

Biological Diagnosis and Physiopathology of von Willebrand's Disease in a Part of the Algerian Population in the East and the South

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Abstract—Von Willebrand's disease is the most common inherited bleeding disorder in humans, it caused by qualitative abnormalities of the von Willebrand factor (vWF). Our objective is to determine the prevalence of this disease at part of the Algerian population in the East and the South by a biological diagnosis based on specific biological tests (automated platelet count, the bleeding time (TS), the time of cephalin + activator (TCA), measure of the prothrombin rate (TP), vWF rate and factor VIII rate, Molecular electrophoresis of vWF multimers in agarose gel in the presence of SDS). Four patients of type III or severe Willebrand's disease were found on 200 suspect cases. All cases showed a deficit in vWF rate ($< 5\%$), and factor VIII ($P < 0, 0001$), and lengthening very significantly high of the TCA ($P < 0, 0001$) and of the bleeding time ($P < 0,0001$), with a normal blood platelet rate ($P = 0,7433$) and a normal prothrombin rate ($P = 0,5808$), an absence of all the multimers of vWF in plasma patients. The severe Willebrand's disease is not only one pathology of primary haemostasis, but it can be accompanied by coagulation's anomaly due to deficit in factor VIII. At this studied population, von Willebrand's disease is less frequent (2%) than other hemorrhagic syndromes identified by the differential diagnosis like the thrombocytopenia (36%).

Keywords—Von Willebrand's disease, differential diagnosis, von Willebrand factor, factor VIII, biological diagnosis, thrombocytopenia.

I. INTRODUCTION

EVERY rupture of the integrity of the vascular circuit at the origin of a blood leak, start a series of cellular and biochemical processes ensuring the obturation of the breach and the control of the haemorrhage [1]. The haemostasis answers the whole of these physiological mechanisms and includes several intricate and interdependent stages which are: primary haemostasis, secondary haemostasis, the fibrinolysis [1]. This mechanism can be modified by several hemorrhagic affections of the primary or secondary haemostasis. The von Willebrand's disease discovered in 1926 by Erik von Willebrand among members of Aeland's family, is one of the most frequent constitutional abnormalities of the haemostasis, its transmission is autosomic, generally is dominant, it is related to an abnormality either quantitative, or qualitative of the von Willebrand factor [2]. The vWF is a large multimeric plasma glycoprotein which plays a central role in haemostasis.

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It has different functional domains which interact with factor VIII, and platelet glucoproteins, it is required both as a carrier and as a stabilizer for the coagulation factor VIII in plasma. Researchers have identified many variations of the disease. There are three groups of von Willebrand's disease: type 1, characterized by a partial quantitative deficit in vWF, type 2 characterized by a qualitative abnormality, this type is subdivided in four molecular variants (2A, 2B, 2M, 2N), type III characterized by a total deficit [2]. It is important to confirm the diagnosis of von Willebrand's disease in a specialized laboratory because an adequate treatment can be prescribed to avoid hemorrhagic complications.

In this study, our objective is to determine the prevalence of the von Willebrand's disease at part of the Algerian population in the East and the South by a biological diagnosis.

It aims:

- To know if it is about the constitutional hemorrhagic disease most frequent.
- To know the biological diagnosis of the von Willebrand's disease.
- To know the differential diagnosis.
- To study the physiopathology of the von Willebrand's disease.

II. MATERIALS AND METHODS

A. Patients and methods

Our study was realized in biotechnology's laboratory of the bioactive molecules and cellular physiopathology, and hematology's central laboratory of the CHU of Batna, on 200 suspect subjects who showed mucocutaneous hemorrhagic symptoms, of the two sex, of various ages, coming from various regions of the Algerian East and South. The study lasted 12 months, from January 2009 to December 2009. The starting point of the biological diagnosis bases on principal and reproducible tests:

The automated platelet count, blood cell counts were made on EDTA- anticoagulated, blood using an electronic coulter,

The bleeding time was measured according to Ivy, The time of cephalin +activator (TCA) this test based on activation of coagulation's contact system, blood for coagulation tests, collected in 3,8% sodium citrate. Measure of the prothrombin rate in the presence of calcic thromboplastin. vWF activity level (plasmatic vWF) measured as ristocetin cofactor after calibration of the international pool against an international standard. Measure of factor VIII rate. Multimeric analysis of von Willebrand factor (in plasmas) using sodium dodecyl sulfate, agarose gel electrophoresis, semidry eletrotransfer, and immunoperoxidase detection.

