online Technical Appendix

Materials and Methods

Design

This prospective randomized controlled clinical trial (RCT) was run in a high-volume academic tertiary referral center. The institutional review board approved the methodology and ethics (IR.TUMS.IKHC.REC.1399.032). It was registered in the Iranian Registry of Clinical Trials (IRCT20200305046700N1).

Patients

All the patients participated voluntarily in this trial and signed the informed consent formula. We enrolled 200 consecutive patients based on the eligibility criteria from our hip subspecialty clinic from March 2020 to January 2022. Forty-eight patients were not included in the final analysis. There were 10 patients who declined to participate and 10 who were excluded due to hemophilia (n=2), impaired coagulation profile (n=4), and inflammatory hip arthritis (n=4). After randomized allocation, one patient was excluded due to impaired coagulation tests on the operation day, 19 patients were lost to follow-up due to not performing postoperative complete blood counts (CBC), and eight patients were excluded from analysis due to high intraoperative mean arterial pressure.
(MAP). Finally, 152 patients, 75 in wax and 77 in control groups, were included in the analysis (Fig. 1).

Inclusion and Exclusion Criteria

The inclusion criteria were patients with hip osteoarthritis (ICD-10-CM\(^1\); M16\(^2\)) who were candidates for primary THA based on clinical and radiological indications, gave their informed consent to participate, and received spinal anesthesia for surgery.

The exclusion criteria were any bleeding disorder (ICD-10-CM; D65-D69\(^3\)) including platelet disorders like von Willebrand disease and coagulation disorders like hemophilia, history of venous/arterial thromboembolic events (e.g. deep vein thrombosis, pulmonary thromboembolism, cerebrovascular accidents, and myocardial infarction), high-risk medical comorbidities (e.g. chronic kidney disease (GFR< 60mL/min), liver, and heart failure (NYHA\(^4\) classes III, IV)), inflammatory hip arthritis (ICD-10-CM; M05-M1\(^5\)), acute hip fracture, developmental dysplasia of hip (DDH) types III and IV, revision THA, use of antithrombotic drugs (ATC\(^6\) code; B01\(^7\)) including vitamin K antagonists (B01AA) like warfarin, platelet aggregation inhibitors (B01AA) like clopidogrel, heparin group (B01AB), Factor X inhibitors (B01AF) like rivaroxaban, and direct thrombin inhibitors (B01AE) like dabigatran, impaired coagulation profile (including INR>1.1, aPTT>1.4, PT>13.5, or thrombocytopenia<150,000/µL), any anesthesia other than spinal,

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\(^1\) International Classification of Diseases, 10\(^{th}\) Revision, Clinical Modification
\(^2\) https://www.icd10data.com/ICD10CM/Codes/M00-M99/M15-M19/M16-
\(^3\) https://www.icd10data.com/ICD10CM/Codes/D50-D89/D65-D69
\(^4\) New York Heart Association
\(^5\) https://www.icd10data.com/ICD10CM/Codes/M00-M99/M05-M14
\(^6\) Anatomical Therapeutic Chemical (ATC) Classification System
\(^7\) https://www.whocc.no/atc_ddd_index/?code=B01
intraoperative mean arterial pressure (MAP) > 85 mmHg, and those intending not to participate or continue participating in the follow-up.

**Intervention Groups**

The intervention was the application of bone wax on the distal femoral neck surface following osteotomy. The bone wax remains in place until the femur is broached. Patients were randomly allocated into two groups of wax, in whom the intervention was applied, and control, who underwent surgery without using bone wax.

**Randomization and Blinding**

A unique number was attributed to each patient in his/her case report form (CRF). The randomized allocation table was prepared using the permuted balanced block randomization and the random number generator function in Microsoft Excel 365. Subsequently, each patient was allocated to either of groups based on the number generated in the table and their unique number.

The patients were blinded towards their intervention groups. The assessor researcher who collected the data and filled the CRFs was also blinded regarding the groups. At the beginning of surgery, the circulator nurse opened a folded label attached to the patient’s CRF, informed the surgeon of the intervention, and discarded the label. The surgeon, although aware of the groups, was not involved in any of the postoperative data collection or analysis steps. The analyzer researcher was also blinded regarding the intervention groups and analyzed the data based on A and B groups. After finishing the analysis, A and B groups were decoded. Therefore, this trial was performed as triple blinded.
Outcome Measures

The primary outcome in this study was the efficacy of bone wax in reducing PBL. Accordingly, we both measured the apparent and calculated the total perioperative blood loss (PBL).

To measure the apparent PBL, we used the following protocol: At first, we measured the net weight ($m_1$) of the surgical sponges, which was to be used during the operation, using a high accuracy (0.01g) digital scale (K1-A Ming Heng, ATOM, India). The surgeon applied maximum possible hemostasis of the surgical field using electrocautery during surgery. The aid was asked to constantly suction the fluid from surgical field leaving none to be dispersed outside. At last, all the surgical sponges were weighed again ($m_2$). The density of blood was considered 1.060 g/mL.

