ASSOCIATION BETWEEN ADAMTS-13 ACTIVITY AND THE PROGNOSIS OF MULTIPLE MYELOMA AFTER BONE MARROW TRANSPLANTATION

Nahed Moawad Ibrahim Ibrahim Rakha[1], Essam Abdel Wahid Hassan[1], Nermeen Adel Nabeeh Abdelghaffar[1], Ahmed Sabri Ali Mubarak[2] and Gehad Hamada Fekry Hafez[1]

1-Department of Internal Medicine and Clinical Haematology, Faculty of Medicine, Ain Shams University.
2- Clinical Haematology department, Nasser Institute, Cairo, Egypt.

Corresponding author:
Ahmed Sabri Ali Mubarak
Mobile**:** 01003717458
e. mail:
ahmedali2017980@gmail.com

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ABSTRACT:

Introduction: *Multiple myeloma is one of the commonest hematological neoplasms. Auto stem cell transplantation is currently the standard plan of care as consolidation in fit eligible patients after receiving induction chemotherapy.*

Aim of the work: *This aim of this study is assessment the significance and validity of pre auto hematopoietic stem cell transplantation plasma level of ADAMTS-13 activity to predict the prognosis (outcome, disease free survival) and complications (including transplant related mortality, relapse) of multiple myeloma patients.*

Patients and Methods: *In this prospective Cohort study, pre transplantation plasma level of ADMATS13 was studied for its potential effect on the survival, incidence of MM relapse and outcome of auto hematopoietic stem cell transplantation. The study took place in a single bone marrow transplantation facility and included 51 patients previously diagnosed with MM, received induction chemotherapy, and admitted for ASCT.*

Results: *Blood samples were collected prior to transplantation and the patients were followed up throughout the transplantation process and till 12 months after transplantation. ADAMTS-13 levels came between 24.6% and 152.4%. The average level was 74.78%, with half of patients below 64.77%. 12 out of 51 patients (23.5%) had ADAMTS-13 level below 50%. 44 out of 51 patients (86.3%) survived though the follow up period without any evidence of relapse. Only 7 patients (13.7%) had MM relapse.*

Conclusion: *The pre transplantation plasma level of ADAMTS-13 has no statistically significant effect on the outcome, relapse incidence or disease-free survival during the first 12 months after transplantation.*

Keywords: *ADAMTS-13, Multiple Myeloma, Bone Marrow Transplantation*

INTRODUCTION:

Adhesion of platelets to damaged blood vessels is managed by circulating glycoprotein in the blood called Von Willebrand Factor (VWF)**(1)**.

Mature VWF is a multimeric protein consists of multiple subunits of VWF, Mature ADAMTS-13 protein is cleaved by the ADAMTS-13 metalloprotease of the ADAMTS family into its subunits. ADAMTS-13 is the main protease that is

responsible of maintaining the distribution of VWF multimers in plasma and controlling its size. In any severe defect of ADAMTS-13 activity, the amounts of VWF multimers concentration increases markedly leading to excessive platelet aggregation and formation micro thrombosis in small arterioles [(2)].

Plasma level of ADAMTS-13 activity lower than 5% of average normal level is associated with thrombotic thrombocytopenic purpura (TTP) [(2)]. The decreased ADAMTS-13 activity is seen in many cases beside TTP as after surgery and in metastatic malignancy [(3)].

prothrombotic phenotype is seen in complete lack of ADAMTS-13 in mice as reported in many studies. Also, there is suggested correlation between decreased activity of ADAMTS-13 in patients with acute systemic inflammation as many patients with inflammation has ADAMTS-13 deficiency [(4)].

However, there are very few information about the role of ADAMTS-13 in Multiple Myeloma (MM). As cancer of lymphoid lineage, MM is characterized by abnormal white blood cells accumulating in the bone marrow and disturbing the growth of normal blood cells. Bone marrow transplantation (BMT) is the treatment of choice for transplant-eligible patients with MM. The major complications after BMT include relapse and infections [(5, 6)].

