

Influence of ABO blood group antigen on activated partial thromboplastin and prothrombin time tests in healthy university students kosti city, White Nile state, Sudan

Abstract

Background: Variances in blood group antigen have been related with susceptibility particular to diseases. Prothrombin time [PT], measures the efficiency of the extrinsic and common coagulation pathways, although Activated partial Thromboplastin time [APTT] test evaluates the intrinsic and common coagulation pathways.

Objective: To explore the influence of blood groups antigen on APTT and PT among Healthy University Students on different ABO blood groups.

Materials and methods: Cross section study including student during the period of three months from September 2020 to December 2020 at Medical campus, University of El Imam El Mahdi, White Nile State, Kosti City, total of 480 students were enrolled. Four milliliters of venous blood was collected from each student, ABO blood grouping was done by the tile method whereas APTT, and PT were analyzed using the manual methods. Data were processed using the Statistical Package for the Social Sciences (SPSS) program version 26 by means of descriptive and inferential statistics.

Results: Blood group O was largest among the test individuals [45%], followed by blood group A [30%] and B [16%], while blood group AB has the smallest percentage of (9%).

Blood group O is significantly higher APTT value [39.07 ± 4.81] second compared to blood groups A [36.60 ± 5.89] second, AB [35.23 ± 4.86] second, and B [34.39 ± 5.30] second, P value < 0.05 .

Likewise, blood group A showed a significantly higher PT value [15.94 ± 1.36] second compared to blood groups O [14.12 ± 1.43] second, B [13.54 ± 1.35] second, and AB [14.67 ± 1.80] second, $P < 0.05$.

Male had a higher APTT level [44.44 ± 7.27] second, PT [15.66 ± 1.88] second, and, compared with the female APTT [35.14 ± 6.49] second, PT [14.08 ± 1.29] second, P value < 0.05 .

Conclusion: Males have a higher APTT and PT levels compared to females. Blood group [O] individuals having a significantly higher APTT, while blood group [A] individuals having higher PT. This proposal that blood group of individuals may affect their intrinsic (APTT) and extrinsic Coagulation mechanisms (PT).

Keywords: ABO blood group antigen, activated partial thromboplastin time, prothrombin time, healthy university students, Kosti, Sudan

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Elham Elamin,¹ Abdelhakam G Tamomh,² AbdElhadi M Agha,¹ Rahma Abd Elfatah M Eltaib,¹ Nada Y Ali,¹ Mohammed Elmadani,³ Alaa F Mohammed,¹ Fatima M Almahdi,¹ Mozdlefa B Musa¹

¹Department of Hematology & Immunohematology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti, Sudan

²Departments of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti, Sudan

³Departments of Epidemiology, Faculty of Public and Environmental Health, University of El Imam El Mahdi, Kosti, Sudan

Correspondence: Elham Elamin, Department of Hematology & Immunohematology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti, White Nile State, Sudan, Tel +249-912948581, Email lhmelamin@yahoo.com, elhamelamin@mahdi.edu.sd

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Introduction

The first discovery of ABO blood group system by Karl Landsteiner in 1900, he identified that O, A, and B blood group types depending on the presence of certain antigen (Ag) on RBC's and antibody (Abs) in plasma; which he earned Nobel Prize.^{1,2} "O" is a German word "Ohne", which means "without" antigens A, B, and AB, was added to the ABO blood group expressed both antigens A and B.³ The ABO blood typing are mostly important for transfusing medicine, hemolytic transfusion reaction and hemolytic diseases of newborn, forensic medicine, and molecular genetics concluded the introduction of Deoxyribonucleic acid (DNA) fingerprints, also the expression of red cell antigen not only on red blood cells, was also found on the surfaces of other human cells, including epithelial, platelets, vascular endothelial cells, the most important being represented by infections, cardiovascular disorders, oncological, and other diseases.⁴⁻⁷

