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

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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 to be 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 (MP) on acquired FVIII inhibitors. A randomized clinical trial was conducted on 11 hemophilia A male patients with a high level of FVIII inhibitors. This pretest–posttest study design consists of an intervention group (six patients) who received 1 mg/body weight/day MP and a control group (five patients) who received a 100 mg/day sugar pill placebo for 6 weeks. Statistical analysis used the independent t-test and paired t-test. The results showed significant FVIII inhibitors rising in pretest–posttest mean comparison of both the intervention group ($p = 0.001$) and control group ($p = 0.001$). Further results showed the intergroups' pretest mean comparison ($p = 0.976$) and intergroups' posttest mean comparison ($p = 0.034$). It can be concluded that low-dose MP significantly suppresses the FVIII inhibitors synthesis pace within 6 weeks instead of inhibiting the FVIII inhibitors synthesis.

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 complications remain barriers to normal daily activities performance among hemophilia A patients (Baek *et al.*, 2020). The current routine therapy to a 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 have 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 and activated recombinant factor VII, desensitization, immune tolerance induction, and a monoclonal antibody that bridge factor IXa and factor X (Meeks and Batsuli, 2016). However,

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the therapies are difficult to obtain and expensive in the limited healthcare resources of low-income countries. Some studies recommend that steroid drugs may be useful as adjuvant therapy for hemophilia A patients with a higher titer of FVIII inhibitors (Cacciotti *et al.*, 2021).

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

MATERIALS AND METHODS

Hemophilia A patients and study setting

This 6-week randomized pretest–posttest clinical trial with a 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 included male patients older than 18 years with a history of FVIII concentrates therapy for more than 9 exposure days (Witmer and Young, 2013). The exclusion criteria are other bleeding disorders comorbidities, other system organs' comorbidities, and being currently on steroid drug therapy. During the 3 months of the simple random sampling period, only 11 hemophilia A patients met the study criteria. Then, the participants were randomly divided into a intervention group ($n = 6$) and a control group ($n = 5$).

Variables and interventions

FVIII inhibitors were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. The data were measured in ng/ml. In the pretest period, the baseline or pretest clinical parameter data were also measured. They included 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/body weight/day MP for the intervention group and a 100 mg/day sugar pill placebo for the control group. The intervention duration was 6 weeks. The patients were educated not to carry out high-risk activities during the intervention period according to the Hemophilia Activities List. The patients were not allowed to consume vitamin E and certain herbal supplements to prevent the platelets from clumping. Aspirin and nonsteroidal anti-inflammatory drugs were prohibited because they may cause bleeding risk.

Data collection

The FVIII inhibitors and laboratory parameters of FBS, HB, WBC, platelet, PT, and APTT were obtained from participants' vein blood. The BIOENZY Human Coagulation FVIII ELISA kits were used to analyze the FVIII inhibitors' titers. It has a sensitivity of 0.28 ng/ml and a standard curve range of 0.5–150 ng/ml. Based on the participant group, either 1 mg/body weight MP or a 100 mg sugar pill was prescribed for as many as 42 drugs for 6 weeks.

Statistical analysis

The paired *t*-test was used to analyze the pretest–posttest FVIII inhibitors mean comparison of the intervention group and control group. The independent *t*-test was used to analyze FVIII inhibitors mean comparison of intergroups' pretest and posttest (Fig. 1). The statistical analyses were conducted using Statistical Package for the Social Sciences 22 statistical software for Windows.

Ethical approval

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 MP or placebo administration did not significantly ($p > 0.05$) affect the clinical and laboratory conditions of the participants (Table 1). MP 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 MP 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-1, interleukin-6, and tumor necrosis factor-alpha. The administration of MP can inhibit these inflammatory cascades, which 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 MP 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). Human leukocyte antigen class II molecules then present the endocytosis results 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 interleukin-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 (Repessé *et al.*, 2013).

