Biochemistry and Biophysics Reports 24 (2020) 100836

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

The relation between mitogen activated protein kinase (MAPK) pathway and different genes expression in patients with beta Thalassemia

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ARTICLE INFO

Keywords:
β thalassemia
MAPK pathway
ARRB1
GNAI2 and DUSP5

ABSTRACT

Background: β-thalassemia is an inherited hemoglobinopathy resulting in quantitative changes in the β-globin chain. Understanding the molecular basis of that disorder requires studying the expression of genes controlling the pathways that affect the erythropoietic homeostasis especially the MAPK pathway. The MAPKs are a family of serine/threonine kinases that play an essential role in connecting cell-surface receptors to DNA in the nucleus of the cell.

Aim: to study the effect of expression of GNAI2, DUSP5 and ARRB1 genes on MAPK signaling pathway in pediatric patients with beta thalassemia.

Methods: Forty children with beta thalassemia major (TM), forty children with beta thalassemia intermedia (TI) and forty age and gender matched healthy controls were enrolled in this study. Detection of GNAI2, DUSP5 and ARRB1 mRNA expression was done by real time polymerase chain reaction (RT-PCR).

Results: revealed increased expression of ARRB1 (Arrestin Beta 1) gene, and decreased expression of both GNAI2 (Guanine nucleotide-binding protein G (i) subunit alpha-2) and DUSP5 (Dual specificity protein phosphatase 5) genes in both patient groups than control groups respectively.

Conclusions: Change in the rate of expression of ARRB1, GNAI2 and DUSP5 may have a role in the pathogenesis of abnormal hematopoiesis in cases of β thalassemia through affecting the MAPK pathway.

1. Introduction

Thalassemias are heterogeneous group of genetic blood disorders, there are two main types, alpha thalassemia and beta thalassemia according to which globin chain is affected. The severity of alpha and beta thalassemia is correlated with the number of affected α-globin and β-globin alleles and on nature of the mutation [1].

β-Thalassemia, one of the most common inherited hemoglobinopathy in the world, is inherited as autosomal recessive disorder affecting the β-globin gene which induce an absence (β^0) or low-level (β^+) synthesis of β-globin protein in erythropoietic cells [2]. Beta-thalassemia includes three main forms: Thalassemia Major, referred to as "Cooley's Anemia" and "Mediterranean Anemia", Thalassemia Intermedia and Thalassemia Minor also called "beta-thalassemia trait" [3].

There are 837 reported HBβ (β-globin gene) mutations, of which 247 have been reported to cause the β-thalassemia phenotype. These can affect any point from expression of the HBB through to protein synthesis [4].

The disorder of β-chain synthesis leads to ineffective erythropoiesis in which erythroid progenitor cells undergo intramedullary apoptosis and do not develop into mature erythrocytes, resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals [5,6].

Although thalassemia is usually asymptomatic or associated with only mild anemia, patients with severe disease require lifelong blood transfusions for survival. Regular transfusion results in normal growth and development until 10–11 years old, after this age risks arise from complications due to transfusion related iron overload [7].

The mitogen activated protein kinase (MAPK) pathway is probably the best characterized signal transduction pathway in cell biology. The

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https://doi.org/10.1016/j.bbrep.2020.100836
Received 1 September 2020; Received in revised form 7 October 2020; Accepted 9 October 2020

