Original Research Article

Impact of Using Low Dose versus High Dose Antithymocyte Globulin Based Conditioning Regimen on the Outcome of Peripheral Blood Stem Cell Transplantation in Children with βeta-thalassemia Major

Abstract

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the only commonly available curative treatment for people with thalassemia major (TM).This work aimed to study the impact of using low dose versus high dose anti thymocyte globulin (ATG)based conditioning regimen on the results of peripheral blood stem cell transplantation in children with βeta- TM.

Methods: This comparative study was established on 40 patients with βeta-thalassemia major (Pesaro class II and/or III) who subjected to (HSCT) from donor who is related. Our patients received the same myeloablative regimen. They were then categorized according to dose of ATG into two groups: group I (Busulfan/Cyclophosphamide/Low dose ATG-30 mg) and group II (Busulfan/Cyclophosphamide/High dose ATG-110 mg).

Results: There was no association between both groups according to the clinical features, transplant related complications in both groups. There was a higher risk of mucositis and infections in high dose group. (P = 0.024 & P = 0.046; respectively). Patients in low dose group achieved faster neutrophil engraftment with median value of (14 days). No difference was found in the incidence of post-transplant viral reactivation, graft versus host disease in both groups.

Conclusions: Pesaro II, III thalassemia patients can safely receive hematopoietic stem cell transplantation (HSCT) with low dose ATG-30 mg regimen and attain the same result

Keywords: Thalassemia, antithymocyte globulin, Hematopoietic stem cell transplantation, Graft-versus-host disease.

Introduction:

βeta-thalassemia is the most prevalent inherited chronic hemolytic anaemia in Egypt, with an estimated 10% carrier prevalence. In Egypt, it is anticipated that 1.5 million live births every year would be affected by thalassemia disease. Approximately 1000 babies are born with βeta-thalassemia major (TM).βeta-thalassemia imposes a social and economic hardship on sufferers' families and the Egyptian government [1].

Allo-HSCT has expanded in prominence to the point that it is now the sole commonly accessible curative option for TM patients globally. The registry-collected data demonstrate unequivocally that various nations, including non-industrialized countries with limited income , routinely do HSCT for TM with excellent outcomes. Due to the unique characteristics of TM disease, a regimen combining busulfan (Bu) and cyclophosphamide (Cy) has been regarded the gold standard for patients receiving HSCT for years [2].

Unfortunately, despite its ability to eradicate thalassemia marrow and facilitate durable engraftment, this regimen has been linked with a higher rate of graft rejection in TM patients and graft versus host disease (GvHD) in non-malignant disorders. Studies presented a low occurance of both graft failure and GvHD in children given human leukocyte antigen (HLA) HSCT from a matched family donor in combination with antithymocyte globulin (ATG) in a Bu/Cy conditioning regimen [3].

The use of ATG represents an in vivo T-cell depletion strategy that simultaneously depletes host T cells that survived the conditioning procedure. This minimises the danger of rejection while decreasing freshly infused donor T cells, perhaps lowering GvHD and passive transfer of memory T cells that reconstitute early immunity [4].

As the dosage of ATG may related to the risk of infectious complications, relapse of the underlying hematologic disease, and/or non-relapse death, precision dosing is critical for optimising GvHD preventive effectiveness and minimising ATG toxicities [5].

The usual dose of ATG for HSCT in thalassemia is 110 mg/kg as a total dose. In an attempt to reduce the dose to minimize ATG side effects without significantly increasing the incidence of acute and chronic GvHD and without adverse effects on other outcomes, this work was designed.

The goal of this work was to study the effect of using low dose versus high dose ATG based conditioning regimen on the result of peripheral blood stem cell transplantation (PBSCT) in children with βeta-TM.

Patients and Methods:

This comparative study was carried out on 40 children with βeta-TM (Pesaro class II, III) who were admitted for allogeneic (PBSCT) from matched related donor.

Children with βeta-TM (Pesaro class I) and hematopoietic stem cell sources other than peripheral blood of matched related donor were excluded from this study.

