Patterns of ABO, Rh D Phenotypes and Secretor Status Among Diabetes Mellitus Subjects in Kano Metropolis

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Abstract
Diabetes Mellitus (DM), commonly referred to as diabetes, is a metabolic disease in which there are high blood sugar levels over a prolonged period of time. Since the discovery of blood group antigens, Scientists have found association between blood group antigens and various diseases and infections. This study aimed to determine the patterns of ABO, Rh D phenotypes and Secretor status among diabetes mellitus subjects in Kano Metropolis. Blood and saliva samples were collected from one hundred and fifty (150) subjects, in which 96 (64%) were diabetes subjects while the remaining 54 (36%) were apparently healthy blood donors who served as controls. The ABO and Rh blood groups were determined using standard tube method, while Secretor status was determined using antigen Inhibition Techniques. Out of the 96 cases, fifty 50 (52%) were males and 46 (48%) were females. In the control subjects, 50 (93%) were males and 4 (7%) were females. The mean ages for the cases and controls were 56.85±12.66 years and 26.98 ±7.00 years, respectively. The results obtained revealed that, the most prevalent ABO blood group were in these orders O>B>A>AB, in both cases and controls with the following frequencies 36 (38%), 30 (31%), 27 (28%) and 3 (3%) for cases and 22 (41%), 15 (28%), 13 (24%) and 4 (7%) for controls, respectively. Rh D positive was more prevalent in both cases and controls with the frequencies of 92 (96%) and 53 (98%), respectively. Rh D negative group of cases and controls were found to be 4 (4%) and 1 (2%) respectively. Non secretors were found to be more prevalent in both cases and controls and account for 68 (71%) and 31 (57%), respectively. The Secretors in the cases and controls subjects were 28 (29%) and 23 (43%), respectively. The association between ABO, Rh D phenotypes and Secretor status was determined using Chi-square test and the p-values were 0.643, 0.714 and 0.147 respectively which were statistically not significant (p>0.05), hence, the study revealed no statistically significant association between ABO, Rh D phenotypes and Secretor status with diabetes disease.

Keywords: ABO blood group, Rh blood group, Secretors, Non-Secretors, Diabetes.

Introduction

The first information on the presence of human blood group was by Karl Landsteiner in 1901, which was the ABO group system.[1] According to antigens present on the surface of red cells, Landsteiner (1900) verified three blood groups namely - A, B and O and established that an individual possessed antibodies against those antigens which are not present on his red cells.[2] Detailed ABO (H) blood group

antigens are expressed on cell surfaces and other tissues including body Secretions like saliva, semen, gastric juice. Ever since the discovery of these antigens, there have been concerted efforts to discover a possible association between ABO (H) antigens and different disease conditions.[3] The ability to secrete water soluble A, B and H antigens was found to be inherited in a Mendelian manner, genetically independent of genes controlling the expression of ABO blood group antigens on the surfaces of red cells. ABH secretion is controlled by two alleles, Se (dominant) and se (recessive). Individuals possessing the dominant allele either in homozygous or heterozygous situation (SeSe/Sese) are termed as secretors, while homozygous recessive individuals (sese) are termed non-secretors.[4] In the most rudimentary sense, it is the secretor gene (FUT2 at 19q13.3) that codes for the activity of the glycosyltransferases needed to assemble aspects of both the ABO and Lewis (Le) blood groups. This is accomplished in concert with the gene for group O, or H (FUT1) and the gene for the Lewis phenotype. These enzymes are then active in places like goblet and mucous gland cells, resulting in the presence of the corresponding antigens in bodily fluids.[5]

Diabetes Mellitus (DM) is a metabolic disorder due to either pancreas not producing enough insulin or the cells of the body not responding to the insulin produced,[6] this gives rise to Type 1 and Type 2 DM respectively.

