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Study Of Thromboelastography (Teg) & Aptt Clot Waveform Analysis In Patients Of Severe Hemophillia A With And Without Inhibitors.

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Abstract

Objective: To find out clinical correlation in patients of Severe Hemophilia A patients with waveform of Thromboelastography (TEG) and aPTT Clot waveform and to evaluate role of TEG and CWA waveforms in patients of Severe Hemophilia A with and without inhibitors. ***Methods:*** This Cross sectional, Observational study was conducted among Patients who are diagnosed known case of Severe hemophilia A with & without inhibitors at Sahyadri Super Speciality Hospital, Karve road, Pune, Maharashtra. Duration of study was August 2021 to February 2022. ***Result:*** Among all the parameters of TEG and aPTT CWA compared to each other and compared to AJBR and HJHS score among both groups of patients, only the following parameters showed statistically significant correlation. In inhibitor positive group (Group B), aPTT clot waveform parameters such as Clotting angle showed statistically significant negative correlation (inverse relationship) with K time (P-value<0.05). In inhibitor positive group (Group B), AJBR showed statistically significant positive correlation (direct relationship) with 1st derivative and 2nd derivative + (P-value<0.05 for all). ***Conclusion:*** in my study, srtong significant Correlation was found only between the (Hypothetical) clotting angle of CWA in inhibitor Positive group with K time of TEG and that of ABJR and 1st and 2nd Derivative + of CWA.

Keywords: Thromboelastography (TEG) &aPTT, Clot Waveform Analysis, Hemophillia, Inhibitors.

INTRODUCTION

Hemophilia A is inherited bleeding disorders resulting from deficiency of coagulation factors VIII. In patients with Severe hemophilia (ie Factor VIII levels <1%), severe spontaneous bleeding may occur in any tissue. Recurrent joint bleeding is typical, especially in the ankles, knee and elbows, and leads to hemophilic arthropathy, with damaged and painful joints with restricted mobility. Factor replacement is necessary during acute bleed as well as for prophylaxis of the same. Following treatment with Factor VIII concentrates, approximately 30% of patients with severe hemophilia A will develop inhibitors, in addition to 5% of patients with mild and moderate hemophilia.[1-5]

Routine coagulation and factor assays, while useful in the classification of severity and treatment monitoring in hemophilia patients, have been shown to be of limited use in managing clinical presentations and outcomes. Routine coagulation tests including platelet count, PT, aPTT, plasma fibrinogen levels and FVIII assays do not fully represent the true in-vivo haemostatic process, which involves the interaction between vessel wall, platelets and fibrinogen, as well as clotting factors within the plasma. There are no rapid standardized laboratory tests that can reliably monitor platelet function or fibrinolysis in Hemophilics. These tests are performed on plasma without platelets and other blood cells. A key limitation of this standard coagulation panel of tests is the fact that it remains a poor predictor of bleeding and mortality. In addition, there is poor correlation between the severity of coagulation defects and the amount of blood products received by patients[6-8]

This prompted the investigation of viscoelastic studies, global coagulation assays in hemophilia care, which have established their utility in various bleeding and thrombotic states.

TEG is a method to evaluate the whole process of blood coagulation as a graph from the beginning of clot formation to fibrinolysis TEG provides pertinent information about adhesiveness, elasticity, and physical properties of clot from both dynamic and global perspectives.

Clot waveform analysis (CWA) is also considered as a global coagulation assay, other than TEG, ROTEM and Thrombin generation assay.

CWA is an optical waveform which depicts the clot formation process by measuring changes in transmittance or absorbance of light beam through the analysed sample. Both quantitative and qualitative CWA parameters have been shown to be associated with pathophysiological processes and have a potential use in clinical applications. Among them, the clinical phenotype of severe haemophilia A (HA) patients could very accurately be predicted by CWA.

Our study aims at studying waveforms of TEG &aPTT Clot Waveform analysis(CWA) in patients of Hemophilia A with and without inhibitors.

MATERIAL AND METHODS

This Cross sectional, Observational study was conducted among Patients who are diagnosed known case of Severe hemophilia A with & without inhibitors at Sahyadri Super Speciality Hospital, Karve road, Pune, Maharashtra. Duration of study was August 2021 to February 2022.

Calculation of Sample Size:
Confidence level: 95%

Margin of error :5%

Population percentage : 0.04%

Sample Size
Sample Size Calculated by using Correlation coefficient between TEG and aPTT duration of blood clotting.