B. Statistical analysis

The results are expressed on mean \pm SEM. To compare the means of the samples, we used the student's t test, if the effective "N" is $<$ to 30 (the calculus were effected by the use of a software Graph Pad Prism 5,00), and the test of means of homogeneity if the effective "N" is $>$ to 30 (the calculus were effected manually), which we calculated in the first time the difference between the two means (controls mean and patients mean), then we calculated in the second time the confidence interval :

$$\left[k_1 = -1.96 * \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}, \quad k_2 = 1.96 * \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}} \right]$$

σ_1 : the variance of the first population. σ_2 : the variance of the second population.

II. RESULTS AND DISCUSSION

4 patients were found, 2 are 8 years old and two other old respectively of 19 and 20 years old, the average age was $13,75 \pm 3,22$, what testifies that this pathology can occur at any age. According to research of Borel [3], type III is rare, and represente 1 to 3% of all the forms of the Willebrand's disease, but represents the most grave form. Our results also confirm that this pathology can touch the two sex; it affects males and females equally. According to Fressinaud and Meyer [2], the autosomic transmission of the von Willebrand's disease predicts an equal frequency among the two sex.

Results obtained show that there is not statistically significant difference of platelets rate ($p=0,7433 >0,05$) among these patients ($243,5 \pm 16,28$) compared to the controls ($237,5 \pm 6,371$). According to Fressinaud and Meyer [2], the platelets numeration must be realized systematically at any patient having a hemorrhagic syndrome. The thrombocytopenia can be seen in a particular type of Willebrand's disease, in the type 2B, however the platelets count is normal in all the other types (oscillate between 150 to $350 \times 10^3 / \text{mm}^3$). On the other hand, no significant difference is recorded ($p= 0,5808$) of prothrombin rate between the controls ($92,69 \pm 1,76$) and the patients ($95,50 \pm 4,50$), (the normal rate of prothrombin varies from 70 % to 100%). According to Charkaoui et al. [4] in his studie effected on the von Willebrand's disease type 3 the prothrombin rate is normal. The TP is normal because it explores the coagulation's exogeneous way.

TABLE I
VARIATION OF THE PLATELETS RATE (PLT) AND THE PROTHROMBIN RATE (TP) AMONG THE WILLEBRAND'S PATIENTS

State / Biological test	Normal Control	Patients	P
PLT rate ($10^3 / \text{mm}^3$)	$237,5 \pm 6,37$	$243,5 \pm 16,28$	0,7433
TP (%)	$92,69 \pm 1,76$	$95,50 \pm 4,50$	0,5808

Normal value: PLT I:150-350 $10^3 / \text{mm}^3$, TP:70- 100%40

The values of vWF recorded for these four patients are $<$ 1% (table II). The vWF rate is very decreased compared to the normal values (de 50 % à 170 %) with a frequency of 1 for

each patient. For type 3 the vWF (vWF Ag and vWF RCo) is undetectable in plasma, platelets and endothelium [2], and according to Pomier [5], in the type III; the vWF is lower than 1%.

This table shows (table.III) a lengthening very significantly high of the bleeding time ($p<0,0001$) is signaled among these patients ($14,75 \pm 0,629$) compared to the controls ($5,43 \pm 0,15$). The TS recorded is superior at the normal (the normal values vary from 4 to 8 mn).