Many studies have revealed an association between some blood groups with certain diseases. It was documented that individuals with blood group A have high risk of having coronary heart disease, prostate cancer and rectal cancer appeared to be associated with A and B blood groups respectively. Group O individuals have propensity to develop duodenal ulcers than A, B, or AB blood groups.[7] However, there still remains a paucity of data that relates diabetes mellitus to blood group phenotypes, and secretor status. Studying the relation between blood group phenotypes, secretor status and diabetes mellitus is germane as it will help better our understanding of the associations that may exist between these heritable traits and their frequencies.

The aim of this study was to determine the patterns of ABO, Rh D phenotypes and secretor status among diabetic mellitus subjects in Kano metropolis.

Materials and methods

Study area and design

The blood and saliva samples for this Case-Control study were collected at Murtala Muhammad Specialist Hospital (MMSH) and Aminu Kano Teaching Hospital Kano, all situated within Kano metropolis, while Sample analyses were conducted in Haematology Teaching Laboratory, Department of Medical Laboratory Science, Bayero University Kano.

Study subjects

A total of one hundred and fifty (150) subjects were recruited into this study, 96 were diabetes subjects and 54 were apparently healthy blood donors who served as controls.

Ethical clearance

Ethical clearance to conduct the research was obtained from the Research Ethics Committee of Aminu Kano Teaching Hospital (AKTH) and Kano state Ministry of Health before the research study. Consent was obtained from the all participants before inclusion in the study in line with revised Helsinki Declaration, 2023 for study involving human subjects.

Inclusion criteria

The inclusion criteria were cases confirmed on the basis of clinical and laboratory tests to have diabetes mellitus and apparently healthy blood donors who served as controls.

Exclusion criteria

Subjects unconfirm to have diabetes mellitus and diabetes mellitus subjects who declined consent to participate were excluded.

Sample collection and processing

Sterile 5ml syringe was used to withdraw 2ml venous blood aseptically from the cubital fossa of all the participants and dispensed into an EDTA container. It was used for the determination of ABO and Rh D blood groups of subjects recruited into the study. Approximately 2 ml of saliva was collected from each participant into a clean, dry universal container following standard protocol for collection of saliva sample. Tubes containing Saliva were kept for 10 minutes in a boiling

water bath at a temperature of 100^0C to denatured the salivary enzymes. It was allowed to cool and then centrifuged for 10 minutes at 1000g, then supernatant was collected and diluted with an equal volume of normal saline. Secretor and non-secretor phenotype was identified using the haemagglutination inhibition technique.[4]

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20. Data was also presented in form of tables and charts. Association between some blood groups phenotype and diabetes mellitus was determined using Chi square test, while odd ratio (OR) and relative risk (RR) was used to ascertain their risk values. All statistical analysis was at 5% level of significance p-value < 0.05.

Results

A total number of one hundred and fifty (150) participants were enrolled into this study for a period of seven months (April through October, 2019). Ninety-six (96) were diabetes subjects while the remaining fifty-four (54) were apparently healthy blood donors which served as the controls. Out of the Ninety-six 96 cases, 50 (52%) were male and 46 (48%) were females. Among the control group, 50 (93%) were males while 4 (7%) were females. The mean ages ± SD of test subjects was 56.85±12.66 years while that of control subjects was 26.98±7.00 years. The tribe distribution is as follows: Hausa recorded the highest number of participant of 69 (72%), 20 (21%) were Fulani, 3 (3%) were Kanuri, 2 (2%) were Yoruba, 2 (2%) were Igbo while the tribe distribution among controls is as follows: 44 (81%) were Hausa, 5 (9%) were Fulani, 1 (2%) Kanuri, 1 (2%) Igbo, 1 (2%) Igala, 1 (2%), 1 (2%) Esan and 1 (2%) Barba (See Table 1).

Table 2: Shows the ABO, Rh D phenotypes of patients and controls. For the patients, 27 (28%) were A, 30 (31%) were B, 3 (3%) were AB and 36 (38%) were O group. For the controls, 13 (24%) were A, 15 (28%) were B, 4 (7%) were AB and 22 (41%) were O. Among the patients, 92 (96%) were Rh D positive while 4 (4%) were Rh D negative. And 53 (98%) of the controls were Rh D positive while 1 (2%) was Rh D negative.

