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PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 24(4): 354-360 (2024)
DOI: 10.31901/24566322.2024/24.04.895

Does ABO/Rh Blood Groups and P1 Antigen Associate with Type II Diabetes Mellitus

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KEYWORDS ABO Blood Group. Diabetes Mellitus Type II. P1 Antigen. Saudi Arabia. Rh Blood Group

ABSTRACT The World Health Organisation has determined that in the Middle East, Saudi Arabia consider as the second-highest diabetes prevalence. ABO blood group system and Rhesus factor (Rh) previously showed an association with diabetes; however, no study found an association between P1 antigen and type-2-diabetes mellitus (TIIDM). This study aims to assess the association of ABO, Rh, and P1 antigens with TIIDM in Jeddah, Saudi Arabia. A case-control study, including 150 cases and 120 controls, was phenotyped and analysed using a Chi-square test. B and P1 antigens were positively associated with TIIDM (χ^2= 4.509, P value = 0.0337) and (χ^2= 4.049, P value= 0.0441), respectively. Rh distribution was equal between both groups, with no association between Rh and TIIDM. There is no association between P1 and Rh antigen in TIIDM.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder, characterised by chronic hyperglycemia that results from defects in either the secretion or function of insulin, or both, and is associated with disturbance of carbohydrate, fat, and protein metabolisms. It affects about 5 to 10 percent of adults worldwide (Alberti and Zimmet 1998). It has been related to several factors, such as obesity, diet, lack of exercise, environmental and genetic factors that play a role in its genetic expression. However, many other factors could be associated with DM. The effects of DM include long-term damage, dysfunction, and failure of various organs (Meo 2016; Alberti and Zimmet 1998 (; Alotaibi et al. 2017). The International Diabetes Federation (IDF) estimated the global number of DM cases to be 366 million in 2011, and numbers are expected to exceed 552 to 578 million by 2030 and 700 million by 2045 (Saeedi et al. 2019; Alotaibi et al. 2017). The World Health Organisation (WHO) has ranked Saudi Arabia as the second highest rate among Middle Eastern countries and seventh globally for the prevalence of DM, representing a major public health concern (Al Dawish and Robert 2020; Saad Salman 2016). The prevalence of TIIDM in Saudi Arabia was 32.8 percent in 2015 and 35.37 percent in 2020, however, the predicted prevalence will rise to 40.37 percent in 2025, and 45.36 percent in the year 2030 (Robert and Al Dawish 2020; Meo 2016).

Antigens of blood groups are hereditary determinants play a vital role in understanding genetics and susceptibility to diseases. ABO is the major human carbohydrate blood group system found on the red blood cells' surface in addition to other tissues. Their frequency distribution varies among races, ethnicities, and socioeconomic groups (Legese et al., 2020). Rhesus (Rh) blood group system is the most polymorphic and clinically significant in transfusion medicine after ABO. It remains the leading cause of hemolytic disease in fetuses and newborns (HDFN) (Dean 2005). The clinical importance of Rh is emphasised by the D antigen, generally described with a positive or negative suffix after the ABO type, which is the most immunogenic of all antigens (Rosenfield 1989). The P1PK blood group system is one of the carbohydrate-based blood group systems (ABO, P1PK, H, I, Lewis, GLOB, and FORS) known as the ABO-like system. P1, P^k, and NOR are the three antigens that comprise the P1PK system. After the discovery of blood groups in 1900, there has been an interest

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among researchers in finding associations between ABO and Rh blood groups and various diseases. (Waseem et al. 2012). The ABO and Rh blood group antigens have been associated with increased susceptibility to various diseases (Bener and Yousafzai 2014; Alanazi et al. 2018). For example, blood group A individuals are more likely to be infected with gastric cancer. In contrast, blood group O individuals are more likely to develop gastric and duodenal ulcers or schizophrenia (Kashfi et al. 2013), while Rh antigen was reported to be a risk factor for other diseases such as noise-induced hearing loss (NIHL). In addition, salivary gland, colorectal, and ovarian tumors, thyroid disorders, coronary heart disease, and DM, especially TIIDM, showed an association with ABO and Rh blood group antigens (Sharif et al. 2014; Klechova and Ts 1980; Zhang et al. 2014; Montavon Sartorius et al. 2018).

