Asian Pac. J. Health Sci., 2015; 2(3):26-30 e-ISSN: 2349-0659, p-ISSN: 2350-0964

The clinical importance of some biological markers for the risk of TIDM in Egypt

N. A. El-Azabawy[1]*, M. M. Youns[1], A. I. Amin[2] and Z. A. Hassan[1]

[1]Department of Biochemistry, Faculty of Pharmacy, Helwan University, Egypt
[2]Hospital Authority and Educational Institutions, Cairo, Egypt

ABSTRACT

Background: Type 1 diabetes mellitus (T1DM) is a polygenic disease caused by multiple susceptibility and protective alleles interacting with each other. However, studies showed that the genes responsible for more than 50% of the genetic predisposition to T1DM, are located in the human leukocyte antigen (HLA) region on chromosome 6. Out of all three HLA classes, the HLA class II loci DQA1, DQB1 and DRB1 contribute most to the genetic predisposition to T1DM. This study determines the HLA-DRB1 allele association with susceptibility and protection to T1DM in Egyptian children. **Results:** The study is a HLA-Class II-DRB1 allele typing, which was carried out with polymerase chain reaction -sequence specific primer. It was conducted on 61 unrelated Egyptian children with T1DM, and 19 unrelated age- and sex- matched healthy subjects. Results of the HLA typing confirmed a positive association of the following alleles with T1DM; DRB1*03:01:01:01, DRB1*03:01:06, DRB1*03:01:12, DRB1*03:01:16, DRB1*03:07, DRB1*03:08, DRB1*03:10, DRB1*03:12, DRB1*03:42, DRB1*03:76, DRB1*03:86, DRB1*08:17, DRB1*11:07, DRB1*11:08:01, DRB1*11:23, DRB1*11:36, DRB1*11:67, DRB1*12:12, DRB1*13:144, DRB1*13:154, DRB1*13:18, DRB1*13:119, DRB1*13:146 and DRB1*15:21. On the other hand, protection against T1DM was conferred by the HLA DRB1*01:03, DRB1*13:45, DRB1*14:04, DRB1*14:31 and DRB1*15:62 alleles. **Conclusion:** Increased frequencies of certain HLA-DRB1 alleles, known to be positively associated with T1DM, which are consistent with other studies. In addition to the well known alleles, our study confirmed the association of some other alleles that might be unique for Egyptians. However, the protective effect of previously reported alleles was not confirmed, instead other HLA-DRB1 alleles are now in question.

Keywords: DRB1 alleles, HLA, Protection, Susceptibility, T1DM,

Introduction

Diabetes mellitus is considered as one of the most rapidly growing diseases worldwide. The International Diabetes Federation estimated in 2011 that 366 million people worldwide suffered from diabetes, which is expected to increase to 552 million people by the year 2030. [1] T1DM is most commonly an autoimmune disease that results from the destruction of insulin secreting beta cells of the islets of Langerhans by auto-reactive T-lymphocytes. [2] It is a clear example of a complex multi-factorial disease resulting from the interaction of several environmental as well as genetic factors. [3] Both genome screens and studies led to the conclusion that T1DM is a genetically polygenic disease caused by multiple susceptibility and protective alleles interacting with each other. [4]

**Correspondence*
N. A. El-Azabawy
Department of Biochemistry, Faculty of Pharmacy, Helwan University, Egypt. **E Mail:** nadine.azabawy@gmail.com