Table 3: Shows the Secretor status of cases and controls. In patients, 28 (29%) were secretors while 68 (71%) were non secretors. For the controls, 23(43%) were secretors while 31 (57%) were non secretors.

Table 4: Shows an association of blood groups antigen phenotypes and risk of diabetes mellitus by comparing with controls. There was no statistically significant association (p>0.05) between theses blood groups (ABO and Rh) phenotype and diabetes mellitus when compared using Chi-square.

Table 5: Shows an association of Secretor status and risk of diabetes mellitus by comparing with controls. There was no statistically significant association (p>0.05) between Secretor status and diabetes mellitus when compared using Chi-square.

Discussion

In this study, the frequencies of ABO blood grouping in the diabetes subjects for groups A, B, AB and O were 27 (28%), 30 (31%), 3 (3%) and 36 (38%) and for the controls were 13 (24%), 15 (28%), 4 (7%) and 22 (41%) respectively. The pattern reported in this study is similar to what was reported by Mohammed *et al*.[8] in their study of 5000 blood donors at Aminu Kano Teaching Hospital. It also goes in line with report of Mukhtar *et al.*[9] in their study of ABO and Rh group phenotypes of Nigerian blood donors at Murtala Muhammad Specialist Hospital Kano, a one year retrospective study which comprises of 12, 233 donors. However, there is striking difference in terms of actual percentages attributable to each group. There is no major difference in the ABO blood groups of the controls and diabetic subjects. This is in support of the findings by Rahman [10] but contradicts those of Okon *et al*.[11] and Kamil *et al*.[12] This may be due to racial and geographical variations.

In this study, the least blood group frequency for both controls and diabetic subject was blood group AB 4 (7%) and 3 (3%) respectively. This result supports the finding that blood group AB is the least frequent among the people of Calabar in southern Nigeria Emeribe *et al*. [13].

Our study is in agreement with Tela *et al*.[14] in a study among malaria infected patients in Jama'are Bauchi State. Similarly, Ali and Nura.[15] reported blood group O to be the dominant group among haemoglobin variants SS and SC but blood group B among AC, which is consonance with general pattern found in this study even though there is slight difference in actual percentages.

In another 2-years review from the record of ABO blood group pattern in neighboring Jigawa state, similar results were reported with blood group O being the dominant while AB being the least.[16] Iyiola *et al*.[17] reported a different pattern from Minna, North Central Nigeria. Even though they found blood group O to be dominant group and AB to be the least, their work found blood group A to be second to O unlike the B that was reported in our study. This could be due to ethnic differences. While the predominant ethnic groups in this study were Hausa and Fulani, their study was from many ethnic groups.[17] Iyiola *et al*.[18] also reported similar pattern with blood group A being second to the dominant O from Ilorin, Kwara State. Ilorin being proximate to Minna geographically may share similar ethnic compositions with the former and the similarity in their pattern of ABO blood group distribution.

Our study is also in concordance with what was reported from studies in southern part of Nigeria [19, 20]. However, while blood group B is the second dominant group from most works from northern part of Nigeria, group A seems to be the second most dominant in the southern part [21] In far distance, some Ethiopian workers reported blood group O to be dominant group and AB to be least in their extensive community-based analysis of information from various ethnic groups [22]. This is similar to what was reported in many Iranian provinces [23].This study confirmed Rh positive 92 (96%) has the highest percentage frequency while Rh negative 4 (4%) in diabetes subjects and controls subjects 53 (98%) Rh positive and 1 (2%) Rh negative has the lowest percentage and frequency. This is in consonance to the study by Hemalatha *et al*.[24] and Behra *et al*.[25] which reported highest percentage of Rh positive subjects and lower percentage of Rh negative subjects.

