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Challenges in the classification of von Willebrand disease with a limited test panel: An experience from an upcoming hemostasis laboratory in southern India

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ABSTRACT

Objectives: The diagnosis and classification of von Willebrand disease (VWD) is an intricate process requiring multiple hemostatic tests. Cutoff values of the tests vary with the subtype. Sometimes, all the tests are not available. This study was done to analyze the clinical and coagulation profile of VWD diagnosed and broadly classified based on a simplified algorithm.

Material and Methods: This was a cross-sectional study done over 6 years. After screening tests, a simplified algorithm taking cutoff for various tests based on updated guidelines using the primary panel of von Willebrand factor antigen assay (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo), and Factor VIII:C was used. Multimer assay was done in a few cases. Data were compiled using summary statistics.

Results: Forty patients fitted the diagnosis of VWD: Type 3 in 13 (32.5%), Type 2N (likely) in 8 (20%), Type 2 (not further categorized) in 9 (22.5%), and VWD, not further categorized in 10 (25%). The mean age was in the second or third decade with female predominance. Diagnosis of Type 3 was relatively straightforward due to markedly deranged parameters. Type 2N was provisionally diagnosed based on bleeding pattern and markedly reduced Factor VIII:C; further, subtyping of Type 2 and categorization of some cases was not possible due to non-availability of some tests.

Conclusion: VWF:Ag assay, VWF:RCo, and Factor VIII:C form the cornerstone for diagnosing major VWD types. The under-representation of milder phenotypes and a greater proportion of severe VWD subtypes observed is likely due to hospital referral bias and may not represent population prevalence.

Keywords: Classification of von Willebrand disease, Factor VIII:C, Multimer assay, von Willebrand disease, von Willebrand factor antigen assay, VWF ristocetin cofactor activity

INTRODUCTION

Von Willebrand disease (VWD) is caused by qualitative or quantitative deficiency of the von Willebrand factor (VWF). VWF is a high molecular weight adhesive multimeric glycoprotein[1,2] synthesized in endothelial cells and megakaryocytes and secreted as a series of multimers in plasma. The basic structural unit is a dimer composed of two subunits linked by a sulfide bond at the "C" terminal region. These dimers are assembled at their "N" regions to form multimers.[3] VWF is one of the few non-erythrocyte proteins that express ABO antigens and studies show that the levels of VWF are reduced in patients with blood group O.[1,4]

There are three major types of VWD. Type I VWD is the partial quantitative deficiency of VWF diagnosed when the VWF plasma level is <30 IU/dL, regardless of bleeding.[5,6] The National Heart, Lung, and Blood Institute (NHLBI) consensus guidelines have recommended diagnosing a category termed "Low VWF." These patients have von Willebrand antigen (VWF: Ag) levels of <50 IU/dL (but >30 IU/dL). They have been found to have less risk of bleeding and associated genetic mutations than Type 1 VWD and many have blood group O.[5] The recent American Society of Hematology (ASH), the International Society on Thrombosis and Hemostasis (ISTH), the National Hemophilia Foundation (NHF), and the World Federation of Hemophilia (WFH) 2021 guidelines recommend the diagnosis of Type 1 VWD for patients with "Low VWF" and associated abnormal bleeding.[6]

Type 2A VWD is characterized by the loss of high molecular weight multimers (HMWM). This results in decreased VWF-mediated platelet adhesion. Type 2B VWD is characterized by a gain in function with an increased affinity of VWF for platelet glycoprotein Ib. These platelet-VWF complexes in the circulation are eventually cleared by ADAMTS13, resulting in diminished VWF activity and mild thrombocytopenia. It can be demonstrated by increased agglutination at a low dose (<0.7 mg/mL)[7] of ristocetin. Type 2B VWD must be distinguished from platelet type VWD where similar interactions occur, but the defect resides in the platelet receptor. Type 2M VWD is characterized by impaired interaction between VWF and platelet receptor GPIb resulting in decreased platelet-mediated VWF adhesion. Multimer assembly is unaffected in this type, hence the notation "M."[1] Type 2N VWD is characterized by the decreased affinity of VWF for factor VIII. VWF acts as a molecular chaperone for factor VIII and increases its half-life. Without the non-covalent association of VWF, factor VIII levels fall below normal and are usually <30 IU/dL.[8] The "N" notation stands for Normandy, where it was first discovered.[9] It is differentiated from hemophilia by its autosomal recessive inheritance pattern and genetic studies. Type 3 VWD is relatively rare compared to Type 1 and Type 2 VWD (approx. 1 in 1 million).[10] It shows a severe deficiency of VWF (<3 IU/dL)[4] with a parallel decrease in factor VIII (<10 IU/dL).[1,11]

