TT	0	0	2	20	0[0-7,98]	0,67
Allelic frequencies	C	8	72,73	8	66,67	/	/
	T	3	27,27	4	33,33	0,54 [0,08-3,24]	0,69

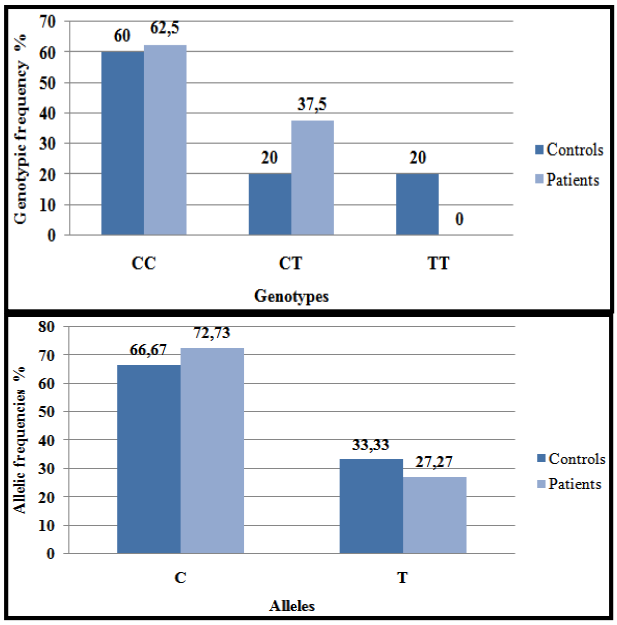

Figure 8: a- Genotypic frequencies of C677T of *MTHFR* in patients and controls. b- Allelic frequencies of C677T of *MTHFR* in patients and controls.

Discussion

Age distribution of the diagnosis

The distribution of patients according to their age ranges shows that β-thalassemia is a pediatric disease revealed, it begins at early childhood and complications appear as evolution. In our results, the mean age of diagnosis is 1.9 years with extremes ranging from 3 months to 10 years, whereas according to Lahlou (2016), Bedir and Miloudi (2006), the average age of diagnosis is 5 years. Romdhane and *al*. (2014) indicate, on the other hand, an average age of 9 years in the Tunisian population with extremes ranging from 2 to 17 years.

Distribution by sex

The results obtained are similar to those of Djamaa (2013) and Romdhane (2014) highlighting a male predominance. Male dominance can't be explained by a relationship between sex and illness since its transmission is autosomal recessive, it affects both sexes equally (Bedir and Miloudi 2006). Our results differ from those obtained by Haddad and Bradai (2016), Sall and al. (2014) reporting a slight female predominance with a sex ratio respectively of 1.3 and 0.87. The number of patients studied (36 patients) does not make it possible to draw a conclusion in this direction.

The consanguinity rate in thalassemic patients

In our series, 20 patients (56%) came from a consanguineous marriage, including 36.11% of the first degree, 5.56% of the 2nd degree and 13.89% of the 3rd degree. These results are supported by those of Bedir and Miloudi (2006) and Djamaa (2013), with consanguinity rates of 68% and 61% respectively. Moreover, according to Djenouni and al. in a study from 1995 to 2002, 23% of patients at CHU Annaba have a history of consanguinity. Consanguinity alone does not seem to be the main cause of thalassemia, but it increases the probability of the appearance of the disease. Its high frequency in the Maghreb countries is explained by the high frequency of consanguineous marriages in these regions. The number of thalassemic children in a family can have a significant impact on the management of patients. In our study, 4 patients, or 11.11% of cases another member in fratery suffering from thalassemia. In a study by Djenouni and al. 39.5% of cases in fratery are reported.

Hematological and electrophoretic profiles of patients

Heterozygous β-thalassemic

Our results are similar to those of Haddad and Bradai (2016) reporting the presence of microcytic pseudo-polycythemia in children with the following average levels: MCV 62.84fL, RBC 5.22 x10^6/µL, and HbA2 5.7%. The results published by Dogaru and al. (2011) have shown that HbA2 levels vary between 3.5 and 7.8%. Sall et al. (2014) explain microcytosis by the occurrence of a deficiency in the synthesis of Hb, resulting in a reduction in its cytoplasmic concentration and an increase in the number of mitoses in order to continue a certain maturation of erythroblasts. This microcytosis is often accompanied by hypochromia and is a biological sign suggestive of hemoglobinopathy such as β-thalassemia. In addition, Desrosiers (2003) explains pseudo-polycythemia by bone marrow reaction that increases RBC synthesis to meet the transport needs of O2. Joly and al. (2014) indicate that the increase in HbA2 is the consequence of a relative increase in the proportion of δ-globin chains relative to β-globin chains.

Homozygous β-thalassemic

Our results show, on the one hand, an intense decrease in the number of red blood cells in patients with severe forms of β-thalassemia (84% of cases), accompanied by severe anemia in all patients (100%), then that microcytic anemia is marked in 84% of cases, hypochromia was marked in only 7 patients.

On the other hand, an increase in the HbF level between 6.4 and 98.6% was observed. These observations are largely confirmed by Bonnello-Palot and al. (2016) report that the severe forms of β-thalassemias are marked by a more or less profound anemia directly linked to the quantitative deficit of the β chain which limits or prevents the formation of the tetramer, the α chain deprived of its partner, precipitates in the erythroblast and in the red blood cell and causes their destruction. According to Mario and Sala (2016), an intermediate or major β-thalassemia is evoked in front of an hypochromic microcytic anemia (Hb <10g/dl), and an increased HbF to compensate for the lack by increasing the synthesis of γ chains. Our results are similar to those published by Loutfi and al. (2016), indicating that two-thirds of diagnosed anemias were microcytic anemias. Similarly, Belhadi (2011) reported an average red blood cell count of 3.899x106/µL in patients, a low level of MVC, MCHC and Hb.

