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Genetic Polymorphisms of Human Platelet Antigens and Risk of Arteriovenous Fistula Thrombosis among Hemodialysis Patients

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Abstract

Objective: The aim of this study was to investigate the genotype frequency of HPA-1 ITGB3 (*rs*5918), HPA-3 ITGB2B (*rs*5911) polymorphisms and D-dimer level in hemodialysis patients with or without history of native arteriovenous fistula thrombosis and to compare all of the previous investigations with healthy control group from the Egyptian population.

Methods: This study was conducted on 90 subjects including 60 hemodialysis patients divided equally into 2 groups; Group I included patients with history of at least one episode of native arteriovenous fistula thrombosis, defined as the absence of blood flow and the impossibility for hemodialysis, and Group II included patients without any episode of arteriovenous fistula thrombosis. In addition, Group III included 30 age and sex matched healthy control subjects.

Results: The results showed statistically significant increase in Group II for genotypes frequency HPA-1*C/C*, HPA-3*A/A*, HPA-3*A/B* (*p* 0.05) & D-Dimer level ($p<0.001$).

Conclusions: The Egyptian race may be protected from arteriovenous fistula thrombi by the presence of HPA-1*C/C* & HPA-3*A/A* genotype. Therefore, the retrieved data support that HPA-1 ITGB3 (*rs*5918) and HPA-3 ITGA2B (*rs*5911) polymorphisms are not associated with native arteriovenous fistula thrombosis in Egyptian hemodialysis patients.

Introduction:

Hemodialysis (HD) is the only option available to patients with end-stage kidney disease (ESRD) who are not eligible for renal transplantation (RT) or for whom a compatible donor cannot be found. However, in order to keep the HD process going, frequent access to the circulation is necessary **(Abdelmaguid et al., 2022).**

Arteriovenous fistula (AVF) thrombosis risk factors in HD patients have been investigated prospectively and in case-control studies. In both pre-existing stenotic segments of AVF and native non-stenotic AVF, thrombophilia and hypercoagulability are risk factors for AVF closure because they impede the pre-existing stenotic segments and promote the formation of blood clots. Furthermore, it has been demonstrated that a hypercoagulable state is associated with certain genetic polymorphisms **(Zhang et al., 2022).**

The three main roles of platelets that are suggested in the etiology of thrombotic disorders are adhesion, activation, and aggregation. Understanding the different components of platelets' membranes enables one to comprehend their function in the different phases of the thrombus formation at the arterial wall level. Platelet membrane glycoproteins (GP) express human platelet antigens (HPA). As of right now, thirteen platelet-specific alloantigen systems have been documented (**Hawkins et al., 2019)**.

The action of plasmin on cross-linked fibrin produces D-dimer, a breakdown product that may suggest increased intravascular cross-linked fibrin deposition or activation of the fibrinolytic pathway. It has been demonstrated that HD patients have higher baseline D-dimer levels than healthy controls, and that these levels grow much more immediately after HD **(Schefold *et al.*, 2020).**

Because people with renal issues not only have poorer kidney clearance of D-dimers but also have active coagulation, elevated D-dimer levels are associated with end-stage renal disease (ESRD) **(Pfortmueller *et al.*, 2017).**

Therefore, the main purpose of this work was to investigate the genotype frequency of HPA-1 ITGB3 (*rs*5918), HPA-3 ITGB2B (*rs*5911) polymorphisms and D-dimer level in HD patients with or without history of native AVF thrombosis and to compare all of the previous investigations with healthy control group from the Egyptian population.

Subjects and Methods

The present study was authorized by the institutional review board of Theodor Bilharz Research Institute (TBRI) and ethical committees of both TBRI (FWA00010609) and faculty of medicine AlAzhar university. An informed consent was obtained from all participants according to Declaration of Helsinki.

This study was conducted on 90 subjects including 60 HD patients divided equally into 2 groups; Group I included patients with history of at least one episode of native AVF thrombosis,

defined as the absence of blood flow and the impossibility for HD, and Group II included patients without any episode of AVF fistula thrombosis. In addition, Group III included 30 age and sex matched healthy control subjects.

The patients participating in this study were attending the HD unit, Nephrology Department, TBRI during the year 2022-2023. They were assessed clinically, radiologically and through routine laboratory tests (CBC including platelet indices, urea and serum creatinine). PCR-RFLP for HPAs technique were done for HPA-1 ITGB3 rs5918 (Roche Diagnostics, Switzerland) **(Kucharska-Newton et al., 2011)** and HPA-3 ITGA2B rs5911 **(Milanowski et al., 2016).** In addition to, quantitative determination of D-Dimer by Human D-Dimer **SimpleStep ELISA® Kit Cat No. (ab260076), Abcam, USA.**

Statistical analysis:

The recorded data were analyzed with the statistical program for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data was reported as mean ± SD and ranges. In addition, qualitative characteristics were reported as numbers and percentages.