Many studies have been carried out to see if there is a possible association between the ABO and Rh blood groups with the development of TIIDM. The findings could have been more consistent and varied from one study to another. In Italy, results showed an increased frequency of blood group B among diabetics. Studies in Germany, Glasgow, and several other recent studies showed no association between TIIDM and blood groups in the diabetics studied (Kashfi et al. 2013). Since TIIDM is prevalent among children and adult Saudi population, predicting the risk factors of this disease could help in its early screening and prevention of complications. As a result, this study will analyse the association between ABO/Rh and P1 antigens and TIIDM development.

The study aimed to achieve the following objectives:

- Determine the ABO/Rh blood groups of all TIIDM and healthy donors
- Determine the P1 antigen of all TIIDM and healthy donors
- Determine the association between ABO/Rh and P1 antigen with TIIDM development

MATERIAL AND METHODS

Study Design and Data Collection

A case-control study was used to assess the association between ABO, Rh, and P1 antigens with TIIDM development by comparing the cases with the control group. Blood samples have been collected from the TIIDM outpatient clinic in the International Medical Centre (IMC), Jeddah, Saudi Arabia, from December 2021 to February 2022. Those patients were already diagnosed with DM and were under treatment. 150 patients with TIIDM consented to be involved in this study, representing the cases, and 120 healthy non-diabetic donors representing the control group.

The inclusion criteria for the study were that the patients should be above 18 years of age, have TIIDM, and not suffer from malignant diseases or other complications. The controls were randomly selected from healthy non-diabetic donors coming for blood donation at the IMC over the study period. The patient's history, gender, age, and diagnosis were taken from the hospital database.

Ethical Approval

The ethical committee of IMC approved this study, IRB registration number 2022-01-186. Recruited participants were informed that their clinical data would be used for research purposes with full confidentiality. Each participant signed a consent form outlining the purpose and potential outcomes of the study.

Sample Processing

One 4 ml Ethylene diamine tetra acetic acid (EDTA) tube sample was collected for ABO, Rh blood grouping, and P1 antigen determination for both TIIDM patients who were coming to the hospital for follow-up on diabetes management and healthy donors who visited the blood bank for blood donation during the study period.

ABO/Rh Antigens

ABO and Rh blood groups' determination was done using the tube technique. Forward (cell) and reverse (plasma) grouping were carried out using this method. The forward grouping detects the presence or absence of A, B, and D antigens on RBCs, while reverse grouping detects the presence or absence of Anti-A and Anti-B in the plasma. In forward grouping, a drop of the patient blood cells is placed in a tube with 500 µl saline as a diluent for making 3-5 percent suspension, then 50 µl of the suspension is placed in three test tubes,

a drop of each anti-A, anti-B, and anti-D is added separately in these tubes. These tubes are centrifuged (Thermo Electron Corporation) for 30 seconds at 3500 RPM, and after that, they are gently shaken to observe agglutination. D antigen needs further steps for detection if it is negative.

P1 Antigen

P1 antigen was determined using an ID-gel card test (DiaClon Anti-P1 monoclonal (6 x P1) BIO-RAD ID card). Preparing the sample suspension by adding 0.5 ml of ID-Diluent 1 with 50 µl of whole blood and incubation for 10 minutes at room temperature (RT). After that, 10 µl or 12.5 µl of the suspension was pipetted into each ID-card well and centrifuged using (BIO-RAD ID-Centrifuge 12 S II), then read the reactions.

