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ORIGINAL RESEARCH

Relationship of ABO and Rhesus D Blood Group Phenotypes with Type 2 Diabetes Mellitus in Kano, Nigeria

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Abstract

Background: ABO and Rhesus D blood group phenotypes have been linked to Type 2 Diabetes mellitus (T2D), but the link has been inconsistent.
Objectives: To determine the association between ABO and Rhesus D blood group phenotypes with T2D in Kano, Nigeria.
Methods: This case-control study recruited 149 consecutive individuals with Type 2 Diabetes mellitus who presented on four different clinic days and 200 prospective blood donors, using systematic random sampling. ABO and Rhesus D phenotypes were determined using monoclonal antisera.
Results: The mean age of the participants was 57 years (26–86 years). ABO phenotypes were significantly associated with T2D ($p = 0.001$), with frequencies of B and AB phenotypes being higher among T2D patients. The frequency of the A phenotype was lowest among individuals with T2D. Similarly, Rhesus D phenotypes were significantly associated with T2D ($p = 0.001$), with the frequency of Rh D negative phenotypes being higher among people with T2D. The frequencies of B^- (24.07% vs 9.30%), AB^- (37.5% vs 00%), and O^- (50% vs 6%) phenotypes were significantly higher among T2D patients compared to controls. Compared to O phenotypes, B phenotypes had twice the odds of having T2D (OR = 2.026; 95% CI = 1.215 – 3.376, $p = 0.007$), while Rhesus D positive phenotypes had 0.14 times the odds of having T2D compared to Rhesus D negatives.
Conclusions: Blood group B^- and AB^- phenotypes are significantly associated with T2D in Kano, Nigeria. More attention should be given to ABO and Rhesus D phenotypes in T2D risk assessment and prevention strategies.

Keywords: ABO phenotypes, Blood group, Rhesus D phenotypes, Type 2 Diabetes mellitus.

Introduction

The prevalence of Type 2 Diabetes mellitus (T2D) is rising globally, making it one of the fastest-growing global health issues in the twenty-first century.[1] Global prevalence of diabetes among adults aged 20 – 79 years rose from 151 million in the year 2000 to 537 million in 2021, representing an increase of over 255% in just under 20 years.[1] Sadly, this figure has been projected to reach 643 million by 2030 and 783 million by 2045.[1] Effectively, close to one billion people, about 12% of the global population, will be living with diabetes by the year 2045.[1]

Even though the current prevalence of diabetes mellitus in sub-Saharan Africa (SSA) is low, at about 4.5% of the population, [1] the region is projected to have the highest percentage increase (129%) by the year 2045.[1] The region also harbours a high proportion of people with undiagnosed diabetes mellitus and those with impaired fasting glucose.[1] Thus, SSA will face the challenges of people living with diabetes mellitus in the near future.[1]

About 90% of people with diabetes mellitus are classified to have T2D. [1] This type of diabetes mellitus is linked to genetic predisposition, environmental factors, and several behavioural and lifestyle factors. Modification of some of these risk factors could provide a means of preventing the disease. For those risk factors that cannot be modified, they can provide a means for risk stratification and prognostication. Blood group is a risk factor that has been linked with T2D, especially the ABO and Rhesus D blood group phenotypes. [2,3] It is thought that people who possess the O phenotypes have a lower incidence of T2D compared to other non-O phenotypes. [4,5] However, this finding has yet to be replicated by a number of studies, [6 - 8] suggesting inconsistency. Indeed, Jassim [8] reported higher glucose levels among O phenotypes compared with non-O phenotypes in an Iraqi study consisting of over 900 diabetic participants and their controls. Similarly, Okon *et al.*[9] reported a higher frequency of O^- O-phenotypes among individuals with diabetes mellitus in a Nigerian study.[9] Similarly, a higher prevalence of O^+ and A^+ groups has been reported among T2D in Lagos, southwest Nigeria, while A^+ and B^- were most prevalent among individuals with Type 1 Diabetes mellitus.[10,11] Furthermore, apart from the reported inconsistencies highlighted above, there are very few Nigerian studies that looked at the possible association between ABO and Rhesus D blood group phenotypes with T2D, and most of these studies were conducted in the southern part of the country. Nigeria is ethnically diverse, and studies in one part of the country may not reflect those in other regions. Indeed, studies have reported ethnic and geographical differences in the distribution and frequency of ABO and Rh blood group phenotypes in Nigeria and other parts of the world. [12, 13]

To the knowledge of the authors, there has not been adequate evaluation of the association between ABO and Rh blood group phenotypes with T2D among the predominantly Hausa-Fulani ethnic group of northwest Nigeria. There is, therefore, the need to explore possible associations between ABO and Rh blood group phenotypes in northwest Nigeria. The aim of this study was to determine the potential association between ABO and Rhesus D blood group phenotypes with T2D at a secondary health facility in Kano, northwest Nigeria.