The Expected correlation coefficient between TEG and aPTT for blood clotting time measurements is 0.70

Following is the formula calculated to calculated the sample size

Sample Size Formula
$N = [(Z_\alpha+Z_\beta)/C]^2 + 3$

Where,

The standard normal deviate for $\alpha = Z_\alpha = 2.57$ (at $\alpha = 1\%$)

The standard normal deviate for $\beta = Z_\beta = 1.287$ ($\beta = 10\%$) power of test is 90%

$C = 0.5 * \ln[(1+r)/(1-r)] = 0.8673$

The Minimum required sample size is 23

The Actual sample size taken by us in this study is 67

Inclusion criteria
Diagnosed Severe Hemophilia A patient, with and without inhibitors

Exclusion criteria
1. Patients who are not willing to participate in the study
2. Patients on ITI
3. Patients on non factor replacment therapy

METHODOLOGY

TEG and CWA will be done on Blood samples from patients of Severe Hemophilia A, both with and without inhibitors.

For TEG: Blood samples will be collected by venepuncture using 20-gauge needles and 2 cc plastic syringe containing no anticoagulant. 1 mL was transferred to a vial containing buffered stabilizers and Kaolin.

The sample collected in Kaolin vial mixed by inversion immediately after collection, and mixing continued by inversion till 360 µL blood transferred to a 37°C pre-warmed disposable cup in Kaolin activated TEG 5000 Thromboelastograph Haemostasis analyzer system (Haemonetics corporation US) machine in less than three minutes and measurements were recorded for no less than 40 min. Results will be obtained and graph with values will be printed for record.

Following TEG parameters will be studied:

Clot time: Period from 0-2mm amplitude (R = Reaction time: It is a measure of clotting factors)

Clot Kinetics: Period from 2-20mm amplitude;

K = Kinetic time: It is a measure of the speed taken to reach a specific level of clot strength.

Alpha Angle (slope between R and K): Measures the speed of fibrin build-up and cross-linking.

Clot strength: MA = maximum amplitude: it represents the ultimate strength of the clot and is a measure of platelet function.

Reference ranges for TEG values as provided by manufacturer are as follows: R value (4s to 8s), K value (0s to 4s), alpha angle (47' to 74'), MA value (54mm to 72mm)

For aPTT Clot Waveform analysis (CWA):
Blood samples will be collected in evacuated anticoagulant tubes

containing 1:9 volume of 3.2% trisodium citrate. Once received in the clinical pathology laboratory, the samples will be checked for the correctness of the identifying bar codes and the quantity of the

sample.

Platelet poor plasma:
Blood samples centrifuged immediately at 2000 g for 15 minutes so as to get platelet poor plasma (PPP). The quality of this plasma will checked twice a day on the Beckman Coulter LH-780 automated haematology system – the plasma should contain less than 10 x 103 platelets/cumm.

Statistical Analysis Method
The data on categorical variables is shown as n (% of cases) and the data on continuous variables is presented as median and minimum – maximum. The inter-group statistical comparison of distribution of categorical variables is tested using Chi-Square test or Fisher's exact probability test if more than 20% cells have expected frequency less than 5. The inter-group statistical comparison of medians of non-normally distributed continuous variables is done using Mann-Whitney U test. Correlation analysis is done using Spearman's method. The underlying normality assumption was tested before subjecting the study variables to Mann-Whitney U test and Spearman's correlation analysis. All results are shown in tabular as well as graphical format to visualize the statistically significant difference more clearly.

In the entire study, the p-values less than 0.05 are considered to be statistically significant. The entire data is statistically analyzed using Statistical Package for Social Sciences (SPSS ver 24.0, IBM Corporation, USA) for MS Windows.

RESULTS

Inter-group comparison of median exposure days (ED)
The median exposure days in Inhibitor Negative Group (Group A) and Inhibitor Positive Group (Group B) was 216 days and 135 days respectively. The minimum – maximum exposure days range in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 5 – 1000 days and 25 – 432 days respectively.

Distribution of median exposure days among the cases studied did not differ significantly between two study groups (P-value>0.05).

Inter-group comparison of median annual joint bleeding rate (AJBR)
The median AJBR in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 10.0 and 8.0 respectively. The minimum – maximum AJBR range in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 0 – 60 and 2 – 45 respectively.

Distribution of median AJBR among the cases studied did not differ significantly between two study groups (P-value>0.05).

Inter-group comparison of median hemophilia joint health score (HJHS)
The median HJHS in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 6.0 and 2.5 respectively. The minimum – maximum HJHS range in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 0 – 40 and 0 – 44 respectively.

Distribution of median HJHS among the cases studied did not differ significantly between two study groups (P-value>0.05).