Data Analysis

All data were analysed using Microsoft Excel and the statistical analysis was expressed in frequencies and percentages. Chi-square test analyses are carried out using GraphPad Prism 9.3.1 to compare frequencies between two or more categories to determine whether any significant association exists between the ABO, Rh, and P1 blood group antigens with the development of TIIDM. The *P*-values were reported as two-tailed. P-values of less than 0.05 were considered the cut-off value for significance.

RESULTS

Distribution of Gender Among Study Participants

The gender distribution among TIIDM patients and healthy non-diabetic donors is shown in (Table 1). A total of 270 participants were involved in the study; 184 (68%) were males, representing the majority of participants, and 86 (32%) were females. Of 184 males, 91 were TIIDM, and 93 were healthy donors. In females out of 86 females, 59 were TIIDM, and 27 were healthy donors.

Distribution of ABO/Rh Among TIIDM Patients and Donors

ABO blood groups' distribution among TIIDM patients included 50 percent (n=75) patients with "O" blood group, 27 percent (n=40) patients with "A" blood group, 19 percent (n=29) patients with "B" blood group, and 4 percent (n=6) patients with "AB" blood group. In contrast, healthy donor samples showed 56 percent (n=67) of the "O" blood group, 26 percent (n=31) of the "A" blood group, 10 percent (n=12) of the "B" blood group, and 8 percent (n=10) are "AB" blood group as shown in (Table 2). According to the ABO blood group distribution, the "O" blood group was the most prevalent, and the "AB" blood group was the least prevalent among both groups. "B" blood group was significantly more common in TIIDM patients than healthy donors (19% versus 10%; $P<0.05$), whereas blood group "O" was more common in the healthy donors group, representing 56 percent. Blood group "A" has a similar distribution in both groups. This distribution has been tested using chi-square test analysis. Only the "B" blood group showed statistical significance at $P<0.05$ ($\mu^2= 4.509$, P value = 0.0337).

Table 1: Distribution of gender among TIIDM and healthy donors

Gender	*No. of participants N (%)*	*TIIDM*	*Healthy donors*
Male	184 (68%)	91	93
Female	86 (32%)	59	27
Total	270	150	120

This table shows the gender distribution of type II diabetic patients and healthy non- diabetic donors involved in this study. Out of 150 patients, 91 were male, representing the majority of participants, and 59 were females. While Out of 120 donors, 93 of them were male, and 27 were females

Distribution of P1 Antigen Among TIIDM Patients and Donors

The phenotyping of P1 antigen showed the presence of P1 antigen in 75 percent (n=112) of TIIDM patients and 63 percent (n=76) of healthy

Table 2: Distribution of ABO blood groups among TIIDM and donors

Blood groups	*TIIDM N (%)*	*Healthy donorsN (%)*	*P value*
A	40 (27%)	31 (26%)	0.877
B	29 (19%)	12 (10%)	0.033
AB	6 (4%)	10 (8%)	0.134
O	75 (50%)	67 (56%)	0.340
Total	150	120	

Table 2 was analyzed using Chi-square test. Only B blood group showed statistically significant at $p<0.05$ ($\chi^2= 4.509$, p-value = 0.0337)

donors. The remaining 25 percent (n=38) of TIIDM patients and 37 percent (n=44) of the healthy donors' control group showed the absence of the P1 antigen on their red blood cell membrane (Fig. 1). Chi-square test results showed there is a statistically significant difference at $p < 0.05$, in the presence of P1- positive antigen in TIIDM patients ($\chi^2 = 4.049, p = 0.0441$).

Distribution of Rh Blood Group Among TIIDM Patients and Donors

Rh blood group distribution among TIIDM comprised 93 percent (n=140) of patients with Rh positive, while the remaining 7 percent (n=10) of them were Rh negative. 92 percent (n=110) of healthy donors were Rh-positive, and the remaining 8 percent (n=10) were Rh-negative. Chi-square test analysis has been done to analyse Rh blood group distribution among the two participant groups. The test showed no significance at $p < 0.05$ ($\chi^2 = 0.270$, p value= 0.603) (Table 3).

