eISSN 2255-0569

ORIGINAL

Characterisation of HLA-DR antigen in patients type 1 diabetes mellitus in patient attending a tertairy hospital in Enugu, south-east Nigeria

Caracterización del antígeno HLA-DR en pacientes con diabetes mellitus de tipo 1 que acuden a un hospital terciario de Enugu, en el sureste de Nigeria

Ijeoma Leticia Okoroiwu[1], Emmanuel Ifeanyi Obeagu[2], Hilda González San Miguel[3], Sebastiana Arroyo Bote[3], Getrude Uzoma Obeagu[4]

1. Department of Medical Laboratory Science, Imo State University, Owerri, Imo State, Nigeria.
2. Department of Medical Laboratory Science, Kampala International University, Western Campus, Uganda.
3. ADEMA-HEALTH group of IUNICS. Balearic Islands University. Spain
4. Department of Nursing Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

Corresponding author
Emmanuel Ifeanyi Obeagu
E-mail: emmanuelobeagu@yahoo.com

Received: 30 - IX - 2022
Accepted: 28 - X - 2022

doi: 10.3306/AJHS.2023.38.01.104

Abstract

Background: The association between HLA-DR antigen and diabetes mellitus was studied in know diabetic patient on medication at University of Nigeria Teaching Hospital Enugu. The study was done to characterize the HLA-DR antigen in patients with type 1 diabetes mellitus attending University of Nigeria Teaching Hospital, Enugu.
Materials and methods: Ethical clearance was obtained from the Hospital Research Ethics Committee, while informed consent was obtained from each member of the studied group. Whole blood was collected from each subject using standard venipuncture into an EDTA anticoagulant bottle and fluoride tube. The fasting glucose level was determined by enzymatic oxidase-peroxidase method and glycated hemoglobin (HbAlc) spectrophotometrically. The HLA-DR antigen alleles were studied using sequence specific primer polymerase chain reaction (SSP-PCR) techniques.
Results: The mean results of glucose and HbAlc levels were normal and showed no significant difference when they were compared according to their alleles. The correlation using Kruskal-Wallis test and Dunn's Multiple comparison test showed no significant difference in all the group; HbAlcvs HLA-DR antigens and glucose levels vs HLA-DR antigens P = 0.7325 and P = 0.6081. However, the alleles HLA- DRB4*01 (17.5%), DRB1* 03 (29.2%) and DRB1*15 (35%) showed higher frequencies in the group studied.
Conclusion: This infers that these alleles may be predictive of diabetes in Enugu.

Key words: HLA-DR antigen, genetic risks, diabetes mellitus, patients.

Resumen

Antecedentes: Se estudió la asociación entre el antígeno HLA-DR y la diabetes mellitus en pacientes diabéticos conocidos que reciben medicación en el University of Nigeria Teaching Hospital de Enugu. El estudio se realizó para caracterizar el antígeno HLA-DR en pacientes con diabetes mellitus de tipo 1 que acuden al University of Nigeria Teaching Hospital de Enugu.
Materiales y métodos: Se obtuvo la autorización del Comité Ético de Investigación del Hospital, y el consentimiento informado de cada miembro del grupo estudiado. Se extrajo sangre total de cada sujeto mediante una venopunción estándar en un frasco anticoagulante con EDTA y un tubo de fluoruro. El nivel de glucosa en ayunas se determinó por el método enzimático de la oxidasa-peroxidasa y la hemoglobina glucosilada (HbAlc) por espectrofotometría. Los alelos del antígeno HLA-DR se estudiaron mediante técnicas de reacción en cadena de la polimerasa con cebadores específicos de secuencia (SSP-PCR).
Resultados: Los valores medios de glucosa y HbAlc fueron normales y no mostraron diferencias significativas cuando se compararon según sus alelos. La correlación mediante la prueba de Kruskal-Wallis y la prueba de comparación múltiple de Dunn no mostró diferencias significativas en todo el grupo; niveles de HbAlc frente a antígenos HLA-DR y niveles de glucosa frente a antígenos HLA-DR P = 0,7325 y P = 0,6081. Sin embargo, los alelos HLA- DRB4*01 (17,5%), DRB1* 03 (29,2%) y DRB1*15 (35%) mostraron mayores frecuencias en el grupo estudiado.
Conclusiones: Esto infiere que estos alelos pueden ser predictivos de la diabetes en Enugu.