Results:

Table (1): Comparison between groups according to D-dimer.

D-dimer	Control Group (N=30)	Group with history of AVF thrombosis (N=30)	Group without history of AVF thrombosis (N=30)	Test value	*P*-value	Sig
Mean ±SD	0.21±0.07C	0.29±0.10B	0.34±0.11A	4.456	0.014	S
Range	0.06-0.34	0.07-0.65	0.04-1.81			

Using: One way Analysis of Variance test was performed for Mean ± SD & Multiple comparison between groups through Post Hoc test: Tukey's test

Different capital letters indicate significant difference at ($p<0.05$) among means in the same row

NS: Non-significant; S: Significant; HS: Highly significant

There was a high statistically significant increase in the mean value of D-dimer in group without history of AVF thrombosis, followed by group with history of AVF thrombosis and the lowest value in control group ($p<0.001$); Tukey's post hoc test revealed significant difference multiple between groups ($p<0.05$) (Table 1).

Table (2): The comparison between group with history of AVF thrombosis and control group according to HPA-3 ITGA2B rs5911 genotypes results.

HPA-3 ITGA2B rs5911	**Control Group (N=30)**	**Group with history of AVF thrombosis (N=30)**	***p.* value**	**Risk assessment**	
				OR (95% C.I)	***p.* value**
B/B	3 (10.0%)	5 (16.7%)	1(Reference)		
A/B	12 (40.0%)	19 (63.3%)	0.001**	15.000(2.477-90.843)	0.002**
A/A	15 (50.0%)	6 (20.0%)	0.282	0.400(0.066-2.437)	0.312

The HPA-3 ITGA2B rs5911 genotype is provided as frequency and percentage, and the data was evaluated using the x2 test. Odd Ratio (OR), Confidence Interval (CI), and P value are derived using log linear regression analysis.

* P-value < 0.05 indicates significance; ** P-value < 0.01 indicates extreme significance.

Regarding HPA-3 ITGA2B rs5911 RFLP results comparing the control group and the group with history of AVF thrombosis, there is a significant increase in A/B genotype frequency in group with history of AFV thrombosis ($p<0.05$) in comparison to control group, while the is no significance in both other genotypes B/B and A/A (Table 2).

Table (3): The comparison between group without history of AVF thrombosis and group with history of AVF thrombosis according to HPA-3 ITGA2B rs5911 genotypes results.

HPA-3 ITGA2B rs5911	**Group with history of AVF thrombosis (N=30)**	**Group without history of AVF thrombosis (N=30)**	***p.* value**	**Risk assessment**	
				OR (95% C.I)	***p.* value**
B/B	5 (16.7%)	5 (16.7%)	1(Reference)		
A/B	19 (63.3%)	12 (40.0%)	0.631	0.727(0.198- 2.674)	0.439
A/A	6 (20.0%)	13 (43.3%)	0.01*	2.292(0.714- 7.353)	0.02*

The HPA-3 ITGA2B rs5911 genotype is expressed as a percent and frequency; the x2 test was used to assess the data. Logistic regression analysis is used to calculate the OR, Odd Ratio, C.I., and P value.

** P value <0.01 is highly significant, while * P value <0.05 is significant.

On comparing both patient groups (group with history of AVF thrombosis and group without history of AVF thrombosis) regarding HPA-3 ITGA2B *rs*5911 RFLP results, A/A genotype showed significant increase in the group without history of AVF thrombosis in comparison with the group with history of AVF thrombosis ($p<0.05$), while both other genotypes B/B and A/B showed no significance ($p>0.05$) (Table 3).

Table (4): The comparison between group without history of AVF thrombosis and group with history of AVF thrombosis.

HPA-1 ITGB3 rs5918	Group with history of AVF thrombosis (N=30)	Group without history of AVF thrombosis (N=30)	*p.* value	Risk assessment	
				OR (95% C.I)	*p.* value
T/T	18 (60.0%)	12 (40.0%)	1(Reference)		
T/C	12 (40.0%)	14 (46.7%)	0.364	1.857(0.485- 7.116)	0.285
C/C	0 (0.0%)	4 (13.3%)	0.01*	3.250(0.533- 19.820)	0.02*

The HPA-1 ITGB3 rs5918 genotype is provided as frequency and percentage, and the data was evaluated using the x2 test. Odd Ratio (OR), Confidence Interval (CI), and P value are derived using log linear regression analysis.