The variances in the main antigens of the ABO blood group, which are usually present on the surface of red blood cells and different epithelial cells, have revealed relatives between ABO blood groups and disease.⁸ Previous study reported that there is a relationship between diseases such as gastric carcinoma, duodenal ulcer, peptic ulcers, diabetes mellitus, bleeding, malignancy, venous thrombosis and urinary tract infection with ABO blood group types.⁹⁻¹³ The hemostatic systems maintain the blood in a fluid state under normal conditions and minimize blood loss via the arrest of bleeding at sites of vascular injury.¹⁴ Disorders of activation hemostatic mechanism resulting in the blocking of a vascular thrombosis, as well as serious hemorrhage.¹⁵⁻¹⁷

The ABO blood group is recognized to influence hemostasis process explaining by von Willebrand factor (vWF)^{18,19}

A, B, and AB blood group of individuals are more susceptible to venous thromboembolism (VTE) than individuals of O blood group

which have greater levels of antigens were inherited co-dominantly over O nearly 25% of vWF and Factor VIII.²⁰⁻²¹

Several studies have investigated the relationship between ABO blood types and hemorrhage, and have established that some patients with certain blood groups are at risk for bleeding from various body sites.²² Activated Partial Thromboplastin Time (APTT) and Prothrombin time (PT) are keys that give an insight into the coagulation status of individuals.^{23,24} The group O has lower levels of the von Willebrand Factor (vWF), and Factor VIII levels, which have been reported to disrupt the functioning of the intrinsic coagulation pathway (APTT), and increase the risk of bleeding.²⁵ Therefore, this study aimed to define whether there is an association between blood type and the coagulation parameters. The study hypothesizes that variation in ABO blood group Antigen affect the intrinsic (APTT), and extrinsic (PT) pathway of coagulation in healthy students of different ABO blood groups in the Medical campus, University of El Imam El Mahdi, Kosti city, Sudan.

Materials and methods

This is cross-sectional study designed to assess the levels of APTT and PT in healthy students of different ABO blood groups in the Medical campus, University of El Imam El Mahdi. Total of 480 undergraduate students from diverse Faculties of the Medical Campus [Medicine, Medical Laboratory Sciences, Public and Environmental Health, Computer Sciences, and Education], Kosti city, White Nile State, Sudan, during the period of September 2020 through to December 2020.

Inclusion criteria

All healthy volunteer students from Medical campus of University of El Imam El Mahdi, Kosti city, Sudan.

Exclusion criteria

Students with known bleeding, students who have been transfused with blood before three months ago, smoker, and/ or who refused to give their consent.

Ethical consideration

The ethical clearance was obtained from the Ethics Committee of the Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Department of Hematology and Immunohematology. Inform consent was obtained from all healthy volunteer students enrolled in this study.

Sample collection and analysis

Four milliliters (ml) of venous blood was collected by venipuncture, and then divided into two tubes (one with 2.25 ml of blood in 0.25ml 3.2% tri-sodium citrate for coagulation tests APTT, PT, and other tube with Ethylene. Demine Tetra-Acetic Acid (EDTA) (1.75 ml of blood for ABO blood group Data was collected using a questionnaire which includes demographic, clinical, and laboratory information. Data was exported into the statistical package for social sciences (SPSS) software, version 26 (Chicago, IL, USA) from Microsoft Excel 7. The P value of <0.05 was considered statistically significant.

Methods

The ABO blood grouping was done by the tile method. A drop of anti -A, B, D was placed on a white pitted tile, and a drop of blood was placed on each of the antisera and mixed with a glass rod. The white tile was rocked gently for 3minutes and was observed for agglutination²⁶

Prothrombin time (PT)

Preparation of Platelet Poor Plasma (PPP)

Platelet Poor Plasma (PPP) is prepared by centrifugation of citrate blood at 2000g for 15 minutes at 4 °C, and the test was performed immediately after samples were prepared.²⁷

Manual method

Deliver 0.1ml of PPP into small test tube (65x10mm) placed in water bath at 37°C. Added 0.1 ml of Thromboplastin, wait for 1-2 min to allow the mixture to worm (Thromboplastin without calcium); then add 0.1 ml of warmed CaCl_2 and mixed well, start stop watch until the CaCl_2 was added. Expressed the PT in second as the mean of duplicated for control and test plasma.²⁸

Activated partial thromboplastin time (APTT)

APTT test measures the clotting time of plasma after the activation of contact Factors, calcification by adding of CaCl_2 to phospholipids.

