Proceedings of the Pakistan Academy of Sciences 50 (4): 315–325 (2013)

ISSN: 0377 - 2969 (print), 2306 - 1448 (online)

Pakistan Academy of Sciences

Research Article

Can We Really Treat Thalassemia Major?

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Abstract: Plant species have proven to be an important source of treatment in different diseases, in particular certain malignant tumors. The cytotoxic efficacy of *Fagonia cretica* has long been a subject of interest. This particular interest in a patient of breast cancer helped us to ascertain the efficacy of this herbal plant in Thalassemia. In initial testing, we obtained encouraging results by using dried aerial parts of the whole plant. It obligated upon us a detailed study of the plant in Thalassemia. A powder of the dried aerial parts of the whole flowering plant was given in doses of 120 mg/kg body weight. The hematological and clinical results in nine Thalassemia Major group, showed a decrease of the Hb F from 97.45 to 10.82% and increase of Hb A from 0.33 to 85.5% ($P<0.05$) after about nine months of treatment, when blood samples for electrophoresis were collected at least six weeks after transfusion. The size of the liver and spleen which were 4-9 cm palpable below costal margins before treatment were not palpable after nine-month treatment. Similarly, in all the six patients, suffering with *Thalassemia intermedia* total hemoglobin improved from 2.13-5.6 g ($P<0.05$). Mean values of hemoglobin before and after the treatment were 8.08 and 11.95 g, respectively. In the four patients suffering with Thalassemia minor mean values of Hb A2 were 5.25 and 3.30, respectively before and after; and the reduction in Hb A2 was significant with 0.13 to 3.77% ($P<0.05$).

Keywords: Thalassemia, *Fagonia cretica*, genetic mutation

1. INTRODUCTION

Enormous literature is available on *Fagonia cretica*, a small wild spiny under-shrub found mostly in dry calcareous rocks and sandy soils throughout Pakistan. *Fagonia cretica* is member of the family *Zygophyllaceae*. Its medicinal value is well documented [2-3]. The plant tastes bitter and is usually used for the treatment of fever, dysentery, asthma, skin infection and liver troubles [2, 3, 5]. Its active ingredients are reported [1-3] and the effect of each ingredient, like bitter alkaloids – especially the Saponin-1 and Saponin-2, has been studied on various animals [4-10]; but, probably, no scientific study has ever been under taken on clinical grounds by a medical professional.

The role of *Fagonia cretica* in cancer treatment has long been discussed and the possible mode of its action has also been suggested in the literature. For example, Lam et al [11] clearly elucidated that "*an aqueous extract of Fagonia cretica induces DNA damage, cell cycle arrest and apoptosis in breast cancer cells via POXO3a and p53expression. Following genotoxic stress, an intact DNA damage response (DDR) is necessary to eliminate lethal and tumorigenic mutations. The DDR is a network of molecular signaling events which control and coordinate DNA repair, cell cycle arrest and apoptosis".* Impairment in the DNA damage response represents a double-edged sword; on the one side loss of repair mechanisms can drive tumorigenesis and on the other can affect sensitivity to genotoxic chemotherapy [11]. *"The tumor suppressor protein, i.e., p53, plays a pivotal role in regulating the cellular response to stress and damage signals. Several of the cell signaling pathways involved in*

Received, June 2013; Accepted, October 2013
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the DDR and cell differentiation converge with p53 and loss of p53 functionality is common in more than 50% of cancers. In response to stress signals, post-translational modifications of p53, such as phosphorylation, drive its nuclear translocation and subsequent target gene transcription. Normally, upon DNA damage, p53 is rapidly stabilized by the DNA damage sensor, ATM, via phosphorylation of serine-15 within the p53 N-terminus activation domain. Consequently, dissociation of the MDM2-p53 repressor complex prevents monoubiquitination of p53 and its degradation. This in turn increases p53 half-life and activates its transcriptional program".

It was for the first time in 1976, perhaps, that we got interested in this herbal product, when a *Hakim* gave this product to a breast cancer patient (Fungating ductal; Fig. 1), in the Seyal Medical Center, Multan, Pakistan. The blood group of the patient was B-ve, and it was very difficult to arrange the supply of blood group after each course of chemotherapy. When she started using *Fagonia cretica,* we evinced good clinical improvement and there was no need of blood transfusion after each course of chemotherapy. Thereafter, we started using this herbal product in all sort of cancer patients and observed that it takes care of almost all the side effects of cytotoxic drugs without compromising the cytotoxic efficiency of the chemotherapy, but instead supplements the cytotoxic efficacy of the drugs. Similar observations were made in Thalassemia. This reported study is based on these observations.