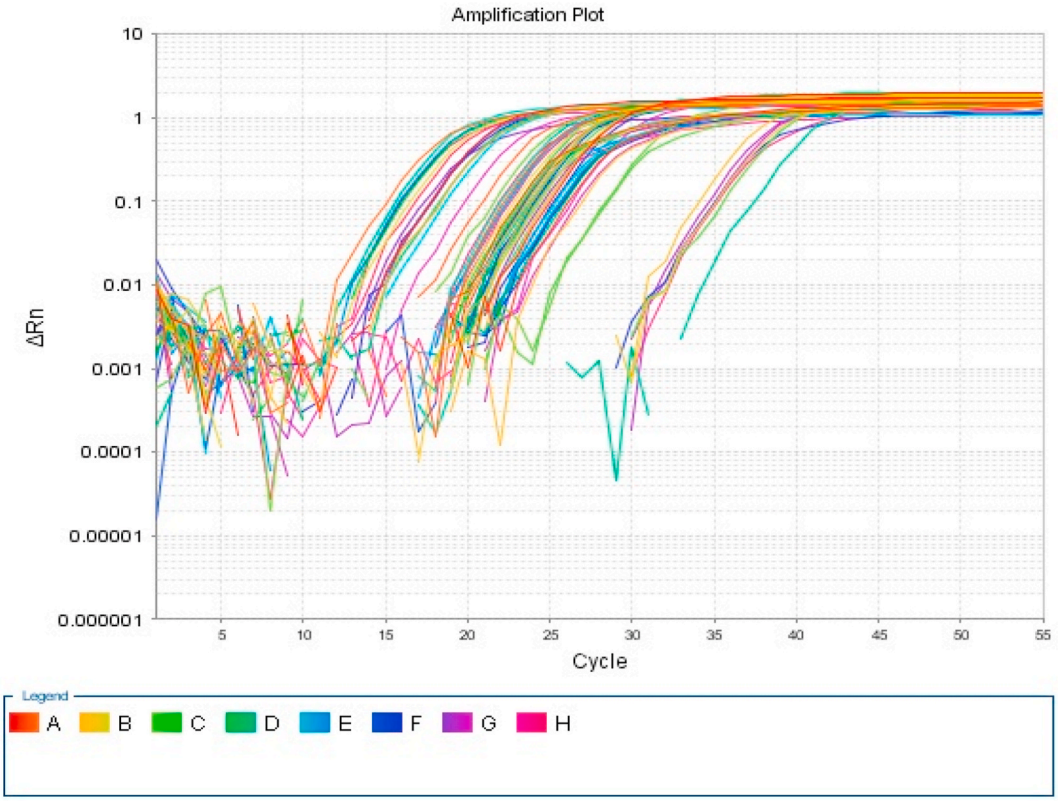

Fig. 1. Shows the amplification plot of genes expression.

MAP kinases are a family of dual-specificity serine/threonine protein kinases; the function of these kinases is to transduce signals from the extracellular receptors to the nucleus where specific genes are activated. These activated genes are implicated in multiple cellular functions, ranging from cell survival and proliferation to cell differentiation and programmed cell death [8,9].

There are three sub-pathways of MAPK, these include extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun amino (N)-terminal kinases 1/2/3 (JNK1/2/3) and p38 isoforms (α, β, γ, and δ) that are most widely studied among the many subfamilies of the MAPK family [10,11]. The ERK signaling pathway mainly exerts its effects on cell proliferation, development, and differentiation induction. In contrast, the JNK and p38MAPK signaling pathways are mainly activated under stress and play roles in growth inhibition, inflammation, and proapoptotic signaling [12,13].

It is well known that hematopoietic stem cells (HSCs) are required to self-renew as well as differentiate into the various hematopoietic lineages [14]. Therefore, appropriate control of HSC self-renewal is essential for the maintenance of hematopoietic homeostasis. Recently, MAPK signaling has been demonstrated to play a key role in the maintenance of HSC quiescence [15].

1.1. Aims of the study

The aim of this study was to study the mRNA expression of GNAI2, DUSP5 and ARRB1 genes within the MAP kinase signaling pathway in beta thalassemia.

1.2. Subjects and methods

This study was carried out on 80 children suffering from β-thalassemia and 40 healthy children as control who not suffering from any hematological disorders. Patients were selected from the Hematology unit, Pediatric department, Menoufia University Hospital. Children included in this study were divided into three groups; **Group I** included 40 children patients with β-thalassemia major (TM), they were 23 males and 17 females with mean age of (7.99 ± 4.27), **Group II** included 40 children patients with β-thalassemia intermedia, they were 23 males and 17 females with mean age of (8.82 ± 4.48) and **Group III** included 40 healthy children not suffering from any hematological disease, age and gender matched as a control group, they were 22 males and 18 females with mean age of (6.87 ± 3.75).

The study was approved by the faculty ethical committee after taking written consent from patients' guardians after a full explanation of the study.

2. Methods

All studied subjects were subjected to complete history taking (including personal history, history of blood transfusion (1st and frequency), history of drug intake and history of splenectomy and its age). Thorough clinical examination stressing on presence of pallor, jaundice and scars of splenectomy.