Our result is close to the findings of Parmanik *et al.* [26] from Nepalese students, in Nepal medical college, Kathmandu. Their subjects were 96.66% Rh positive and 3.33% Rh negative. In Rh blood group distribution, O blood group has the highest frequency of Rh positive 33 (36%) followed by blood group B 30 (33%), blood group A 26 (28%) and blood group AB 3 (3%) in diabetes subjects. While it is O blood group has the highest frequency of Rh negative 3 (75%) then blood group A 1 (25%) in the control subjects. Blood group B and AB has no Rh negative in our study. This is in contrast to the findings of Eweidah *et al*.[27] in Al-Jouf province of Saudi Arabia which reported that blood group B has the highest frequency of Rh negative 4.5%, followed by O 2.0%, A 1.8% and AB 0.5%. Another study by Hemalatha *et al*.[24] reported that O blood group has the highest frequency of Rh positive 39.9 % while AB has the lowest frequency 7.7% they also reported that blood group O has the highest frequency of Rh negative 66.7% which is similar to our findings.

In our study, the frequency distribution of ABH secretors and non-secretors were 28 (29%) and 68 (71%) respectively in the diabetes subjects, 23 (43%) and 31 (57%) in the controls subjects respectively. This is in contrast to the Bangladeshi population study, 60% of study population was ABH secretor and 40% non-secretor. In addition, in a study carried out in Institute of Paramedical Sciences, Khyber Medical University Peshawar (KMU IPMS) in 2018. 188 healthy adult students of both genders were recruited, out of which 155 (82%) were males and 33 (18%) were females Out of 155 were male, 107 (69%) were found secretors while 48 (31%) were non-secretors. On the other hand, out of 33 female participants 22 (67%) were secretors while 11 (33%) were non-secretors. Similarly, a study was conducted by Saboor *et al*.[28] in which they reported that the frequency of secretor was 64% and 36% was non-secretor Similarly, another study conducted in India indicates 72.4% secretors and 27.6% non-secretor status [29] .

Conclusion

This study revealed that blood groups (ABO and Rh) distribution and secretor status among diabetic subjects had not deviated from the normal distribution in healthy individuals. No statistically significant association was obtained between ABO, Rh D, secretor status and diabetes mellitus and hence the inheritance of ABO, Rh D and secretor status phenotype does not constitute a risk factor for the development of diabetes mellitus.

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Conflict of interest: None

Table 1: Shows Socio-demographic distribution of cases and controls

	Patients: Number (%)	Controls: Number (%)
Age		
Mean±SD	56.85±12.66 years	26.98±7.00 years
Gender		
Male	50 (52%)	50 (93%)
Female	46 (48%)	4 (7%)
Total	96 (100%)	54 (100%)
Ethnic group		
Hausa	69 (72%)	44 (81%)
Fulani	20 (21%)	5 (9%)
Kanuri	3 (3%)	1 (2%)
Yoruba	2 (2%)	0
Igbo	2(2%)	1 (2%)
Igala	0	1 (2%)
Esan	0	1(2%)
Barba	0	1 (2%)
Total	**96 (100%)**	**54 (100%)**

Table 2: Distribution of ABO, Rh D phenotypes of diabetes subjects (n = 96) and controls (n= 54)

ABO	Patients: Number (%)	Controls: Number (%)
A	27 (28%)	13 (24%)
B	30 (31%)	15 (28%)
AB	3 (3%)	4 (7%)
O	36 (38%)	22 (41%)
Rh D positive	92 (96%)	53 (98%)
Rh D negative	4 (4%)	1 (2%)

Table 3: Shows the distribution of secretor status of diabetes subjects and controls

Secretor status	Patients: Number (%)	Controls: Number (%)
Secretor	28 (29%)	23 (43%)
Non secretor	68 (71%)	31 (57%)

Table 4: Shows the Risk values associated with inheritance of ABO and Rh D phenotype in diabetes subjects

Blood Group	Patients: Number (%)	Controls: Number (%)	P value	OR	RR
A	27 (28)	13 (24)	0.7013	1.234	1.076
B	30 (31)	15 (28)	0.7131	1.182	1.061
AB	3 (3)	4 (7)	0.2522	0.4032	0.6590
O	36 (38)	22 (41)	0.7290	0.8727	0.9517
Rh positive	92 (96)	53 (98)	0.6544	0.4340	0.7931
Rh negative	4 (4)	1 (2)	0.6544	2.304	1.261

Legend: OR=Odd Ratio, RR=Relative Risk, Rh=Rhesus, p value > 0.05= not significant.