Before the extraordinary success of the Genome-wide association studies (GWAS) only six susceptibility genes were discovered. However, GWAS resulted in the identification of a number of new genes associated with T1DM, reaching 60 genes in 2012. [5] Studies showed that the most important genes, responsible for more than 50% of the genetic predisposition to T1DM, are located in the HLA region on chromosome 6. [6] This region consists of a total of 224 genes, arranged in separate clusters forming three different classes: class I, class II and class III. [7] Even though HLA class I was found to be associated with T1DM, the HLA class II loci DQA1, DQB1 and DRB1 contribute most to the genetic predisposition to T1DM. [8] However, the risk caused by certain HLA alleles as well as the protection obtained from other alleles varies across different ethnical groups and geographical regions. [9]Egypt is considered as one of the developing countries in the Middle -East with the highest rates of diabetes. [10] A study performed on the Egyptian population suggests

that the number of diagnosed and undiagnosed people with diabetes will reach 8.80 million by the year 2025. [11] This high prevalence rate makes the study of the HLA genotype in the Egyptian population necessary. [12]This study was designed to determine HLA-DRB1 allele association with the susceptibility and/or protection to T1DM in a random group of Egyptian children.

Materials and methods

Subjects

This study was conducted on 61 unrelated Egyptian children diagnosed with T1DM. They were presented to the pediatric inpatient's wards of the National Institute for Diabetes and Endocrinology, El KasrEleiny, Cairo, Egypt. The study included 32 males and 28 females. The mean age of the patients is 11.87± 0.48.The control group involved 19 unrelated age- and sex-matched healthy subjects without T1DM or any other autoimmune disease. After the protocol was approved by the local ethical committee, written consents were obtained from the parents of the patients and controls.

HLA class II- DRB1 allele typing

Genomic DNA extraction was done for all samples using a DNA purification kit (PureLink® Genomic DNA mini Kit Cat. K1820-01, Lot No. 1398040, Invitrogen,Life technologies). HLA Class II-DRB1 allele typing was carried out with a polymerase chain reaction-sequence-specific primer using MICRO SSP™ HLA DNA typing trays (Lot number #004, One Lambda, Inc.). Test conditions were according to the manufacturer's instructions.

Statistical analysis

All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 21. Data was statistically described in terms of frequencies and percentages for qualitative data. For comparing categorical data, Fisher Exact test was used. Genotype and allele frequencies were compared between the disease and the control groups using Fisher exact test. Odds ratio (OR) with 95% confidence intervals was calculated. A probability value (P value) less than 0.05 was considered statistically significant.

Results

Characteristics of the Participants:

By comparing the data obtained from the patients and controls, it was obvious that the patients had elevated fasting blood glucose levels (247.58± 13 vs. 80.68± 1.37mmol/litre; P < 0.001) and glycated hemoglobin (HbA1c) levels (10.84±0.21 vs. 5.26±0.05 as a percent of total hemoglobin; P < 0.001). The kidney and liver functions however, didn't show any significant difference between patients and controls.

Frequencies of HLA-DRB1 alleles:

The HLA-DRB1 allele frequencies found in 61 T1DM patients and 19 healthy controls are given in Table 1. Significant differences were detected between T1DM patients and controls in the frequencies of DRB1*03:01:01(85.2% vs. 36.6%, P <0.001), DRB1*03:01:06 (65.6% vs. 5.3%, P < 0.001), DRB1*03:01:12 (85.2% vs. 36.6%, P <0.001), DRB1*03:01:16 (77.0% vs. 31.6%, P = 0.001), DRB1*03:07 (83.6% vs. 36.8%, P < 0.001), DRB1*03:08 (70.5% vs. 15.8%, P < 0.001), DRB1*03:42 (83.6% vs. 31.6%, P < 0.001), DRB1*03:76 (75.4% vs. 26.3%, P < 0.001), DRB1*03:86 (73.8% vs. 15.8%, P < 0.001), DRB1*08:17 (72.1% vs. 10.5%, P < 0.001), DRB1*11:07 (72.1% vs. 21.1%, P < 0.001), DRB1* 11:08:01 (68.9% vs. 15.8%, P < 0.001), DRB1*11:23 (82.0% vs. 36.8, P < 0.001) DRB1*11:36 (83.6% vs. 36.8, P < 0.001), DRB1*11:67 (73.8% vs. 21.1%, P < 0.001), DRB1*12:12 (68.9% vs. 10.5%, P < 0.001), DRB1*13:119 (86.9% vs. 31.6%, P < 0.001) and DRB1*15:21 (67.2% vs. 15.8%, P < 0.001). In addition, the allelic frequencies DRB1*03:10 (86.9% vs. 57.9%, P 0.017), DRB1*03:12 (86.9% vs. 47.4%, P 0.001), DRB1*13:144 (90.2% vs. 57.9%, P0.003), DRB1*13:154 (86.9% vs. 63.2%, P0.039), DRB1*13:18 (90.2% vs. 57.9%, P0.003) and DRB1*13:71 (86.9% vs. 47.4%, P0.001) showed also significant differences between patients and controls.