Blood sample and laboratory investigations: five milliliters (ml) of venous blood were withdrawn from the cubital vein and divided as follows: two ml of blood were put in a tube containing EDTA for complete blood count (CBC) measured with Pentra – 80 automated blood counter (ABX– France – Rue du Caducee-Paris Euromedecine-BP-7290.34184 Montpellier-Cedex 4.), estimation of HbF% by Hb electrophoresis (automated analyzer (minilite) MNL320350-Italy) and for detection of GNAI2, DUSP5 and ARRB1 mRNA expression by RT-PCR. The remaining 3 ml blood was transferred into plain tube, **separated by centrifugation and stored at -20°C** for determination of ALT, AST, urea, creatinine on auto-analyzer (SYNCHRON CX5) from Beckman (Beckman, instrument Inc., Scientific Instrument Division, Fullerton, CA92634 - 3100) and ferritin by ELISA kits, Ramco Laboratories Inc, Stafford, Texas, USA.

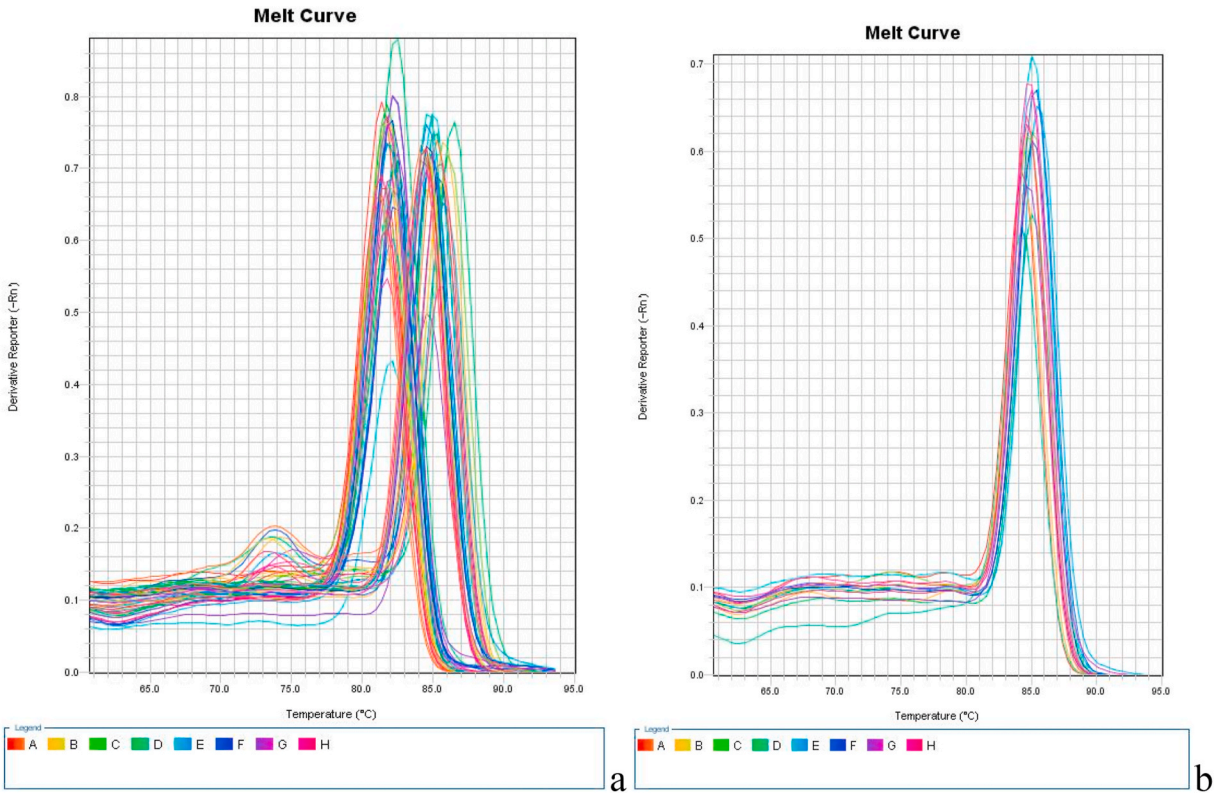

Fig. 2. a, shows melting curve of GNAI2 and DUSP5 genes expression. b, shows melting curve of ARRB1 gene expression.