Patients are classified into two equal group. Both groups received myeloablative regimen; oral busulfan: (16 mg/kg total dose in 4 divided doses on days -11 to - 8 if >8years or 20 mg/kg/total dose in 4 divided on days -11 to -8 if <8years) and Cy (200 mg/kg total dose; 50 mg/kg/day once daily on days -5 to -2). While group I received low dose ATG (30 mg/kg total dose; 10 mg/kg on days -3, -2, -1) and group II received high dose ATG (110 mg/kg total dose; 11 mg/kg on days -5 to -1 & days +1 to +5).

For Patients Pesaro Class III:

Pre-conditioning intensive hyper-transfusion program and cytoreduction with hydroxyurea 30 mg/kg daily and azathioprine 3mg/kg daily start at 3 months pre-transplant.

All cases involved in the study underwent to the following:

Complete data and clinical examination taking with assessment of Pesaro risk classes.

Laboratory investigations involving: complete blood count, blood group, bone marrow aspiration, HLA typing, viral marker screening, renal and liver function tests, electrolytes, random blood glucose, C-reactive protien, cultures, serum ferritin level and hemoglobin electrophoresis, hepatitis C antibodies & polymerase chain reaction (PCR). hepatitis B (s & e antigen), hepatitis B (s, e, c antibodies), human Immune Deficiency (HIV) antibodies, cytomegalovirus antibodies (IgG, IgM), Epstein-Barr virus antibodies (IgG, IgM), herpes simplex virus antibodies (IgG, IgM), toxoplasma antibodies (IgG, IgM) and haemoglobin electrophoresis. Imaging including: ECHO, Chest X-ray, Electrocardiogram and Fibro Scan/ liver biopsy.

FibroScan measurements of liver stiffness were associated with various stages of fibrosis histologically. The diagnostic yield of FibroScan measurements was compared to the histological stage of fibrosis (Metavir score) [6].

All donors – after identification of HLA matched sibling – were subjected to pre-transplantation evaluation including the same hematological, biochemical, virology screen, ECHO, and bone marrow aspiration. The following variables were used to assess the outcome of allo-HSCT: time to engraftment, acute and/or chronic GvHD, infection incidence and type, and regimen-related toxicities.

Stem Cell Transplantation Procedure:

Hickman catheter was inserted for all patients. All patients were nursed under strict protective isolation in barrier nursing rooms with high efficiency particulate air filters. Oral prophylactic sulfamethoxazole/trimethoprim for Pneumocystis jiroveci prophylaxis was given. Any febrile episode due to uncontrolled infection was treated by upgrading to broader spectrum anti-microbials according to standard protocol. Mobilization of stem cells from the donor was performed using granulocyte-stimulating factor (G-CSF) at a dose of 10 µgm/Kg/day for 5 days (D-4 to D-0). All cases received uromitexan a total dose of 1.2 × the daily dose of Cy dose; 4 hours before cy, on time of cy, Q4 hours after Cy for 4 doses (total of 6 doses) guard against hemorrhagic cystitis. Patients received seizures prophylaxis by phenytoin at a dose 8mg/kg/d with loading dose: 20 mg/kg starting before 1st dose of Bu. All patients got prophylaxis against GvHD using methotrexate. (MTX) at a dose of 15 mg/m^2 IV on day + 1 then 10 mg/ m^2 on days +3, +6 and +11, with folinic acid rescue at a dose of 15 mg/ m^2 IV tds for just 24 hours and the day after MTX injection.

Statistical Analysis:

SPSS version V20 was used to arrange, tabulate, and perform statistical analysis on the acquired data (Armonk, NY: IBM Corp).The range, mean, median, and standard deviations of numerical values were determined. The (t) test was used to determine the differences between mean values. The Mann Whitney test was used to determine the difference between median values. For categorical variables, the number and percentage were determined, and the chi square test was used to determine differences across subcategories. ($\chi 2$). The odds ratio and its 95% confidence interval were determined for risk estimation. Pearson's correlation coefficient (r) was performed to determine the correlation between variables. The significance level was set at $p < 0.05$..