Table 5: Shows the Risk values associated with inheritance of Secretor status in diabetes subjects

Secretor Status	Patients: Number (%)	Controls: Number (%)	P value	OR	RR
Secretor	28	23	0.1083	0.5550	0.7993
Non-Secretor	68	31	0.1083	1.802	1.251

Legend: OR=Odd Ratio, RR=Relative Risk, p value > 0.05= not significance

References

1. Garraty, G., Dzik, W., Issitt, P. D., Lubin, D. M., Reid, M.E. and Zelinski, T. (2000). Terminology for blood group antigens and genes historical origins and guideline in the new millennium. *Journal of Transfusion*. **40**:477–489.
2. Mohammad, A., Tanvir, A., Mohammad, A. R., Fahima, C., Rasheduzzaman, R., Ashraful, K. and Regina, L. (2011). Individuals with Le(a+b-) Blood Group Have Increased Susceptibility to Symptomatic *Vibrio cholerae* 01 Infection. *Neglected tropical diseases.* **5**(12). e1413.
3. Waseem, A. G., Iqbal, M., Khan, O., A., and Tahir, M. (2012). Association of Diabetes Mellitus with ABO and Rh blood groups. *Annals of Pakistan Institute of Medical Science.* **8**(2): 58-60.
4. Olorunshola, K. V., and Audu, L. (2013). AB (H) secretor status of sickle cell disease patients in Zaria, Kaduna State, Nigeria. *Nigerian Journal of Physiology.* **28:** 29-34.
5. Bals, R., Woeckel, W. and Welsch, U. (1997). Use of antibodies directed against blood group substances and lectins, together with glycosidase digestion to study the composition and cellular distribution of glycoproteins in the large human airways. *Journal of Anat*omy.**190**:73-84.
6. World Health Organization. (2015). Diabetes fact sheets. Retrieved December 20th 2015 from www.who.int/mediacentre/factsheets/en/
7. Shoback, edited by David, G. and Gardner, D. (2011). Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw- Hill.
8. Mohammed, T., Muhammad, B., Aisha, K. G., Alhaji, S., Chima, O., and Kwaru, A. (2012). ABO and rhesus blood groups among blood donors in Kano, North- Western Nigeria. *Nigerian Journal of Basic and Clinical Sciences*, **9**(1): 11-13.
9. Mukhtar, I. G., Muhammad, S. M., Yakasai, B. W. and Salisu, A. I. (2018). ABO and Rh blood group phenotype of Nigerian blood donors at Murtala Muhammad Specialist Hospital, Kano. *Bayero Journal of Medical Laboratory Science,* **3**(1): 48-53.
10. Rahman, M. (1997). Non-association of ABO blood groups with diabetes mellitus in Bangladesh. *Bangladesh Medical Research Council Bulletin,***2**: 144-146.
11. Okon, U. A., Antai, A. B., Osim, E. E. and Ita, S. O. (2008). The relative incidence of diabetes mellitus in ABO/Rhesus blood groups in South-Eastern Nigeria. *Nigerian Journal of Physiological Science,* **23**(1-2):1-3.
12. Kamil, M., Al-Jamal, H. A. and Yusoff, N. M. (2010). Association of ABO blood groups with diabetes