Palabras clave: Antígeno HLA-DR, riesgos genéticos, diabetes mellitus, pacientes.

Introduction

According to their distinct roles in the immune system, HLA molecules are typically divided into three classes: HLA-1, HLA-II, and HLA-III[1]. Subclasses are further separated into the major classes: There are six types of HLA-I molecules: HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G. There are also eight types of HLA-II molecules: HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB, HLA-DPAI, HLA-DPBI, HLA-DQA1, HLA-DQBI, HLA-DRA, HLA-[2]. The complement system's components are encoded by HLA-III molecules. Class I and class II molecules have different functions and structural characteristics: Class I molecules are found in almost all nucleated cells and are made up of a single a chain that is non-covalently attached to a short polypeptide (32-microglobulin) and delivers intracellular peptides from the virus to CD8+ cytotoxic T lymphocytes, which causes the lysis of the infected cells. Immune system cells with particular functions, such as dendritic cells, macrophages, and B cells, express class II molecules[3].

The main objective of HLA-DR is to introduce peptide antigens to the immune system that may be of foreign origin in order to stimulate or inhibit T-(helper)-cell responses that ultimately result in the generation of antibodies against the same peptide antigen. DR are commonly found in antigen-presenting cells, such as macrophages, B-cells, and dendritic cells. Since the DR "antigen" on the cell surface is frequently produced in response to stimulation, DR is also a sign of immunological stimulation[4].

A strong association of certain HLA class II (DR and DQ) subtypes with T1D has been very consistently demonstrated across all population groups studied, though specific risk alleles (and especially their frequencies) vary across ethnic groups and/or geographical regions. The major genetic risk factors for type 1 diabetes mellitus (T1D) map to the major histocompatibility complex region on chromosome 6p21.3[5].

In NIDDM patients, clusters of HLA-DR positive macrophages have been seen to surround islet amyloid deposits. HLA class II has been strongly related to Type 1 Diabetes, although there is limited research connecting HLA class II to NIDDM. HLA DRB1*04 and DRB1*07 have been found as several HLA alleles linked to NIDDM, while DR3, DR4, DR7, DRII, and DR13 6–9 have also been linked to the condition[6-9].

Material and methods

Sample size

Blood sample was collected from 120 participants, all of which are individuals with type 1 diabetes mellitus. Approximately 5 ml of blood was collected using standard venipuncture techniques. 2 mL was placed in sodium fluoride tubes for fasting blood glucose and 3 mL was placed in EDTA tubes for hemoglobin ale (HbAlc) and HLA-DR assays. Fluoride tube samples were tested at room temperature within 6 hours of collection. EDTA samples were stored in a refrigerator at a temperature of -4 °C before being assayed within 48 hours.

Eligibility criteria

Written consent was obtained from the subjects after reviewing the Participant Information Sheet. The subjects of this study were diabetic subjects from the University Teaching Hospital of Nigeria, Enugu and were of Nigerian descent. Subjects (n = 120) consisted of women and men of various ages.

Ethical clearance

The study was approved by the Institutional Review Board of the University of Nigeria Teaching Hospital Enugu and the subjects received a written informed outlet to initiate the study approved by the Institutional Review Board of the hospital. The study complies with the Declaration of Helsinki.

Methodology

All reagents used are commercially prepared and analytically tested

Measurement of glucose level

A. Spectrum Reagent (Ref 250)

Enzymatic glucose oxidase - peroxidase method was used for the estimation of glucose.

Procedure:

Three test tubes labeled blank, standard and sample were placed and 1 ml of reagent was added to each.10 µl plasma was added to standard tubes and 10 µl plasma was added to appropriately labeled sample tubes. Nothing was added to tubes marked blank, except glucose reagent they were then mixed and incubated at 37°C. for 10 minutes. The absorbance of the samples and standards against the reagent blank was measured at 420 nm within 30 minutes.