Methods

Study population, design, and sampling technique

The study was conducted at the Diabetes Clinic of Murtala Muhammad Specialist Hospital, Kano, Nigeria, from 24 April to 05 June 2023. The hospital is a secondary health facility with one of the highest patient turnouts in northern Nigeria and a good representation of the dominant Hausa-Fulani ethnic group of northwest Nigeria.

The study population consisted of individuals with Type 2 diabetes mellitus attending the clinic who fulfilled the inclusion criteria and consented to participate in the study. Healthy controls without diabetes were prospective blood donors who presented at the Blood Bank of the hospital during the period of the study. The study is a case-control descriptive study, and a systematic random sampling technique was used to recruit the participants. The total number of patients with Type 2 Diabetes mellitus registered at the clinic (700) and the estimated sample size of 133 to arrive at sampling interval of five. A random

number table was then used to select a number between one to five, which is three. We began participant selection from the third patient in the clinic and continued thereafter with every fifth patient until the required sample size was obtained. The patients were arranged according to the time of arrival at the clinic.

Inclusion and exclusion criteria

All patients with Type 2 diabetes mellitus who consented to the study were recruited for the study. We excluded Type 1 Diabetes mellitus, T2D with complications, and those with co-morbidities (hypertension, other endocrine disorders). Similarly, prospective blood donors who had abnormal fasting blood glucose were excluded from the study.

Sample size determination

The minimum sample size was determined using the formula: [14]

$n = N/1+N(e^2)$ where,

n = minimum sample size,

N = an estimate of the number of T2D patients expected to attend the clinic during the period of the study = 200,

e = degree of precision = 0.05.

$n = 200/1+200(0.05)^2 = 133$.

Data collection

A data capture form was used to collect the socio-demographic information of the participants. The glucose oxidase method determined the fasting blood glucose level using an on-site Accu-Chek® glucometer (Roche Diabetes Care, Inc., Burgdorf, Bern, Switzerland). The glucometer was validated in the pilot and two previous studies. ABO and Rhesus (D) blood group phenotypes were determined using potent monoclonal anti-A, anti-B, and anti-D reagents obtained from (Plamatec Lab. Ltd., Bridport, UK). The tile agglutination method was used as described by Bhatnagar. [15]

Statistical analysis

The data were analysed using the Statistical Package for Social Sciences version (IBM, SPSS), version 23.0. The Independent t-test was used to compare the mean values of quantitative variables. The Chi-square test of association was used to determine the association between ABO and Rhesus D phenotypes with T2D. Binary logistic regression was used to determine the odds of having T2D among the various ABO Rhesus D phenotypes. *P* value ≤ 0.05 was considered statistically significant.

Ethical clearance

Ethical approval for the study was obtained from the Health Research Ethics Committee of the Kano State Ministry of Health, with approval number SHREC/2022/3741, dated 23 January 2023. All the participants signed an individual informed consent form before the commencement of the study.

Results

A total of 349 participants were recruited for the study, comprising 149 T2D patients and 200 apparently healthy non-diabetic controls. The mean age of the participants was 56.67 years (26-86 years). There was a statistically significant age difference among the participants, with the individuals with T2D being older than the controls ($t = 5.61$, $p = 0.002$). Similarly, the T2D group had statistically significantly higher mean fasting blood glucose (FBG) than the controls (8.59±3.35mmol/l vs. 4.56±0.23mmol/l; $t = 3.32$; $p = 0.003$). The socio-demographic characteristics of the participants are presented in Table I.

Table I: Age and Fasting Blood Glucose of the T2D participants and controls

Variable	*Controls Mean±SD*	*Diabetics Mean±SD*	*Mean diff. (95% CI)*	*t-test*	*p-value*
Age (years)	43.45 ±5.44	56.59±11.69	13.14 (10.33 - 21.45)	5.61	0.002
FBG (mmol/l)	4.56±0.23	8.59±3.35	4.03 (3.01 - 6.57)	3.32	0.003

FBG - Fasting Blood Glucose, T2D - Type 2 Diabetes mellitus.

Table II shows the associations between ABO and Rhesus D blood group phenotypes and T2D. The ABO blood group phenotypes were significantly associated with T2D (X^2 = 16.27, p = 0.001), with frequencies of B and AB phenotypes being disproportionately higher among T2D participants compared to the controls.