Results:

Table 1 presents that there is an insignificant association between both groups as regard splenomegaly, hepatomegaly and tanner staging with delayed puberty in both groups. As regards Pesaro risk classes; patients of both groups were ranged from Pesaro II to Pesaro III with low dose ATG group had 17 patients (85%) Pesaro II and 3 patients (15%) Pesaro III, while high dose ATG group had 15 patients (75%) Pesaro II and 5 patients (25%) Pesaro III with an insignificant difference between both groups (Table 1). As regards diagnosis/transplant lag; there is an insignificant difference between both groups with a mean value of (4.100 ± 1.774 vs. 4.250± 2.291) years in low and high dose ATG groups respectively **(Table 1).**

Table 1: Comparison between the two groups as regards patients' pre-transplantation clinical data

Pre-transplant clinical data	ATG dose				Chi-Square	
	G1 (Low dose)		G2 (High dose)			
	N	%	N	%	X^2	P-value
Splenomegaly	19	95.00	18	90.00	0.360	0.548

Hepatomegaly		12		60.00	14		70.00	0.440	0.507
Splenectomy		0		0.00	1		5.00	1.026	0.311
Tanner stage	**Stage I**	19		95.00	17		85.00	1.111	0.292
	Stage II	1		5.00	3		15.00		
Pesaro	**II**	17		85.00	15		75.00	0.625	0.429
	III	3		15.00	5		25.00		
T-Test								**t**	**P-value**
Diagnosis/transplant Lag (years)	**Range**	1	-	8	1	-	9	-0.231	0.818
	Mean ±SD	4.100	±	1.774	4.250	±	2.291		

ATG: antithymocyte globulin

As regards serum ferritin and degree of liver fibrosis there was an insignificant difference between both group. **As regards viral screening;** there was no statistically significant association between both groups apart from cytomegalovirus (CMV) IgG was 8 patients (40%) in low dose ATG group in comparison with 16 patients (80%) in high dose ATG group with statistically significant difference between both groups (P = 0.010) **(Table 2).**

Table 2: Comparison between the two groups as regards patients' pre-transplantation laboratory and imaging data

Patients' Pre-transplantation Laboratory and Imaging Data		**ATG dose**				**Mann-Whitney Test**	
		G1 (Low dose)		**G2 (High dose)**		**Z**	**P-value**
Serum Ferritin (ng/ml)	**Range**	548 - 3500		230 - 5922		1.895	0.058
	Median	2550.00		1458.00			
Chi-Square		**N**	**%**	**N**	**%**	**X^2**	**P-value**
Degree of Liver Fibrosis	**F0**	13	65.00	10	50.00	3.209	0.523
	F1	4	20.00	7	35.00		
	F2	2	10.00	2	10.00		
	F3	1	5.00	0	0.00		
	F4	0	0.00	1	5.00		
Viral Screening	**+ve EBV IgM**	0	0.00	0	0.00	-	-
	+ve EBV IgG	0	0.00	0	0.00	-	-
	+ve CMV IgM	0	0.00	1	5.00	1.026	0.311
	+ve CMV IgG	8	40.00	16	80.00	6.667	**0.010***
	+ve Toxoplasma_IgM	0	0.00	0	0.00	-	-
	+ve Toxoplasma_IgG	0	0.00	3	15.00	3.243	0.072
	+ve HSV _IgM	0	0.00	0	0.00	-	-
	+ve HSV _IgG	0	0.00	0	0.00	-	-
	+ve HCV Ab	1	5.00	4	20.00	2.057	0.151
	+ve HCV PCR	0	0.00	2	10.00	2.105	0.147
	+ve HBs Ag	0	0.00	0	0.00	-	-
	+ve HBs Ab	5	25.00	9	45.00	4.286	0.058

	+ve HBc Ab	0	0.00	2	10.00	2.105	0.147
	+ve HIV Ab	0	0.00	0	0.00	-	-

ATG: antithymocyte globulin, F0: no fibrosis, F3: numerous septa without cirrhosis, F1: portal fibrosis without septa, F4: cirrhosis, F2: portal fibrosis with few septa, * Statistically significant (P<0.05), EBV: Epstein-Barr virus, CMV: cytomegalovirus, HSV: herpes simplex virus, HCV: Hepatitis C-virus, HBs: hepatitis B surface antigen, HBc: hepatitis B core antibody, HIV: human immunodeficiency virus

Regarding donors' age and sex, there was an insignificant difference between both groups. **As regard viral screening**, there was no statistically significant difference between both groups with low dose ATG group had 5 donors (25%) positive for CMV IgG, in comparison to high dose ATG group that exhibited 13 donors (65%) positive for CMV IgG with statistically significant difference between both groups (P value 0.011) **(Table 3)**.