The diagnosis and classification of VWD is an intricate process. Although several tests are needed for classifying VWD; however, the introductory panel for the diagnosis of VWD should include VWF: Ag assay, VWF ristocetin cofactor activity (VWF:RCo), and Factor VIII:C.[12] Since the classification can be based on the same tests with differing cutoff values; hence, an algorithm is useful. Further, confirmatory tests are needed for accurate subtyping. This study was done to analyze the clinical and coagulation profile of VWD, diagnosed, and broadly classified based on a limited panel of tests and a simplified algorithm.

MATERIAL AND METHODS

This was a cross-sectional study conducted in the coagulation laboratory of the hematology section of the department of pathology in a tertiary care teaching hospital in southern India over 6 years (January 2016–December 2021) with approval from the Institute Ethics Committee. Patients from various departments, suspected to have VWD based on clinical history, and screening laboratory tests were initially enrolled and underwent confirmatory tests.

The methods/instruments used for the tests done were as follows: Platelet count (PC) by automated hematology analyzer (XT 2000i, Sysmex Corporation, Kobe, Japan); bleeding time (BT) by Ivy's method; prothrombin time (PT); activated partial thromboplastin time (aPTT), mixing studies, factor assays by automated coagulation analyzers (STA compact CT, Diagnostica Stago, Asnières-sur-Seine, France and ACLTOP500, Instrumentation Laboratory, Bedford, Massachusetts, USA); VWF:Ag assay either by Enzyme-Linked Immunosorbent Assay (ELISA) (Raybiotech Life, Georgia, the United States for cases enrolled in 2016) or by automated coagulation analyzers (for cases enrolled later), VWF:RCo either by light transmittance aggregometry (490-2D, Chronolog Corporation, Havertown, PA, USA) or by automated coagulation analyzer (ACLTOP500) using reagents from respective companies, platelet function studies by light transmittance aggregometry (as mentioned above), and multimer assay using Hydragel 5 von Willebrand Multimers kits (Sebia, Lisses, France).

VWF:Ag assay, VWF:RCo and FVIII levels formed the cornerstone of diagnosis. Ratios of function to antigen, which included VWF:RCo/VWF:Ag and FVIII:C/VWF:Ag, were derived. Multimer assay was done in a few cases. A simplified diagnostic algorithm was devised for the diagnosis and classification of VWD [Figure 1]. Ristocetin-induced platelet aggregation (RIPA) and VWF: Collagen binding assay (VWF:CB) were not standardized and could not be used. VWF: Factor VIII binding (VWF:FVIIIB) and VWF: Platelet binding (VWF:PB) were not available.

Laboratory evaluation was incomplete for some of the cases enrolled early in the study period as some of the tests were added later. However, with the available data, cases fitting with the diagnosis of VWD were analyzed and classification was made to the best possible extent. The clinical presentation and available coagulation studies of the cases are presented using summary statistics, mean (SD), median (range), and frequency (%).