AIM OF THE WORK:

This aim of this study is to assess the significance and validity of pre auto hematopoietic stem cell transplantation plasma level of ADAMTS-13 activity to predict the prognosis (outcome, disease free survival) and complications (including transplant related mortality, relapse) of multiple myeloma patients.

PATIENTS AND METHODS:

This study is a prospective cohort study, bone Marrow Transplantation Unit at Nasser Institute Hospital. Patients will be followed up for at least 12 months post-transplantation. 51 Multiple Myeloma patients underwent autologous bone marrow transplantation were included in this study.

Inclusion criteria:

All patients diagnosed with multiple myeloma and underwent ASCT, either males or females, all cases without randomization, disease status before transplantation is in complete remission, very good partial response, or partial response and either received full or reduced dose conditioning regimen.

Exclusion criteria:

Patients who underwent second ASCT and patients with history of TTP.

Methods:

Pre-transplant assessment:

Before enrolment, patients underwent: ADAMTS-13 activity assessment, comprehensive clinical examination, CBC, full chemistry panel, virology (HBV, HCV, Toxoplasma, CMV, and HIV), bone marrow aspirate, serum protein electrophoresis, serum protein immunofixation, beta-2 microglobulin, imaging studies (abdominal and pelvic ultrasound, echocardiography, chest and Paranasal sinuses CT scan), dental consultation, creatinine clearance (24-hour urine collection).

Conditioning regimen:

All patients will be conditioned with high-dose Melphalan (100 mg/m^2 from D-3 to D-2). Patients will receive filgrastim 10 mcg/kg/day starting from D+6 till engraftment.

ADAMTS-13 Assessment:

ADAMTS-13 activity will be determined pre-transplantation. The citrated plasma samples will be assayed for ADAMTS-13 activity using recombinant VWF86-ALEXA FRET substrate. The standard curve will be constructed using normal plasma of known concentrations of ADAMTS-13 in the kit. The activity of ADAMTS-13 in plasma will be determined by interpolating the fluorescence change from the standard curve, and the activity will be expressed as ng/ml according to the curve. According to this method, the local reference range of ADAMTS-13 (above the 2.5th percentile and below the 97.5th percentile) will be determined.

Statistical analysis:

The collected data were revised, coded, tabulated, and introduced to a PC using the Statistical package for Social Science (SPSS for windows; SPSS Inc, Chicago, IL, version 25) developed by IBM (The International Business Machines Corporation). Two-tailed p-values of < 0.05 will be considered statistically significant. Normally distributed data will be represented by means and standard deviations and skewed data by medians and ranges. Student's t-test or Mann-Whitney U test will be used to compare continuous data and the Chi-squared or Fisher's Exact test will be used to compare categorical data. Kaplan-Meier method will be used to estimate the survival functions, and the survival functions will be compared using the log rank test.

Ethical considerations:

The Ain Shams University faculty of medicine's regional ethical committee granted their approval for our study (MS/ 637/2022).

RESULTS:

Table 1: Descriptive statistics of studies cases.

	N	%
Age in years		
Median (range)	55 (38-65)	
Mean ± SD	53 ± 7.46	
Sex		
Male	28	54.9%
Female	23	45.1%
Disease status prior to transplantation		
CR	30	58.8%
VGPR	19	37.3%
PR	2	3.9%
Outcome after transplantation		
Alive in remission	44	86.3%
Relapse	7	13.7%
Dose of melphalan		
Full dose	40	78.4%
25% reduction	11	21.6%
Serum creatinine in mg/dl		
Median (range)	1 (0.5-2.8)	
Mean ± SD	1.1 ± 0.49	
Creatinine clearance in ml/min		
Median (range)	88 (19-160)	
Mean ± SD	89 ± 36.4	
Ejection fraction by %		
Median (range)	65 (51-75)	
Mean ± SD	64 ± 5	

