Research Article

The Associations between HLA DQB1 different Alleles and β-thalassemia Major

Amel M. Kamal Eldin, Emad A. Abd El Naem, Mostafa A. Elsayed and Nada O. Zaki Mahmoud
Department of Clinical Pathology, Faculty of Medicine, Minia university

Abstract
Thalassemias are the commonest inherited hemoglobinopathies in the world. Approximately 68,000 children are born with various thalassemia syndromes each year. β-Thalassemia represents a major public health problem in Egypt. beta thalassemia. It is caused by reduced or absent synthesis of beta globin chains. **Aim of the work:** Determine frequencies & association of HLA DQB1 alleles in β-thalassemia major patients. **Patients:** forty-five unrelated randomly selected β-thalassemia major patients, and forty-five unrelated randomly selected healthy individuals, composed the control group with age matched to patients of group I. **Study procedure**: Routine lab investigations & HLA DQB1 genotyping by real time PCR. The Kit were supplied by DNA-TECHNOLOGY (catalog no.334-1), Russia. **Conclusion:** HLA DQB1*0601 give significance importance as an etiological risk factor for β-thalassemia major; HLA DQB1*0302 give significance importance as a preventive risk factor for β-thalassemia major.
Key words: beta thalassemia major, HLA class II, Real time PCR.

Introduction
β-thalassemia occurs when there is a quantitative reduction of β globin chains that are usually structurally normal. They are caused by mutations that nearly all affect the β globin locus and are extremely heterogeneous. Almost every possible defect affecting gene expression at transcription or post-transcriptional level, including translation, have been identified in β thalassemia. These genetic defects lead to a variable reduction in β globin output ranging from a minimal deficit (mild β+ thalassemia alleles) to complete absence (β° thalassemia) (Thein and Diseases, 2018).

However, blood transfusion is not a treatment, long life blood transfusion is the chief supportive management of thalassemia. The beneficial outcome of regular blood transfusion is to sustain growth and development during childhood (Cappellini et al., 2018).

Currently, bone marrow transplantation is the only effective treatment for thalassemia patients. Patients without risk factor as liver fibrosis, hepatomegaly, and increasing iron accumulation have a disease-free survival more than 90% when stem cell transplant from HLA-identical sibling. In fact, approximately 30% of patients can find HLA-identical within the same family (Shenoy and Thompson, 2016).

Due to limited identification of compatible sibling donor and high prevalence of β-thalassemia in Egypt it was important to start applying of HLA molecular typing techniques to contribute to the global information on the level of therapeutic strategies (Cappellini et al., 2018).

The term HLA refers to the (Human Leukocyte Antigen) System, which is controlled by genes on the short arm of chromosome six. The HLA loci are part of the genetic region known as the Major Histocompatibility Complex (MHC). The MHC has genes (including HLA) which are integral to normal function of the immune response (Matzaraki et al., 2017).

HLA genotyping is significant in both clinical and research purposes in order to understand the mechanism of associated diseases, to discover new informative markers used as tool for risk evaluation of disease and organ transplantation (Karnes et al., 2017).

Subjects and Methods:
The present study was carried out in the

Clinical Pathology Department, Faculty of Medicine, El-Minia University Hospital. It was conducted through the period from May 2019 to January 2020. This study included 2 groups, group I included 45 children with previous history of beta thalassemia major, group II included 45 apparently healthy children with matched age to group I patients (as control). All individuals in the study were subjected to the following: Comprehensive medical history taken, Clinical examination, radiological imaging(Abdominal and pelvic ultrasound), and Laboratory Investigations which included: Routine investigations(CBC, blood glucose level, liver function tests and renal function tests, Hemoglobin electrophoresis & serum ferritin were taken from patient file and special investigations (identification of HLA DQB1 genotyping by Real time PCR).

Results

-**03* allele which was found in 28 thalassemic patients (30.1%) & 41 normal controls (45.6%) *with P-value is* ***0.031***

-*0302 allele was found in 6 patients (6.5%) & 24 normal controls (26.7%) with P-value is <**0.001** figure (1).