B. Determination of Glycated Haemoglubin (HBALC) by tecodiagnostics, U S.A (Teco Diagnostics Reagent)

Procedure:

a. Hemolysate Preparation:

Into tubes (13 x 100 mm) labeled: Standard, Control, Sample 1, etc 500ul Lysing Reagent was dispensed and l00ul of the well-mixed blood sample, standard or control was placed unto the appropriately labeled tube and mixed well. They were allowed to stand for 5 minutes.

b. Glycohemoglobin Preparation

Into 13 x 100 mm glass tubes labeled: Standard, Control, Sample 1, etc, 3.0ml of GlycohemoglobinCation-

exchange Resin was dispensed to each. Before use, the resin was mixed by inverting at least 10 times. The bottle was swirled after addition to each 5 tubes. From Step a3,100ul of the hemolysate was added. The Filter Separators was positioned in the tubes so that the rubber sleeve is approximately 1 cm above the liquid level. The tubes were placed on the rocker and mixed continuously for 5 minutes. The tubes were then removed from the rocker. The Filter Separator was pushed into the tubes until the resin is firmly packed. The supernatant was poured into a cuvette for absorbance measurement. The instrument was adjusted to zero absorbance at 415 nm with deionized water as the blank. The absorbance values for Standard, Control, Sample 1, etc were read and recorded.

Total Hemoglobin Fraction:

Into test tubes labeled Standard, Control, Sample 1, etc, 5.0 ml deionized water was dispensed. From Step a3, 20ul of the hemolysate was placed into the appropriately labeled tubes and mixed. The instrument was adjusted to zero absorbance at 415 nm with deionized water as the blank. The absorbance values for standard, control and sample were read and recorded. These readings are for total hemoglobin.

Calculations

The result was determined in the following step:

$$\%\text{Glyco.} = \frac{\text{R (unknown)}}{\text{(R standard)}} \text{ x standard cone}$$

Where R:

$$\text{R unknown} = \text{Ratio (unknown)} = \frac{\text{Abs.of Glyco (unknown)}}{\text{Abs.of Total Hb (unknown)}}$$

$$\text{R (standard)} = \text{Ratio (standard)} = \frac{\text{Abs.of Glyco (standard)}}{\text{Abs.of Total Hb (standard)}}$$

C. Processing of whole blood to guanidium isothiocynate (GITC) lysate

Procedure:

Whole blood sample was transferred into a 15ml tube that was labeled serially. Cold lx RCLB buffer (10 ml) was added to each sample and the tube was properly mixed by inversion. The 15 ml tube (sample tube) was placed on ice for 10 minutes. The tubes were wiped carefully and centrifuged at 4000 rpm for 7 minutes. The supernatant was carefully decanted into the waste bucket while making sure the-cell pellet was not lost. Cold lx RCLB buffer (10 ml) was added to the cell pellet, mixed by vortexing and steps 3-5 were repeated. Sterile PBS (10 ml) was added to the cell pellet, mixed by vortexing and centrifuged at 4000 rpm for 7 minutes. The supernatant was decanted; 5ml of sterile PBS was then added into each tube, mixed by vortexing and centrifuged at 4000 rpm for 5 minutes. The supernatant was decanted into the waste bucket (care was taken not to discard the pellet), and the 15 ml tubes were drained on a clean towel. While draining, the GITC buffer was prepared by adding l0µl ßME to the lml of GITC. Activated GITC (lml) containing ßME was added to the white cellpellets in the tube. The GITC lysate was homogenized using blunt end 18G needle and 5ml syringe 18-times. The GITC lysate was transferred into 2ml cryovial using sterile Pasteur pipettes. And labeled accordingly for immediate nucleic acid extraction or storage at minus 20°C. For quality control Purpose, lml of GITC buffer containing (3ME was transferred into a cryovial, labeled as control and was treated as a sample during nucleic acid extraction. All GITC lysate sample were stored at the minus 20°C freezer.