2.1. Detection of GNAI2, DUSP5 and ARRB1genes expression

RNA was extracted from fresh EDTA treated blood sample using direct-zol RNA Mini protocol for purification of total cellular RNA according to the manufacturer's instructions. Single-stranded cDNAs were created utilizing Quanti Tect Reverse Transcription Kit (Qiagen, Applied Biosystems, USA): the reverse-transcription master mix was prepared by mixing 1 μl of Quantiscript reverse transcriptase, 4 μl of Quantiscript RT buffer and 1 μl of RT Primer, then mixed with 4 μl RNase-free water Mix, then 10ul of extracted RNA was added to each tube containing reverse transcription master mix, to achieve reverse-transcription reaction of 20 μl total volume and stored on ice. The programming of warm cycler condition was: hold for 1 h at 42 °C, hold for 5 min at 95 °C to inactivate Quantiscript Reverse Transcriptase then for 5 min at 4 °C.The reverse-transcription reactions were stored at −20 °C for real-time PCR.

Second Step- PCR done by: cDNA amplification with SYBR Green II with low ROX for detection of GNAI2, DUSP5, And ARRB1 genes expression (QuantiTect SYBR Green PCR Kit, Applied Biosystems, USA). The reaction mix for each gene was prepared as follow: 10 μl of 2xQuantiTect SYBR Green PCR Master Mix with low ROX, 1 μl for each forward and reverse primers of the gene, 3 μl of template cDNA, 5 μl of RNase-free water to give a final total reaction volume of 20 μl.

The forward (F) and reverse (R) primers for each gene are: GNAI2 (F: TCTGGCATCAGGGAGGCTTTCA,R:TTCCCTTGGTGGCTTTCCTAGC), DUSP5 (F:CTACCCACTCAACAGTCTCAGAGC, R:AAATCCCA-GAAGTCCACAGCTT) and ARRB1 (F:GCGAGCACGCTTACCCTTT,R: CAAGCCTTCCCCGTGTCTTC). Beta actin is used as the endogenous control gene.

The programming of real-time cycler was: hold for 15 min at 95°Cfor initial activation of HotStar Taq DNA Polymerase, hold for 30 min at 42 °C, then, 3 step cycling of denaturation at 95 °C for 15 S, annealing at 60 °C for 30 S and extension at 72 °C for 34 S. 45 cycles with a total time of 210 min was performed. Melting curve analysis of the PCR products was performed using 7500 software version 2.0.1, the melting curve cycling program is 95 °C for 15s, 55 °C for 1 min fluorescence data collection, 95 °C for 30s and 55 °C for 15 s. Fig. 1: shows the amplification plot of genes expression and Fig. 2: a, shows melting curve of GNAI2 and DUSP5 genes expression. b, shows melting curve of ARRB1 gene expression **Data analysis using Applied Biosystems 7500, software version 2.0.1.**

Quantification is done by the comparative Ct (cycle threshold) method that uses arithmetic formulas to achieve the result for relative quantitation, where the amount of the target is normalized to an endogenous reference (beta actin). This involves comparing the Ct values of the samples of interest with a control. The Ct values of both the calibrator and the samples of interest are normalized to an appropriate endogenous housekeeping gene (beta actin).

2.2. Statistical analysis

The collected data were tabulated and analyzed by SPSS (statistical package for social science) version 22.0 on IBM compatible computer.

Two types of statistics were done: Descriptive statistics in which percentage(%), mean, standard deviation (SD), and median were used while for analytical statistics: Chi-square test ($\chi 2$): was used to study association between two qualitative variables, Fischer exact test for 2 x 2 tables, Student t-test: used for comparison between two groups having quantitative parametric variables, Mann-Whitney test(nonparametric test): used for comparison between two groups, ANOVA (F) test (parametric test): used for comparison between more than two groups, Kruskal-Wallis (H) test (nonparametric test): used for comparison between three or more. Spearman correlation was used to correlate between two quantitative variables. A p-value $\leq$ 0.05 was considered significant.

