



## ARAŞTIRMA / RESEARCH

### Long term effect of general and regional anesthesia on bone turnover markers and fracture healing in adult patients

Erişkin hastalarda genel ve rejyonel anestezinin kemik belirteçleri ve kırık iyileşmesi üzerine uzun dönem etkileri

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#### Abstract

**Purpose:** Bone metabolism can be monitored quantitatively by measuring bone turnover markers in serum and/or urine. We aimed to investigate long-term effect of the type of the anesthesia on bone turnover markers and fracture healing.

**Materials and Methods:** Thirty patients with American Society of Anesthesiologist physical status I-II whom were aged 40-70 years, scheduled for hip fracture were recruited. Patients were divided into the two groups as general anesthesia and regional anesthesia. Only morphine and tramadol were used for postoperative analgesia till the 12<sup>th</sup> weeks. Serum bone-specific alkaline phosphatase, osteocalcin,  $\beta$ -C terminal telopeptide and urine  $\beta$ -C terminal telopeptide levels were measured at preoperative, 4<sup>th</sup> week and 12<sup>th</sup> week of fracture.

**Results:** A total of 25 patients were eligible for the study. There were no statistically significant between groups for values of bone turnover markers at the time point of preoperative, 4<sup>th</sup> and 12<sup>th</sup> weeks. With using linear regression analysis, serum  $\beta$ -CTX levels at 12<sup>th</sup> week can be predict by 4<sup>th</sup> weeks  $\beta$ -CTX levels (R<sup>2</sup>: 0.944) and urine  $\beta$ -CTX levels at 12<sup>th</sup> week can be predict by first week level.

**Conclusions:** This pilot study showed that both general and regional anesthesia has similar effect on bone turnover markers and fracture healing.

**Keywords:** Bone turnover markers, fracture healing, general anesthesia, regional anesthesia

#### Öz

**Amaç:** Kemik metabolizması serum ve/veya idrarda kemik belirteçlerinin ölçülmesi ile kantitatif olarak izlenebilmektedir. Anestezi tipinin kemik belirteçleri ve kırık iyileşmesi üzerine uzun dönem etkilerini araştırmayı amaçladık.

**Gereç ve Yöntem:** Amerikan Anestezi Cemiyeti klinik durum klasifikasyonu I-II olan 40-70 yaş arası kalça fraktürü nedeniyle opere olacak 30 hasta çalışmaya dahil edildi. Hastalar genel ve rejyonel anestezi olarak iki gruba ayrıldı. Postoperatif analjezi için 12<sup>th</sup> haftaya kadar sadece tramadol kullanıldı. Serum kemik spesifik alkalen fosfataz, osteokalsin,  $\beta$ -C terminal telopeptide ve idrar  $\beta$ -C terminal telopeptide düzeyleri postoperatif 4<sup>th</sup> ve 12<sup>th</sup> haftalarda ölçüldü.

**Bulgular:** Toplam 25 hasta çalışmaya uygun bulundu. Postoperatif 4<sup>th</sup> ve 12<sup>th</sup> haftalardaki kemik belirteçleri açısından gruplar arasında istatistiksel olarak anlamlı fark bulunamadı. Lineer regresyon analizi uygulandığında 4<sup>th</sup> haftadaki  $\beta$ -CTX düzeyi ile 12<sup>th</sup> haftadaki serum  $\beta$ -CTX seviyelerinin (R<sup>2</sup>: 0.944) ve ilk haftadaki idrar  $\beta$ -CTX düzeyleri ile 12<sup>th</sup> haftadaki idrar  $\beta$ -CTX seviyelerinin tahmin edilebileceği tespit edildi.

**Sonuç:** Bu pilot çalışma genel ve rejyonel anestezinin kemik belirteçleri ve kırık iyileşmesi üzerine benzer etkisi olduğunu göstermiştir.

**Anahtar kelimeler:** Kemik belirteçleri, kırık iyileşmesi, genel anestezi, rejyonel anestezi

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## INTRODUCTION

Bone metabolism can be followed by serum and urine turnover markers<sup>1,2</sup>. Formation and resorption are balanced during normal homeostasis of bone. At the stage of bone formation process, formation markers such as bone-specific alkaline phosphatase (BAP), osteocalcin (OC) and N-terminal propeptide of type I collagen released from osteoblasts and broken matrix components. Bone resorption markers such as crosslinked C-(CTX) and N-(NTX) telopeptides of type I collagen indicate bone catabolic process. Both formation and resorption rates rise up at the repair process of bone fractures<sup>3</sup>. Increasing in bone metabolism can be detected as elevation on bone turnover markers (BTM). Higher levels of BTM are associated with bone loss and elevated risk of future fracture<sup>4,5</sup>.