On the other hand, the alleles DRB1*01:03(31.6% vs. 3.3%, P0.002), DRB1*13:45(89.5% vs. 63.9%, P = 0,045), DRB1*14:04 (36.8% vs. 11.5%, P0.018), DRB1*14:31 (36.8% vs. 8.2%, P 0.006) and DRB1*15:62 (36.8% vs. 8.2%, P0.006) were found significantly higher in controls than in patients. Differences in the frequencies of other DRB1 alleles between patients and controls were insignificant. (Table 1)

Table 1: Significant HLA-DRB1 allele frequencies in patients and controls

	Patients(61)		Control (19)		P-value	OR	95% CI
DRB1*03:01:01:01	52	85.2%	6	31.6%	< 0.001	12.519	3.776-41.501
DRB1*03:01:06	40	65.6%	1	5.3%	< 0.001	34.286	4.276-274.930
DRB1*03:01:12	52	85.2%	6	31.6%	< 0.001	12.519	3.776-41.501
DRB1*03:01:16	47	77.0%	6	31.6%	0.001	7.274	2.334-22.666
DRB1*03:07	51	83.6%	7	36.8%	< 0.001	8.743	2.761-27.682
DRB1*03:08	43	70.5%	3	15.8%	< 0.001	12.741	3.302-49.162
DRB1*03:10	53	86.9%	11	57.9%	0.017	4.818	1.487-15.612
DRB1*03:12	53	86.9%	9	47.4%	0.001	7.361	2.290-23.664
DRB1*03:42	51	83.6%	6	31.6%	< 0.001	11.050	3.391-36.004
DRB1*03:76	46	75.4%	5	26.3%	< 0.001	8.587	2.650-27.825
DRB1*03:86	45	73.8%	3	15.8%	< 0.001	15.000	3.855-58.366
DRB1*08:17	44	72.1%	2	10.5%	< 0.001	22.000	4.584-105.580
DRB1*11:07	44	72.1%	4	21.1%	< 0.001	9.706	2.818-33.433
DRB1*11:08:01	42	68.9%	3	15.8%	< 0.001	11.789	3.066-45.338
DRB1*11:23	50	82.0%	7	36.8%	< 0.001	7.792	2.497-24.315
DRB1*11:36	51	83.6%	7	36.8%	< 0.001	8.743	2.761-27.682
DRB1*11:67	45	73.8%	4	21.1%	< 0.001	10.547	3.047-36.509
DRB1*12:12	42	68.9%	2	10.5%	< 0.001	18.789	3.940-89.609
DRB1*13:119	53	86.9%	6	31.6%	< 0.001	14.354	4.238-48.620
DRB1*13:144	55	90.2%	11	57.9%	0.003	6.667	1.928-23.055
DRB1*13:146	55	90.2%	12	63.2%	0.01	5.347	1.522-18.787
DRB1*13:154	53	86.9%	12	63.2%	0.039	3.865	1.173-12.732
DRB1*13:18	55	90.2%	11	57.9%	0.003	6.667	1.928-23.055
DRB1*13:45	39	63.9%	17	89.5%	0.045	0.209	0.044-0.988
DRB1*13:71	53	86.9%	9	47.4%	0.001	7.361	2.29-23.664
DRB1*14:04	7	11.5%	7	36.8%	0.018	0.222	0.066-0.753
DRB1*14.31	5	8.2%	7	36.8%	0.006	0.153	0.041-0.565
DRB1*15.21	41	67.2%	3	15.8%	< 0.001	10.933	2.851-41.922
DRB1*15:62	5	8.2%	7	36.8%	0.006	0.153	0.041-0.565