3. Results

Demographic features showed a non-significant statistical difference

Table 1
Comparison between the three studied groups according to demographic data and different clinical parameters.

Parameter	Major (n = 40)		Intermedia (n = 40)		Control (n = 40)		Test of Sig.	p
	No.	%	No.	%	No.	%		
Sex	23	57.5	23	57.5	22	55	$\chi 2 = 0.068$	0.967
Male	17	42.5	17	42.5	18	45		
Female								
Age (years)	1.5–17		0.75–17		0.5–15		H = 4.064	0.131
Min. – Max.	4.27 ± 7.99		4.48 ± 8.82		3.75 ± 6.87			
Mean ± SD.	8.0		9.0		6.50			
Median								
							χ^2	**p**
Pallor	40	100.0	40	100.0	0	0.0	120.0*	<0.001*
Jaundice	35	87.5	8	20.0	0	0.0	73.138*	<0.001*
Organomegally	33	82.5	5	12.5	0	0.0	73.094*	<0.001*
Splenectomy	32	80.0	40.0	100.0			8.889*	^{FE}p=
Negative	8	20.0	0	0.0				0.005*
Positive								
No of transfusion/year	14.0–2.0		4.0–1.0				U = 49.0*	<0.001*
Min. – Max.	3.44 ± 9.15		0.74 ± 2.60					
Mean ± SD.	9.50		3.0					
Median								

χ^2: Chi square test H: Kruskal Wallis test FE: Fisher Exact U: Mann Whitney test.

Table 2
Comparison between the three studied groups according to laboratory investigations.

	Major (n = 40)		Intermedia (n = 40)	Control (n = 40)	Test of Sig.	p
RBCs (million/mcl)						
Min. – Max	1.64–3.88		2.27–5.03	4.14–5.63	F=	<0.001*
Mean ± SD	2.73 ± 0.48		3.22 ± 0.74	4.84 ± 0.41	154.837*	
Median	2.66		3.0	4.77		
Sig. bet. Grps.	p1 = 0.001*,p2<0.001*,p3<0.001*					
Hb (g/dl)						
Min. – Max	5.0–9.60		5.50–11.50	9.90–13.90	F=	<0.001*
Mean ± SD	7.10 ± 1.14		8.54 ± 1.46	11.77 ± 0.99	155.216*	
Median	7.0		8.50	11.50		
Sig. bet. Grps.	p1<0.001*,p2<0.001*,p3<0.001*					
HCT (%)						
Min. – Max	15.0–28.10		15.10–34.60	32.0–40.0	F=	<0.001*
Mean ± SD	19.97 ± 3.47		25.22 ± 4.87	35.46 ± 2.50	177.195*	
Median	19.25		25.15	35.25		
Sig. bet. Grps.	p1<0.001*,p2<0.001*,p3<0.001*					
MCV (fl))	63.0–83.0	79.0–63.40		79.50–65.0	F=	0.043*
Min. – Max.	71.59 ± 5.01	4.20 ± 4.07		4.11 ± 73.48	3.231*	
Mean ± SD.	**72.60**	75.40		74.10		
Median						
Sig. bet. Grps.	p1 = 0.045*,p2 = 0.146,p3 = 0.859					
MCH (pg))	29.0–20.70	31.0–21.30		45.0–21.70	F = 1.237	0.294
Min. – Max.	2.09 ± 25.52	1.90 ± 26.07		4.66 ± 26.63		
Mean ± SD.	25.70	26.0		26.0		
Median						
MCHC (g/dl)	43.0–31.90	35.10–32.0		36.21–32.0	H = 13.961*	0.001*
Min. – Max.	2.23 ± 35.23	0.75 ± 33.79		1.14 ± 34.24		
Mean ± SD.	34.70	33.90		34.25		
Median						
Sig. bet. Grps.	p1<0.001*,p2 = 0.044*,p3 = 0.085					
HbF (%)						
Min. – Max	91.0–0.0		96.80–0.0	0.80–0.0	H = $^{68.090}$*	<0.001*
Mean ± SD	21.11 ± 26.75		23.62 ± 43.74	0.21 ± 0.21		
Median	10.40		43.20	0.10		
Sig. bet. Grps.	p1<0.001*,p2<0.001*,p3<0.001*					
Ferritin (ng/ml)						
Min. – Max	5340.0–71.40		851.0–86.0	88.0–30.0	$^{H=101.331}$*	<0.001*
Mean ± SD	1144.4 ± 2140.7		135.4 ± 245.4	48.33 ± 15.71		
Median	1803.5		210.5	42.65		
Sig. bet. Grps.	p1<0.001*,p2<0.001*,p3<0.001*					