But some studies showed that increasing BTMs are not independently associated with the risk of hip or nonspine fracture<sup>4,6</sup>. A large meta-analysis showed that the GR (gradient of risk) for hip fracture is similar both sexes and bone mineral density (BMD) is a risk factor for fracture. Also fracture risk with BMD is age dependent<sup>7</sup>.

So many factors such as age, gender, fracture type and size, osteoporosis, different methods of surgical stabilization, BMD and drug therapies may affect fracture healing<sup>8</sup>.

Regional techniques for orthopedic surgery have many advantages both patients and surgeons<sup>9</sup>. Particularly, regional anesthesia can provide good pain control and better recovery process<sup>10</sup>. However, long term effect of type of anesthesia on BTM and fracture healing unknown.

In this pilot study, we aimed to investigate effect of type of anesthesia on BTM and fracture healing. Primary objective of this pilot study is evaluation to effect of regional and general anesthesia on BTM in patients who underwent orthopedic surgery with hip fracture during fracture healing process. Secondary objective is effect of the type of anesthesia on fracture healing.

## MATERIALS AND METHODS

Our study protocol was registered at clinicaltrials.gov (principal investigator's name: EB, and identifier: NCT02621255) on November, 2015 and approved by ethics committee (decision number: 41/10 and

Date: June 18, 2015). After obtaining written informed consent, 30 patients with ASA I to II between to ages of 40-70 years, scheduled for hip fracture were recruited. Exclusion criteria were the presence of malignancy, inflammatory disease, polytrauma, kidney failure, drug usage which can affect bone metabolism [estrogen, nonsteroidal anti-inflammatory drugs (NSAID), calcium, anticonvulsants and vitamin D] and fracture history in the past and ASA III-IV patients.

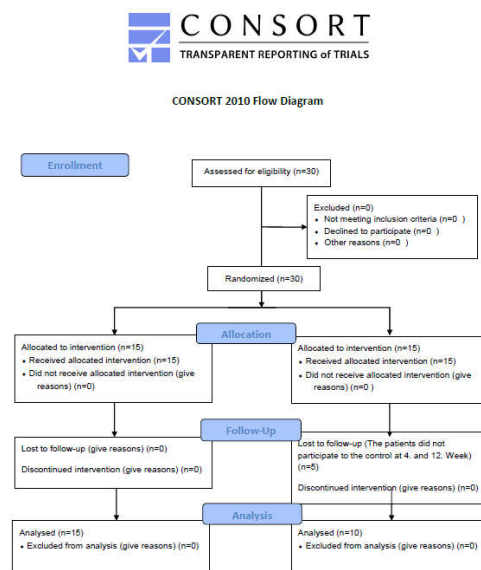


Figure-1: Consort flow diagram

First blood samples were taken in a fasting state after 24 h of injury preoperatively. All patients were randomly allocated in to the two groups as group general anesthesia (Group G) and group regional anesthesia (Group R) with computer-generated randomization list.

After admitted to the operation room, all patients were monitored by non-invasive arterial blood pressure, electrocardiogram, and peripheral oxygen saturation (SpO<sub>2</sub>) (Draeger-Primus Anesthesia Device Monitor, Draeger Medical Systems, Inc., Denver MA). An intravenous (IV) cannula (18 G) was inserted.

General anesthesia was performed for first group of patients (Group G). Propofol 2 mg/kg and rocuronium 0.6 mg/kg intravenously (IV) were applied for anesthesia induction. Anesthesia

maintenance was provided with sevoflurane 1-2 % and NO<sub>2</sub>-O<sub>2</sub> 50/50 % mixture. 1 h before end of the surgery morphine 1 mg kg IV was given for postoperative analgesia. Patient controlled analgesia PCA was provided with morphine (1 mg bolus morphine, 20 min lockout time). PCA will be continued until 24 h at the postoperative period.