Discussion

The incidence of T1DM is increasing rapidly throughout the world with a rate that is highly variable among different ethnic groups. [13]Egypt is a country located in North Africa between the Mediterranean world, the Arab world and Black Africa. As a result of this special location the genetic components of the Egyptian population is somehow a combination of Arabs, Bedouins and to a small extent Berber. Therefore Egyptians are classified between Caucasians and Africans. However they are considered to be closer to Caucasians. [14]Studies proved that HLA-DR-DQ represent about 40-50% of the disease susceptibility. [8]Different population-based association studies have shown a strong relationship between HLA-DR3 and -DR4 and T1DM in Caucasoid populations. [13]Analysis of the frequencies of the HLA-DRB1 alleles in our patients compared to the control group revealed that DRB1*03:01:01:01, *03:01:06, *03:01:12 and *03:01:16 were positively associated with T1DM ($P < 0.001$, $P < 0.001$, $P < 0.001$ and $P = 0.001$ respectively). These observations are consistent with the findings in previous studies conducted on different Caucasoid and Arab populations which showed a strong disease association with the DRB1*03:01 alleles. These studies included the study of Benseffaj on the Moroccan population [15], the Bahraini study of Al-Harbi *et al*. [16] as well as the Slovakian study by Buc *et al*. [9].In addition to the well known DRB1*03:01 alleles our patients showed a strong association between other DRB1*03 alleles and T1D; such as DRB1*03:07 ($P < 0.001$), DRB1*03:08 ($P < 0.001$), DRB1*03:10 ($P = 0.017$), DRB1*03:12 ($P = 0.001$), DRB1*03:42($P < 0.001$), DRB1*03:76 ($P < 0.001$) and DRB1*03:86 ($P < 0.001$).Our study however, didn't reveal any association of DR4 alleles. Although there seemed to be a difference between patients and

controls, yet this difference was not clinically significant (P = 0.054). A previous study done on the Egyptian population in 2011 also showed an insignificant difference between diabetic patients and controls for DRB1*04 subtypes. [17] This was also the case in the Romanian study carried on by Durbală in 2009. [18] Two Lebanese studies also showed a strong association for DR3 alleles with no significant association for DR4 alleles. [19, 20]. Nevertheless, a larger study must be done in order to be able to investigate the predisposing effect of HLA-DR4 alleles on the Egyptian population. Furthermore our study exposed the predisposing role of a number of other genesincluding,DRB1*08:17(P<0.001),DRB1*11:07(P < 0.001), DRB1*11:08:01(P < 0.001), DRB1*11:23(P < 0.001), DRB1*11:36 (P < 0.001), DRB1*11:67 (P < 0.001), DRB1*12:12 (P < 0.001), DRB1*13:119 (P < 0.001), DRB1*13:144 (P = 0.003), DRB1*13:146 (P = 0.01), DRB1*13:154 (P = 0.039), DRB1*13:18 (P = 0.003), DRB1*13:71(P = 0.001) and DRB1*15:21 (P < 0.001). Interestingly, the number of alleles thought to be involved with T1DM in the Egyptian population in this study is numerous. However, when taking in consideration the fact that Egypt is considered as one of the countries with highest rates of diabetes, the extremely high numbers of predisposing alleles might be explained. On the other hand, a negative association to T1DM was reported with the following DRB1 alleles: DRB1*01:03, DRB1*13:45, DRB1*14:04, DRB1*14:31 and DRB1*15:62 (P = 0.002, P = 0.045, P = 0.018, P = 0.006 and P = 0.006 respectively). Although these results were not expected, since previous population-based studies carried on Caucasians and Arabs, usually showed DRB1*11:01 and DRB1*15:01. In addition a former study performed on the Egyptian population also revealed DRB1*11:01, DRB1*10:01 and DRB1:15:01 to play a protective role against T1DM [17], unlike our study, which did not show a significant difference between controls and patients for these alleles. However a few studies proved DR13 as well as DR14 sub-alleles to have a protective role. One of these studies were conducted on Saudi patients [21] and showed a significant negative association between T1DM and DRB1*13:07. Another study from Turkey [13] also revealed the T1DM-protective effect of DRB1*13:01 and DRB1*14:01 alleles. The Bahraini population as well showed in a study a strong negative association between DRB1*13:07:01 and T1DM [16].According to the results of this study, we can conclude that the Egyptian population showed some similarities with other populations regarding the alleles known to be positively associated with T1DM. However, the study reported some alleles that might be unique for Egyptian children. On the other hand, the previously reported alleles with known protective effect were not reported in this study.