Family tree of the representative family

Individual II.4 first married with his cousin who was healthy (1st degree consanguinous marriage), all their children (III.5, III.6, and III.7) are carriers of β-thalassemia. After the death of his wife, he remarried with another woman (II.3) in a non-consanguineous marriage, this second wife was a carrier of β-thalassemia, her children III.3 and III.4 are homozygous β-thalassemic. II.2 the sister of II.3 is also a carrier of β-thalassemia and is married to a carrier (II.1), one of their children is a carrier (III.1) and the other is a carrier (III.2).

These observations are consistent with what is reported in the literature concerning, on the one hand, the mode of transmission of β-thalassemia which is autosomal recessive since several children affected (homozygotes) and belonging to both sexes, are from unaffected parents (heterozygotes). Thus, the presence of the two mutated alleles of the gene is necessary for the disease to manifest itself. On the other hand, consanguinity is not the main cause of the onset of the disease but increases its probability.

Genetic profile of β-thalassemia

Case-control analytical study of the genotypic and allelic profiles of the T3801C polymorphism of the *CYP1A* gene

The genotypic frequency distribution of the polymorphism T3801C of *CYP1A1* shows that the wild genotype (TT) is the most common in the diseased population. The heterozygous genotype (TC) is second in both populations (healthy and sick). In addition, the mutated homozygous genotype (CC) was found only in patients. The OR and p-value calculations show that the T3801C polymorphism of *CYP1A1* is not a risk factor for β-thalassemia (p value> 0.05). These results are inconclusive given the small number of our sample. Regarding the allelic frequencies of *CYP1A1* T3801C, the OR and p-value calculations show no correlation between *CYP1A1* T3801C polymorphism and β-thalassemia. To our knowledge, no study, allowing us to confirm or invalidate our results, has been established concerning the relation between the T3801C polymorphism of *CYP1A1* and β-thalassemia.

Case-control analytical study of genotypic and allelic profiles of C677T polymorphism of *MTHFR* gene

The genotypic frequency distribution of the *MTHFR* C677T polymorphism in our sample shows that the healthy homozygous genotype (CC) is the predominant genotype in both populations (patients and controls). The heterozygote genotype (CT) is predominant in the diseased population. As for the homozygous mutant (TT), it is present only in the healthy population. The OR and p-value calculations suggest that the *MTHFR* C677T polymorphism does not appear to be involved in the occurrence of β-thalassemia.

The distribution of *MTHFR* C677T allelic frequencies indicates that the C allele is predominant in the diseased population, whereas the T allele is more common in the healthy population. The OR and *p-value* calculations indicate that there is no association between the polymorphism in question and β-thalassemia. Our results are in agreement with several studies. Indeed, Mustafa and al. (2010), in a Kuwait study of 50 β-thalassemic patients and 50 healthy controls, showed that 32% of patients were heterozygous and 4% were homozygous for the *MTHFR* C677T mutation. They revealed that the polymorphism in question does not appear to be a risk factor in thrombotic events. Similarly, an Iranian study by Rahimi and al. (2008), which included 151 patients with β-thalassemia major and 7 patients with β-thalassemia intermediate, including 82 men and 76 women with a mean age of 13.6 +/- 6.3 years, and 180 controls (103 men and 77 women) with an average age of 16.8 +/- 2.1, reported that the prevalence of *MTHFR* polymorphism C677T was slightly higher in patients (50%) than in healthy controls (48.3%), and that thrombophilic mutations are not associated with thrombotic events in β-thalassemic patients, suggesting that these findings need to be confirmed by a larger sample study. In a Jordanian study, evaluating the prevalence of the C677T mutation of *MTHFR* in β-thalassemic patients, Al-Sweedan and al. (2009) found that this mutation was slightly higher, but not significant, in patients with major β-

thalassemia than controls. B-thalassemia major is thus a chronic hypercoagulable disease independent of predisposing genetic factors. In two other studies of the Eastern Mediterranean region conducted by Zalloua and al. (2003) and Iolascon and al. (2001), the presence of the C677T mutation of *MTHFR* was not significantly correlated with thrombotic risk. While no association between C677T polymorphism and β-thalassemia has been reported, we suggest that the allele C at position 677 of *MTHFR* is highly conserved in our study population. These results can only be conclusive if the size of the study population will be larger.

CONCLUSION

Our results showed that β-thalassemia is a pediatric revelation disease with male predominance, and that its appearance is not solely due to consanguinity. The results obtained by studying the hematological and biochemical profiles of the two types of β-thalassemias (homozygotes and heterozygotes) are totally consistent with what is reported in the literature. Moreover, the genotypic exploration of the two polymorphisms (*MTHFR* C677T polymorphism and *CYP1A1* T3801C polymorphism) indicates that they are not associated with β-thalassemia. However, the relatively small size of the cohorts used for these studies does not reveal the real effect of these polymorphisms on this pathology. It would be interesting to identify mutations of the *β-globin* gene in our samples and to continue studying the influence of these polymorphisms on β-thalassemia using a larger sample.

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