Table II: Association between ABO and Rhesus blood group phenotypes and T2D

Blood Groups		*Controls n (%)*	*T2D cases n (%)*	X^2	*p-value*
ABO blood group				16.27	0.001
	A	45 (22.50)	17 (11.41)		
	B	43 (21.50)	54 (36.24)		
	AB	12 (6.00)	16 (10.74)		
	O	100 (50.00)	62 (41.61)		
Rhesus blood group				46.07	0.001
	Rh positive	185 (92.50)	94 (63.09)		
	Rh-negative	15 (7.50)	55 (36.91)		

Conversely, the proportion of A phenotypes was disproportionately lower in the T2D group compared to the controls. There was not much difference in the proportion of O phenotypes between the comparative groups. Similarly, Rhesus D phenotypes were also significantly associated with T2D (X^2 = 46.07, p = 0.001), with the frequency of Rhesus D negative phenotypes being higher in the T2D group compared to the controls.

There were significant associations between T2D and B^- (X^2 = 3.614, p = 0.005), AB^- (X^2 = 5.727, p = 0.017), and O^- (X^2 = 42.044, p = 0.001) phenotypes. Specifically, the frequencies of B^- (24.07% vs 9.30%), AB^- (37.5% vs 00%), and O^- (50% vs 6%) phenotypes were significantly higher among T2D patients compared to the controls. A similar pattern of higher proportion of A^- phenotypes among T2D participants compared to the controls was also observed, but the association was not statistically significant (X^2 = 3.055, p = 0.174) (Table III).

Table IV shows the outcome of binary logistic regression to assess the odds of having T2D among the various ABO and Rhesus D phenotypes. Compared to O phenotypes, B phenotypes had two times the odds of having T2D (OR = 2.026; 95%CI = 1.215 – 3.376, p = 0.007). This suggests that the B phenotype is associated with a higher risk of having T2D compared to other phenotypes. For Rhesus D phenotypes, Rhesus D positive phenotypes had only 0.14 times the odds of having T2D compared to Rhesus D negative phenotypes.

Table III: Association between combined ABO and Rhesus phenotypes and T2D

Blood group phenotypes		Non-DM n (%)	DM n (%)	X^2	p-value
A				3.055	0.174
	A^+	40 (88.89)	12 (70.59)		
	A^-	5 (11.11)	5 (29.41)		
B					
	B^+	39 (90.70)	41 (75.93)	3.614	0.005
	B^-	4 (9.30)	13 (24.07)		
AB				5.727	0.017
	AB^+	12 (100.0)	10 (62.40)		
	AB^-	0 (0.0)	6 (37.50)		
O				42.044	0.001
	O^+	94 (94.00)	31 (50.00)		
	O^-	6 (6.00)	31 (50.00)		

DM - Diabetes mellitus, T2D - Type 2 Diabetes

Table IV: Binary logistic regression between T2D and ABO and Rhesus D blood group phenotypes

Variable	Beta	SE	OR	95% CI for OR Lower	Upper	*P* value
B	0.706	0.261	2.026	1.215	3.376	0.007*
AB	0.766	0.415	2.151	0.954	4.848	0.065
Rh D positive	-1.976	0.318	0.139	0.074	0.258	0.001*

OR - Odd ratio, CI - Confidence interval

Discussion

This study looked at the possible association between ABO and Rhesus D blood group phenotypes with T2D at a secondary health facility in Kano, northwest Nigeria. ABO blood group phenotypes appear to be associated with T2D. Specifically, the frequencies of B and AB phenotypes were disproportionately higher among T2D compared to the non-diabetic controls, while the frequencies of A and O phenotypes were lower among T2D. This implies that B and AB phenotypes are associated with a higher prevalence of T2D while A and O phenotypes are associated with a lower disease prevalence. A number of studies have reported similar findings of higher frequency of B phenotypes among T2D compared to non-diabetic controls. [4,7,16,17] Two systematic reviews and meta-analyses involving over thirty studies have reported an increased frequency of B phenotypes among T2D and a higher risk of the disease among B phenotypes than O phenotypes. [4,5] Similarly, Legese *et al.*[16] and Buckwalter[7] reported a higher frequency of B phenotypes in T2D in African and American cohorts, respectively. In a longitudinal study involving over 80,000 women that were followed up from 1990 to 2008 (the E8N cohort), B phenotypes were reported to be associated with an increased risk of T2D. [17] Despite the widely reported increased risk of T2D associated with B phenotypes, some studies have not found any association between T2D and B phenotypes.[6,18,19] While Navabi *et al.*[18] reported A phenotypes as having higher odds of T2D among an Iranian cohort compared to other phenotypes, Aggarwal *et al.*[6] reported increased frequencies of AB and O phenotypes

