**The effects of low-dose methylprednisolone as adjuvant therapy for hemophilia A patients
with factor VIII inhibitors**

Sri Marwanta^{1*}, Soetrisno¹, Bambang Purwanto¹, Brian Wasita², Tonang Dwi Ardyanto³, Faizal
Muhammad⁴, Eti Poncorini Pamungkasari⁵, Dono Indarto⁶, Eillien Ramadhani Fauzi⁴

¹Doctoral Program of Medical Sciences, Faculty of Medicine, Sebelas Maret University,
Surakarta, Indonesia, 57126

²Pathology Anatomy Department, Dr. Moewardi General Hospital, Sebelas Maret University,
Surakarta, Indonesia, 57126

³Clinical Pathology Department, UNS Teaching Hospital, Faculty of Medicine, Sebelas Maret
University, Surakarta, Indonesia, 57126

⁴Internal Medicine Department, UNS Teaching Hospital, Faculty of Medicine, Sebelas Maret
University, Surakarta, Indonesia, 57161

⁵Public Health Department, Faculty of Medicine, Sebelas Maret University, Surakarta,
Indonesia, 57126

⁶Biomedics and Physiology Department, Faculty of Medicine, Sebelas Maret University,
Surakarta, Indonesia, 57126

***Correspondence Author**

Sri Marwanta

Doctoral Program of Medical Sciences, Faculty of Medicine, Sebelas Maret University. Jl. Ir.
Sutami No.36, Jebres, Surakarta, Central Java, Indonesia 57126

Email: marwantaspdmkes@student.uns.ac.id

Phone number: (+62) 8122584412

ABSTRACT

Hemophilia A is an inherited bleeding disorder caused by factor VIII (FVIII) deficiency. In most minor-to-moderate bleeding events, FVIII concentrates therapy can result in significant lifesaving. However, chronic FVIII concentrates therapy can cause FVIII inhibitors synthesis and replacement therapy inefficacious. Immunosuppressive therapy can suppress FVIII inhibitors synthesis and make FVIII concentrates therapy more responsive. This study aims to determine the effects of low-dose methylprednisolone on acquired FVIII inhibitors. A randomized clinical trial was conducted on 11 hemophilia A male patients with high level of FVIII inhibitors. This pretest-posttest study design consists of intervention group (6 patients) who received 1 mg/bodyweight/day methylprednisolone, and control group (5 patients) received 100 mg/day sugar pill placebo for 6 weeks. Statistical analysis used independent t-test and paired t-test. Results showed significant FVIII inhibitors rising in pretest-posttest mean comparison of both intervention group ($p = 0.001$) and control group ($p = 0.001$). Further result showed inter-groups pretest mean comparison ($p = 0.976$) and inter-groups posttest mean comparison ($p = 0.034$). We conclude that low-dose methylprednisolone significantly suppresses the FVIII inhibitors synthesis pace within 6 weeks instead of inhibits the FVIII inhibitors synthesis.

Keywords: factor VIII, factor VIII inhibitors, hemophilia A, methylprednisolone.

INTRODUCTION

Hemophilia A is a congenital hemorrhagic disorder caused by factor VIII (FVIII) genetic abnormality (Castaman and Matino, 2019). It is a disorder that is complex to treat. Hemophilia A accounts for about 80% of approximately 400.000 hemophilia cases worldwide (Machin *et al.*, 2018). Meanwhile, only 25% of hemophilia A cases receive adequate therapy (Kadhim *et*

al., 2019). Arthropathy and cerebrovascular complication remain barriers to normal daily activities performance among hemophilia A patients (Baek *et al.*, 2020). Current routine therapy to treat bleeding event depends on replacement therapy of clotting factor concentrates since childhood, meanwhile, chronic factor concentrates therapy causes the development of FVIII inhibitors (Santagostino *et al.*, 2018). Studies showed that 3-13% of mild and most cases of moderate-to-severe hemophilia A patients developed FVIII inhibitors (Yamanouchi *et al.*, 2018). This neutralizing antibody certainly causes inadequate replacement therapy and poor medical outcomes (Walsh *et al.*, 2016).

Treatment of patients with FVIII inhibitors including porcine FVIII, activated prothrombin complex concentrate, activated recombinant factor VII, desensitization, immune tolerance induction, and monoclonal antibody that bridge factor IXa and factor X (Meeks and Batsuli, 2016). However, those therapies are difficult to obtain and expensive in limited healthcare resources of low-income countries. Some studies recommended that steroid drugs may be useful as adjuvant therapy for hemophilia A patients with high titer of FVIII inhibitors (Cacciotti *et al.*, 2021).