mellitus.*Libyan Journal of Medicine*, **5**:4847–4851.
13. Emeribe, A. O, Igweagu, C. A. and Ossim, E. E. (1992). ABH secretor status in saliva of Calabar municipal residents.*Eastern Africa of Medical Journal*.**69** (1). 27- 30.
14. Tela, I. A., Modibbo, M. H., Adamu, L. H., and Taura, M. G. (2015). Prevalence of Malaria Infection Among ABO Blood Groups In Jama'are, Bauchi, Nigeria. *RA Journal of Applied Research*, **1**(7): 255-262.
15. Ali, N. I. and Nura, G. (2017). Association of Some Haemoglobin Variants with ABO and Rhesus D Antigen Phenotype in Kano Metropolis.*International Journal of Biomedicaland Clinical Sciences*.**2**(2): 12–16.
16. Aliyu, I., Michael, G., Ibrahim, H., Ibrahim, Z. F., and Isah, A. O. (2018). Blood group pattern and its distribution among blood transfusion recipients in a seminurban setting in North West Nigeria. *Sudan Medical Monitor*.**12(**1): 9–12.
17. Iyiola, O. A., Igunnugbemi, O. O. and Bello, O. G. (2012). Gene frequencies of ABO and Rh(D) blood group alleles in Lagos, South-West Nigeria. *Egyptian Journal of Medical Human Genetics*, **13(**2): 147–153.
18. Iyiola, O. A., Igunnugbemi, O. O., Raheem, U. A. and Anifowoshe, A. T. (2011). Gene frequencies of ABO and Rh (D) blood group alleles in Ilorin. *Nigerian Journal of Pure and Applied Science*.**4**(1): 6–14.
19. Odokuma, E. I, Okolo, A. G. and Aoamaka, P. C. (2007). Distribution of ABO and Rhesus blood groups in Abraka, Delta State.
20. Enosolease, M. E. and Bazuaye, G. N. (2008). Distribution of ABO and Rh-D blood groups in the Benin area of Niger- Delta: implication for regional blood transfusion. *Asian Journal of Transfusion Science*, **2**(1): 3-5.
21. Bamidele, O., Arokoyo, D. S., Akinbola, A. O. (2013). Distribution of ABO and rhesus blood groups among medical students in Bowen University, Iwo, Nigeria. *Annals of Biological Research*, **4**(11): 1–6.
22. Tesfaye, K., Petros, Y., and Andargie, M. (2015). Frequency distribution of ABO and Rh (D) blood group alleles in Silte Zone, Ethiopia. *Egyptian Journal of Medical Human Genetics*.**16**(1): 71–76.
23. Torabizade, M. J., Paridar, M., Mahmodian Shoushtari, M., Kiani, B., Nori, B., Shahjahani, M., and Jalali Far, M. A. (2016). Distribution of ABO blood groups and Rhesus factor in a Large Scale Study of different cities and ethnicities in Khuzestan province, Iran. *Egyptian Journal of Medical HumanGenetics*.**17**(1): 105–109.
24. Hemalatha, N. R., Bhagya, V. (2015). Frequency and Distribution of Blood Groups among Medical Students in Davanagere. *Journal of Public Health Medical Research*, **3**(1):1-4.
25. Behra, R. and Joshi, Y. R. (2013) Distribution of ABO blood group and Rh D factor in western Rajasthan. *National Journal of Medical Research*, **3**: 73-75.
26. Parmanik, T., Parmanik, S. (2000) *Eastern Mediterranean Health Journal*. **6**(1): 156-158.
27. Eweidah, M. H., Rahiman, S., Ali, H. MD., Al-shamary, A. M. D. (2011) Distribution of ABO and Rhesus (RHD) blood groups in Al-Jouf province of the Saudi Arabia. *Anthropologist*, **13**(2): 99-102.
28. Saboor, M., Ullah, A., Qamar, K. and Mir, A. (2014). Frequency of ABH secretors and non-secretors: A cross sectional study in Karachi. *Pakistan Journal of Medical Science*, **30**(1):189–193.
29. Poonam, W. (2017). To find out the prevalence of ABH secretor and non-secretor status on the basis of saliva test and its clinical significance among the population of Gwalior region. *Indo American Journal of Pharmaceutical Sciences,***5**: 201-205

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