Combined epidural and spinal anesthesia was performed for second group of patients (Group R). Lumbar epidural-spinal catheter was inserted at the L3-4 or L4-5 level. Bupivacaine 15 mg+ 20 µg fentanyl applied to the spinal space and epidural catheter inserted to the epidural space. If block is not enough 0.5 % bupivacaine 10 ml was given for adequate anesthesia during surgery. Postoperative analgesia provided with morphine PCA (1 mg bolus morphine, 20 min lockout time, 10 mg limit for 4 h) and PCA continued until 24 h at the postoperative period.

After 24 h analgesia was provided with tramadol 100 mg per orally twice a day. Postoperative first walking time was recorded for all patients.

NSAID was not applied any phase of study. Use of NSAID up to 12 weeks was forbidden for all patients. All patients were warned that they could only use tramadol until 12 weeks for pain relief.

Fracture healing was followed by clinical evaluation and radiography at the postoperative period.

BAP, OC and β-CTX levels were measured at the 24 h of injury, 4<sup>th</sup> weeks and 12<sup>th</sup> weeks.

β-CTX levels were measured from serum and urine. BAP and OC levels were measured from serum. Also blood urea nitrogen (BUN), creatinine, calcium, potassium were measured at the same time points.

### Specimen collection

Fasting blood and first urine samples were obtained from patients and collected in 5 mL vacuum collection tubes with no anticoagulant (Becton Dickinson Vacutainer® ref. 369032). The tubes with no anticoagulant was allowed to clot at room temperature for 15-20 min and separated by centrifugation at 3000 r.p.m for 20 min. and the sample was frozen (-20°C) and analyzed within six month.

When specimens were available, they were thawed, mixed, again centrifuged (at 3000 r.p.m for 20 min) and analyzed at room temperature. The urine and serum samples were thawed once.

### Biochemical marker measurements

The collected serum and urine samples were studied using a Human C- telopeptide of type I collagen (CTX- I) ELISA kit (Catalogue No. : 201-12-1350) and serum samples were studied using a Human bone alkaline phosphatase (BALP) ELISA kit (Catalogue No. : 201-12-1494) according to the manufacturer's instructions (Sunred Biological Technology Co., Ltd. Shanghai PR China). For the samples terminating with the enzyme substrate reaction, the samples were spectrophotometrically measured at a wavelength of 450 nm with Triturus Instruments (Barcelona, Spain). Detection ranges of this kit were 6ng/ml - 1500ng/ml for CTX- I and 3U/L - 900U/L for BALP.

Serum osteocalcin levels were analyzed with electrochemiluminescence immunoassay (ECLIA) using a standard assay kit for in vitro diagnostics (kit catalogue number: 12149133122). Reactions and quantification were performed by using a fully-automatized Cobas e 411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Reference values were 0-1000 ng ml<sup>-1</sup> for males and females.

Serum creatinine was analyzed with Beckman Coulter kits (CA), which were manufactured to use on Beckman UniCel DXC 800 Synchron (Beckman Coulter Inc., CA, USA) auto-analyzer. Creatinine: Jaffe method (rate-blanked kinetic alkaline picrate) measured at 520 nm, at 37°C (ref: 472525). Reference values were 0.3-1 mg/dl for 0 to 19 age's males, 0.7-1.2 mg/dl for 20 to 120 age's males and 0.4-1 mg/dl for 20 to 120 age's females.

### Radiological assessment

Radiological assessment of fracture union was carried out by same orthopedic surgeon and radiologist who interest in musculoskeletal imaging. Direct radiography was taken immediately after the fracture and at 4<sup>th</sup> and 12<sup>th</sup> weeks.

At the point of 12<sup>th</sup> weeks bone mineral density (BMD) was detected with bone densitometry. T-score < -2.5 was accepted as osteoporosis according to the World Health Organization criteria for diagnosis<sup>11</sup>.

### Statistical analysis

All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package

(IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp). Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and minimum-maximum where appropriate. The normality of distribution for continuous variables was confirmed with the Shapiro Wilk test.

Chi-square test was used to compare categorical variables between the groups. For comparison of continuous variables between two groups, the Student's t-test or Mann-Whitney U test was used depending on whether the statistical hypotheses were fulfilled or not.

To evaluate the change in the measurements obtained in the time interval, the Repeated Measurements Analysis was applied. To evaluate the correlations between measurements, Pearson Correlation Coefficient or Spearman Rank Correlation Coefficient was used depending on whether the statistical hypotheses were fulfilled or not. For each BTM, Linear regression analysis was applied to predict the 12<sup>th</sup> week values of it. The statistical level of significance for all tests was considered to be 0.05.