D. Blood genomic DNA (gDNA) purification/ extraction using GeneJETgDNA purification kit by Thermo Scientific, Lithunia.

Procedure:

For each sample, 200 µl GITC lysate was transferred to a 2 ml Eppendorf tube, 400 µl lysate, 20 µl proteinase K was added. Mix well by vortexing. Samples were then incubated at 56°C for 10 minutes with occasional vortexing. Add 200 µl absolute ethanol (100%) and mix by vortexing. The prepared lysate was transferred to the GeneJETgDN extraction column placed in a collection tube. Centrifuge the column at 6000 rpm for 1 minute. Collection tubes containing flow-through solution were discarded. Place the GeneJETgDNA extraction column into a new 2 mL collection tube. Wash buffer I, 500 µl was added. It was then centrifuged at 8000 rpm for 1 minute. The flow-through was discarded and the extraction column was returned to the collection tube. 500 µl of wash buffer II (with ethanol added) was added to the GeneJET gDNA extraction column. Centrifuged at 12,000 rpm for 3 minutes. The flow-through collection tube was discarded and the GeneJET gDNA extraction column was transferred to a sterile 1.5 mL microcentrifuge tube. Add 200 µl of Elution Buffer to the center of the membrane of the GeneJET gDNA Extraction Column to elute the gDNA. Then incubate at room temperature for 2 minutes and centrifuge at 8000 rpm for 1 minute. 8. The extraction column was discarded and the purified DNA was stored at -20°C.

E. Sequence specific primers polymerase chain reaction (SSP-PCR) by Olerupssp AB, Sweden.

Human leukocyte antigen (HLA) was previously determined using a lymphotoxicity test. However, this test has been superseded by polymerase chain reaction (PCR)-based DNA typing techniques due to error rates and lack of resolution at the allelic level. In most PCR-based techniques, the PCR process is only required as an amplification step for the desired target

DNA and as a post-amplification step to distinguish between different alleles. In contrast, in the SSP-PCR methodology (sequence-specific primers – SSP), discrimination between different alleles is made during the PCR process. This shortens and simplifies the post-amplification step to a simple gel electrophoresis detection step. The results of the SSP test can be either positive or negative, so there is no need to interpret the results complicatedly. Moreover, the typing resolution of SSP-PCR is higher than other PCR-based typing techniques. This is because each primer pair defines two sequence motifs encoded in cis. H. on the same chromosome. Additionally, the synthetic properties of the SSP reagents resulted in improved stability and reduced lot-to-lot variability.

Procedure:
For each sample, the following reaction mixture where carried out. 4µ of DR primer mixes + 5µ PCR master mix + 2µ of gDNA using a well labeled microplate. Caped carefully and minifuged for 30 sec. It was then transferred to thermal cycler (PCR machine) and run using the program um expOOl; at the following thermal profile. 95°c for 5 min.

I. 95°C for 30 sec.
II. 64°C for 50 sec.
III. 72°C for 50 sec.
IV. 72°C for 5 min.
V. Step ii-iv for 35 cycles

The reaction mixture was then removed after completion of the run. Ready for gel electrophoresis.

F. Agarose gel electrophoresis.

2% Agarose gel preparation

4 g of agarose powder was dissolved in 200 mL of Tris base, acetic acid, and EDTA (TAE) buffer in a 250 mL Scholtduran bottle. The mixture was well stirred and microwaved at medium temperature for 8 minutes. It was cooled to about 36°C. and 10 µL of ethidium bromide solution was added. Then shake to obtain a homogeneous mixture. Inserted the electrophoresis comb into the gel mold. The comb creates wells in which samples are placed. Warm agar was then poured into the mold, allowed to gel, and chilled for 20 minutes. The cold gel was placed on a dark surface to facilitate sample placement in the wells. Each sample was mixed with a small amount of dye using a micropipette. Samples were then loaded into the wells. Place the sample-loaded gel in the electrophoresis chamber. The sample side must face the black or negative terminal. TAE buffer was injected into both sides of the chamber so that the buffer level covered the top of the gel. The electrophoresis lid was placed in the chamber and the power was turned on. Make sure the voltage is 100V and it works for 30 minutes. The power was turned off and the electrophoresis lid was removed. Then the gel was taken out and placed in a transilluminator. A transilluminator is a UV lightbox. An image of the gel was taken and ethidium bromide fluoresced and various allelic bands were seen.