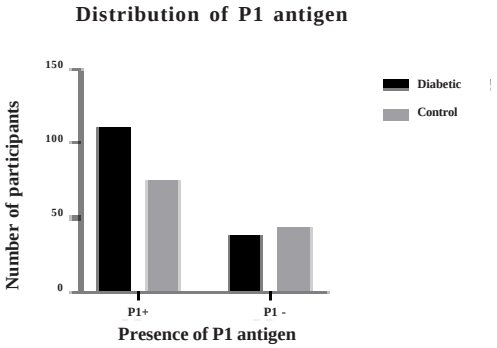

Fig. 1. Distribution of P1 antigen among TIIDM and healthy non-diabetic donors

Fig. 1. Chi-square test analysis showed a statistically significant difference, as shown in the figure at $p < 0.05$, in the presence of P1 antigen in type II diabetic patients (÷^{2}= 4.049, p= 0.0441)

Association of Rhesus (Rh) Blood Group and P1 Antigen in TIIDM

Rh blood group distribution among TIIDM comprised 93 percent (n=140) of patients with Rh positive, while the remaining 7 percent (n=10) of them were Rh negative. The distribution of P1 antigen among TIIDM comprised 75 percent (n=112) of patients who were P1 antigen positive, and 25 percent (n=38) were P1 antigen negative. Figure 2 shows the Rhesus (Rh) blood group's association with the P1 antigen in TIIDM. Chi-square test analysis showed no association between the Rh blood group and P1 antigen in TIIDM at $P<0.05$. ($\chi^2 = 1.332$, P value = 0.2485).

Table 3: Distribution of Rhesus (Rh) blood group among TIIDM and healthy non-diabetic donors

Rh grouping	*TIIDMN (%)*	*Healthy donorsN (%)*
Rh Positive	140 (93%)	110 (92%)
Rh-Negative	10 (7%)	10 (8%)
Total	150	120

Table 3 shows the distribution of Rh blood groups among type II diabetic patients and the control group; it shows an equal distribution between the groups: 93% vs. 92% are Rh positive, respectively, and 7% vs. 8% are Rh negative, respectively. The chi-square test showed no statistically significant result is shown at $p<0.05$

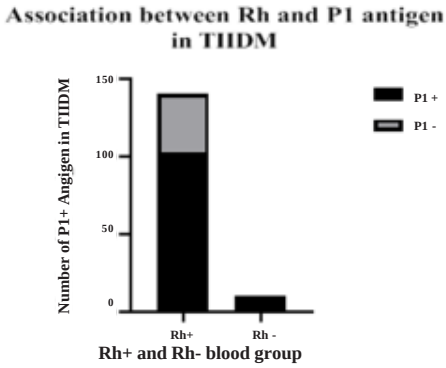

Fig. 2. Association of Rhesus (Rh) blood group and P1 antigen in TIIDM

Fig. 2. Chi-square test analysis showed no association between the Rh blood group and P1 antigen in TIIDM at $p<0.05$ (÷^{2}= 1.332, p-value = 0.2485)

DISCUSSION

This study has supported that there is an association between ABO/Rh and P1 blood group antigens with TIIDM development. According to different studies, blood group B was significantly higher among TIIDM patients than in the control group (19 versus 10%). Bener and Yousafzai (2014) conducted a study in Qatar and found that the frequency of blood group B was significantly higher among diabetic patients compared to the non-dia-

betic population. Two other studies, one from Malaysia and the other from India, revealed similar findings (Kamil et al. 2010; Jaggi and Yadav 2014). In addition, another study was conducted in Pakistan by Qureshi and Bhatti (2003), demonstrating an association between diabetes and ABO blood group, with the B blood group having the highest prevalence of diabetes.