Table 3: Comparison between the studied groups as regards donors' characteristics

Donors' characteristics		**ATG dose**				**Mann-Whitney Test**	
		G1 (Low dose)		**G2 (High dose)**		**Z**	**P-value**
Age (Years)	**Range**	5 - 24		2 - 25		1.507	0.132
	Median	9.5		5.5			
Chi-Square		**N**	**%**	**N**	**%**	**X^2**	**P-value**
Sex	**Female**	10	50.00	8	40.00	0.404	0.525
	Male	10	50.00	12	60.00		
Viral screening	**+ve CMV IgM**	0	0.00	0	0.00	-	-
	+ve CMV IgG	5	25.00	13	65.00	6.465	**0.011***
	+ve Toxoplasma IgM	0	0.00	0	0.00	-	-
	+ve Toxoplasma IgG	0	0.00	0	0.00	-	-
	+ve EBV IgM	0	0.00	0	0.00	-	-
	+ve EBV IgG	0	0.00	0	0.00	-	-
	+ve HBs Ab	4	20.00	6	35.00	3.956	0.057
	+ve HCV Ab	0	0.00	0	0.00	-	-
	+ve HCV PCR	0	0.00	0	00.00	-	-
	+ve HIV Ab	0	0.00	0	0.00	-	-

***Statistically significant (P<0.05), ATG: antithymocyte globulin, EBV: Epstein-Barr virus, CMV: cytomegalovirus, HCV: Hepatitis C-virus, HBs: hepatitis B surface antigen, HBc: hepatitis B core antibody, HIV: human immunodeficiency virus**

As regards sex and ABO matching and donor / recipient CMV disparity: there was an insignificant difference between both groups **(Table 4)**.

Table 4: Comparison between the studied groups as regards donor /recipient match

Donor /recipient match		**ATG dose**				**Chi-Square**	
		G1 (Low dose)		**G2 (High dose)**			
		N	**%**	**N**	**%**	**X^2**	**P-value**
Sex matching	**Matching**	9	45.00	11	55.00	0.400	0.527
	Non-Matching	11	55.00	9	45.00		

Sex nonmatching	**Male/Female**	5	45.45	5	55.56	0.202	0.653
	Female/Male	6	54.55	4	44.44		
ABO Matching	**Matching**	10	50.00	8	40.00	0.404	0.525
	Non-Matching	10	50.00	12	60.00		
ABO nonmatching	**Major**	3	30.00	6	50.00	0.917	0.632
	Minor	6	60.00	5	41.67		
	Bidirectional	1	10.00	1	8.33		
Donor /recipient CMV disparity	**Matching**	14	70.00	13	65.00	0.114	0.736
	Non-Matching	6	30.00	7	35.00		
Non-Matching	**Negative/Positive**	4	66.67	5	71.43	0.034	0.853
	Positive/Negative	2	33.33	2	28.57		

CMV: cytomegalovirus, ATG: antithymocyte globulin, ABO: blood groups A, B, AB, O

As regards the dose of stem cell infused: there is an insignificant difference between studied groups **(Table 5)**.

Table 5: Comparison between the studied groups as regards the dose of stem cells infused

Stem Cell dose ($\times 10^6$/kg)	**ATG dose**						**T-Test**	
	G1 (Low dose)			**G2 (High dose)**			**t**	**P-value**
Range	3	-	18	3.8	-	15.5	1.833	0.075
Mean ±SD	6.415	±	3.035	8.130	±	2.880		

ATG: antithymocyte globulin

As regards mucositis: Mucositis was significantly increased in high dose ATG group (p = 0.024). **As regards infection:** In low dose ATG, 9 patients (45%) did not get infection, 7 patients (35%) suffered from bacterial infection, 3 patients (15%) had viral infection, and one patient (5%) had fungal infection while in high dose ATG group, only 3 patients (15%) did not get infection, 14 patients (70%) suffered from bacterial infection, 2 patients (10%) had fungal infection, and one patient (5%) had both bacterial and fungal infection with substantially difference between both groups (P= 0.046). Moreover, no statistically significant difference was found between the two groups as regards the incidence of occurrence of other complications **(Table 6, Figure 1 Figure 2)**.