Since long, biologists believed that they understood how genetic mutations cause the disease. But recent our work has revealed an important 'twist in the tale' and uncovered surprising, even counterintuitive, ways by which alterations in DNA not only can make people sick but also can alter the original parent sick gene to normal [11-14]. The classic views assumed that what are termed "silent" mutation were inconsequential to health, because such changes in DNA would not alter the composition of the proteins encoded by genes. Proteins function in virtually every process carried out by cells, from catalyzing biochemical reactions to recognizing foreign invaders. Hence, if a protein's makeup ends up being correct, any small glitches in the process leading to its construction could not do body harm, but instead will provide a relief to the sick masses [15-16].

The high incidence of Thalassemia is related to selective advantage of the carrier state to malaria infection. Initially, Thalassemia or the sickle cell mutation apparently arose repeatedly in regions riddled with malaria during the late 15th century in Africa and the Middle East [13]. As a result these diseases are more common in the areas where malaria is endemic, like the Mediterranean region, through tropical countries including like Sub-Saharian Africa, the Middle East, Pakistan, India, South East Asia, and Indonesia [12, 13].

Thalassemias are classified according to their severity into major, intermediate, and minor forms. Thalassemia intermediate is characterized by anemia and splenomegaly though not of such severity as to require regular transfusion. Thalassemia minor is the symptomless carrier state. While these descriptive terms do not have a precise genetic meaning, they remain useful in clinical practice [14].

During the last couple of years it has become clear that Thalassemia is extremely heterogeneous and that its clinical picture can result from the interaction of many different genetic defects, all of which result from a reduced rate of production of one or more of the globin chain(s) of hemoglobin. Because Thalassemia occurs in populations in which structural hemoglobin carriers are common, it is not at all unusual for an individual to receive a Thalassemia gene from one parent and a gene for a structural hemoglobin variant from the other. These different interactions produce an extremely complex and clinically diverse series of genetic disorders, which range in severity from death in utero to extremely mild, symptomless, hypochromic anemia [14-16].

Genetics have discovered thousands of mutations responsible for the incidence of diseases in humans, but founder mutations stand apart [13]. The victims of genetic disorders die before reproducing, stopping the spread of the original mutant genes from reaching the future creations. But the founder mutations often spare their carriers and therefore can spread from the original founder to his or her descendents. Perhaps the best-known

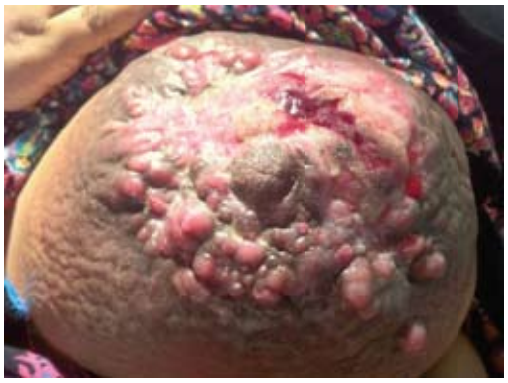

Fig. 1 In 1977 a 56 year old lady with a Fungating Breast Cancer. She was refused surgery and radiotherapy because of the very big size of the tumor. She was given combination chemotherapy along with Crude Extract of **Fagonia cretica** She recovered completely and died in 2004 This picture was taken 3 months after the treatment with Casemia. Initially, the breast looked like a weeping cauliflower, thus picutre was not taken as I could never believe such a fascinating response and eventually it healed completely.