Last but not least further studies on large scales are required for a more precise understanding of the HLA genotype in the Egyptian population.

References

1. Reginald Oputa and Sonny Chinenye. Diabetes mellitus: a global epidemic with potential solutions, African Journal of Diabetes Medicine 2012; 20(2):33-35.
2. Bart Roep. The role of T-cells in the patho-genesis of Type 1 diabetes: from cause to cure, Diabetologia 2003; 46:405-21.
3. Hakon Hakonarson and Struan FA.Grant. Genetic Associations with Type 1 Diabetes: What Do We Know? CML-Diabetes 2010; 27(2): 37-46.
4. L Leigh Field, Zenia Larsen, Flemming Pociot, JØrn Nerup, Rose Topias & Vagn Bonnevie – Nielsen. Evidence for locus (IDDM16) in the immunoglobin heavy chain on chromosome 14q32.3 producing susceptibility to type 1 diabetes, Genes Immun 2002; 3:338-44.
5. Marina Bakay, Rahul Pandey and Hakon Hakonarson. Genes Involved in Type 1 Diabetes: An Update, Genes 2013; 4:499-521.
6. Tom L.Van Belle, Ken T. Coppieters and Matthias G. Von Herrath. Type 1 Diabetes: Etiology, Immunology, Therapeutic Strategies, Physiol Rev 2011; 91: 79-118.
7. Narinder K. Mehra, Neeraj Kumar, Gurvinder Kaur, Uma Kanga and Nikhil Tandon. Biomarkers of susceptibility to type 1 diabetes with special reference to the Indian population, Indian J Med Res 2007; 125: 321-344.
8. Youssef M.Mosaad, Fatma A. Auf, Shereen S. Metwally, Ashraf A. Elsharkawy, Amani K. El-Hawary, Rasha H. Hassan, Ziyad E. Tawhid and Farha A. El-Chennawi. HLA-DQB1* alleles and genetic susceptibility to type 1 diabetes mellitus, World Journal of Diabetes 2012; 3(8):149-155.
9. Ivana Shawkatova, Dagmar Michalkova, Lubomir Barak, Henrieta Fazekasova, Daniel Kuba and Milan Buc. HLA class II allele frequencies in type 1A diabetes mellitus Slovak patients, Bratisl Lek Listy 2006; 107(3): 76-79.

10. Nanees Ismail, Omar Kasem, Mohamed Abou-El-Asrar and Mona El Samahy, Epidemiology and Management of Type 1 Diabetes Mellitus at the Ain Shams University Pediatric Hospital, J Egypt Public Health Assoc 2008; 83(1&2):107-132.