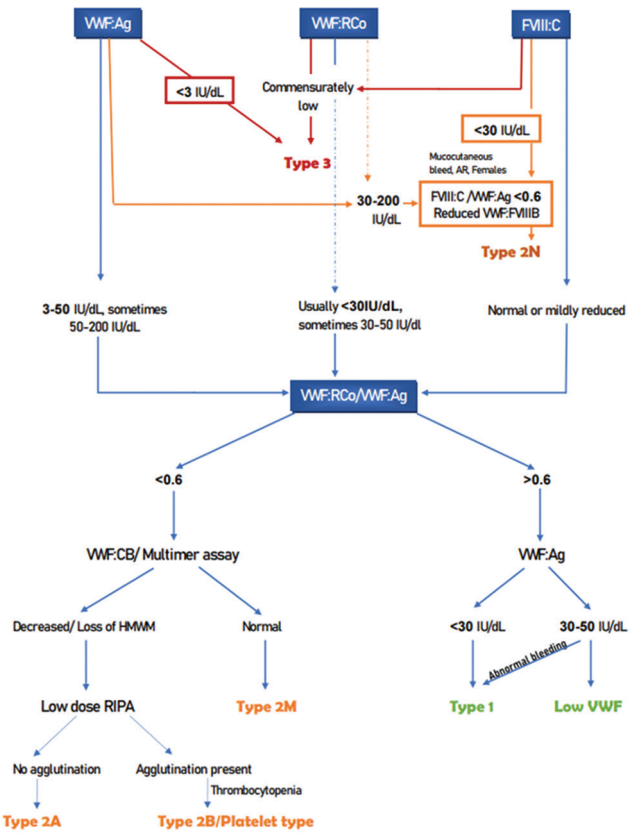

Figure 1: A simplified diagnostic algorithm for the diagnosis and classification of major von Willebrand disease subtypes.

RESULTS

A total of 94 patients, 59 (62.8%) females and 35 (37.2%) males, ages ranging from 7 months to 74 years and presenting with either history or screening coagulation profile suggestive of dual hemostatic defect were initially analyzed. After further workup, 40 patients were diagnosed to have some form of VWD; the break-up being: Type 3 in 13 (32.5%), Type 2N (likely) in 8 (20%), Type 2 (not further categorized) in 9 (22.5%), and VWD, not further categorized in 10 (25%). The coagulation parameters of the various subtypes are summarized in [Table1].

Type 3

There were 13 cases, with ages ranging from 3 to 52 with a mean of 29.4 (15.9) years, of whom nine (69.2%) were females and four (30.8%) were males. The bleeding patterns were epistaxis, oral bleeds, easy bruising, and abnormal uterine bleeding. Two cases had a family history of bleeding disorder. BT was prolonged (>5 min 30 s) in 6/8 (75%) cases with a median of >15 min (2->15 min). PC was elevated (>450 × 10^9/L) in 23.1% (3/13) cases with values ranging from 527 to 678 × 10^9/L, possibly secondary to bleeding manifestations. PT was normal in all the cases, and aPTT was prolonged (>6 s of control) in all the cases with a mean value of 58.1 (13.7) s. Factor VIII was deficient in all the cases with a median of 5.6% (1–24%). VWF:RCo and VWF:Ag levels were markedly reduced in all the cases with median values of 0% (0–4%) and 1% (0–3%), respectively. A platelet aggregation study done in six cases showed ristocetin defect in all (100%) with ristocetin aggregation ranging from 0% to 16%. Multimer assay done in 7 cases showed a total absence of multimers in all (100%), confirming the diagnosis of Type 3 [Figure 2].

Type 2N (likely)

There were eight cases, ages ranging from 1 to 30 with a mean of 20.1 (12.1) years, of whom 6 (75%) were female and 2 (25%) were male. The bleeding patterns were epistaxis, bleeding gums, and menorrhagia. BT was prolonged in two cases (25%). PC and PT were normal in all the cases; aPTT was prolonged in all (100%) cases with a mean of 54.7 (5.5) s. Factor VIII level was reduced in all the cases with a median value of 4% (1–17%). VWF:RCo was in the normal range in all the cases with a median of 75 (56.2–128). VWF: Ag level was also in the normal range with a median of 88.9% (50–183%). The FVIII:C/VWF:Ag ratio where it could be derived was <0.6 in all cases. A platelet aggregation study done in three cases showed ristocetin defect in one case (aggregation of 19%) and a normal pattern in two cases. Multimer assay done in two of these cases showed a normal pattern. Unfortunately, we were not able to do VWF: FVIIIB, which would have been confirmatory.