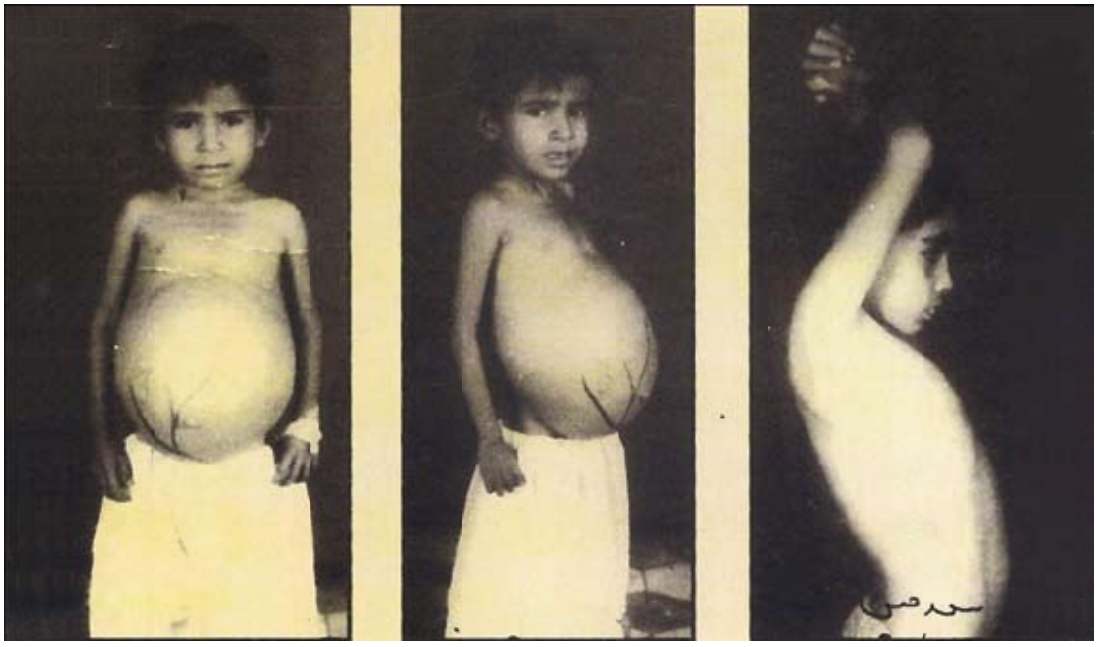

Fig. 2A April 2nd 1996 [Before Treatment].

April 2nd 1996.

Fig. 2B April 4th 1997 [After Treatment].

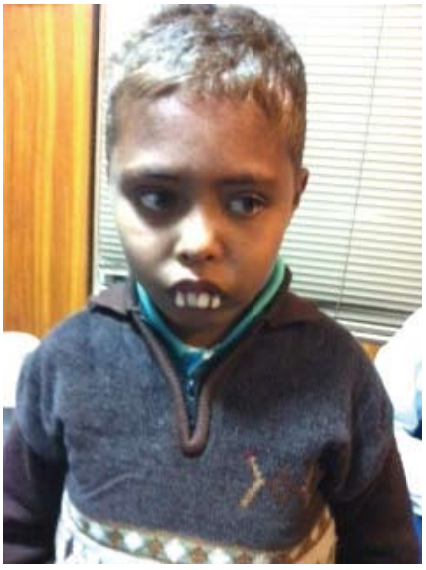

Fig. 3 1st October 2012
Before Treatment
Hb 3.8 Gm: Hb F 98.6%.

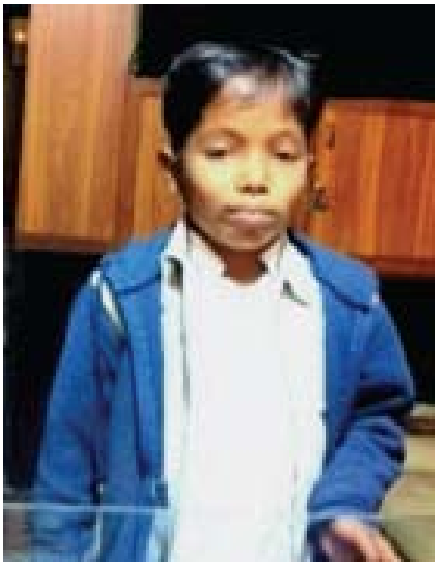

Fig. 4 21st January 2013
After 3 ½ month
Hb 7.1 Gm.

Fig. 5 15th April 2013
After about 6 ½ month
Hb 8.7 Gm Hb F 81.7%.

example of a double-edged genetic mutation is the one responsible for Thalassemia. A single copy gene helps the carrier to survive malarial infection [13, 18-19]. But two copies doom the bearer to pain and short life. But during the past century there have been lot of improvement in the treatment of Thalassemia. With repeated regular transfusions and chelating therapy the individuals with beta Thalassemia Major can survive beyond the age of forty. Bone marrow transplant is another choice of treatment but it has its own limitations [20-23]. Our 20-year research in Thalassemia gives convincing information about the effectiveness of this wild thorny plant, i.e., *Fagonia cretica,* in changing the genetic pattern of this long inherited disease.

The effectiveness of *Fagonia cretica* in the treatment of Thalassemia was a chance occurrence about 20 years back, when a critically ill Thalassemia patient responded in a miraculous way. We gave this herbal preparation out of curiosity, with the belief that it probably acts at the molecular level. Ever since we have used this herbal medicine in Thalassemia patients and found quite satisfactory results (data not reported). It was therefore planned to conduct a scientific study on the subject.

2. MATERIAL AND METHODS

The reported study comprised of two components. The first component is based on a case study of three patients. The second component has been designed as a usual treatment case controlled study based on the encouraging results of the first component.