Gamma globulin in gm/dl Median (range) Mean ± SD	 0.9 (0.6-1.8) 1 ± 0.35	
Total protein in gm/dl Median (range) Mean ± SD	 7.2 (6.5-8.6) 7.2 ± 0.55	
Gamma globulin / total protein by % Median (range) Mean ± SD	 12.8 (8.4-23.1) 14.5 ± 4.5	
Plasma cells ratio in BMA by % Median (range) Mean ± SD	 3 (1-9) 3.3 ± 1.92	
Plasma cells ratio in BMB by % Median (range) Mean ± SD	 4 (1-9) 4 ± 2.1	
Lytic lesions Present Absent	 7 44	 86.3% 13.7%
Hb in gm/dl Median (range) Mean ± SD	 11.2 (8.2-14.2) 11.4 ± 1.61	
HCV Ab Positive Negative	 2 49	 3.9% 96.1%
CD34+ cell yield in 10^6/kg Median (range) Mean ± SD	 2.5 (1.5-10.6) 3.01 ± 1.68	
ADAMTS-13 level (percent of normal average) Median (range) Mean ± SD	 64.77 (24.6-152.4) 74.78 ± 35.2	

CR: Complete remission; VGPR: Very good partial remission; PR: Partial remission; BMB: Bone marrow biopsy; BMA: bone marrow aspirate; HCV: hepatitis C virus; Hb: Hemoglobin

Following up patients till 12 months after transplantation showed that only 7 cases (13.7%) had medullary relapse, 44 patients were alive without relapse, and none of the patients died of non-relapse causes. ADAMTS-13 levels were tested before transplantation. ADAMTS-13 levels came between 24.6% and 152.4% The average level was 74.78%, with half of patients below 64.77%. Other descriptive data of the patients.

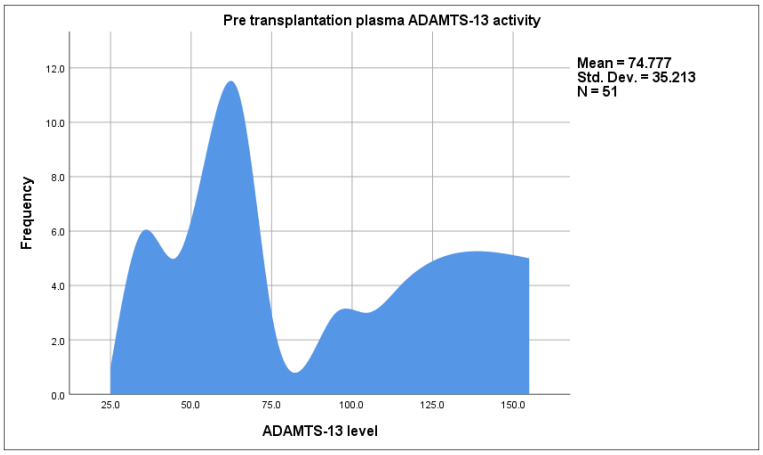

Figure 1: Frequency of pre transplantation plasma levels of ADAMTS-13.

Table 2: Relation between ADAMTS-13 level and patients' characteristics.