Methylprednisolone is a prednisone-derived drug with potent anti-inflammatory and immunosuppressive effects even in low doses. Methylprednisolone also has minimal side effects on water retention, sodium retention, weight gain, and gastric irritation on long-term therapy compared to other steroid derivatives (Permana *et al.*, 2019). It is a cost-effective drug and widely available in every healthcare setting. Hence, this study aims to investigate the effect of low-dose methylprednisolone on hemophilia A patients with factor VIII inhibitors.

MATERIALS AND METHODS

The hemophilia A patients and study setting

This 6-weeks randomized pretest-posttest clinical trial with control group design was conducted from September 2020 to March 2021 at the Hematology Polyclinic of Dr. Moewardi General Hospital, Surakarta, Indonesia. The participant inclusion criteria including male patients more than 18 years old with a history of FVIII concentrates therapy more than 9 exposure days (Witmer and Young, 2013). The exclusion criteria are other bleeding disorders comorbidities, other system organs comorbidities, and currently on steroid drug therapy. During 3 months of the simple random sampling period, only 11 hemophilia A patients met the study criteria. Then, the participants were divided into intervention group (n = 6) and control group (n = 5) randomly.

Variables and interventions

FVIII inhibitors were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. Those data were measured in ng/ml. In the pretest period, baseline or pretest clinical parameter data were also measured. They were including body weight, fasting blood glucose (FBS), hemoglobin (Hb), white blood cells (WBC), platelet, prothrombin time (PT), and activated partial thromboplastin time (APTT). The FVIII inhibitors and clinical parameter data were tested in both pretest and posttest studies.

The interventions included 1 mg/bodyweight/day methylprednisolone for the intervention group and 100 mg/day sugar pill placebo for the control group. The intervention duration was 6 weeks. During the intervention period, the patients were educated not to perform high-risk activities according to the Hemophilia Activities List. The patients were not allowed to consume vitamin E and certain herbal supplements which may prevent the platelets from clumping. Aspirin and non-steroidal anti-inflammatory drugs were prohibited because they may cause bleeding risk.

Data collection and analysis

The FVIII inhibitors and laboratory parameters of FBS, HB, WBC, platelet, PT, and APTT were obtained from participant vein blood. The BIOENZY Human Coagulation Factor VIII ELISA Kits were used to analyze the FVIII inhibitors titer. It has a sensitivity of 0.28 ng/ml and a standard curve range of 0.5 ng/ml - 150 ng/ml. Based on the participant group, either 1 mg/bodyweight methylprednisolone or 100 mg sugar pill were prescribed as many as 42 drugs for 6 weeks.

Paired t-test was used for statistical analysis of pretest-posttest FVIII inhibitors mean comparison of each intervention group and control group. Independent t-test was used for statistical analysis of FVIII inhibitors mean comparison of inter-groups pretest and inter-groups posttest. The statistical analyses were conducted using SPSS 22 statistical software for windows.

Ethical consideration

This study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital, No.1.069/XI/HREC/2019. The study was also conducted following the Helsinki Declaration. All the participants received a study protocol explanation and gave written informed consent. The ethical approval is available for review by the Editor-in-Chief of this journal.

RESULTS AND DISCUSSION

The clinical parameters baseline or pretest and posttest data were checked to ensure that the methylprednisolone or placebo administration did not significantly ($p > 0.05$) affect the clinical and laboratory conditions of the participants (**Table 1**). Methylprednisolone has a wide range of physiologic effects, including endocrine, immunologic, and hematologic disorders (Yang *et al.*, 2020). The only significant difference between the intervention and control groups in the

posttest APTT parameters ($p = 0.039$) indicated that methylprednisolone affected the intrinsic pathway of blood coagulation. FVIII inhibitors or autoantibodies synthesis in hemophilia A patients is a multifactorial process and involves cytokines and immune regulatory molecules (Tieu *et al.*, 2020). This T-cell-mediated hypersensitivity is mediated by inflammatory cytokines like interleukin (IL) 1, IL-6, and tumor necrosis factor-alpha. The administration of methylprednisolone can inhibit these inflammatory cascades, which then significantly shortened the APTT duration in the intervention group (Ebrahimi *et al.*, 2016).