Case 1: In 1992, a 12-year old girl suffering from Thalassemia major was having weekly transfusion besides having chelating agents. She was critically ill and was not responding to any treatment as the transfusions often resulted in severe blood reactions and the chelating agents caused a lot of distress. She had a lot of ascites with enlarged liver and spleen over and above 10 cm palpable below costal margins. She was given dried powder of *Fagonia cretica* whole flowering plant (in doses of 120 mg kg^{-1} body weight). She started exhibiting good clinical improvement in a couple of weeks with reduction in the size of spleen, liver and ascites. Her Hb F was 97.4% and Hb A2 2.6%, when she was first diagnosed Thalassemia Major at the age of six months. When she was almost free of the disease symptoms and was no longer transfusion-dependent for more than three months, her Hb A was 98%; Hb A2 was 1.6% and Hb F was 0.4 %.

Case 2: A seven years old boy was suffering Thalassemia major (Fig. 2-A) when first reported. His hemoglobin was 4.2 G, liver and spleen were 7 cm and 11 cm, respectively, palpable below costal margin; also, that there was a lot of ascites. He had been diagnosed with Thalassemia major when he was just six months old; at that age his hemoglobin was 6.3 Gm, Hb A 1.2%, Hb A2 2.4%, and Hb F 96.4%. He was given powdered *Fagonia cretica*. He showed good clinical improvement and after about one year (Fig. 2B) he was no more blood dependent. His Hb was 12.4 Gm, Hb A 87.6%, Hb F 10.2% and Hb A2 was 2.2%.

Case 3: A 13-year boy had been diagnosed with Thalassemia major when he was just three months old. Transfusions invariably caused severe blood reactions. His clinical condition gradually deteriorated. On the first visit his spleen was quite enlarged and palpable seven cm below the costal margin and liver five cm. There was a lot of ascites. The bony deformities were also evident (Fig. 3). His blood pictures on the first and subsequent visits are given in Table 1. His clinical improvement with this treatment was quite evident after about three and half months (Fig. 4) and more so after about six and a half months (Fig 5). During the course of treatment with *Fagonia cretica* he was not using other drugs. He is still using *Fagonia cretica* without any chelating agent or supportive medicines like folic acid or calcium supplements. Also, he never had blood transfusion after he started the herbal treatment. His Hb F dropped from 98.6% to 81.7% whereas Hb A increased from nil to 17.6% (Table1).

A usual case control study was planned to show the effects of the whole aerial parts of flowering plant of *Fagonia cretica* in all the three categories of Beta Thalassemia i.e., major; intermedia and minor. There were around 32 patients of Thalassemia major, six of intermediate and four of minor. The dropout rate was quite high in Thalassemia major

Table 1. Details about a patient of Beta Thalassemia Major (without transfusion).

Parameter	1st diagnosed at the age of 3 months	1st Oct 2012	21st January 2013	15th April 2013	Normal Value
Hb	4.9	3.8	7.1	8.7	13.00 18.00
Total RBC	2.1	1.7	3.8	3.1	4.50 6.50
Hct	16.0	1.5	24.0	29.0	38.00 52.00
MCV	74.0	76	52.0	92.0	75.00 95.00
MCH	22.0	20	22.0	28.0	26.00 32.00
MCHC	30.0	22	28.0	30.0	30.00 35.00
Platelet Count	199.0	160	174.0	216.0	150.00 400.00
Nucleated RBCs	10.0	-	-	-	
RBC MORPHOLOGY					
Hypochromia	++	+++	+++	++	
Microcytosis	++	+++	+++	++	
Macrocytosis	++	+++	+++	++	
Anisocytosis	+++	+++	+++	+++	
Poikilocytosis	++	+++	+++	++	
Schistocytes	+	++	++	+	
HEMOGLOBIN ELECTROPHORESIS:					
Hb F:%	98.6 %			81.7%	≤ 1%
Hb A2:%	1.4 %			0.7%	< 3.5%
Hb A: %	----			17.6%	
Spleen:	Palpable below costal margin	7 cms	Not Palpable		
Liver:		5 cms	Not Palpable	.	

group, as most of the patients were discouraged, because there was no clinical data available to support our observations. The other reason of excluding a group of patients from the study was, because they could not produce the initial reports of diagnosis for Thalassemia. Thus, only nine patients in Thalassemia major were left; the results of various tests are given in Table 2. The plants of *Fagonia cretica* were collected during March, April and early May, when these were flowering. Aerial parts of whole plants were dried under shade and then crushed to a fine powder. The powder thus prepared was administered orally @ 120 mg kg^{-1} body weight.

3. RESULTS

The age of nine patients of Thalassemia in major varied from two to 13 years. Clinical investigations of the Thalassemia major patients included total hemoglobin content, size of the liver and spleen palpable below costal margin, and electrophoresis. These patients were advised to continue with the medicines like folic acid and chelating agents and avoid the food already prescribed by the physicians. The patients were monitored at weekly intervals by their attending physician. Finally, when the patients had shown good clinical improvement after about nine months and they were no more transfusion dependent, the electrophoresis was done to compare the results.