Inter-group comparison of median functional independence score in hemophilia (FISH)

The median FISH in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 32.0 and 32.0 respectively. The minimum – maximum FISH range in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 21 – 32 and 21 – 32 respectively.

Distribution of median FISH among the cases studied did not differ significantly between two study groups (P-value>0.05).

Inter-group comparison of type of factor administration

Of 55 cases in inhibitor negative group (Group A), 32 (58.2%) had on demand administration and 23 (41.8%) had prophylaxis administration. Of 12 cases in inhibitor positive group (Group B), 10 (83.3%) had on demand administration and 2 (16.7%) had prophylaxis administration.

Distribution of type of factor administration among the cases studied did not differ significantly between two study groups (P-value>0.05).

Table 1) Inter-group comparison of medians of parameters of aPTT clot waveform analysis.

	Group A [Inhibitor Negative] (n=55)		**Group B [Inhibitor Positive] (n=12)**		**P-value**
Parameters	**Median**	**Min – Max**	**Median**	**Min – Max**	
1st Derivative	45.87	22.93 – 374.69	42.93	19.40 – 78.25	0.109^{NS}
2nd Derivative -	14.31	0.17 – 489.69	11.89	4.24 – 34.35	0.070^{NS}
2nd Derivative +	41.65	14.68 – 928.46	38.53	13.74 – 117.29	0.086^{NS}
Clotting angle (A-B)	65.00	46.00 – 87.00	58.50	45.00 – 76.00	0.064^{NS}
T Max	80.43	10.64 – 1109.25	64.51	23.64 – 264.18	0.454^{NS}
Values are median and min - max, P-value by Mann-Whitney U test. P-value<0.05 is considered to be statistically significant. NS – Statistically non-significant.					

Inter-group comparison of median parameters of aPTT clot waveform analysis

The median 1st Derivative in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 45.87 and 42.93 respectively. The minimum – maximum 1st Derivative range in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 22.93 – 374.69 and 19.40 – 78.25 respectively.

Distribution of median 1st Derivative among the cases studied did not differ significantly between two study groups (P-value>0.05).

The median 2nd Derivative - in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 14.31 and 11.89 respectively. The minimum – maximum 2nd Derivative - range in inhibitor negative group (Group A) and inhibitor positive group (Group B) was 0.17 – 489.69 and 4.24 – 34.35 respectively.

Distribution of median 2nd Derivative - among the cases studied did not differ significantly between two study groups (P-value>0.05).

The median 2nd Derivative + in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 41.65 and 38.53 respectively. The minimum – maximum 2nd Derivative + range in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 14.68 – 928.46 and 13.74 – 117.29 respectively.

Distribution of median 2nd Derivative + among the cases studied did not differ significantly between two study groups (P-value>0.05).

The median Clotting angle (A-B) in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 65.00 and 58.50 respectively. The minimum – maximum Clotting angle (A-B) range in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 46.00 – 87.00 and 45.00 – 76.00 respectively.

Distribution of median Clotting angle (A-B) among the cases studied did not differ significantly between two study groups (P-value>0.05).

The medianTMax in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 80.43 and 64.51 respectively. The minimum – maximum TMax range in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 10.64 – 1109.25 and 23.64 – 264.18 respectively.

Distribution of median TMax among the cases studied did not differ significantly between two study groups (P-value>0.05).

Table 2) Inter-group comparison of medians of parameters of Thromboelastography (TEG).

Parameter	Group A: Inhibitor Negative (n=55)Median (Min–Max)	Group B: Inhibitor Positive (n=12)Median (Min–Max)	P-value
R Time (min)	19.80 (0.20–87.00)	33.95 (2.80–123.50)	0.120 NS
K Time (min)	7.10 (0.10–51.20)	5.70 (0.80–19.50)	0.416 NS
Alpha angle	31.00 (2.10–237.00)	29.85 (1.00–75.40)	0.589 NS
MA	32.90 (2.20–67.70)	53.35 (3.70–65.23)	**0.004****

Inter-group comparison of median parameters of TEG

The median R time in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 19.80 and 33.95 respectively. The minimum – maximum R time range in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 0.20 – 87.00 and 2.80 – 123.50 respectively.

Distribution of median R time among the cases studied did not differ significantly between two study groups (P-value>0.05).

The median K time in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 7.10 and 5.70 respectively. The minimum – maximum K time range in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 0.17 – 489.69 and 40.80 – 19.50 respectively.

Distribution of median K time among the cases studied did not differ significantly between two study groups (P-value>0.05).