Manual method

Prewarmed CaCl_2 reagent in the water bath at 37°C for at least 10 min. In clean test tube, 0.1 ml of plasma from test or control samples was added in water bath at 37°C, mixed with equal volumes of APTT reagents (phospholipids reagent and the kaolin), wait for 1-3 min, after that, 0.1 ml of pre-warmed CaCl_2 was added and start a stopwatch immediately. The time for clot formation was observed and recorded; the results of APTT were expressed in second as the mean of duplicated for control and test plasma.²⁸

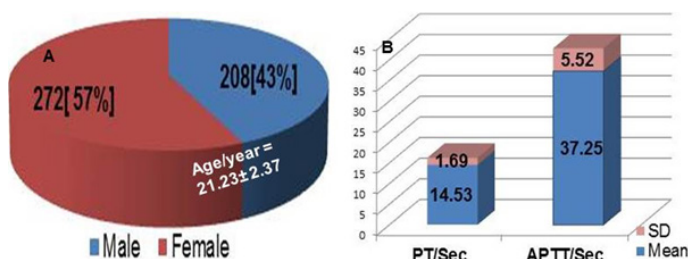


Figure 1 General characteristics [A] students no= 480, 208 (43%) males, and 272 (57 %) females, age represented as mean $\pm$ standard deviation (SD), was 21.23 ± 2.37 /year. [B] PT = 14.53 ± 1.69 second, and APTT = 37.25 ± 5.52 Second.

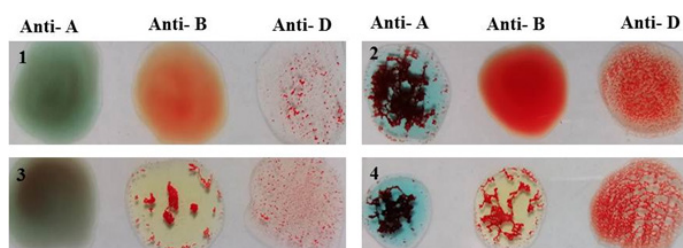


Figure 2 Blood Group Phenotype; 1- [O] Positive (+ve), 2- [A] +ve, 3- [B] +ve, and 4- [AB] +ve.

Discussion

ABO blood groups are valuable in the studies of universal genetic to resolution medical, and a immunologic safety of blood during a transfusion.²⁹ Activated partial Thromboplastin (APTT), and Prothrombin Tim (PT) tests are commonly performed to screen coagulation factor deficiencies to give a considerate the coagulation status of individuals.^{30,31} To answer the research hypothesis; that if

the variation in ABO blood group Antigen affects the intrinsic and extrinsic pathway of coagulation factors. Furthermore, to study if the gender and age affect the levels of APTT, and PT. A Total of 480 healthy volunteer students from University of El Imam El Mahdi medical campus included, 208 (43%) male, and 272 (57%) females, at the same age with represented as mean $\pm$ standard deviation (SD), was 21.23 ± 2.37 year tested for a blood group, which are presented as frequency and percent according to gender, ABO Blood group O is higher 92 (43%) male, and 124 (57%) female with total number 216 (45%), followed by than blood group A students stated 64 (44%) male, 80 (56%) female with total number 144 (30%), group B students reported that 32 (42%) male, 44 (58%) female with total number 76 (16%), and AB students presented smallest number stated 20 (45%) male, 24 (55%) female with total number 44 (9%) Table 1, The distribution design of the ABO blood antigen varies among different populations in the world. As predictable, blood group O was the predominant ABO blood group in the present study which agrees with the predictable findings of this study.^{32,33}