Type 2, Not further categorized

There were nine cases, with ages ranging from 4 to 21 with a mean of 12.7 (7.7) years, of whom five (55.6%) were female and 4 (44.4%) were male. The bleeding patterns were epistaxis, easy bruising, and menorrhagia. BT was prolonged in 2/8 (25%) cases. PC was elevated (>4.5 × 10^9/L) in 1/9 (11.1%) cases; none had thrombocytopenia. PT was normal in all the cases, and aPTT was prolonged in 7/9 (77.8%) cases with a mean value of 40.1 (6.8) s. VWF:RCo was reduced in all the cases with a median value of 13% (0–45%). In two cases where VWF:RCo value was 0%, the VWF:Ag level was 18% and 64%, respectively, hence not fitting with Type 3. VWF:Ag level showed a range of values with a median of 56% (18–95%). There was one case with VWF:Ag <30% and three cases in the range of 30–50%. The remaining five cases had values in the normal range of >50%. Platelet aggregation study showed ristocetin defect in 4/8 (50%) cases and median ristocetin aggregation of 26% (3–9%). Multimer assay done in two of these cases showed

Table 1: Clinical and coagulation parameters of the various subtypes of VWD.

Parameters/Diagnosis	VWD Type 3 (13)	VWD Type 2N likely (8)	VWD Type 2 (9)	VWD, Not further categorized (10)
Age in years, Mean (SD)	29.4 (15.9)	20.1 (12.1)	12.7 (7.7)	15.7 (10.3)
Gender, Male%: Female%	30.8:69.2	25:75	44.4: 55.6	30:70
BT in min, Median (Range)	>15 (2–>15)	4:30 (3–10:30)	3:45 (1:30–>15)	3:00 (1:30–5)
aPTT in sec, Mean (SD)	58.1 (13.7)	54.7 (5.5)	40.1 (6.8)	30.9 (2.5)
FVIII level in %, Median (Range)	5.6 (1–24)	3 (1–17)	35 (31–44)	NA
VWF: RCo in %, Median (Range)	0 (0–4)	75 (56.2–128)	13 (0–45)	31 (NA)
VWF: Ag in % or ng/mL, Median (Range)	1 (0–3)	88.9 (50–183)	56 (18–95)	47 (37–49.6)
Ristocetin aggregation				
Deficient (<30%)	6/6 (100%)	1/3 (33.3%)	4/8 (50%)	1/4 (25%)
Median (Range)	7.5 (0–16)	42 (19–85)	26 (3–95)	55 (17–79)
Multimer assay (Total done/Pattern)	7, Absent in all	2, Normal pattern	2, Lack of HMWM (Type 2A/B)	NA

NA: Not available, VWD: von Willebrand Disease, BT: Bleeding time, aPTT: Activated partial thromboplastin time, VWF: RCo: VWF ristocetin cofactor, VWF: Ag: von Willebrand factor antigen assay, HMWM: High molecular weight-multimers, SD: Standard deviation

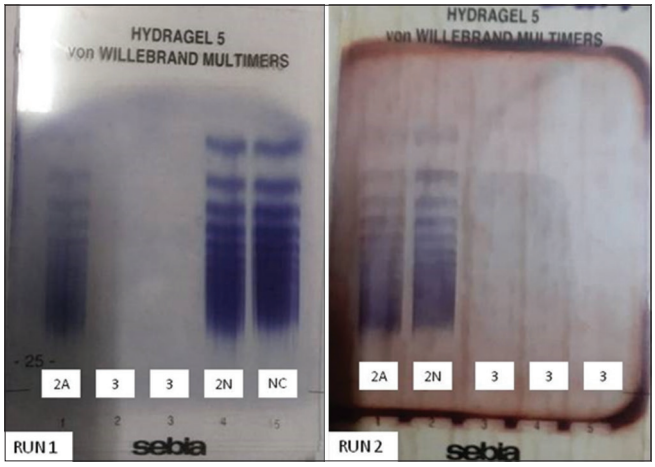

Figure 2: Hydragel VW Multimers Run 1 and 2 showing a normal control (NC) in Lane 5 of Run1 with a normal pattern of multimers; Lanes 2 and 3 of Run 1 and Lanes 3, 4 and 5 of Run 2 showing complete absence of multimers consistent with Type 3 von Willebrand disease (VWD); Lane 1 of Run1 and Lane 1 of Run 2 showing preferential absence of high-molecular weight-multimers consistent with Type 2A/B VWD and Lane 4 of Run 1 and Lane 2 of Run 2 showing a normal pattern of multimers consistent with Type 2N VWD.

a deficiency of HMWM consistent with pattern Type 2A/B. Low-dose RIPA could have distinguished Type 2A from 2B. None of the cases had thrombocytopenia favoring the former.