* P-value < 0.05 indicates significance; ** P-value < 0.01 indicates highly significance.

On comparing group with history of AVF thrombosis and group without history of AVF thrombosis regarding HPA-1 ITGB3 rs5918 RFLP results, C/C genotype showed statistically significant increase in the group without history of AVF thrombosis in comparison with the group with history of AVF thrombosis ($p<0.05$), while the other 2 genotypes T/T and T/C showed no significance ($p>0.05$) (Table 4).

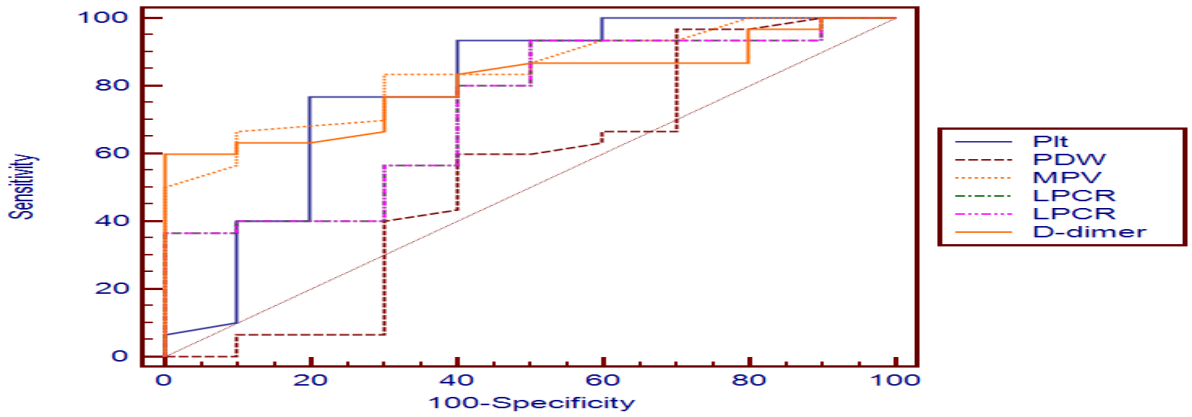

Fig. (1): Receiver-operating characteristic (ROC) curve for prediction of thrombosis, using the platelets indices and D-dimer.

Discussion:

For patients with ESRD who need renal replacement therapy, arteriovenous fistulas, or AVFs, are a crucial treatment strategy. Although there are a number of vascular access techniques, such as catheters and grafts, native fistulas are the most recommended since they have a lower risk of infection and consequences. **(Manook *et al*., 2013)**.

However, a number of issues that jeopardize venous access can influence AVFs. The most frequent side effects include thrombosis, stenosis, aneurysm formation, and infection. Of these, thrombosis and stenosis are most closely associated with AVF access failure. **(Wasse *et al*., 2012)**.

To our knowledge this study is pioneer in studying HPA-1 ITGB3 (*rs*5918), HPA-3 ITGB2B (*rs*5911) polymorphisms genotypes frequency among the Egyptian HD patients and its association with AVF thrombosis risk.

The results of the presented study showed that HPA-1C/C genotype showed statistically significant increase in group without AVF thrombosis ($p<0.05$). Thus, this indicates that the HPA-1C/C genotype may have a protective value for the Egyptian population against thrombosis of AVF.

Conversely to the current conclusions, the study by **Karami *et al.*, 2018** in Iran**,** found a meaningful association between HPA-1T/C genotypes frequency and thrombophilia. However, **Al-Astal and Sharif, 2014** stated that in Gaza strip- Palestine there was no significant association found in HPA-1T/C genotypes frequency neither as a prothrombotic gene, nor as protective against thrombophilia.

In the current study, on comparing group with history of AVF thrombosis with control group regarding HPA-1T/C genotypes frequency, it showed no statistical significance ($p<0.05$). This is in agreement with **Petrişor *et al.*, 2016** who reported that HPA-1T/C genotypes are not related to thrombophilia.

In the present study, regarding HPA-3A/B genotype frequency it was found that the genotype HPA-3A/A showed statistically significant increase in group without history of AVF thrombosis ($p<0.05$) which implies that the Egyptian race may be protected from AVF thrombosis by the presence of HPA-3A/A genotype. However, **Hadhri *et al.*, 2013** reported that HPA-3A/A genotype is an independent risk factor for AVF thrombosis ($p < 0.05$) with frequency of 62.2% in the Tunisian AVF thrombosis group.