	ADAMTS-13 level		Test of significance
	> 50%	< 50%	
	N = 39	N = 12	
Sex Male Female	 20 (71.4%) 19 (82.6%)	 8 (28.61%) 4 (17.4%)	$\chi^2 = 0.88$ P = 0.35
Conditioning regimen Full dose melphalan 25% dose reduction	 31 (77.5%) 8 (72.7%)	 9 (22.5%) 3 (27.3%)	$\chi^2 = 0.11$ P = 0.74
Disease status CR VGPR PR	 24 (80%) 14 (73.7%) 1 (50%)	 6 (20%) 5 (26.3%) 1 (50%)	$\chi^2 = 1.07$ P = 0.59
Lytic lesions Absent Present	 34 (77.3%) 5 (71.4%)	 10 (22.7%) 2 (28.6%)	$\chi^2 = 0.16$ P = 0.74
HCV Ab Negative Positive	 37 (75.5%) 2 (100%)	 12 (24.5%) 0	$\chi^2 = 0.64$ P = 0.42
Outcome CR Relapsed	 33 (75%) 6 (85.7%)	 11 (25%) 1 (14.3%)	$\chi^2 = 0.36$ P = 0.54
Age Median (min - max)	56 (38 - 65)	54.5 (38 - 63)	t = 0.24 P = 0.82
Creatinine Median (min - max)	0.9 (0.5 – 2.8)	1.1 (0.6 – 2.2)	z = 0.79 P = 0.43
Creatinine clearance Median (min - max)	95 (24 - 160)	80 (19 -122)	z = 1.34 P = 0.18
Ejection fraction Median (min - max)	65 (55 - 75)	65 (51 - 72)	z = 0.51 P = 0.61
Gamma globulin Median (min - max)	0.9 (0.6 – 1.8)	0.9 (0.7 – 1.7)	z = 0.35 P = 0.73
Total protein Median (min - max)	7.1 (6.5 – 8.6)	7.2 (6.6 – 8.3)	z = 0.67 P = 0.5
Gamma globulin ratio Median (min - max)	12.9 (8.5 – 23.1)	12.7 (9.7 – 22.7)	z = 0.23 P = 0.82
Plasma cells ratio in BMA Median (min - max)	3 (1 - 8)	3 (1 - 9)	z = 0.251 P = 0.8
Plasma cells ratio in BMB Median (min - max)	4 (1 - 9)	3.5 (1 - 9)	z = 0.01 P = 0.99
HB Median (min - max)	11.3 (8.2 – 14.2)	10.9 (8.7 – 13.7)	z = 1.1 P = 0.27
CD34+ cell yield in 10^6/kg Median (min - max)	2.5 (1.5 – 9.1)	2.4 (1.7 – 10.6)	z = 0.18 P = 0.86
χ^2: Chi-square test, t: Student t-test, z: Mann–Whitney U test			

CR: Complete remission; VGPR: Very good partial remission; PR: Partial remission; BMB: Bone marrow biopsy; BMA: bone marrow aspirate; HCV: hepatitis C virus; Hb: Hemoglobin

Many factors were tested for their potential effect on pre transplantation plasma level of ADAMTS-13. These factors included age, gender, disease status, presence of lytic lesions, HCV Ab status, serum creatinine level, creatinine clearance, ejection fraction, gamma globulin levels, total protein level, Gamma globulin to total protein ratio, plasma cells ration in BMA & BMB, Hb concentration, and $CD34^+$ cell

yield during stem cell collection. None of these factors has been shown to have any statistically significant effect on pre transplantation plasma level of ADAMTS-13.

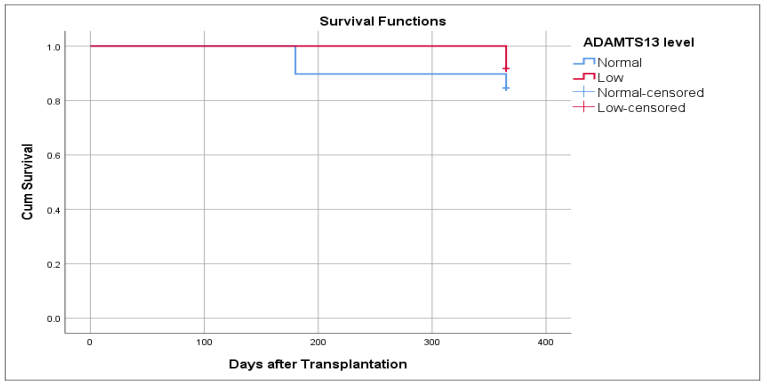

Figure 2: Kaplan Miere curve showing median disease-free survival among Studied cases according to different plasma ADAMTS-13 levels.

Table 3: Median disease-free survival according to plasma ADAMTS-13 level.

	Median DFS (95% CI)	log rank χ^2	P value
ADAMTS-13 level > 50%	346 (326 - 365)	0.409	0.522
ADAMTS-13 level < 50%	365 (365 - 365)		
Overall	350 (335 - 365)		