F: **ANOVA test**, **H**: **Kruskal Wallis test**.
p_1: p value for comparing between **Major** and **Intermediate**.
p_2: p value for comparing between **Major** and **Control**.
p_3: p value for comparing between **Intermediate** and **Control**.

between the studied groups as regards age and gender. On comparison between both patient groups as regard clinical data, there was increased number of children having jaundice, organomegally, splenectomy and number of blood transfusion per year in TM group than TI group (Table 1). Blood indices showed a highly significant statistical decrease of red blood cell (RBC) count, hemoglobin (Hb) and hematocrit (HCT)

Table 3
Comparison between the three studied groups according to RQ.

RQ	Major (n = 40)	Intermedia (n = 40)	Control (n = 40)	H	p
GNAI2					
Min. – Max.	0.0–1.62	0.0–2.28	1.80–7.70	93.631*	<0.001*
Mean ± SD.	0.65 ± 0.41	1.56 ± 0.44	4.20 ± 1.98		
Median	0.40	1.57	3.43		
Sig. bet. Grps.	$p_1 < 0.001$*,$p_2 < 0.001$*,$p_3 < 0.001$*				
DUSP5					
Min. – Max.	0.33–6.78	1.19–1.92	1.23–7.13	89.925*	<0.001*
Mean ± SD.	0.95 ± 0.96	1.54 ± 0.25	3.54 ± 1.88		
Median	0.85	1.56	3.07		
Sig. bet. Grps.	$p_1 < 0.001$*,$p_2 < 0.001$*,$p_3 < 0.001$*				
ARRB1					
Min. – Max.	1.13–9.14	1.75–4.15	0.01–1.80	87.750*	<0.001*
Mean ± SD.	4.77 ± 2.31	2.55 ± 0.60	0.69–0.54		
Median	4.15	2.48	0.52		
Sig. bet. Grps.	$p_1 = 0.001$*,$p_2 < 0.001$*,$p_3 < 0.001$*				

H: Kruskal Wallis test.
p_1: p value for comparing between **Major** and **Intermediate**.
p_2: p value for comparing between **Major** and **Control**.
p_3: p value for comparing between **Intermediate** and **Control**.

values in both patient groups when compared with control group and a non-significant difference between studied groups as regards MCV, MCH and MCHC. Hb F was significantly increased in both patient groups than control group and in TI group than TM group. Also, serum ferritin showed a highly significant increase in TM group than other groups and in TI group than control group (Table 2).

Expression of ARRB1 (Arrestin Beta 1) genes is markedly increased in TM group than TI and control groups, while expression of both GNAI2 (Guanine nucleotide-binding protein G (i) subunit alpha-2) and DUSP5 (Dual specificity protein phosphatase 5) genes showed a significant decrease in TM group than TI and control groups respectively (Table 3). Expression of ARRB1 genes showed a significant negative correlation as regard serum ferritin, Hb F% and number of transfusion per year and showed significant negative correlation as regard RBCs, Hb and HCT while expression of both GNAI2 and DUSP5 genes showed a significant positive correlation as regard RBCs, Hb and HCT and showed a significant negative correlation as regard serum ferritin, Hb F% and number of transfusion per year (Table 4).

4. Discussion

Erythropoiesis describes the processes involved in the lineage commitment, maturation and terminal differentiation of a hematopoietic stem cell (HSC) into a mature red blood cell (RBC); many signaling pathways regulate these processes including the MAPK pathway [16–18]. Traditionally, the pathogenesis of β-thalassemia has been attributed to ineffective erythropoiesis due to intramedullary apoptosis and delayed maturation of erythroid progenitor cells [5,6].

Our work aimed to study the effect of expression of GNAI2, DUSP5 and ARRB1 genes on MAPK signaling pathway in pediatric patients with beta thalassemia.