-**0601* allele was found in 9 patients (9.7%) & 2 normal children (2.2%) with P-value **0.034.**

- *0401-0402 allele & *0503 allele show nonsignificant increase in patients compared to control group with P-value (0.059) table (1).

- No significant difference was established between two groups in HLADQB1*02.

Table (1): Comparison between the studied groups regarding HLA DQB1 allele frequency:

HLA-DQB1 allele	Case (n=45)		Control (n=45)		p value
***02**	28	(30.1%)	25	(27.8%)	0.728
***03**	28	(30.1%)	41	(45.6%)	**0.031***
*0301	18	(19.4%)	17	(18.9%)	0.936
*0302	6	(6.5%)	24	(26.7%)	**<0.001***
*0303	2	(2.2%)	0	(0.0%)	0.162
*0305	2	(2.2%)	0	(0.0%)	0.497
***04**					
*0401_0402	5	(5.4%)	0	(0.0%)	0.059
***05**	13	(14.0%)	9	(10.0%)	0.408
*05	2	(2.2%)	0	(0.0%)	0.497
*0501	4	(4.3%)	9	(10.0%)	0.134
*0502_0504	2	(2.2%)	0	(0.0%)	0.497
*0503	5	(5.4%)	0	(0.0%)	0.059
***06**	19	(20.4%)	15	(16.7%)	0.513
*0601	9	(9.7%)	2	(2.2%)	**0.034***
*0602_8	9	(9.7%)	13	(14.4%)	0.322

***p < 0.05 considered significant**
N. of studied alleles in cases=93
N. of studied alleles in controls =90

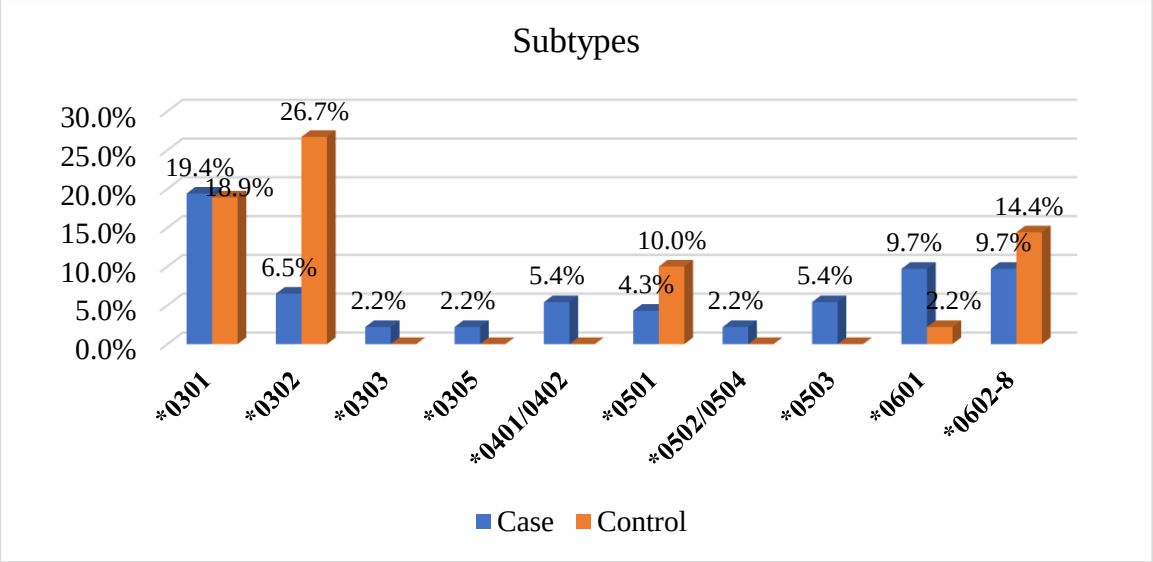

Figure (1): Analytical charts of genotypic difference between patients and controls

Discussion

The thalassemia's are a group of inherited hematologic disorders caused by defects in the synthesis of one or more of the hemoglobin chains. β-thalassemia is caused by reduced or absent synthesis of beta globin chains. Imbalances of globin chains cause hemolysis and impair erythropoiesis (Saliba et al., 2020).