TABLE II
DISTRIBUTION OF THE PATIENTS ACCORDING TO THE vWF RATE AMONG THE WILLEBRAND'S PATIENTS

vWF (%)	FREQUENCY	vWF%
$<$ 1%	4	100
TOTAL	4	100

Normal value: 50 – 170 %

TABLE III
VARIATION (O) OF THE BLEEDING TIME (TS) AMONG THE WILLEBRAND'S PATIENTS

State / Biological test	Normal Control	Patients	P
TS mn	$5,43 \pm 0,15$	$14,70 \pm 0,62$	$<0,0001$

Normal values: TS : 4– 8mn

Type III is defined by a very lengthened TS [6], in spite of the realization difficulties of this test (Ivy method) the lengthening of the bleeding time remained an important criterion of diagnosis.

According to Davies and al. [7], the TS explores the primary phase of the haemostasis. This process implies the platelets adhesion to the subendothelium; this interaction is done by the intermediary of von Willebrand factor.

The vWF is a very large multimeric protein found in the blood plasma, and at high concentration in vesicles in both the platelets and endothelial cells. It is therefore always available. Endothelial cells are the cells that line all blood vessels. In platelets adhesion two interactions are important: 1/binding of vWF two collagen in the wall of the vessel; 2/binding of vWF to a receptor protein on the platelet membrane: glycoprotein Ib/IX (GPIb/IX).vWF thus enables platelets to adhere to collagen. Adhesion to collagen via vWF causes the initial activation of platelet, initiating a signalling pathway that results in the release of Ca^{+2} from the dense granules and platelet activation [8]. Upon platelet activation, another major receptor of the platelet membrane becomes functional. It is glycoprotein GPIIb/IIIa (GP IIb/IIIa). Unlike GPIb/IX. This is sensible, since its function is to enable platelets to stick to each other. Once platelets are activated, GPIIb/IIIa becomes functional on the surface, and it binds fibrinogen. Fibrinogen present all the time in solution in plasma, is a long demeric molecule, and it has two GPIIb/IIIa binding sites, one at each end. Activated platelets thus adhere to each other and form aggregates [7]. The vWF multimers mediate platelet adhesion at sites of vascular injury by binding to connective tissue and to platelets, therefore, deficit in vWF can cause bleeding [9], [10]. The lengthening of the bleeding time is according to the deficit in vWF.

The examination of these results (table.IV) allows to remark the reduction very significantly high ($p < 0,0001$) of FVIII rate among these patients ($3,50 \pm 0,28$) compared to the controls ($100,3 \pm 3,56$). The F VIII rate is very decreased ($< 5\%$) compared to the normal values (from 60 % to 150 %). Trzeciak and Bordet [11] reported that in the Willebrand's disease type3 the factor VIII rate is very lowered. Patients who affected by severe Willebrand's disease have F VIII rates about 2 to 7% of the normal one [2].

According to Fressinaud and Meyer [2], the von Willebrand factor (vWF), is a plasma glycoprotein with essential platelet-dependent functions in primary haemostasis and a carrier for factor VIII (FVIII) in the circulation (also binds and stabilizes blood clotting factor (F) VIII), these functions makes these two molecules dependent.

TABLE IV
VARIATION OF FACTOR VIII RATE AND CEPHALIN + ACTIVATOR TIME (TCA)
AMONG THE WILLEBRAND'S PATIENT

State / Biological test	Normal Control	Patients	P
TCA (sec)	$29,81 \pm 0,42$	$76,25 \pm 4,80$	$< 0,0001$
FVIII (%)	$100,3 \pm 3,56$	$3,50 \pm 0,28$	$< 0,000$

Normal values: TCA: 28-36 sec, FVIII: 60- 150 %.

Therefore the deficit in vWF causes a deficit in FVIII. (The bleeding disorder resulting from an almost complete lack of vWF and consequently markedly reduced levels of FVIII).

Our results reveal, a lengthening very significantly high ($p < 0,0001$) of TCA rate among our patients ($76,25 \pm 4,80$) compared to the controls ($29,81 \pm 0,42$). According to Charkaoui and al. [4], the TCA is lengthened with secondary deficit in factor VIII (the normal TCA varies from 28 sec to 36 sec).

The TCA explores the endogeneous way of coagulation. It is very sensitive; it explores the factors of the contact phase (kininogen of higher molecular weight, prekallikrein, F XII, F XI) as well as the factors II, V, VIII, IX, X and the fibrinogen [12].