We applied the paired t-test to compare various determinants of blood tests and size of the liver and spleen. The total Hb significantly improved from 2.888-4.88 g ($P<0.05$). Mean hemoglobin content in patients before the treatment was 6.84 g, whereas after nine months it rose to 10.72 g. Similarly, before the treatment the average Hb A was 0.33% and

Table 2. Details about nine patients of Beta Thalassemia Major.

Sr. No.	Age in months	Sex	Total Hb in Gm		Hb A %		Hb A2%		Hb F%		Liver		Spleen	
											Palpable in cms below costal margin			
			Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1.	30	male	8.2	12-14	-	96.5	2.8	1.6	97.2	1.9	2	-	3	-
2.	72	male	6.8	11.3	-	96.4	2.8	3.6	97.2	-	3	-	5	-
3.	60	female	7.2	9.4	-	98.4	1.2	1.4	98.8	0.2	2	-	3	-
4.	15	male	5.2	8.5	-	92	1.6	2.2	98.4	5.8	3	-	3	-
5.	156	male	7.8	11.6	3.0	93.5	1.2	2.3	95.8	4.2	3	-	5	-
6.	51	male	9.2	11.2	-	94.6	3.6	2.6	96.4	2.8	3	-	5	2
7.	24	male	7.19	11.29	-	97.7	2.1	2	97.9	0.3	-	-	5	-
8.	3 6	male	6.2	12.4	-	96.8	3.2	2.7	96.8	0.5	-	-	-	-
*9.	156	male	3.8	8.7	-	17.6	1.4	0.7	98.6	81.7	6	±	10	±

Before: At the time of first diagnosis **After :** 9 months after the treatment
*This patient never used transfusion during the course of treatment.

Table 3. Means and 95% confidence intervals.

Parameter	Mean		95% Confidence interval	
	Before	After		
Hb in Gm	6.84	10.72	2.88-4.88	significantly improved
Hb A %	0.33	87.05	66.69-106.74	significantly improved
Hb A2 %	2.21	2.12	0.54-0.72	non-significant, reduced
Hb F %	97.45	10.82	66.00-106	significantly reduced
Liver Cms below costal margin	2.44	00	1.03-3.84	significantly reduced
Spleen Cms below costal margin	4.33	0.22	2.02-6.19	significantly reduced

87.5% and the minimum improvement was above 66% (P<0.05), whereas reduction in Hb F was from 97.45% to 10.82% and minimum reduction was 66% (P<0.05). However, there is non-significant difference in Hb A2 before and after the treatment.

The size of liver was reduced significantly as the mean value for liver size before and after was 2.44 and 0.00 cm palpable below costal margin; the reduction was 1.03 to 3.84 (P<0.05; Table 3). Similarly, mean values for spleen were 4.33 cm before the treatment and 0.22 cm palpable below costal margin; reduction in spleen size was from 2.02 to 6.19 (P<0.05; Table 3).

The six patients in Thalassemia intermedia group were given the herbal treatment for about nine months. Their total hemoglobin, Hb F, Hb A2, Hb A, size of the spleen and liver palpable below costal margin were measured at start of the study and again at the end of study (Table 4). The hemoglobin significantly improved from 2.13 to 5.6 g (P<0.05). Mean values for hemoglobin before and after were 8.08 and 11.95, respectively. Mean values for Hb F before and after the treatment were 14.68 and 7.40. The Hb F was reduced from 3.4 to 11.76 (P<0.05). Average value for Hb A2 were 4.31 and 3.31, before and after the treatment, respectively, and the reduction was 0.30–1.70 (P<0.05). The mean values for HbA were 82.00 and 89.58 and Hb A increased substantially from 2.0 to 13.6 (P<0.05). Average values for spleen before and after the treatment were 2.16 and 0.3 and the reduction in the size of spleen is 1.04 – 2.62 (P<0.05). The reduction in size of liver was insignificant as the mean size at the start of the study was 0.66 (Table 5).

The measurements for four patients of Thalassemia minor are given in Table 6. The first three patients used *Fagonia cretica* for three months only and the fourth patient, aged 28 years, for six months. The paired t-test was used to compare the means of different parameters. Mean values for hemoglobin before and after the treatment were 9.89 g and 11.5 g, respectively. The improvement in Hb was from 2.95 to 6.4 (P<0.05). Mean values of Hb A2 were 5.25 and 3.30, respectively, before and after; and the reduction in Hb A2 was from 0.13 to 3.77 (P<0.05). The average values for HbA

Table 4. Details about six patients of Intermedia.