Results

The study group included 120 participants (61 women and 59 men). The average age of participants at the time of the study was 48.8 ± 32.0 years. Group mean, median, standard deviation, minimum, maximum, 25% percentile, 75% percentile, lower limit of 95% confidence interval for mean, upper limit for 95% confidence interval for mean, and standard error for fasting plasma glucose levels Decided. Depending on the HLA-DR type, as shown in **table I**.

The mean, median standard deviation, minimum value, maximum value, 25% percentile, 75% percentile, lower 95% confidence interval of mean, upper 95% confidence interval of mean and standard error of glycated hemoglobin value were group according to their HLA-DR type as shown in **table II**.

Table I: Summary Sugar and HLA DR Type.

	DRB 1* 03:01	DRB 1* 03:02	DRB 1* 04:01	DRB 1* 07:01	DRB 1* 15:01	DRB 1* 15:02	DRB 3* 01:01	DRB 1* 16:01	DRB 4* 01:01
Number of Values	19	16	6	6	28	17	4	6	21
Minimum 25%	3.800	3.800	4.100	3.200	3.000	3.400	4.100	3.500	3.300
Percentile	4.125	4.000	4.100	3.200	3.800	4.500	4.100	3.500	3.750
Median 75%	4.500	4.800	4.200	3.500	4.500	5.600	23.05	6.500	4.100
Percentile	6.100	13.60	5.300	5.100	7.800	6.800	42.00	7.900	5.100
Maxim	14.10	18.80	5.300	5.100	20.90	10.90	42.00	7.900	7.100
Mean	6.300	8.214	4.533	3.933	6.91	5.929	23.05	5.633	4.522
Std. Deviation	3.553	5.809	0.6658	1.021	5.169	2.444	26.80	2.203	1.188
Std. Error	1.256	2.196	0.3844	0.5897	1.558	0.9237	18.95	1.272	0.3961
Lower 95% CI of mean	3.329	2.842	2.879	1.396	3.219	3.668	-2.17.7	0.1607	3.603
Upper 95% CI of mean	9.271	13.59	6.187	6.471	10.16	8.189	263.8	11.11	5.436
Sum	50.40	57.50	13.60	11.80	73.60	41.50	46.10	16.90	40.70

Table II: Summation for AIC and HLA DR type.

	DRB 1* 03:01	DRB 1* 03:02	DRB 1* 04:01	DRB 1* 07:01	DRB 1* 15:01	DRB 1* 15:02	DRB 3* 01:01	DRB 1* 16:01	DRB 4* 01:01
Number of Values	19	16	6	6	28	17	4	6	21
Minimum 25%	4.600	3.000	3.10	8.100	4.300	4.700	3.400	4.200	2.400
Percentile	4.850	3.600	3.100	8.100	4.900	5.100	3.400	4.200	2.650
Median 75%	8.450	4.500	6.000	8.700	5.600	5.900	6.050	6.700	5.500
Percentile	12.03	8.000	8.900	8.800	7.700	6.400	8.700	11.90	12.20
Maxim	15.10	10.40	8.900	8.800	12.60	9.400	8.700	11.90	12.40
Mean	8.563	5.800	6.000	8.533	6.591	6.129	6.050	8.600	6.800
Std. Deviation	3.883	2.681	2.900	0.3786	2.435	1.561	3.748	3.966	4.318
Std. Error	1.373	1.013	1.674	0.2186	0.7342	0.5899	2.650	2.290	1.439
Lower 95% CI of mean	5.316	3.320	-1.204	7.593	4.955	4.685	-27.62	-1.252	3.481
Upper 95% CI of mean	11.81	8.280	13.20	9.474	8.227	7.572	39.72	18.45	10.12
Sum	68.50	40.60	18.00	25.60	72.50	42.90	12.10	25.80	61.20

The distribution of HLA-DRB1, HLA-DRB3, HLA-DRB4 alleles is summarized in **table III**. A total of 5 different HLA-DRB1 alleles, a HLA- DRB3 and HLA-DRB4 were detected. With HLA-DRB4*01 HLA- DRB1*03 and DRB1*15 were presents in highly frequencies of 21 (17.5%), 35 (29.2%) and 42(35%) respectively.

Table III: Frequency distribution of HLA-DRB1, DRB3, and DRB4 in participant.