Patients with β-thalassemia have been typically categorized as minor, major, or intermedia on the basis of their α-globin or β-globin chain imbalance, severity of anemia, and clinical picture at presentation (Taher et al., 2018).

Human Leucocyte Antigen (HLA) genotyping is beneficial clinically and in researches to understand the mechanism of associated diseases as well as organ transplantation. HLA matching in beta-thalassemia patients has a great role in therapeutic interventions through hematopoietic stem cell transplantation (Bertaina et al., 2018).

Studies that correlates genetic relationship with HLA in β-thalassemia major would be helpful in clearing up pathogenesis in addition to minimizing symptoms and/ or future treatment planning (Bou-Fakhredin et al., 2020).

This study demonstrates that, HLA DQB1 *0601 was significantly higher in thalassemic children when compared to normal controls (P value 0.034) . This result was in agreement with results of (Zeiny, 2016) who demonstrate that HLA-DQB1*06 allele may be a risk factor for major β-thalassemia.

Also, the data reveal that HLA DQB1*03 was more frequent in control group than thalassemic with (P value 0.031) which may be a preventive allele for β-thalassemia.

There is no significant difference was established between two groups in HLA DQB1*02.
HLA DQB1*04 & HLA DQB1*05 show nonsignificant increase in thalassemic patients when compared to controls (P value 0.059 & 0.408 respectively). this was agreed with (Zeiny, 2016) whose study revealed that HLA DQB1*05 was more in frequency in patient and could be an etiological risk factor for β-thalassemia.

References:

1. Thein, S.L., 2018. Molecular basis of β thalassemia and potential therapeutic targets. Blood Cells, Molecules, and Diseases, 70, pp.54-65.
2. Cappellini, M.D., Porter, J.B., Viprakasit, V. and Taher, A.T., 2018. A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies. Blood reviews,32(4),pp.300-311.

3. Shenoy, S. and Thompson, A.A., 2016. Unrelated donor stem cell transplantation for transfusion-dependent thalassemia. Annals of the New York Academy of Sciences, 1368(1), p.122.
4. Matzaraki, V., Kumar, V., Wijmenga, C. and Zhernakova, A., 2017. The MHC locus and genetic susceptibility to autoimmune and infectious diseases. Genome biology, 18(1), p.76.
5. Karnes, J.H., Bastarache, L., Shaffer, C.M., Gaudieri, S., Xu, Y., Glazer, A.M., Mosley, J.D., Zhao, S., Raychaudhuri, S., Mallal, S. and Ye, Z., 2017. Phenome-wide scanning identifies multiple diseases and disease severity phenotypes associated with HLA variants. Science Translational Medicine, 9(389).
6. Saliba, A.N., Atoui, A., Labban, M., Hamade, H., Bou-Fakhredin, R., Mufarrij, A. and Taher, A.T., 2020. Thalassemia in the emergency department: special considerations for a rare disease. Annals of Hematology, pp.1-11.
7. Taher, A.T., Weatherall, D.J. and Cappellini, M.D., 2018. Thalassaemia. The Lancet, 391(10116), pp.155-167.
8. Bertaina, A. and Andreani, M., 2018. Major histocompatibility complex and hematopoietic stem cell transplantation: beyond the classical HLA polymorphism. International journal of molecular sciences, 19(2), p.621.
9. Bou-Fakhredin, R., Tabbikha, R., Daadaa, H. and Taher, A.T., 2020. Emerging therapies in β-thalassemia: toward a new era in management. Expert Opinion on Emerging Drugs, pp.1-10.
10. Zeiny, S.M., 2016. The correlation between HLA class II and β-thalassemia major in Al-Karama teaching hospital. Journal of the Faculty of Medicine Baghdad, 58(4), pp.366-370.