In Hail, Saudi Arabia, a study has been conducted to examine the relationship between the inheritance of ABO blood group and Rh factors and the development of TIIDM in the male population. The results revealed that blood group B-positive patients had a much higher percentage of diabetic patients than controls. In comparison, the blood group of O-positive patients had a slightly lower percentage among diabetic patients than the control group (Farshori et al. 2016). On the other hand, a study conducted among the Algerian population found that diabetic patients had lower frequencies of blood groups A and B than healthy populations (Dali et al. 2011). On the contrary, a study from Nigeria reported a strong association between blood group A and diabetes (Okon et al. 2008). Blood group O was more common in healthy non-diabetic donors (56%). In comparison, it was less common in TIIDM patients (50%), showing a negative association between the O blood group and TIIDM in this study population. However, in other populations, blood group O has been conveyed as the highest blood group distribution among diabetic patients in Iraq, Japan, Pakistan, and Algeria (Waseem et al. 2012; Jassim 2012; Kanazawa et al. 1983; Dali et al. 2011).

This study showed that the Rhesus factor (Rh) was not associated with TIIDM due to an equal distribution of Rh- positive and Rh- negative between the two groups, and the chi-square test shows no significant association at *p-value* <0.05. Studies in India and Algeria reported similar findings (Aggarwal et al. 2018; Dali et al. 2011). However, a study in Pakistan indicated that Rh- Rh-negative blood groups and TIIDM had a significant association (Waseem et al. 2012). These findings are also similar to the results of Alanazi et al. (2018), who found a statistically significant association between Rh factor and developing DM, where more frequencies of diabetic patients are Rh-negative. Conversely, in 2008, research in Iran showed that Rh-positive blood groups are positively associated with TIIDM (Al-Ali 2008). Since diabetes is rising rapidly in the Saudi population and to the researchers' knowledge, few studies have been done to find an association between ABO blood groups and the development of TIIDM in the Saudi population; the researchers decided to study the association between ABO and Rh blood groups and P1 antigen with the development of TIIDM as a first study to be conducted among Saudi population in Jeddah city.

Statistically, the researchers found a significant difference in the presence of P1 antigen between TIIDM patients and controls. Chi-square test analysis showed that P1-positive antigen is statistically higher in TIIDM at $P < 0.05$. This study was the first to find this correlation in Saudi Arabia. The association between the Rh blood group and P1 antigen in type II diabetic patients has been tested, and no association was found. The possible explanation of these conflicting results regarding the association of ABO blood groups and P1 antigen with the development of TIIDM could be due to racial or geographical variations that could play a role in the genetic expression of the disease (Waseem et al. 2012; Daniels 2002).

CONCLUSION

This study concludes that there is a positive association between the ABO blood group and P1 antigen with the development of TIIDM. Blood group B has a positive association with TIIDM, while blood group O has a negative association with the disease. Blood groups A and AB were equally distributed among diabetic and non-diabetic healthy donors.

TIIDM patients have a significant association with P1 antigen. However, no association was found between the Rh factor and TIIDM development, and there was no association between the Rh blood group and P1 antigen in TIIDM patients.

RECOMMENDATIONS

Considering the findings in this study, large-scale research is recommended to be done to provide better analysis and conclusions with more insights into the exact mechanism of association between ABO and P1 antigens with the development of TIIDM. Complete investigations of the ABO-like blood group system and their associa-

tion with the development of TIIDM are highly recommended.

LIMITATIONS

This research was conducted only at the diabetic outpatient clinic of International Medical Centre, Jeddah, Saudi Arabia. Its focus is solely on patients with TIIDM. The clinical history, including family history and medication, has not been included in this study.

Although there are many factors associated with DM, this study concentrates specifically on the relationship between the ABO blood group and P1 antigen.

ACKNOWLEDGMENT

None

CONFLICT OF INTEREST

The authors declare that this study has been presented as a poster at ISBT 2023, Cape Town, South Africa

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FUNDING

This research was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia, grant number G: 223-290-1440.

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Paper received for publication in November, 2023
Paper accepted for publication in July, 2024