Patient	1- 14MM		2- 13MF		3-15MM		4-14MM		5- 24MM		6-14MM	
Parameters	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Hb in Gm	7.8	13.1	7.6	12.5	9.2	13.1	7.8	13.1	8.8	10.1	7.3	9.8
RBC MORPHOLOGY												
Hypochromia	+++	-	+++	-	++	-	+++	-	+++	+	+++	+
Microcytes	++	+	+++	+	++	Few	++	Few	++	+	+++	++
Macrocytosis	++	+	+++	Few	++	Few	+++	+	++	+	++	+
Anisocytosis	++++	+	+++	+	+++	+	+++	+	+++	+	+++	+
Poikilocytosis	++	+	+++	+	+++	+	++	+	+++	+	+++	+
Schistocytes	+	-	++	+	+	+	++	+	++	+	_	_
NRBC	+	Few	-	-	+	-	+	+	+	_	_	_
Spherocytes	+	-	-	-	-	-	-	-	-	-	-	-
HEMOGLOBIN ELECTROPHORESIS												
Hb F: %	19.6	4.2	11.6	5.3	12.8	7.5	11.7	5.2	13.8	9.4	18.6	11.0
Hb A2: %	4.3	3.3	5.1	3.6	5.2	4.4	4.7	2.7	3.7	3.1	2.9	2.8
Hb A: %	76.1	92.5	83.3	91.1	88.0	88.1	83.6	92.1	82.5	87.5	78.5	86.2
Palpable below costal margin in cm												
Spleen	1	-	2	-	1	-	2	-	4	1	3	1
Liver	2	-	-	-	-	-	-	-	-	-	2	-

Table 5. Means and 95% confidence intervals.

	INTERMEDIATE		
	Mean		**95% confidence level**
	Before	**After**	
HB	8.08	11.95	2.13-5.60 significantly improved
HBF	14.68	7.10	3.40-11.78 significantly reduced
HBA2	4.31	3.31	0.29-1.70 non-significant, reduced
HBA	82	89.58	2.00-13.16 significantly improved
Spleen Cms below costal margin	2.16	0.33	1.04-2.62 significantly reduced
Liver Cms below costal margin	0.66	0	-0.41-1.75 significantly reduced

before and after were 94 and 96, respectively, and improvement in Hb A was up to 4.00 ($P<0.05$; Table 7).

4. DISCUSSION

Our more than 20-year experience with the use of *Fagonia cretica* has given a new hope in the treatment of Thalassemia. We observed that in almost all our patients, who regularly used the herbal medicine as recommended, the electrophoresis after about nine months of treatment evinced normal pattern. The size of the spleen and liver were reduced to normal and the ascites also disappeared. They exhibited impressive clinical improvement. In patient # 3, 5 and 6, hemoglobin used to drop below 10 g which necessitated fresh blood transfusion at about six weeks interval; therefore, we adopted the discipline of electrophoresis after six weeks of the transfusion, i.e., just before the next transfusion in all patients.

Table 6. Details about four patients of Thalassemia Minor**.**

Parameters	1	3 Female*	2	5 Female*	3	3 Female*	4	28 Male
	19/06/2012 Before	02/10/2012 After	19/06/2012 Before	02/10/2012 After	19/06/2012 Before	02/10/2012 After	07/10/2012 Before	30/05/2013 After
Hb in Gm	9.3	10.6	9.9	9.5	9.7	9.7	10.4	16.4
RBC MORPHOLOGY								
Hypochromia	++	+	++	+	++	+	++	-
Microcytes	++	+	++	+	++	+	+	-
Macrocytosis							+	-
Anisocytosis	+	+	++	+	+	+	++	+
Poikilocytosis	+	-	+	-	-	-	+	-
Schistocytes	-	-	-	-	-	-	+	-
NRBC	-	-	-	-	-	-	+	-
Spherocytes	-	-	-	-	-	-	+	-
HEMOGLOBIN ELECTROPHORESIS								
Hb F:%	0.7%	0.7%	0.8%	0.8%	0.6%	0.6%	0.8%	0.5%
Hb A2:%	5.2%	3.5%	**5.0%**	4.5%	5.1%	2.7%	5.7%	2.5%
Hb A:%	94.1%	95.8%	94.2%	94.7%	94.3%	96.7%	93.5%	97%

*Patient 1-3 had treatment for only three months

Table 7. Means and 95% confidence intervals.