Results revealed a statistically increased HbF in both patient groups of thalassemia than control group with marked increase in TI group than TM group.

Previous studies reported elevated fetal hemoglobin (HbF) expression in cases of β-thalassemia and this elevation may ameliorate the clinical symptoms of β-globin disorders in these cases [19–21]. Several studies work to explain the cause of increased HB F expression in β-thalassemia; **Pace et al.,** [22] and **Mabaera et al.,** [20]reported that the p38 MAPK signaling pathways use histone deacetylase inhibitors to induce HbF expression, while **Tohru et al.,** [21] stated that activation of the cAMP signaling pathway prove to be an important signaling mechanism to reactivate HbF expression in erythroid cells in part by inhibiting BCL11A gene expression. On other hand **Galanello et al**. [23], reported that plasma erythropoietin levels correlate with HbF levels in β-thalassemia intermedia and this in agreement with **Bhanu et al.,** [24] who found that increase in cytokines including erythropoietin, stem cell factor, and tumor growth factor-β in β-thalassemic patients; strongly enhance Hb F activity in primary erythroid cells.

As regards serum ferritin, this study revealed marked elevation of serum ferritin in TM group than TI group and this may be attributed to the repeated blood transfusion for patients with TM than TI leading to iron overload. Despite the life-saving nature of long-term blood transfusion, iron intoxication due to dysregulated cellular iron metabolism is the leading cause of prolonged complications in patients with β-TM [25,26]. Normally, iron is stored intracellularly in the form of ferritin. Under conditions of iron overload, excess iron accumulates within tissues such as the liver, heart, lungs, and endocrine glands. These unbound iron particles contribute to the release of free radicals, which damage membrane lipids and other macromolecules and lead to cell death and, eventually, organ failure [27,28].

Studying gene expression in this work showed increased expression of ARRB1 gene in TM and TI groups than control group while there were decreased expression of both DUSP5 and GNAI2 in TM and TI groups than control group.

β-Arrestins (βarrs) are ubiquitously expressed proteins that were first described for their role in desensitizing G protein-coupled receptors

Table 4
Correlation between gene expression and different parameters in total subjects.

	GNAI2		DUSP5		ARRB1	
	r_s	p	r_s	p	r_s	p
Age (years)	−0.197*	0.031*	−0.105	0.252	0.117	0.201
RBCs (million/mcl)	0.677*	<0.001*	0.623*	<0.001*	−0.672*	<0.001*
Hb (g/dl)	0.734*	<0.001*	0.672*	<0.001*	−0.703*	<0.001*
HCT (%)	0.753*	<0.001*	0.687*	<0.001*	−0.706*	<0.001*
MCV (fl)	0.189*	0.038*	0.123	0.180	−0.118	0.199
MCH (pg)	0.067	0.466	0.021	0.816	−0.062	0.503
MCHC (g/dl)	−0.139	0.131	−0.228*	0.012*	0.108	0.242
HbF (%)	−0.365*	<0.001*	−0.302*	0.001*	0.431*	<0.001*
Ferritin (ng/ml)	−0.881*	<0.001*	−0.853*	<0.001*	0.842*	<0.001*
No of transfusion/year	−0.735*	<0.001*	−0.752*	<0.001*	0.561*	<0.001*

r_s: Spearman coefficient.
*: Statistically significant at $p \leq 0.05$.

(GPCRs) [29]. There are two βarr isoforms, β-arrestin1 and β-arrestin2, both are expressed ubiquitously and share 78% sequence homology [30].

Platelet activating factor receptor (PAFR) desensitization was β-arrestin-1-dependent, with receptor internalization requiring both β-arrestin-1 and dynamin-2. This translocation and binding of β-arrestin-1 to the PAFR provided a platform for recruitment of a p38 MAPK signal some (ASK1/MKK3/p38 MAPK) and its subsequent activation [31,32].

P38 activation was reported to be induced by erythropoietin (EPO) [33–35], and several reports have suggested that p38 is necessary for the initiation of erythroid differentiation. Pharmacological inhibition of p38 suppressed EPO-induced differentiation of SKT6 cells, and p38 antisense oligonucleotides inhibited Epo-induced hemoglobinization [36]. Other study reported that lack of p38 activity was found to interfere with stabilization of Epo mRNA in human hepatoma cells undergoing hypoxic stress, resulting in diminished EPO gene expression [37,38].