Six weeks after MP 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, the study recommended the use of prednisone–cyclophosphamide as
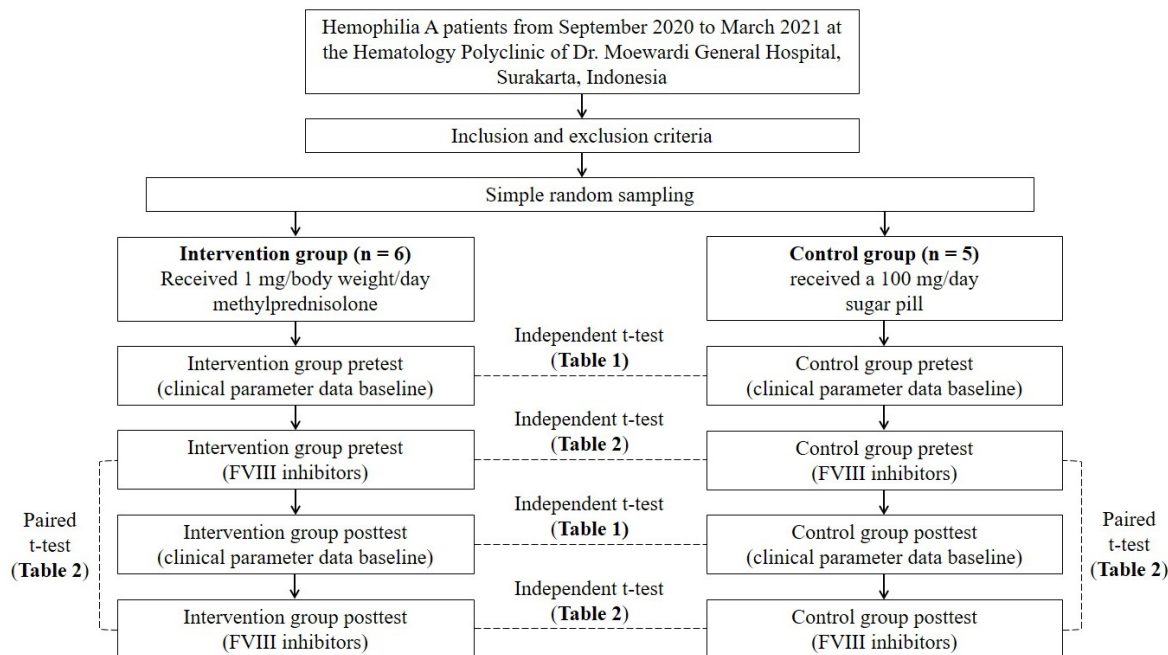
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│ Hemophilia A patients from September 2020 to │
│ March 2021 at the Hematology Polyclinic of   │
│ Dr. Moewardi General Hospital,               │
│ Surakarta, Indonesia                         │
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                      ↓
┌─────────────────────────────────────────────┐
│         Inclusion and exclusion criteria     │
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│             Simple random sampling           │
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Intervention group (n = 6) Received 1 mg/body weight/day methylprednisolone		Control group (n = 5) received a 100 mg/day sugar pill
Intervention group pretest (clinical parameter data baseline)	Independent t-test (Table 1)	Control group pretest (clinical parameter data baseline)
Intervention group pretest (FVIII inhibitors)	Independent t-test (Table 2)	Control group pretest (FVIII inhibitors)
Intervention group posttest (clinical parameter data baseline)	Independent t-test (Table 1)	Control group posttest (clinical parameter data baseline)
Intervention group posttest (FVIII inhibitors)	Independent t-test (Table 2)	Control group posttest (FVIII inhibitors)

Paired t-test (Table 2) [intervention side] · Paired t-test (Table 2) [control side]

Figure 1. Graphical flow chart diagram of the study.

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

Clinical parameter data	Control (n = 5)		Intervention (n = 6)		p-value*
	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
Hb (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
Hb (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 (seconds)	11.6	1.5	11.8	1.8	0.826
APTT (seconds)	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.
*Independent *t*-test.

second-line therapy for FVIII inhibitors in patients who failed the prednisone adjuvant therapy (Sarah *et al.*, 2016).

CONCLUSION

The low dose of 1 mg/body weight/day MP 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 healthcare settings. Although low-dose MP did not inhibit the FVIII inhibitors synthesis, this adjuvant therapy is recommended for hemophilia A patients who received long-term FVIII concentrates therapy. Further research is needed to investigate the other effective therapeutic dose of MP. Similar

Table 2. FVIII inhibitors' pretest–posttest mean comparison of the intervention group and control group, FVIII inhibitors' intergroups pretest and posttest mean comparison.

FVIII inhibitors (ng/ml)	Control (*n* = 5)		Intervention (*n* = 6)		*p*-value[*]
	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[#]	0.001		0.001		

FVIII inhibitors, factor VIII inhibitors; SD, standard deviation.
[*]Independent *t*-test.
[#]Paired *t*-test.

interventional clinical research with more than 30 hemophilia A patients or different steroidal anti-inflammatory drugs is also needed to support this study result.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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