In all seven cases where VWF:RCo/VWF:Ag ratio could be derived, it was <0.6. In two cases, where VWF:RCo was not available, the ratio could not be derived. One of them had a multimer assay with a pattern consistent with Type 2A/B. The other patient had VWF:Ag level of 56%, FVIII of 35% and showed ristocetin aggregation defect, and hence, the best fit was Type 2.

The FVIII:C/VWF:Ag ratio was <0.6 in one case and >0.6 in the remaining cases where it could be derived. The former has been put under Type 2 and not Type 2N as the VWF:RCo value was low (13%) and the VWF:RCo/VWF:Ag ratio was <0.6.

VWD, Not further categorized

There were ten cases, ages ranging from 3 to 29 with mean of 15.7 (10.3) years, of whom seven (70%) were female and 3 (30%) were male. All had presented with bleeding episodes, the patterns being epistaxis, menorrhagia, cutaneous bleeding, hematuria, and gastrointestinal bleeding. The BT, PT, and aPTT (mean 30.9 [2.5]) were normal in all the cases. PC was mildly reduced (1.3 lakhs/cu.mm) in one case and normal in the rest. VWF:Ag level done in all the cases was in the range of 30–50% with a mean of 45.4 (5) and median of 47% (37–49.6%). VWF:RCo was available in only one case with a value of 31%, and VWF:RCo/VWF:Ag ratio was >0.6, fitting it with a diagnosis of Type 1 VWD. In the remaining cases, it was not possible to categorize further due to a lack of VWF:RCo values. However, due to the pattern of bleeding and VWF:Ag levels, these patients fit with VWD rather than low VWF. None of these cases would be Type 3 based on the VWF:Ag levels and they are unlikely to be Type 2N due to normal aPTT levels. They are likely to be VWD Type 1 or Type 2, other than Type 2N. Platelet aggregation studies done in five cases showed ristocetin aggregation defect in one (20%) case with ristocetin aggregation of 17% and a normal aggregation pattern in four (80%) cases. Multimer assay was not done in any of these cases.

DISCUSSION

In this study, we came across a spectrum of subtypes of VWD. The confirmatory diagnosis was often after two to three rounds of testing in a phased manner. Some of the tests were added during the study period, and hence, the laboratory evaluation was better for the cases in the later part of the study period. Moreover, complete testing in some patients could not be done due to a lack of follow-up visit.

Formerly, to measure VWF:Ag levels, ELISA was used. Since it was time-consuming, latex agglutination assays have been developed and are in use at present.[13] The test values can be reported in percentage of normal, IU/mL or IU/dL. Some studies have reported in terms of ng/mL or μg/mL.[14] According to the WHO 6th International Standards (IS), 1 ampoule (1 mL) of human plasma (07/316) contains 1.00 IU of VWF:Ag and 1.04 IU of factor VIII.[15] According to a study by Pipe *et al*., around 100–200 ng/mL factor VIII concentration in blood is equivalent to 1 IU/mL.[16] Since 1 mL of human plasma contains almost equal amounts of VWF:Ag and factor VIII, extrapolating this, 100 ng/mL VWF:Ag can be considered equivalent to 1 IU/mL which is equal to 100 IU/dL. Furthermore, according to the NHLBI, IU/dL is the percentage of normal (pooled normal plasma).[5] A cutoff of 30 IU/dL is currently being used for the diagnosis of Type 1 VWD. For the diagnosis of Type 3 VWD, the VWF:Ag level must be <3 IU/dL, and for low VWF, a value of 30–50 IU/dL has been recommended. In Type 2 VWD (except Type 2N), the value is usually <50 IU/dL, though it can range anywhere between <30 and 200 IU/dL.[5] We have used similar cutoff values in our diagnostic algorithm.

VWF: RCo is a functional test used to measure the activity of VWF. The current ASH, ISTH, NHF, and WFH 2021 guidelines recommend platelet binding tests such as VWF:GPIbM (gain-of-function mutant GPIb binding) and VWF:GPIbR (ristocetin-triggered GPIb binding).[6,17] The current VWF:RCo test that we are doing in our laboratory using ACL TOP 500 with IL reagents is based on the latter principle.