The intervention group continued to undergo a significant increase in FVIII inhibitors levels ($p = 0.001$) within 6 weeks, as did the control group (**Table 2**). These indicated that administration of methylprednisolone neither inhibited nor stopped the synthesis of FVIII inhibitors. The FVIII antibodies formation requires the FVIII endocytosis process through the role of antigen-presenting cells (Astermark, 2015). The endocytosis results are then presented by human leukocyte antigen class II molecules to the surface of the cluster of differentiation 4+ (CD4+) T cells. Moreover, T-regulatory cells (Tregs) have an inhibition role in the FVIII antibody formation process (Khalilian *et al.*, 2020). Tregs inhibit the antigen-specific B-cell differentiation into memory B cells and CD4+ activation by IL-10 (Delignat *et al.*, 2019). Thus, there will be a potentiation effect to inhibit FVIII antibodies synthesis by an immunosuppressive drug. However, the relationship between FVIII inhibitors formation in hemophilia and Tregs function is still inconclusive. It is believed to be due to HMOX-1 gene polymorphism in hemophilia patients (Repešć *et al.*, 2013).

Six weeks after methylprednisolone and placebo administration, there was a significant difference ($p = 0.034$) in the mean levels of FVIII inhibitors between the intervention and control groups. The APPT posttest laboratory parameters between the intervention and the control groups are also significant ($p = 0.039$) (**Table 1**). A study also showed that prednisone adjuvant therapy alone could control bleeding episodes in hemophilia patients with FVIII

inhibitors. Moreover, that study recommended the use of prednisone-cyclophosphamide as second-line therapy for FVIII inhibitors in patients who failed in prednisone adjuvant therapy (Sarah *et al.*, 2016).

CONCLUSION

The low-dose of 1 kg/bodyweight/day methylprednisolone as adjuvant therapy in hemophilia A patients with FVIII inhibitors can suppress the synthesis pace of FVIII inhibitors within 6 weeks. Its long-term administration has minimal side effects. Furthermore, it can be a cost-effective adjuvant therapy in limited health care settings. Although low-dose methylprednisolone did not inhibit the FVIII inhibitors synthesis, this adjuvant therapy is recommended for hemophilia A patients who received long-term FVIII concentrates therapy.

ACKNOWLEDGMENTS

The authors are grateful to the internal medicine specialists of Dr. Moewardi General Hospital for providing feedback and technical support.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

FUNDING

The authors did not receive financial support for the research, authorship, and publication of this article.

REFERENCES

Astermark J. FVIII inhibitors: Pathogenesis and avoidance. *Blood*, 2015:2045–2051.

Baek HJ, Park YS, Yoo KY, Cha JH, Kim YJ and Lee KS. Health-related quality of life of moderate and severe haemophilia patients: Results of the haemophilia-specific quality of life index in Korea. *PLoS One*, 2020; 15(9):e0238686.

Cacciotti C, Chan A and Bhatt M. Oral corticosteroid therapy as an adjuvant treatment for acute bleeding in hemophilia patients with high titer inhibitors. *J Pediatr Hematol Oncol*, 2021; 43(2):e237–e239.

Castaman G and Matino D. Hemophilia A and B: Molecular and clinical similarities and differences. *Haematologica*, 2019; 104(9):1702–1709.

Delignat S, Russick J, Gangadharan B, Rayes J, Ing M, Voorberg J, Kaveri S V. and Lacroix-Desmazes S. Prevention of the anti-factor VIII memory B-cell response by inhibition of bruton tyrosine kinase in experimental hemophilia A. *Haematologica*, 2019; 104(5):1046–1054.

Ebrahimi L, Kheirandish M and Foroughi M. The effect of methylprednisolone treatment on fibrinolysis, the coagulation system, and blood loss in cardiac surgery. *Turkish J Med Sci*, 2016; 46(6):1645–1654.

Kadhim KAR, Al-Lami FH and Baldawi KH. Epidemiological Profile of Hemophilia in Baghdad-Iraq. *Inq (United States)*, 2019; 56(20):1–8.

Khalilian S, Motovali-Bashi M and Rezaie H. Factor VIII: Perspectives on immunogenicity and tolerogenic strategies for hemophilia a patients. *Int J Mol Cell Med*, 2020:33–49.

Machin N, Ragni M V and Smith KJ. Gene therapy in hemophilia A: A cost-effectiveness analysis. *Blood Adv*, 2018; 2(14):1792–1798.