The median Alpha angle in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 7.10 and 5.70 respectively. The minimum – maximum Alpha angle range in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 2.10 – 237.00 and 1.00 – 75.40 respectively.

Distribution of median Alpha angle among the cases studied did not differ significantly between two study groups (P-value>0.05).

The median MA in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 32.90 and 53.35 respectively. The minimum – maximum MA range in Inhibitor negative group (Group A) and inhibitor positive group (Group B) was 2.20 – 67.70 and 3.70 – 65.23 respectively.

Distribution of median MA among the cases studied is significantly higher in inhibitor positive cases (Group B) compared to inhibitor negative cases (Group A) (P-value<0.05).

Table 3) Correlation analysis between some selected parameters of aPTT clot waveform analysis and parameters of TEG.

Correlation between	Group A [Inhibitor Negative] (n=55)		Group B [Inhibitor Positive] (n=12)	
	R-value	**P-value**	**R-value**	**P-value**
2nd derivative + And R time	-0.157	0.252^{NS}	-0.074	0.820^{NS}
2nd derivative + And K time	-0.121	0.385^{NS}	0.105	0.759^{NS}
2nd derivative + And Alpha angle	0.171	0.211^{NS}	-0.515	0.087^{NS}
2nd derivative + And MA	0.083	0.548^{NS}	-0.490	0.106^{NS}
Clotting angle And R time	-0.176	0.198^{NS}	-0.407	0.189^{NS}
Clotting angle And K time	-0.178	0.198^{NS}	-0.661	0.027^{*}
Clotting angle And Alpha angle	0.173	0.207^{NS}	0.211	0.511^{NS}
Clotting angle And MA	0.130	0.343^{NS}	0.105	0.745^{NS}

T max And R time	0.043	0.756^{NS}	-0.375	0.230^{NS}
T max And K time	0.112	0.423^{NS}	-0.077	0.821^{NS}
T max And Alpha angle	0.005	0.973^{NS}	-0.193	0.549^{NS}
T max And MA	0.041	0.766^{NS}	-0.238	0.456^{NS}
Correlation analysis using Spearman's method. P-value<0.05 is considered to be statistically significant correlation. **P-value<0.01, NS – Statistically non-significant.				

Correlation analysis between some selected parameters of aPTT clot waveform analysis and parameters of TEG

In both inhibitor negative group (Group A) and inhibitor positive group (Group B), aPTT clot waveform parameters such as 2nd derivative + did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

In inhibitor negative group (Group A), aPTT clot waveform parameters such as Clotting angle did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all). In inhibitor positive group (Group B), aPTT clot waveform parameters such as Clotting angle showed statistically significant negative correlation (inverse relationship) with K time (P-value<0.05).

In both inhibitor negative group (Group A) and inhibitor positive group (Group B), aPTT clot waveform parameters such as T Max did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

Table 4) Correlation analysis between AJBR, HJHS and parameters of aPTT clot waveform analysis.

Correlation between	**Group A [Inhibitor Negative] (n=55)**		**Group B [Inhibitor Positive] (n=12)**	
	r-value	**P-value**	**r-value**	**P-value**
AJBR And 1st derivative	-0.123	0.370^{NS}	0.789	0.002^{**}
AJBR And 2nd derivative-	-0.135	0.325^{NS}	0.545	0.067^{NS}
AJBR And 2nd derivative +	-0.076	0.583^{NS}	0.722	0.008^{**}
AJBR And Clotting angle	-0.107	0.437^{NS}	0.367	0.241^{NS}
AJBR And T Max	0.002	0.987^{NS}	0.354	0.259^{NS}
HJHS And 1st derivative	0.075	0.588^{NS}	0.317	0.315^{NS}
HJHS And 2nd derivative-	0.064	0.641^{NS}	0.390	0.210^{NS}
HJHS And 2nd derivative +	0.106	0.443^{NS}	0.335	0.287^{NS}
HJHS And Clotting angle	0.106	0.441^{NS}	-0.493	0.103^{NS}
HJHS And T Max	0.056	0.690^{NS}	0.441	0.151^{NS}
Correlation analysis using Spearman's method. P-value<0.05 is considered to be statistically significant correlation. **P-value<0.01, NS – Statistically non-significant.				

Correlation analysis between AJBR, HJHS and parameters of aPTT clot waveform analysis

In both inhibitor negative group (Group A) and inhibitor positive group (Group B), HJHS did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

In inhibitor positive group (Group B), AJBR showed statistically significant positive correlation (direct relationship) with 1st derivative and 2nd derivative + (P-value<0.05 for all).

Table 5) Correlation analysis between AJBR, HJHS and parameters of TEG.