S/N	Allele	F	%
	HLA-DRBI		
1	03	35	29.2
2	04	6	5
3	07	6	5
4	15	42	35
5	16	6	5
	HLA-DRB3		
6	01	4	3.3
	HLA-DRB4		
7	01	21	17.5

Table IV

Table Analyzed	**A1c Vs HLA DR type**		
Kruskal-Wallis test			
P value	0.7323		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	9		
Kruskal-Wallis statistic	5.234		
Dunn's Multiple Comparison Test	**Difference in rank sum**	**Significant? P<0.05?**	**Summary**
DRB1*03-01 vs DRB1*03;02	13.13	No	ns
DRB1*03-01 vs DRB1*04:01	9.458	No	ns
DRB1*03-01 vs DRB1*07:01	-3.875	No	ns
DRB1*03-01 vs DRB1*15:01	7.307	No	ns
DRB1*03-01 vs DRB1*15:02	8.054	No	ns
DRB1*03-01 vs DRB3*01:01	11.88	No	ns
DRB1*03-01 vs DRB1*16:01	0.2917	No	ns
DRB1*03-01 vs DRB4*01:01	9.014	No	ns
DRB1*03:02 vs DRB1*04:01	-3.667	No	ns
DRB1*03:02 vs DRB1*07:01	-17.00	No	ns
DRB1*03:02 vs DRB1*15:01	-5.818	No	ns
DRB1*03:02 vs DRB1*15:02	-5.071	No	ns
DRB1*03:02 vs DRB3*01:01	-1.250	No	ns
DRB1*03:02 vs DRB1*16:01	-12.83	No	ns
DRB1*03:02 vs DRB4*01:01	-4.111	No	ns
DRB1*04:01 vs DRB1*07:01	-13.33	No	ns
DBR1*04:01 vs DRB1*15:01	-2.152	No	ns
DRB1*04:01 vs DRB1*15:02	-1.405	No	ns
DRB1*04:01 vs DRB3*01 :Q1	2.417	No	ns
DRB1*04:01 vs DRB1*16:01	-9.167	No	ns
DRB1*04:CT vs DRB4*01:01	-0.4444	No	ns
DRB1*07:01 vs DRB1*15:01	11.18	No	ns
DRB1*07:01 vs DRB1*15:02	11.93	No	ns
DRB1*07:01 vs DRB3*01:01	15.75	No	ns
DRB1*07:01 vs DRB1*16:0l	4.167	No	ns
DRB1*07:01 vs DRB4*01:01	12.89	No	ns
DRB1*15:01 vs DRB1*15:02	0.7468	No	ns
DRB1*15:01 vs DRB3*01:01	4.568	No	ns
DRB1*15:01 vs DRB1*16:01	-7.015	No	ns
DRB1*15:01 vs DRB4*01:01	1.707	No	ns
DRB1*15:02 vs DRB3*01:01	3.821	No	ns
DRB1*15:02 vs DRB1*16:01	-7.762	No	ns
DRB1*15:02 vs DRB4*01:01	0.9603	No	ns
DRB3*01:01 vs DRB1*16:01	-11.58	No	ns
DRB3*01:01 vs DRB4*01:01	-2.861	No	ns
DRB1*16:01 vs DRB4*01:01	8.722	No	ns