Meeks SL and Batsuli G. Hemophilia and inhibitors: Current treatment options and potential new therapeutic approaches. *Hematol (United States)*, 2016; 2016(1):657–662.

Permana D, Barliana M and Hamijoyo L. Dosage and Duration of Methylprednisolone Therapy Affect the Occurrence of Cushing Habitus in Patients with Systemic Lupus Erythematosus. *J Pharm Bioallied Sci*, 2019; 11(8):S628–S634.

Repressé Y, Peyron I, Dimitrov JD, Dasgupta S, Moshai EF, Costa C, Borel-Derlon A, Guillet B, D'Oiron R, Aouba A, Rothschild C, Oldenburg J, Pavlova A, Kaveri S V. and Lacroix-Desmazes S. Development of inhibitory antibodies to therapeutic factor VIII in severe hemophilia A is associated with microsatellite polymorphisms in the HMOX1 promoter. *Haematologica*, 2013; 98(10):1650–1655.

Santagostino E, Young G, Carcao M, Mannucci PM, Halimeh S and Austin S. A contemporary look at FVIII inhibitor development: still a great influence on the evolution of hemophilia therapies. *Expert Rev Hematol*, 2018:87–97.

Sarah L, Prantik D and Gary B. Systemic therapy in acquired haemophilia - A single institute experience. *Ulster Med J*, 2016; 85(3):187–192.

Tieu P, Chan A and Matino D. Molecular mechanisms of inhibitor development in hemophilia. *Mediterr J Hematol Infect Dis*, 2020:e2020001.

Walsh CE, Jiménez-Yuste V, Auerswald G and Grancha S. The burden of inhibitors in haemophilia patients. *Thromb Haemost*, 2016; 116(S 01):S10–S17.

Witmer C and Young G. Factor VIII inhibitors in hemophilia A: Rationale and latest evidence. *Ther Adv Hematol*, 2013; 4(1):59–72.

Yamanouchi J, Tokumoto D, Ikeda Y, Maruta M, Kaneko M, Hato T and Yasukawa M.

Development of an fviii inhibitor in a mild hemophilia patient with a phe595cys mutation.

Intern Med, 2018; 57(21):3179–3182.

Yang R, Xiong Y, Ke H, Chen T and Gao S. The role of methylprednisolone on preventing

disease progression for hospitalized patients with severe COVID-19. Eur J Clin Invest, 2020;

50(11):e13412.

Table 1. Clinical parameter data baseline or pretest and posttest of both control and intervention group.

Clinical parameter data	Control (n = 5)		Intervention (n = 6)		p-value ^a
	Mean	SD	Mean	SD	
Pretest					
Body weight (kg)	51.0	5.8	60.2	12.0	0.155
FBS (mg/dL)	112.8	13.1	107.8	11.9	0.527
Hemoglobin (g/dL)	11.9	1.4	12.1	1.6	0.864
WBC (10 ³ /dL)	6.4	1.5	6.2	1.7	0.833
Platelet (10 ³ /dL)	324.0	104.1	297.3	122.0	0.709
PT (s)	11.6	2.1	12.3	2.1	0.573
APTT (s)	49.4	7.4	50.7	5.6	0.754
Posttest					
Body weight (kg)	51.0	5.4	60.3	12.0	0.161
FBS (mg/dL)	118.6	10.4	112.3	10.9	0.358
Hemoglobin (g/dL)	12.1	1.3	12.5	1.5	0.640
WBC (10 ³ /dL)	6.1	1.5	5.9	1.7	0.812
Platelet (10 ³ /dL)	308.4	88.1	308.5	127.7	0.999
PT (s)	11.6	1.5	11.8	1.8	0.826
APTT (s)	46.6	5.5	40.5	5.0	0.039

APTT, activated partial thromboplastin time; FBS, fasting blood sugar; PT, prothrombin time; SD, standard deviation; WBC, white blood cells.

^aIndependent t-test

Table 2. FVIII inhibitors pretest-posttest mean comparison of each intervention group and control group, FVIII inhibitors inter-groups pretest and posttest mean comparison.

FVIII inhibitors (ng/ml)	Control (n = 5)		Intervention (n = 6)		p-value ^a
	Mean	SD	Mean	SD	
Pretest	19.0	5.8	19.2	9.4	0.976
Posttest	64.4	14.7	48.7	4.7	0.034
p-value^b	0.001		0.001		

FVIII inhibitors, factor VIII inhibitors; SD, standard deviation.

^aIndependent t-test

^bPaired t-test