Correlation between	**Group A [Inhibitor Negative] (n=55)**		**Group B [Inhibitor Positive] (n=12)**	
	r-value	**P-value**	**r-value**	**P-value**
AJBR And R time	-0.074	0.593^{NS}	-0.046	0.887^{NS}
AJBR And K time	-0.161	0.244^{NS}	-0.307	0.359^{NS}
AJBR And Alpha angle	0.055	0.691^{NS}	0.014	0.965^{NS}
AJBR And MA	0.084	0.541^{NS}	-0.067	0.836^{NS}
HJHS And R time	0.153	0.264^{NS}	0.146	0.652^{NS}
HJHS And K time	0.082	0.554^{NS}	0.249	0.461^{NS}
HJHS And Alpha angle	-0.122	0.373^{NS}	-0.346	0.271^{NS}
HJHS And MA	0.128	0.352^{NS}	-0.349	0.266^{NS}
Correlation analysis using Spearman's method. P-value<0.05 is considered to be statistically significant correlation. NS – Statistically non-significant.				

Correlation analysis between AJBR, HJHS and parameters of TEG

In both inhibitor negative group (Group A) and inhibitor positive group (Group B), AJBR and HJHS did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

DISCUSSION

We divided our data among two groups Inhibitor Positive Group A and inhibitor Negative Group B and compared various paramenters of TEG and aPTT Clot Waveform to each other and to AJBR, FISH and HJHS score

Inter-group comparison of median parameters of aPTT clot waveform analysis :

Distribution of median 1st Derivative among the cases studied did not differ significantly between two study groups (P-value>0.05).

Distribution of median 2nd Derivative - among the cases studied did not differ significantly between two study groups (P-value>0.05).

Distribution of median 2nd Derivative + among the cases studied did not differ significantly between two study groups (P-value>0.05).

Distribution of median Clotting angle (A-B) among the cases studied did not differ significantly between two study groups (P-value>0.05).

Distribution of median TMax among the cases studied did not differ significantly between two study groups (P-value>0.05).

Inter-group comparison of median parameters of TEG

Distribution of median R time among the cases studied did not differ significantly between two study groups (P-value>0.05).

Distribution of median K time among the cases studied did not differ significantly between two study groups (P-value>0.05).

Distribution of median Alpha angle among the cases studied did not differ significantly between two study groups (P-value>0.05).

Distribution of median MA among the cases studied is significantly higher in inhibitor positive cases (Group B) compared to inhibitor negative cases (Group A) (P-value<0.05).

Correlation analysis between some selected parameters of aPTT clot waveform analysis and parameters of TEG
In both inhibitor negative group (Group A) and inhibitor positive group (Group B), aPTT clot waveform parameters such as 2nd derivative + did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

In inhibitor negative group (Group A), aPTT clot waveform parameters such as Clotting angle did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

In inhibitor positive group (Group B), aPTT clot waveform parameters such as Clotting angle showed statistically significant negative correlation (inverse relationship) with K time (P-value<0.05).

In both inhibitor negative group (Group A) and inhibitor positive group (Group B),aPTT clot waveform parameters such as T Max did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

Correlation analysis between AJBR, HJHS and parameters of aPTT clot waveform analysis
In both inhibitor negative group (Group A) and inhibitor positive group (Group B), HJHS did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

In inhibitor positive group (Group B), AJBR showed statistically significant positive correlation (direct relationship) with 1st derivative and 2nd derivative + (P-value<0.05 for all).

Correlation analysis between AJBR, HJHS and parameters of TEG
In both inhibitor negative group (Group A) and inhibitor positive group (Group B), AJBR and HJHS did not show statistically significant correlation with TEG parameters such as R time, K time, Alpha angle and MA (P-value>0.05 for all).

The reason behind no statistical significant correlation between AJBR, HJHS and parameters of TEG and aPTT CWA can be attributed to Phenotypicheterogeniety among Hemophillics.

CONCLUSION

There are multiple factors taken into account during vesico-elastic monitoring of Clot formation study like TEG vs that of aPTT clot waveform analysis, like role of Coagulation factors, Contact Factors, Kaolin, Fibrinogen.

Also, given the phenotypic Heterogeniety of Hemophillia, in my study, strong significant Correlation was found only between the (Hypothetical) clotting angle of CWA in inhibitor Positive group with K time of TEG and that of ABJR and 1st and 2nd Derivative + of CWA.

Larger studies are required to affirm or refute my findings and there is a long way to go to utilise the aPTT clot wave form analysis in routine clinical practises while managing Severe HemophillaA patients while on prophylaxis or on demand therapy

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