MINOR				
	Mean		**95% confidence level**	
	Before	**After**		
HB	8.08	11.50	6.4-2.95	significantly improved
HBF	.67	.65	.05-.10	non-significant
HBA2	5.25	3.30	.18-3.77	significantly reduced
HBA	94	96	4.02	significantly improved

Amongst the first diseases to be studied at the genetic level, Thalassemia still remains a challenge for understanding the pathogenetic basis of inherited disorders as well as the molecular mechanism involved in the regulation of gene expression. Different studies have contributed to define the complex pathophysiological mechanisms underlying this disease and have made possible prevention programs based on large scale screening and prenatal diagnosis in populations at high risk [21, 23, 24]. Soon after gestation, the embryonic, fetal and later after birth the physiological changes in oxygen requirements are accompanied by the switching of globin gene expression [15, 16]. This process represents one of the most intriguing and studied regulatory mechanisms of gene expression which leads to progressive and sequential changes in the expression of embryonic, fetal and adult globin genes (Fig. 6) and thus allows synthesizing different types of hemoglobin tetramers. However, the detailed mechanisms that control this process are still not fully understood. Michela Grosso [20] gave a detailed elucidation of Thalassemia at molecular level.

Human hemoglobin synthesis requires two switches: from embryonic to fetal hemoglobin at 6 week of gestation and from fetal to adult production at birth (Fig. 6). *The first genes to be expressed are those of the ζ-chain (α-like) and ε-chain (β-like), synthesized in the embryonic yolk sac until 4-5 weeks of gestation, which lead to the formation of Hb Gowers I (ζ2ε2) [26]. Then, with the change of the liver as the main erythropoietic compartment, synthesis of α and γ chains is activated. At this stage the embryonic Hb Gowers II (α2ε2) and Hb Portland*

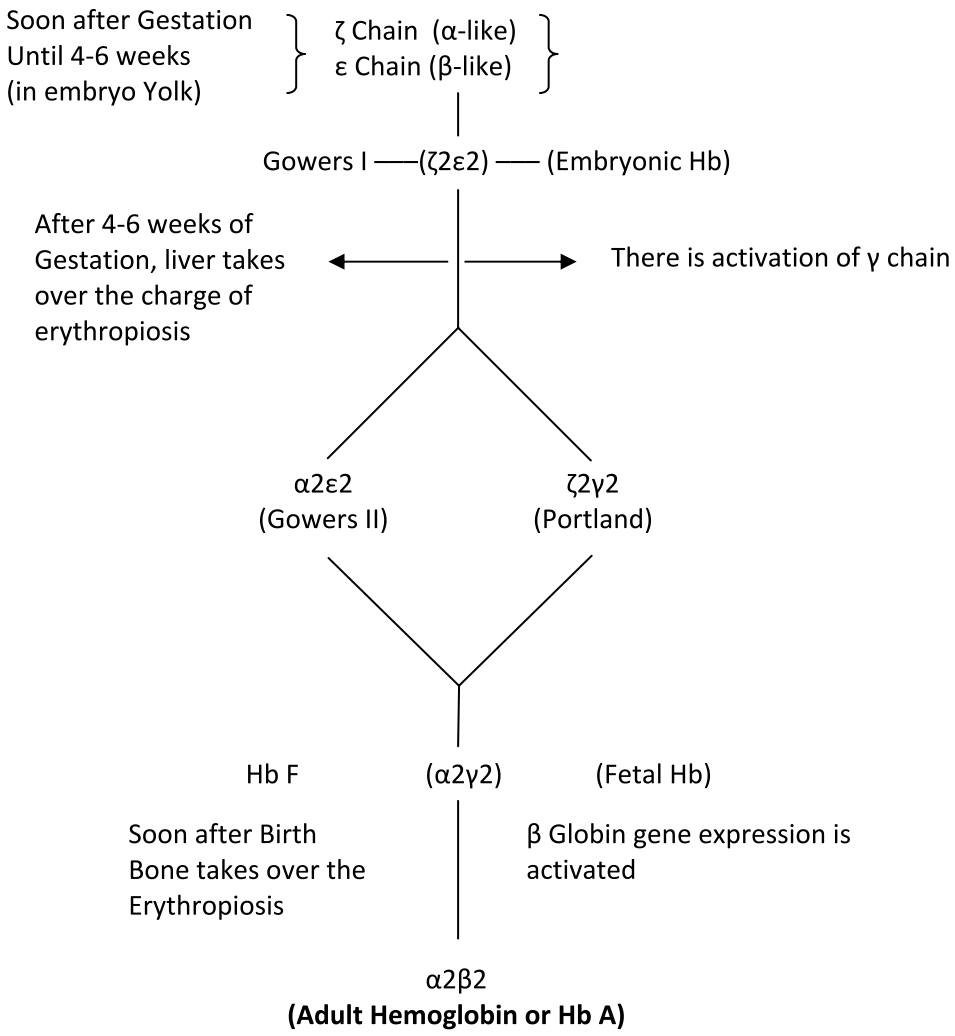

Fig. 6. Globin Gene Expression at different stages of life.