among individuals with diabetes compared to non-diabetic controls. Indeed, Abu-Bakare *et al.*[19], an earlier study among the Nigerian cohort found no association between ABO phenotypes and diabetes mellitus. Therefore, the association between ABO phenotypes and T2D appears inconsistent. This could partly be due to the wide variation in the distribution and frequency of ABO blood group phenotypes across different ethnic groups and geographical locations. However, despite this seeming inconsistency, B phenotypes are associated with a higher risk of T2D and O phenotypes with a lower disease risk in northwest Nigeria. Indeed, this study found B phenotypes to have two times the odds of having T2D compared to other phenotypes on binary logistic regression.

The association between A and AB phenotypes with T2D is even more polarised than between B and O phenotypes with T2D. Some studies reported an increased frequency of A phenotypes among T2D and hence higher odds of having T2D [17,18]. Some reported lower frequency among T2D [20], while others found no association.[19] A similar pattern was noted for AB phenotypes, ranging from higher frequency among people with T2D [6] to lower frequency [7] and no association.[19] Therefore, the association between A and AB phenotypes and T2D is mixed. This could be due to a lower frequency of AB phenotypes among most populations, thereby pausing the challenge of under-representation. A study with a large proportion of AB phenotypes could shed more light on the true nature of the association between AB phenotypes and T2D.

The present study also found Rhesus D phenotypes to be significantly associated with T2D, with the frequency of negative phenotypes being disproportionately higher among T2D compared to non-diabetic controls. Similarly, Rhesus D favourable phenotypes have only 0.14 odds of having T2D compared to Rhesus negative phenotypes. Only a few were spooked at the possible associations between Rhesus D phenotypes and T2D. However, in a systematic review and meta-analysis of 15 publications, Meo *et al.*[4] reported no association between Rhesus D phenotypes and T2D. Rhesus D antigen is very prevalent in SSA, including in Nigeria. [12,21,22] The disproportionately high prevalence of Rhesus D antigen in SSA could explain why the present study found a significant association between Rhesus D phenotypes and T2D.

Following several combinations of the ABO and Rhesus D phenotypes cross-tabulated with T2D status, the result was hugely influenced by Rhesus D negative phenotypes with frequencies of AB^-, B^-, and O^- phenotypes being significantly higher in T2D individuals compared to controls. This is partly similar to what was reported by Okon *et al.*[9] in a Nigerian study. They found a higher frequency of O^- phenotypes among individuals with T2D compared to non-diabetic controls. However, in contrast to the present study's findings, they also found a higher frequency of A^+ phenotypes among people with T2D than controls. Furthermore, Yahaya *et al.*[10] found O^+ and A^+ to be the most prevalent phenotypes among T2D patients in Lagos, southwest Nigeria; they also reported A^+ and B^- to be the most prevalent phenotypes among patients with Type 1 Diabetes. [11] Taken together, the findings in the present study concerning the association of ABO and Rh blood group phenotypes and T2D in northwest Nigeria and those from the southern part of Nigeria suggest significant ethnic and geographical differences. Even though Yahaya *et al.* [10] included all three major ethnic groups in Nigeria in their study, over 70% of the participants were of Yoruba ethnicity, suggesting possible underrepresentation of other ethnicities. This calls for more localised studies among specific ethnicities and regions in Nigeria. It also indicates that the association between ABO and Rh blood group phenotypes with T2D should be interpreted in the context of the ethnic and

geographical backgrounds of the individual concerned.

Limitations

The participants were drawn from one centre and may, therefore, not be truly representative of Kano residents. However, the fact that the centre is a referral facility that receives patients from different parts of the state, including the neighbouring states, could make up for this limitation. Using a glucose oxidase-based glucometer to measure the fasting blood glucose (FBG) among the controls might have influenced the pattern of glucose intolerance in the controls. Still, the fact that the glucometer was validated and used in previous studies may attenuate this likely influence. Despite these limitations, our study has provided data on the possible association between ABO and Rhesus D phenotypes and T2D in Kano, Nigeria, that others can build on.

Conclusion

ABO and Rhesus D blood group phenotypes are significantly associated with T2D in Kano, northwest Nigeria, with the proportion of B and AB phenotypes being significantly higher among T2D and B phenotypes having 2.15 odd of having T2D and Rhesus D positive phenotypes, 0.14 odd, compared to other phenotypes. More attention should be given to ABO and Rhesus D phenotypes in T2D risk assessment and risk stratification.

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