Table 1 Frequency of ABO blood group among the students according to Gender (n= 480)

ABO group phenotype	Gender		Total frequency, n (%)
	Male, n (%)	Female, n (%)	
O	92 [43]	124 [57]	216 [45]
A	64 [44]	80 [56]	144 [30]
B	32 [42]	44 [58]	76 [16]
AB	20 [46]	24 [55]	44 [9]
Total	208 [43]	272 [56]	480 [100]

Meanwhile Table 2, Table 3 Compare between APTT, and PT among the different Test ABO blood groups, our results revealed that students with group A have higher PT (15.94 ± 1.36 second), Followed by AB, (14.67 ± 1.80 second) group O (14.12 ± 1.43 second), and B (13.54 ± 1.35 second) statistically significant p. value < 0.05 . This result was supported by study done by Choi *et al* 2015, Ail and MF, in Iraqi 2018 done by Okeke CO, Okoro US, Babatunde.³⁴⁻³⁶ Blood group O statistically higher APTT when compared with non-O [A, B, and AB blood groups], while blood group A is significantly higher PT when compared with other blood groups³⁵ Blood group O individuals have the tendency to hemorrhage and other (A, AB, and B); thus, have less risk of VTE when lower APTT level on non-O blood have linked to increased risk of coronary heart disease and VTE. This finding explaining by blood group O individuals have a lower plasma concentration of vWF than individuals with other blood group types.¹⁸ The presence of ABH antigenic structures on circulating vWF has been identified as the molecular basis of this phenomenon, which modulate the activity of this protein through different degrees of glycosylation.³⁵ Fourel et al.³⁶ Reported that sex has a significant influence on APTT, with lower mean values in females than in males, while Abdullah *et al*, and Aral *et al* proposed that PT levels differ between ages and gender.^{37,38}

In conclusion this study found that, males have a higher APTT and PT level compared to females. PT and APTT levels differ among individuals of different ABO blood groups, blood group O individuals having a significantly higher APTT, while blood group A having higher PT. This proposal that blood group of individuals affect their intrinsic and extrinsic Coagulation mechanisms. Males have a higher APTT and PT level compared to females.

This study recommended that further researches should be done deeply taking into account the knowledge of cultural, and ethnicity, and to confirm the relationship between ABO blood grouping antigen

types and hemostatic mechanism of coagulation factors. Furthermore, evaluated vWF antigen and factor VIII activity in healthy Sudanese individual.

Table 2 Comparison of APTT, PT and ABO blood groups among

Students (n= 480). ABO Type	APTT/Sec	P. value	PT/Sec	P. value
O	39.07 ± 4.81		14.12 ± 1.43	
A	36.60 ± 5.89	* <0.001	15.94 ± 1.36	* <0.001
B	34.49 ± 5.30	* <0.002	13.54 ± 1.35	* 0.002
AB	35.23 ± 4.86	* <0.002	14.76 ± 1.80	* 0.002
Total (480)	37.25 ± 5.52	* <0.001	14.52 ± 1.69	* 0.002

APTT, activated partial thromboplastin time; **PT**, prothrombin time;

Sec, second; **P. value**, probability value; *statistical significant <0.05 ,

PT& APTT group O student Verse Group A, B, AB, and total number respectively, Data expressed as Mean $\pm$ SD.

Table 3 Effect of gender in a activated Partial Thromboplastin (APTT), and Prothrombin Time (PT) among students (n=480)

Test	Gender		P. value
	Male, (n= 208, 43%)	Female, (n= 272, 57%)	
APTT/Sec	44.44 ± 7.27	35.14 ± 6.49	* <0.001
PT/ Sec	15.66 ± 1.88	14.08 ± 1.29	* <0.001

APTT, activated partial thromboplastin time; **PT**, prothrombin time;

Sec, second; **P. value**, probability value, *statistical significant <0.05

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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