11. William H. Herman, Ronald E. Aubert, Mohammad Ali, Edwar S. Sous and Ahmed Badran. Diabetes mellitus in Egypt: risk factors, prevalence and future burden, Eastern Mediterranean Health Journal 1997; 3(1):144-148.

12. Bart J. Van der Auwera, Frans C Schuit, Ilse Weets, Ann Ivens, Jan E. Van Autreve and Frans K. Gorus. Relative and absolute HLA-DQA1-DQB1 linked risk for developing type I diabetes before 40 years of age in the Belgian population: implications for future prevention studies. Hum Immunol 2002; 63: 40-50.

13. Mehmet Keskin, Ayşe Aygün, Sacide Pehlivan, Özlem Keskin, Yılmaz Kor, Ayşe Balat. and Yavuz Coşkun. Trends in the Frequency of HLA DR-DQ Haplotypes Among Children and Adolescents with Type 1 Diabetes Mellitus in the Southeast Region of Turkey, Journal of Clinical Research in Pediatric Endocrinology 2012; 4:4

14. Samir A. Gaber, Gina Mazzola, Monica Berrino, Lorena Canale, Maura Cornaglia, Isis Ghali, Emilio Sergio Curtoni and Antonio Amoroso. Human leucocyte antigen class II polymorphisms and genetic susceptibility of IDDM in Egyptian children, Diabetes Care 1994;17:1341-1344.q1

15. Nadia Benseffaj, Chehrazade Brick, Ouafa Atouf, Asmaa Drissi Bourhanbour, Ouadghiri Sanae and Malika Essakalli. Human leukocyte antigen (HLA) polymorphism and type 1 diabetes in the Moroccan population, African Journal of Biotechnology 2012; 11(95):16126-16131.

16. Einas M. Al-Harbi, Abdul-Jabbar Abbassi, Hala Tamim, Fayza al-Jenaidi,Mariam Kooheji, Madeeha Kamal, Salwa Al-Mahroos, Faisal Al-Nasir, Ayesha A. Motala and Wassim Y. Almawi. Specific HLA-DRB and -DQB Alleles and Haplotypes Confer Disease Susceptibility or Resistance in Bahraini Type 1 Diabetes Patients, Clinical and Diagnostic Laboratory Immunology 2004; 11(2): 292-296.

17. Elham Ragab Abd El-Samea, Farha El Chennawy and Mamdouh Radwan El-Nahas. Association of Auto Antibodies and Genetic Factors in Type 1 Diabetes Mellitus, New York Science Journal 2011; 4(5): 1-10

18. Irina Durbală. Assessment of the risk for Type 1 Diabetes Mellitus conferred by HLA class II genes, Annals of the Romanian Society for Cell Biology 2009; 14(2): 123-129.

19. Rana J. El Wafai, Hania Nakkash Chemaisse, Rajaa F. Makki and Hana Fakhoury. Association of HLA Class II Alleles and CTLA-4 Polymorphism with Type I Diabetes, Saudi Journal of Kidney Diseases and Transplantation 2011; 22(2): 273-281.

20. Fayza A. Al-Jenaidi, Saria F.Wakim-Ghorayeb, Abduljabbar Al-Abbasi, Mona R.Arekat, Noha Irani-Hakime, Pierre Najm, Khadija Al-Ola, Ayesha Motala and Wassim Y. Almawi. Contribution of Selective HLA-DRB1/DQB1 Alleles and Haplotypes to the Genetic Susceptibility of Type 1 Diabetes among Lebanese and Bahraini Arabs, The Journal of Clinical Endocrinology & Metabolism 2005; 90(9): 5104-5109.

21. Manan Hakbany, Angham Al Mutair and Sitelbanat Awadalla. Genetic and diabetic auto-antibody markers in Saudi children with type 1 diabetes, Humman Immunology 2010; 71(12): 1238-42.

Source of Support: Nil
Conflict of Interest: None