Table IV

Table Analyzed	**A1c Vs HLA DR type**		
Kruskal-Wallis test			
P value	0.6081		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	9		
Kruskal-Wallis statistic	6.350		
Dunn's Multiple Comparison Test	**Difference in rank sum**	**Significant? P<0.05?**	**Summary**
DRB1*03-01 vs DRB1*03;02	-3.321	No	ns
DRB1 *03-01 vs DRB1*04:01	5.750	No	ns
DRB1*03-01 vs DRB1*07:01	15.58	No	ns
DRB1*03-01 vs DRB1*15:01	2.023	No	ns
DRB1*03-01 vs DRB1*15:02	-2.250	No	ns
DRB1*03-01 vs DRB3*01:01	-5.500	No	ns
DRB1*03-01 vs DRB1*16:01	0.5833	No	ns
DRB1*03-01 vs DRB4*01:01	9.028	No	ns
DRB1*03:02 vs DRB1*04:01	9.071	No	ns
DRB1*03:02 vs DRB1*07:01	18.90	No	ns
DRB1*03:02 vs DRB1*15:01	5.344	No	ns
DRB1*03:02 vs DRB1*15:02	1.071	No	ns
DRB1*03:02 vs DRB3*01:01	-2.179	No	ns
DRB1*03:02 vs DRB1*16:01	3.905	No	ns
DRB1*03:02 vs DRB4*01:01	12.35	No	ns
DRB1*04:01 vs DRB1*07:01	9.833	No	ns
DBR1*04:01 vs DRB1*15:01	-3.727	No	ns
DRB1*04:01 vs DRB1*15:02	-8.000	No	ns
DRB1*04:01 vs DRB3*01 :Q1	-11.25	No	ns
DRB1*04:01 vs DRB1*16:01	-5.167	No	ns
DRB1*04:CT vs DRB4*01:01	3.278	No	ns
DRB1*07:01 vs DRB1*15:01	-13.56	No	ns
DRB1*07:01 vs DRB1*15:02	-17.83	No	ns
DRB1*07:01 vs DRB3*01:01	-21.08	No	ns
DRB1*07:01 vs DRB1*16:0l	-15.00	No	ns
DRB1*07:01 vs DRB4*01:01	-6.556	No	ns
DRB1*15:01 vs DRB1*15:02	-4.273	No	ns
DRB1*15:01 vs DRB3*01:01	-7.523	No	ns
DRB1*15:01 vs DRB1*16:01	-1.439	No	ns
DRB1*15:01 vs DRB4*01:01	7.005	No	ns
DRB1*15:02 vs DRB3*01:01	-3.250	No	ns
DRB1*15:02 vs DRB1*16:01	2.833	No	ns
DRB1*15:02 vs DRB4*01:01	11.28	No	ns
DRB3*01:01 vs DRB1*16:01	6.083	No	ns
DRB3*01:01 vs DRB4*01:01	14.53	No	ns
DRB1*16:01 vs DRB4*01:01	8.444	No	ns

Using Kruskal-Wallis test and Dunn's multiple comparison test statistical tool to analyzed glycated hemoglobin verse HLA-DR type by comparison, the P value was 0.73 23 (P>0.05). There was no significant difference between glycated hemoglobin and HLA-DR Type. Theses is shown in **table IV**.

Discussion

Human major histocompatibility complex (MHC) or human leukocyte antigen (HLA) is a cell surface protein that plays an important role in the adaptive immune response. These proteins form complexes with antigenic peptides presented on the cell surface. This complex is recognized by T-cell receptors and triggers adaptive immune responses by inducing cell death and/or antibody production. A strong association of specific human leukocyte antigen (HLA) class II (DR and DQ) subtypes with T1D was demonstrated very consistently across all populations analyzed. Although specific risk alleles (and especially their frequencies) vary by ethnic group and/or geographic region[5]. For example insulin dependent diabetes mellitus (IDDM) has been clearly associated with certain specific HLA class II alleles (like with HLA DR3 and DR4 in India 7 and DRB1*04 in Saudi Arab[10], Various HLA alleles associated with non-insulin dependent diabetes mellitus (NIDDM) have been identified as HLA DRB1 *04 and DRB1*07 while HLA antigens associated with the disease include DR3, DR4, DR7, DRll and DR13. The complex multifactorial etiology of NIDDM requires extensive research to identify predisposing factors in order to implement preventive measures such as dietary management and regular exercise. WHO estimates that Nigeria has the highest number of people with diabetes in Africa, with an estimated burden of about 1.7 million, rising to 4.8 million by 2030[8-9].

Few studies have been conducted in Nigeria to determine the association between diabetes and genetic markers such as HLA-DR. Michael etc. DR3 has been found to be the most common antigen in her type 1 diabetic patients

living in Yoruba, southwestern Nigeria. Their study found no association between type 2 diabetes and HLA[11].