The intrinsic limbs of the pathway include activation of factor XI to factor XIa by thrombin, with the ultimate generation of more thrombin using factor IXa and factor VIIIa to activate factor X. This pathway model also explains the mechanism of how two of the important cofactors, factor V and factor VIII, are activated by thrombin. It was known that both factor V and factor VIII had to be partially proteolyzed or activated to participate in the formation of a blood clot [13]. Activation of factors V and VIII by thrombin results in a further burst of coagulation activity through increased activity of the tenase and prothrombinase complexes. Fibrinogen is the ultimate substrate protein of the coagulation cascade and forms the principal structural protein of the fibrin clot [14]. Therefore a lengthening of the TCA is generally observed, due to deficit in F VIII. The deficit in F VIII rate reflects indirectly a deficit in vWF [15].

The severe Willebrand's disease is not only one pathology of primary haemostasis, but it can be accompanied by coagulation's anomaly due to deficit in factor VIII.

The multimeric analysis of the normal plasma (Molecular electrophoresis of vWF multimers in agarose gel 1, 5%) indicates that band patterns produced by these pooled plasmas were remarkable similar; within all controls, at least 15 bands were clearly identifiable. And there are essentially no identifiable multimers in the plasma with von Willebrand's disease type III. This absence is caused may be by the mutations of vWF gene. Willebrand's disease type 3 includes virtually complete deficiency of vWF. Willebrand's disease type 3 is inherited as a recessive trait, and heterozygous relatives usually have mild or no bleeding symptoms. The vWF mutations that cause Willebrand's disease type 3 are usually nonsense mutations or frameshifts because of small insertions or deletions. Large deletions splice site mutations and missense mutations are less common [16]. At the time of this study the differential diagnosis enabled us to eliminate 72 cases (36%) of thrombocytopenia. There is a significant difference of the platelets rate between the controls mean ($239,62 \pm 3,50$) and one of patients ($117,09 \pm 3,18$), the platelets rate is decreased for these patients (thrombocytopenia) and we remark also a significant difference of the bleeding time between the controls mean ($5,55 \pm 0,10$) and the patients mean ($16,47 \pm 1,02$), the bleeding time is prolonged. Thrombocytopenia is a frequent bleeding disorder, with peripheral or central etiologies. However, thrombocytopenia is more frequently an acquired disease; it is defined as a decrease in platelet count below $150 \times 10^3 / \text{mm}^3$ [17].

III. CONCLUSION

This study allows us to demonstrate: The prevalence of von Willebrand's disease in a part of the Algerian population in the East and the South is 2% compared to the studied population, it is less frequent than other hemorrhagic syndromes like the thrombocytopenia (36%). The lengthening of the TCA generally observed, due to deficit in F VIII and the lengthening of the bleeding time is according to the deficit in vWF. The molecular electrophoresis of vWF multimers in agarose gel in the presence of SDS, shows an absence of all the multimers of vWF in plasma patients of Willebrand's disease type III, this absence is caused by the mutations of vWF gene which encodes important functional domains of this protein.

TABLE V
VARIATION OF TS, TCA, AND PLT, TP, vWF RATE AMONG THE
THROMBOCYTOPENIA PATIENTS

State / Biological test	Normal Control	Patients
PLT rate ($10^3 / \text{mm}^3$)	$239,6 \pm 3,50$	$117,09 \pm 3,18$
TS (mn)	$5,55 \pm 0,10$	$16,47 \pm 1,02$
TCA (sec)	$29,82 \pm 0,18$	$30,33 \pm 0,31$
TP (%)	$93,05 \pm 0,81$	$92,16 \pm 1,14$
vWF (%)	$91,42 \pm 1,20$	$94,44 \pm 1,94$

The Willebrand's disease remains unknown in Algeria and generally seen at the time of a haemorrhage in case of surgical intervention or a delivery, and it presents a great biological heterogeneity and genetic which sometimes make difficulties of diagnosis.

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