For the diagnosis of Type 2 VWD (except 2N), a VWF:RCo/VWF:Ag ratio of <0.5–0.7 is considered[5] and the recent combined guideline[6] has recommended a higher cutoff of 0.7 rather than 0.5. We have used the cutoff of 0.6 in our algorithm like the UK Hemophilia Center Doctors guidelines approved by BCSH which recommends a cutoff of <0.6.[18] When the ratio is <0.6, VWF:CB or multimer assay can be done to rule out Type 2M VWD. VWF:CB is more sensitive to HMWMs and, hence, decreased in Type 2A/2B VWD. Furthermore, multimer assay shows loss of HMWMs in Type 2A/2B VWD. Patients with 2A and 2B VWD can be differentiated using low-dose RIPA or genetic testing for 2B VWD variants, in which when positive can be diagnosed as 2B VWD.[6,19,20] Type 2B VWD, in addition, may feature thrombocytopenia and must be demarcated from platelet type VWD by VWF:PB, where VWF binding to formalin-fixed donor platelets is measured. VWF:PB will be increased in Type 2B, whereas it will be normal in platelet type VWD.[5] They can also be differentiated by RIPA mixing studies at lower doses of ristocetin (0.5 mg/mL) where the demonstration of addition of a plasma factor inducing platelet aggregation at such low doses of ristocetin would reinforce the phenotypic laboratory diagnosis of VWD2B.[21]

In Type 2N, VWF:Ag and VWF:RCo values are normal, though they may be decreased sometimes. The FVIII/VWF:Ag ratio is <0.5–0.7.[12] In our algorithm, we have kept a cutoff of FVIII/VWF:Ag ratio <0.6. As the FVIII level is usually <30 IU/dL, it is often misdiagnosed as mild hemophilia. In males, genetic studies often may be required to distinguish between these two diseases. Diagnosis of Type 2N must be suspected where there is marked FVIII reduction in a case with mucocutaneous bleeding pattern or with prolonged BT, especially in a female patient or in cases with apparently non-X-linked inheritance pattern. In our experience, the diagnosis of Type 3 was relatively more straightforward due to a marked reduction of both VWF:Ag and VWF:RCo and commensurately low FVIII levels.

Although, Type 2 VWD has been extensively studied and subclassified, for Types 2A and 2M VWD, a desmopressin trial is advised for routine management.[22] When the response is poor, mostly VWF concentrate or cryoprecipitate is recommended. However, the desmopressin trial includes its own set of contraindications.[6] Hence, in a resource-limited setup, weighing the cost-to-benefit ratio, subtyping of Types 1, 2N, 2 (not categorized), and 3 VWD without further typing may possibly be adequate.

Although Type 1 VWD is the commonest subtype, we had Type 3 as the most common subtype. In another study from this region, among 202 patients, 107 patients were Type 3, 62 were Type 1, and 33 patients were Type 2VWD.[23] In two other studies from northern India, the prevalence of subtypes described were Type 1 in 17 (42.5%), Type 2 in 11 (27.5%), and Type 3 in 12 (30.0%) among 40 patients[24], and 21.9% Type 1, 43.7% Type 2, 1.6% acquired VWD, and 32.8% Type 3 among 64 patients of VWD.[25] However, our finding of more severe subtypes cannot be extrapolated as true population prevalence. This could be due to hospital bias, with the severe bleeding phenotypes presenting to the hospital compared to the milder ones. Moreover, among the patients presenting to the various outpatient departments, the most severe phenotypes are likely to have been referred to the coagulation laboratory for evaluation. This would also explain why aPTT prolongation was seen in a sizable proportion of our patients. None of the patients in the VWD, not further categorized

group, that were likely to be either VWD Type 1 or Type 2 other than Type 2N, showed aPTT prolongation.

The limitations of the study include a lack of complete clinical details including family history and complete testing in some patients. Moreover, this is a hospital referral-laboratory-based data with limited availability of some confirmatory tests. The strength of the study is that it highlights how an algorithmic approach helped in the diagnosis and broad subtyping of VWD despite these challenges.

CONCLUSION

A simplified algorithm based mainly on VWF:Ag assay, VWF:RCo, and Factor VIII:C helped in diagnosing major VWD types. The greater proportion of severe VWD subtypes and under-representation of milder phenotypes is likely due to hospital referral bias and may not represent population prevalence.

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Declaration of patient consent

The Institutional Review Board (IRB) permission was obtained for the study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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