*β-Arrestin-1*genemRNA expression in the present study showed a marked increase in TM group than TI group which was also increased more than control group. Overexpression of *β-Arrestin-1* gene may ameliorate the effects of severe anemia and hypoxia in cases of β-thalassemia by stimulating erythropoietin synthesis and these findings are in agreement of Nişli **et al.,** who found elevated serum EPO in both TM and TI groups than control group and also reported that In the TM patients, the serum EPO concentration was not consistently correlated with clinical signs of erythropoietic activity [39].

MAPK phosphatases (MKPs) also called Dual-specific (Thr/Tyr) MAPK phosphatases (DUSPs) are protein phosphatases that dephosphorylate both tyrosine and serine/threonine residues of MAP kinases, among them DUSP5 and DUSP6 are known to be induced by ERK signaling, and thereby involved in a negative feedback loop that tightly controls phosphorylated ERK (pERK) [40–42].**Geest et al., and Mori et al.,** have demonstrated that ERK MAPK activity is essential for survival of erythroid CD34 progenitor cells and inhibition of ERK1/2 activity completely abolished expansion and subsequent differentiation of CD34 erythrocyte progenitors as a result of induction of apoptosis [43,44].

Our study revealed that DUSP5 mRNA expression is decreased in both TM and TI groups and this decease may not affect the ERK kinase activity which may be explained by a previous work who has shown that DUSP5 interacts with ERK and is responsible for its nuclear anchoring, but this binding is not accompanied by the catalytic activation of the phosphatase, also the basal activity of DUSP5 in the absence of ERK activation is greater than that of DUSP6 before and even after its activation by ERK [45]. In contrast to these results **Buffet et al.,** stated that induction of DUSP5 by the MEK-ERK pathway serves as an important feedback loop that controls activation of ERK1/2 [46].

GTP-binding guanine nucleotide regulatory proteins (G proteins; either stimulatory [Gs] or inhibitory [Gi]) are heterotrimeric, composed of α-, β-, and γ-subunits. The Gi family of G proteins is defined by the α-subunits and includes Gαi1, Gαi2, Gαi3, Gαz, GαoA and GαoB isoforms [47].

One of these isoforms is the Gαi2 (or GNAI2; Guanine nucleotide-binding protein G (i) subunit alpha-2) which showed decreased expression in TM and TI groups than control group in our study, have proven to affect the MAPK signaling pathway by different mechanisms. One of these mechanisms is by stimulating the Ras-dependent ERK signaling without directly involving the activation of Ras by inhibiting the activity of Rap-1 [48,49] and the other mechanism is by attenuating the activation of the p38MAPK module [50].

5. Conclusion

From the above study it is well documented that regulation of erythropoiesis requires urgent control from MAPK pathway and that the rate of expression of the three studied genes affects directly the MAPK pathway. This occurs through activation or inactivation of P38 and ERK sub-pathways which have roles in differentiation, survival and apoptosis of erythropoietic cells, and so in cases of β thalassemia. Further study is needed at the protein level of these genes in cases of β thalassemia.

Ethical approval and consent to participate

The Institutional Review Board (IRB) of the Menoufia Faculty of Medicine approved the study. Research work was performed in accordance with the Declaration of Helsinki. A written patient consent was taken from the parents and caregivers after explaining all aspects of the study, with the right to withdraw at any time.

Consent for publication

- All authors are in agreement with the content of the manuscript.
- On behalf of my colleagues, we have pleasure for dealing with your Journal. I would like to thank you and I hope that our research work is under your kind care and observation.

Availability of supporting data

All data are available upon request.

Funding

This article was not funded

Authors' contributions

All authors co-operated in: conceptualization, design of the work, data collection, resources detection, formal analysis, interpretation of data, the creation of new software used in the work, validation and methodology, revision. YE and MA do the lab investigation, AE writing methodology and results section, SE received, collecting data. AM performing data interpretation according to hematological disease, final revision and responsible for submission of paper .

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

We acknowledge the central laboratory unit, faculty of Medicine, Menoufia University for their valuable help and cooperation and providing us with the necessary instruments for completion of the study.

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