In Bahrain, DRBl*040101(p - 0.019) and DRB1 *070101 ($p < 0.001$) were positively associated with the disease while DRB1*110101 ($p = 0.014$) and DRB1 *160101 ($p = 0.038$) were reported to be negatively associated[8]. Almawi *et al*. have found DRB 1*070101 ($p = 0.001$) to be positively associated with N1DDM in Bahraini and Lebanese population[9].

However, since HLA is one of the most polymorphic genes known and there are large differences in HLA allele frequencies between different ethnic groups and geographic regions, the differences in these findings are expected increase. In the current study, the most common of DRB 1*15, DRB 1*03, and DRB4+01 may be factors contributing to the increased risk of developing diabetes in the study population. Mean results for glucose and her HbA1c levels were normal and showed no significant difference when compared according to their alleles. Correlations using the Kruskal-Wallis test and Dunn's multiple comparison test showed no significant differences across groups. HbAlc vs HLA-DR antigen and glucose levels vs. HLA-DR antigen P=0.7325 and P=0.6081. However, alleles HLA-DRB4*01 (17.5%), DRB1*03 (29.2%) and DRB1*15 (35%) showed higher frequencies in the investigated group. This suggests that these alleles may predict Enugu's diabetes.

Conclusion

HLA-DRB1 + 15, DRB1*03 and DRB4*01 are genetic markers that may be associated with an increased risk of developing diabetes in the Nigerian Enugu population. High-resolution typing can further resolve specific DRB1*15, DRB1*03, and DRB4*01 alleles associated with disease and identify individuals at high risk of developing type 1 diabetes mellitus may help improve our understanding of genetic risk factors as prognostic/ predictive value in diabetes and may contribute to the development of new therapeutic targets in the future.

Conflict of Interest

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

References

1. Han HX, Kong FH, Xi YZ. Progress of studies on the function of MHC in immuno-recognition, J. *Immunol* (Chinese). 2003; 16 (4): 15-7.

2. Minh NN, Jianmin M, Gary BF, Jagath, CR. Di-Codon Usage for Classification of Genes". *Bioystems*. 2009; 20: 1-17

3. Sanchez-Mazas A, Jean-Francois L, Mathias C. Distinct evolutionary strategies of human leucocyte antigen loci in pathology-rich environments. *Phil. Trans. R. Sac. B.* 2011; 367:830-9.

4. Fernando MM, Stevens CR, Walsh EC, De jager PL, Goyette P. Defining the role of the MHC in autoimmunity.a review and pooled analysis. *FLOS Genet*. 2008;4:1-24

5. Puliese A. Genetics of type I diabetic. *EndocrinolMetabClin N AM*. 2004; 33:1-16.

6. Miklossy J, Martins R, D.arbinian N, Khalili K, McGeer PL. Type 2 diabetes: local inflammation and direct effect of bacterial toxic components. *Open Pathol J*. 2008; 2:86-95.

7. Rao PV, Ushabala P, Ahuja MM. Genetics of diabetes mellitus: a review of Indian population studies. *Int J DiabDev Countries*. 1990; 10:41-7.

8. Motala AA, Busson M, Al-Harbi EM, Khuzam MAA, Al-Omari EMD, Arekat MR. Susceptible and protective human leucocyte antigen class II alleles and haplotypes in Bahraini type 2 (non-insulin dependent) diabetes mellitus patients. *ClinDiagn Lab Immunol*. 2005;12:213-20.

9. Almawi WY, Wakim-Ghorayeb SF, Arekat MR, Najm P, Keleshian SH, Al-Sayed N. Association of selective LILA class II susceptibility conferring and protective haplotypes with type 2 diabetes in patients from Behrain and Lebanon. *ClinVaccin*. 2006

10. Al-Hussein KA., Rama NR, Ahmad M, Rosemuller., Tilanus MG. HLA-DPB 1*0401 is associated with dominant protection against type 1 diabetes in the general Saudi population and in subjects with a high-Risk DR/DQ haplotype. *Rur J Immunogenet*. 2003;30:115-24.

11. Michael JM, Olufunsho OF, Ishmael AN, Andrew FB, Junaid IA, Marilyn M, et al. D. HLX-DR Associations in Black Type I Diabetics in Nigeria. Further Support for Models of Inheritance. *Diabetes*. 1986;35: 583-9