The volume of blood within the long-gauze pads ($V_1$) was calculated as follows:

$$V_1 [mL] = \frac{m_2 [g] - m_1 [g]}{\text{blood density} [g/mL]}$$

The blood inside the suction canister ($V_2$) was calculated by subtracting the volume of irrigation serum from the whole volume of suctioned fluids at the end of surgery. Because we routinely did not use surgical drains postoperatively, the volume of apparent postoperative blood loss ($V_3$) was zero. Therefore, we calculated apparent PBL as:

$$\text{Apparent PBL} = V_1 + V_2 + V_3 = V_1 + V_2$$

For total PBL, taking hidden blood loss into account, we used the formulae proposed by Good et al, which is based on the pre- (Hb$_{preop}$) and postoperative serum Hb (Hb$_{postop}$), estimated blood volume (EBV), and the Hb content of one unit of packed red blood cells (PRBCs) (Hb$_t$):
Hb\text{loss}[g] = EBV[mL] \times \left( Hb_{\text{preop}}[g/dL] - Hb_{\text{postop}}[g/dL] \right) \times 0.01[dL/mL] + Hb_{t}[g]

Total PBL(mL) = \frac{Hb_{\text{loss}}[g] \times 100(mL/dL)}{Hb_{\text{preop}}[g/dL]}

We calculated total PBL on postoperative days (POD) 3 and 5. For EBV, we used the formulae proposed by Nadler et al\textsuperscript{34}:

EBV [mL] = (0.0003669 \times \text{height}^3[cm]) + (32.19 \times \text{weight [Kg]}) + 604 \text{ (males)}

EBV [mL] = (0.0003669 \times \text{height}^3[cm]) + (32.19 \times \text{weight [Kg]}) + 183 \text{ (females)}

Our secondary outcomes were the need for blood transfusion (PRBCs) and the adverse events or complications, which could be related to the use of bone wax.

Pre- and Postoperative Protocol

The patients were admitted one day before surgery. Base lab tests including CBC, PT, PTT, and INR were done. The demographic, clinical, and laboratory data of the patients were recorded in their CRFs. After appropriate templating, THA was performed by the senior author, which is a high-volume hip surgeon. Thirty minutes before surgery, all the patients received 10 mg/Kg of intravenous TXA and cefazolin (<60Kg: 1g, 60-80Kg: 2g, >80Kg: 3g). Spinal anesthesia was administered with a target MAP below 80 mmHg during surgery.

For postoperative anticoagulant prophylaxis, aspirin (325mg BD) was administered for six weeks according to AAOS guideline\textsuperscript{35}. No antibiotic was administered. The patients were ambulated with full weightbearing the evening after surgery. Postoperative transfusion indications, although rarely needed, were serum Hb below 7g/dL and symptomatic anemia. Patients were discharged on POD2.
unless their medical conditions did not permit (rarely). A CBC was requested to be done on the third and fifth PODs in the same laboratory. The patients were visited one week, one month, and three months after the surgery. Any postoperative complication was noted and recorded in the CRF.

Surgical Technique

After standard prep and drape, THA was performed through DAA using the standard technique on an ordinary operation table in the supine position. Following anterior capsulotomy, double osteotomy of femoral neck was performed using power saw. Immediately after removing the neck fragment, an ample amount of bone wax (W31C, Ethicon, US) was applied to the distal cut surface only in wax group. The wax remained in place during the subsequent head removal, acetabulum reaming, cup impaction and fixation, and posterior capsular release for proximal femoral exposure. When the femur was to be broached, the wax was completely removed and discarded. The stem was inserted, and stability was tested. After complete hemostasis, the wound was irrigated and closed in a layered fashion. No drain was used. In this trial, uncemented stems (Fitmore, M/L Taper, and Wagner cone) and uncemented cups (Continuum and Trilogy) were used (Zimmer Biomet®, Warsaw, US).

Sample Size

As no previous similar study was found, we calculated the sample size based on the results of the study by Moo et al on the efficacy of bone wax in reducing blood loss 72 hours after total knee arthroplasty. Considering a significance level of 0.05 ($\alpha=0.95$), a power of 0.9 ($\beta=0.1$), and an allocation ratio ($k$) of 1, the sample size ($n_1=n_2$) was calculated so that if the difference between
the mean 72-hour blood loss of two groups ($\Delta = \mu_1 - \mu_2 = 1183.5 - 987.9$) became greater than 196 mL, it would be statistically significant. The minimum size of each group was determined to be 64. However, due to possible exclusions and lost cases to follow-up, we considered a sample size of 90 in each group for randomization.

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n_1 = \frac{(\sigma_1^2 + \sigma_2^2)\left(z_{1-\alpha} + z_{1-\beta}\right)^2}{\Delta^2}
\]

**Statistical Analysis**

Continuous variables except PBL were presented as mean ± standard deviation (range), and categorical variables as absolute (relative) frequency. Normality was assessed by the Shapiro-Wilk test. Independent-samples $t$-test was used to compare continuous variables except for PBL. Due to non-normal distribution, it was compared by Mann-Whitney U test and presented as median (interquartile range, IQR). Chi-squared and Fischer’s exact tests were used for categorical variables. All the statistical tests were performed by SPSS version 26. The tests were all two-sided, and the significance level was 0.05.