(ζ2γ2) are progressively and completely substituted by the fetal hemoglobin Hb F (α2γ2). Around birth, when the bone marrow becomes the main erythropoietic site, β-globin gene expression is activated to synthesize the adult Hb A (α2β2), which at birth is about 20% of total hemoglobin.

The switch from fetal to adult hemoglobin is completed within the first two years of life and leads to the pattern in which adult globin expression Hb A (α2β2) comprises about 97%, HbA2 (α2δ2) 2-3% and Hb F (α2γ2) less than 1% of total hemoglobin, respectively [15, 26]. The control of tissue and developmental expression of specific globin genes is exerted by physical interactions between different globin gene promoters and the Locus Control Region 'LCR' (LCR is a relatively large cluster region in the β-gene, encompassing -20Kb) through binding of both ubiquitous and erythroid-specific transacting factors. The sequential expression of different globin genes requires coordinated mechanisms of gene silencing and gene competition for the LCR sequences, as well as chromatin remodeling and complex chromosomal looping and tracking processes [15, 26].

The switching of the expression of β-globin genes is not only a fascinating and complex model used for studying regulation mechanisms of gene expression, but its full understanding could also have important therapeutic implications in the treatment of β-Thalassemia. Indeed, the clinical picture of this condition can improve in the presence of sufficiently high levels of 'Hb F' in β-Thalassemia syndromes, in fact, hereditary persistence or drug-mediated reactivation of γ-globin chain output may

result in a reduction of the α and β globin chain imbalance, which represents the main pathogenetic factor influencing the severity of this condition [15, 26].

Locus Control Region persistent expression of fetal hemoglobin may be associated with specific genotypes or induced by appropriate drug treatments. In fact, fetal globin genes can be reactivated by demethylation of regulatory sequences generated by hydroxyurea or 5-azacytidine or y histone deacetylation induced by treatment with short- chain fatty acids [15]. However, besides toxic side effects of these drugs, response to treatment is transient and highly variable. Thus a better understanding of the switching processes and regulatory mechanisms of the β-globin gene may provide a new therapeutic approach in the treatment of Thalassemia.

Our clinical data do not go deep down to the molecular level but our hematological and clinical observations strongly support that has an effect on the *switching processes and regulatory mechanisms of the β-globin gene* providing a powerful relationship of molecular basis of Thalassemia. Our results clearly elucidate the decrease in the fetal hemoglobin and also Hb A2 level, besides increasing Hb A and the total hemoglobin concentration in the blood. A few patients suffering from Thalassemia major showed gradual drop in the hemoglobin level even though their electrophoresis reports were almost normal. The persistence of anemia could possibly due to the clusters of inclusion bodies in the bone marrow causing poor erythropoiesis as discussed before [14-16].

Perhaps for the first time an observation is made that a medicine may change the genetic pattern. The results already discussed also indicate recovery of the distorted RBCs morphology, i.e., Microcytosis, Macrocytosis, Anisocytosis, Poikilocytosis, Schistocytosis, Nucleated RBC and Spherocytosis, in all the three groups of patients

Patients of Thalassemia **intermedia** showed gradual improvement in total hemoglobin without transfusion with marked improvement in their clinical condition with reduction in the size of the spleen. Similarly, the **Thalassemia minor** patients exhibited reduction in the Hb A2. Our observations strongly support that a detailed study should be conducted to show the influence of this herbal product at the molecular level and also elucidate exactly the clinical course of erythropiosis in the bone marrow during the course of treatment in different patients.

Also, we need to work more aggressively to know the active constituent(s) of this herbal plant which are effective in the treatment of Thalassemia. A lot of work has already been done to dig out the active ingredients of *Fagonia cretica*. The chemical constituents are Triterpenoid Saponins: Saponin 1 and Saponin 11, besides it contains beta- sitosterol; ceryl-alcohol; chinovic acid; water soluble saponins, i.e., glucose rhamose; xylose; arabinose; fagogenine and lipids 0.3-1.14%: Campesterol; aglycone; fagonin; oleanolic acid; betulic acid, the later four are derived from the saponins fraction [1-3]. It is beyond the scope of this paper to discuss each chemical constituent of the herbal plant, but we do need to determine and isolate the active ingredients alone or in combination which are effective in all categories of Thalassemia.

5. ACKNOWLEDGEMENTS

This research was conducted and shared by Ayesha Marion Foundation in Dr A Q Khan Thalassemia Research Center. I am highly indebted to Dr. A.Q. Khan for his most sincere support and persuasive guidance for research on the subject.

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