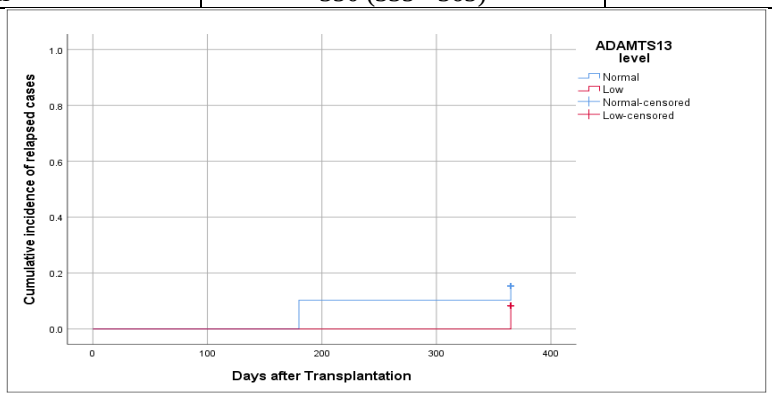

Figure 3: Cumulative incidence of relapsed MM cases over time according to ADAMTS-13 level.

Table 4: Hazard ratio of incidence of relapse MM cases after ASCT in pre transplantation ADAMTS-13 levels.

		Outcome		Total	Test of significance
		Alive	Relapsed		
ADAMTS-13 level	> 50%	33 (84.6%)	6 (15.4%)	39	$\chi^2 = 0.36$
	< 50%	11 (91.7%)	1 (8.3%)	12	p = 0.51
Total		44	7	51	HR = 0.50

Pre transplantation plasma level of ADAMTS-13 has been shown to have no statistically significant effect on disease-free survival or incidence of relapse of MM patients during the first year after auto stem cell transplantation.

DISCUSSION:

Multiple myeloma (MM) is a cancer of the plasma cells, a type of white blood cell that helps the body fight infection. In multiple myeloma, the plasma cells become cancerous and multiply out of control. This can lead to several problems, including bone pain, anemia, and kidney problems [(7)]. After leukemia, Multiple myeloma is considered to be the second most common hematological malignancy [(8)].

Typically, patients are treated with three to four cycles of induction therapy, then proceed to stem cell harvest and for autologous hematopoietic stem cell transplantation (ASCT). Till now, high-dose therapy followed by autologous stem cell transplantation are the standard care of eligible fit patients after receiving induction chemotherapy [(9)].

In this prospective cohort study, we studied the relation between levels of ADAMTS-13 in plasma and outcome of ASCT in patients diagnosed with MM. Blood samples were obtained from the patients before receiving ASCT conditioning regimen, then the patients were followed up throughout the transplantation and till 12 months after transplantation at follow up appointments at outpatient clinics. Re-evaluation of MM status is assessed every 3 months after transplantation. MM relapse is established according to Standard International Myeloma Working Group (IMWG) response criteria.

The study was done in a single bone marrow transplantation facility and included 51 patients previously diagnosed with MM, received induction chemotherapy, and admitted for ASCT. ADAMTS-13 levels were tested before transplantation. ADAMTS-13 levels came between 24.6% and 152.4% the average level was 74.78%, with half of patients below 64.77%.

According to Terrell et al. [(10)], there is no universal range of normal levels of ADAMTS-13. The normal differs between countries according to different ethnic populations, age, gender, and the assay type used for testing.

Peyvandi et al. [(11)] also agreed to this fact and considered normal ADAMTS-13 activity in healthy adults generally ranged between 50% and 160%.

In this study, pre transplantation levels of ADAMTS-13 came between 24.6% and 152.4%. 12 out of 51 patients (23.5%) had pre transplantation plasma ADAMTS-13 level below 50%. Many factors in this study were tested for any statistically significant effect on levels of ADAMTS-13 prior to transplantation. Age, gender, disease status, presence of lytic lesions, HCV Ab status, serum creatinine level, creatinine clearance, ejection fraction, gamma globulin levels, total protein level, Gamma globulin to total protein ratio, plasma cells ration in BMA & BMB, Hb concentration, and $CD34^{+}$ cell yield during stem cell collection had no statistically significant effect on ADAMTS-13 levels.

Mariotte et al. [(12)], Scully et al. [(13)], Terrell et al. [(10)], and Reese et al. [(2)] showed that average ADAMTS-13 varies between different ethnic groups, and this average level isn't related to higher incidence of acquired severe ADAMTS-13 deficiency and development of TTP.