In the current study, regarding HPA-3 A/B genotypes frequency, it was found that the genotype HPA-3A/B showed statistically significant increase in group without AVF thrombosis ($p < 0.05$) which may indicate that the presence of HPA-3A/B genotype is a risk factor for ESRD, in contrary to these results, the study by **Wu *et al.*, 2012** stated that there is no difference between ESRD and control groups among the Chinese population**.**

In comparison between studied Egyptian healthy individuals and other different ethnic healthy populations regarding the frequency of different genotypes of HPA-1 it was found that the frequency of HPA-1 genotypes T/T, T/C and C/C was 60%, 30% and 10% respectively, while, the study by

Karami *et al.*, 2018 in Iranian population showed discrepancy in the frequencies of the above-mentioned genotypes T/T, T/C and C/C to be 86.36%, 10.91% and 2.73% respectively**.**

In comparison regarding the frequency of different genotypes of HPA-3 in Egyptian healthy individuals and other studies, the data obtained from this study found that the frequency of HPA-3 genotypes A/A, A/B and B/B was 50%, 40% and 10% respectively. This is conflicting with the results from Egyptian study by **Azza *et al.*, 2019** who stated that the frequency of HPA-3 genotypes was 98%, 2% and 0% respectively. While in a Brazilian study by **Silva-Malta *et al.*, 2018** the frequency was 40%, 43.81% and 16.19% respectively**.** In Tunisian study by **Smaoui *et al.*, 2013** the prevalence of HPA-3 genotypes was 43%, 46.2% and 10.8 % respectively**.**

While D-dimer is nearly always raised in venous thromboembolism, its specificity and positive predictive value are rather modest because it can also rise in a number of other situations, such as acute sickness, recent trauma or surgery, active malignancies, severe atherosclerosis, pregnancy and CKD**.** Moreover, Renal impairment is another significant confounding factor associated with a high incidence of elevated D-dimer levels in critical illness **(Shiekh *et al.*, 2021).**

The presented study showed a high statistically significant mean value of D-dimer in group without history of AVF thrombosis, followed by group with history of AVF thrombosis and the lowest value was in control group (p<0.001). Further more, According to Receiver-operating characteristic (ROC) curve that was performed for the prediction of thrombosis, D-dimer showed high statistical significance as a predictor for the process of thrombosis (p<0.001) (Figure 1).

Similarly, outcomes given by **Milburn *et al.*, 2013 who stated** that the existence of a procoagulant state in HD patients regardless off the occurrence of AVF thrombosis, with increased levels of D-dimer and thrombin /anti thrombin compared to healthy controls**.**

To sum up, the data provided indicates that platelet HPA-1 ITGB3 and HPA-3 ITGB2B polymorphisms are not linked to native AVF thrombosis in Egyptian HD patients. However, larger-scale research and investigations in other ethnic groups are still needed to confirm the significance of these polymorphisms to the development and progression of AVF thrombosis and to determine the effects of preventive therapy in lowering the risk of thrombotic complications on HD linked to these polymorphisms.

Overall, the literature on genetic variants of HPAs and the risk of AVF thrombosis in HD patients provides useful insights into understanding the underlying mechanisms and components that contribute to this condition.

Firstly, a number of investigations have found that specific genetic variants of HPAs are associated with a higher risk of AVF thrombosis in patients with HD. These genetic differences may have an impact on platelet function, which may result in a prothrombotic condition and even thrombotic

episodes. By identifying those who may be more prone to thrombosis, these genetic relationships can be used to inform targeted therapies and preventive measures.

Furthermore, research indicates that genetic variants may combine with environmental factors, comorbidities, and age to further increase the risk of AVF thrombosis. The complexity of thrombotic events in HD patients is highlighted by our data, underscoring the necessity of a thorough comprehension of the interactions between hereditary and non-genetic variables. To improve risk stratification, more research should be devoted to clarifying these relationships and creating prediction models that take into account both genetic and non-genetic factors.

Lastly, studies on the relationship between HD patients' risk of AVF thrombosis and genetic variations in human platelet antigens have the potential to advance personalized treatment, strengthen risk assessment, and increase patient outcomes. Healthcare professionals can better manage vascular access issues by customizing treatment and preventive efforts based on the identification of genetic markers linked to higher thrombotic risk. In order to improve the care given to HD patients, more study can delve deeper into gene-environment interactions and examine the influence of genetic variants on other vascular access-related outcomes.

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