Table 6: Comparison between the studied groups as regards regimen-related toxicity

Regimen-related toxicity		ATG dose				Chi-Square	
		G1 (Low dose)		G2 (High dose)			
		N	%	N	%	X^2	P-value
Mucositis	No	6	30.00	0	0.00	9.436	**0.024***
	GI	8	40.00	11	55.00		
	GII	2	10.00	4	20.00		
	GIII	4	20.00	5	25.00		
Infections	Culture negative	9	45.00	3	15.00	9.667	**0.046***
	Bacterial	7	35.00	14	70.00		
	Viral	3	15.00	0	0.00		
	Fungal	1	5.00	2	10.00		
	Bacterial/Fungal	0	0.00	1	5.00		
Other complications	Renal	2	10.00	1	5.00	8.700	0.368
	Engraftment syndrome	1	5.00	2	10.00		
	HTN	2	10.00	1	5.00		

ATG: antithymocyte globulin, HTN: hypertension, GI, GII, GIII: grade I, II, III, *: statistically significant P value

As regards total days of neutropenia: In low dose group, the days of neutropenia range from 6 to 26 days with a mean value of (15.600 ± 5.995) while in high dose group, they range from 8 to 30 days with a mean value of (17.30 ± 4.828) with an insignificant difference between both groups **(Table 7)**.

Table 7: Comparison between the studied groups as regards the total days of neutropenia

		ATG dose						T-Test	
		G1 (Low dose)			G2 (High dose)			t	P-value
Total Days of Neutropenia	Range	6	-	26	8	-	30	0.771	0.445
	Mean ±SD	15.600	±	5.995	17.30	±	4.828		

ATG: antithymocyte globulin

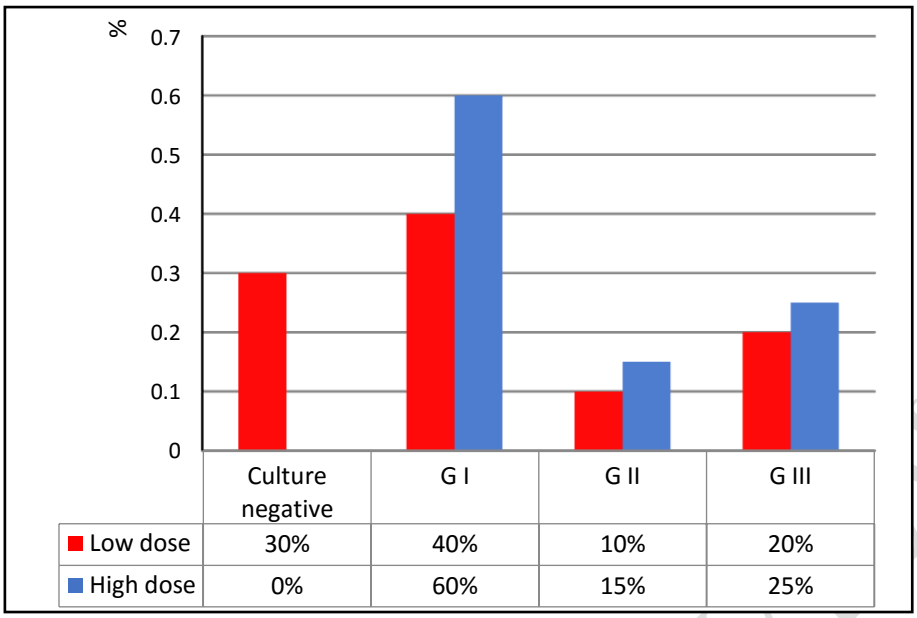

Figure 1: Comparison between the studied groups as regards incidence & grades of mucositis