44 out of 51 patients (86.3%) survived though the follow up period without any evidence of relapse. Only 7 patients (13.7%) had MM relapse and none of the patients died of non-relapse causes related to transplantation. These 7 relapsed cases occurred during the first 12 months after transplantation and considered to be early relapse. The incidence of late relapse couldn't be assessed due to relatively short follow up period.

This early relapse rate is similar to results of large study done by Bygrave et al.[(14)]. The study included 1349 patients who completed ASCT, and 179 patients (12.9%) had early relapse during the first year after transplantation.

There wasn't any mortality in the first year after transplantation due to causes other than relapse. The disease-free survival is considered to be 86.3%. The overall survival couldn't be properly assessed because the patients were not followed up after the relapse.

Most of studies done to assess survival in MM patients after ASCT had longer follow up period. According to Lehners et al. [(15)], the disease-free survival was 74% after three years of follow up and the survival was 59% after 5 years of follow up.

ADAMTS-13 level prior to ASCT was tested for any effect on the outcome of MM patients. There is no statistically significant effect of low ADAMTS-13 level on the outcome of MM patients after ASCT ($p = 0.9$). Also, there no statistically significant difference among the disease-free survival according to ADAMTS-13 level ($p = 0.5$). The hazard ratio or low ADAMTS-13 level to develop relapsed MM was 0.5 compared to normal ADAMTS-13 level, but this ratio has no statistically significant value as ($p = 0.51$).

There are no published papers explaining the effect of ADAMTS-13 level on the outcome of MM after ASCT. Age, gender, disease status, presence of lytic lesions, HCV Ab status, serum creatinine level, creatinine clearance, ejection fraction, gamma globulin levels, total protein level, Gamma globulin to total protein ratio, plasma cells ration in BMA & BMB, Hb concentration, and $CD34^+$ cell yield during stem cell collection were tested for any possible effect on outcome of MM after ASCT and showed not to have any statistically significant effect.

These results are similar to study done by Pourmoussa et al. [(16)], except that male sex was related with predictive factors for early relapse before 18 months after ASCT.

Conclusion:

In multiple myeloma patients who underwent auto stem cell transplantation, the pre transplantation plasma level of ADAMTS-13 has no statistically significant effect on the outcome, relapse incidence or disease-free survival during the first 12 months after transplantation.

Conflict of interest:

No conflict of interest

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العلاقة بين نشاط ADAMTS-13 ونتائج زرع نخاع العظمي الذاتي للميلوما المتعددة

ناهد معوض ابراهيم ابراهيم رخا [2] وعصام عبد الواحد حسن [2] و نرمين عادل نبيه عبد الغفار[2]

و أحمد صبري علي مبارك [1] و جهاد حمادة فكري حافظ[2]

قسم أمراض دم وزرع النخاع بمستشفى معهد ناصر[1] وقسم أمراض الباطنة وامراض الدم الاكلينيكية وزرع النخاع[2]

كلية الطب جامعة عين شمس القاهرة

يعد مرض الميلوما المتعددة من أكثر أنواع سرطانات الدم انتشارا على مستوى العالم، ولا يزال زرع خلايا الدم الجذعية الذاتية هو الإجراء المتبع في المرضى بعد تلقى العلاج الكيماوي.

تم إجراء هذا البحث لاستكشاف أهمية نشاط ADAMTS-13 لتوقع نسب البقاء بدون مرض ومعدلات الارتداد في مرضى الميلوما المتعددة وفقاً لمستوى نشاط الـ ADAMTS-13 قبل عملية زرع خلايا الدم الجذعية الذاتية.

خضع 51 مريض ميلوما متعددة لهذه الدراسة وتم تقييم مستوى نشاط ADAMTS-13 قبل إجراء عملية الزرع، مع متابعة المرضى أثناء عملية الزرع وحتى 12 شهرا بعد العملية.

وقد تبين أن مستوى نشاط الـ ADAMTS-13 قبل عملية زرع خلايا الدم الجذعية الذاتية، ليس له تأثير ذو أهمية إحصائية على نتائج الزرع، أو معدلات ارتداد المرض، أو البقاء بدون مرض خلال فترة المتابعة.