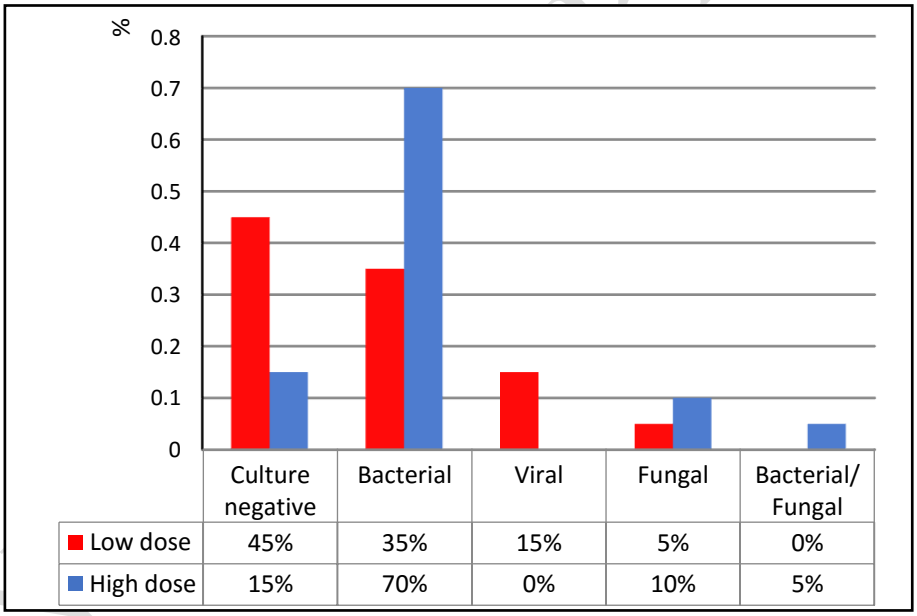

Figure 2: Comparison between the studied groups as regards incidence & type of infection

Discussion:

The best dose of ATG for GvHD prophylaxis that minimizes infection risk without worsening GvHD is unknown till now [7]. Additionally, dosage ATG is less defined in young patients than it is in adults [3].

In Egypt, the mean cost of a transplant is around 15,000 USD, and it is completely covered by medical insurance or the Ministry of Health **[8]**.

Even though the combination of ATG-110mg/kg plus a Bu/Cy conditioning regimen is the most followed conditioning regimen, in our center, for allo-HSCT in βeta-TM, the need for lower dose and less expensive with safety profile and favorable outcome is mandatory.

Although two groups in our study received similar dose of Bu, mucositis score > GI was more frequent in high dose ATG group than low dose ATG group (45%) vs. (30%), respectively. This could be explained by high incidence of infections and delayed neutrophil engraftment in high dose group.

The infectious complications in our study did not appear to be significantly different from those reported globally. The incidence of infections was significantly higher in the high dose ATG group (85%) vs. (55%) in low dose group. This might be because of more immunosuppressive effect from high dose ATG, and longer neutropenic period compared to low dose group with range (6-28 vs. 8-30 days, respectively).

These results are in agreement with those of **Choi et al., [9]** performed a retrospective randomized trial comparing two different doses of ATG and found that although a higher ATG dose was associated with better GvHD prevention, It raised the chance of infectious complications, as was also shown in the current study's findings.

Moreover, in a randomized clinical study by **Bonifazi et al**, [10] evaluating ATG effect in preventing GvHD after allo-HSCT, In a subset getting high doses, there was an increased occurrence of fatal infections, but not in a subgroup receiving lower dose ATG compared to no ATG. This is due to impaired T-cell reconstitution after allo-HSCT has been linked to an increased risk of opportunistic viral reactivations and infections, intracellular bacteria, and fungal infections.

Our results showed an insignificant difference in both groups as regards the total days of neutropenia with mean value of (15.600 ± 5.995 vs. 17.30 ± 4.828) days in low and high dose ATG respectively. We couldn't find any available published data regarding this point to compare our results with.

According to these findings, we propose that Pesaro II, III thalassemia patients can safely receive HSCT with low dose ATG-30 mg regimen and achieve the same outcome as Pesaro II, III thalassemia patients who received the standard high dose ATG-110 mg regimen.

Limitations of the present study:

- The insufficient number of patients involved was inadequate to assemble conclusive data.
- Short follow up period. Therefore, the risk of delayed graft failure cannot be determined.

Conclusion:

Pesaro II, III thalassemia patients can safely receive HSCT with low dose ATG-30 mg regimen and attain the same excellent result like Pesaro II, III thalassemia patients who got the standard high dose ATG-110 mg regimen. These data are encouraging in terms of sustained engraftment with no difference in the outcome, minimal toxicity and low cost.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Ethical Approval and Consent :

All procedures involving human subjects were conducted in compliance with the ethical criteria established by the study's ethics committee and its later amendments or comparable ethical standards (code: 32592/9/18) and an informed written consent were taken after informing the patients about the procedure.

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