## RESULTS

Thirty patients were recruited to this pilot study but 25 patients could complete to the study, and data from 25 patients were used in the statistical analysis. (Figure 1) Demographic data was similar between two groups. (Table 1)

**Table 1. Demographic Data of the Groups and Type and Duration of Surgery**

	Group I (n=18)	Group II(n=17)	p
Age (yr)	58.2±17	61.4±11	0.069 <sup>a</sup>
Gender, n (%)			0,5 <sup>b</sup>
Male	7(39)	9(53)	
Female	11(61)	8(47)	
Type of surgery			
Totally hip replacement	12(67)	11(65)	
Partially hip replacement	6(33)	6(35)	
Postoperative discharge time			
2 <sup>nd</sup> day	1 (5)	2 (12)	
3 <sup>rd</sup> day	8 (44)	7 (41)	
4 <sup>th</sup> day	4 (22)	4 (23)	
5 <sup>th</sup> day	4(22)	3 (17)	
6 <sup>th</sup> day	1 (5)	1 (5)	

Percentages are presented as % within group.; <sup>a</sup> Independent sample T test.; <sup>b</sup> Chi-Square test.

**Table 2. Bone Turnover Markers and BMD-T score levels**

Formation Markers	Group G (N=15)			Group R(N=10)		
	Preoperative	4.th week	12.th week	Preoperative	4.th week	12.th week
S-Bone ALP(U/L)	28.8±15.07	28.8±12.27	30.38±12.51	29.02±12.14	29.64±7.72	31.4±11.31
(P value)	(0.605)	(0.461)	(0.765)			
Osteocalcin	24.34±11.79	25±7.9	27.48±9.3	20.94±10.63	23.82±7.5	23.75±6.7
(P value)	(0.428)	(0.849)	(0.311)			
Resorption Markers						
S-CTX(ng/L)	332.26±190.4 3	362.26±179.49	359.53±194.57	442.6±266.49	478.8±216.02	473.3±197.3
(P value)	(0.285)	(0.160)	(0.238)			
U-CTX(ng/L)	436.4±179.9	477.46±73.73	513.06±53.74	426±189.37	426.4±168.4	427.5±162.64
(P value)	(0.567)	(0.765)	(0.160)			
Mean T score		-0.65±0.73		-0.74±1.78		
BMD		0.85±0.01		0.83±0.09		
(p value)		(0.935)				

Data are presented as Mean±SD; Mann Whitney U test was used; S-Bone ALP; serum bone alkaline phosphatase, S-CTX; serum C-telopeptide, U-CTX; urine C-telopeptide, BMD; bone mineral density.

Study patients were 10 males (40%) and 15 female (60%). There was no statistically significance between

groups for the levels of serum BAP, serum OC, serum  $\beta$ -CTX and urine  $\beta$ -CTX at the time of 24<sup>th</sup> h,

4<sup>th</sup> week and 12<sup>th</sup> weeks. Linear regression analysis was showed that serum  $\beta$ -CTX levels at 12<sup>th</sup> week can be predict by 4<sup>th</sup> weeks  $\beta$ -CTX levels (R2: 0.944) and urine  $\beta$ -CTX levels at 12<sup>th</sup> week can be predict by first week level. BUN, creatinine, calcium and potassium levels were normal and similar between groups at the time point of 24<sup>th</sup> h, 4<sup>th</sup> and 12<sup>th</sup> weeks. All study patients were walked at the first 24<sup>th</sup> hours postoperatively

Radiographic evaluations were normal for all groups till the 12<sup>th</sup> weeks. There was no difference for radiographically assessment of fracture healing for all patients. At the time point of 12<sup>th</sup> weeks, BMD was similar between groups. BMD was  $0.85 \pm 0.01$  for group G and  $0.83 \pm 0.09$  for group R ( $p=0.605$ ). T score was  $-0.65 \pm 0.73$  for group G and  $-0.74 \pm 1.78$  for group R ( $p=0.935$ ). Bone turnover markers, BMD and T scores were showed in Table 2.

## DISCUSSION

In this pilot study, long term effect of general and regional anesthesia on BTM and fracture healing were investigated. All patients were followed up for 12 weeks and found that the levels of serum BAP, serum OC, serum  $\beta$ -CTX and urine  $\beta$ -CTX were similar between groups of general and regional anesthesia. All values of the BTM increased over the time and this acceleration was similar previous studies<sup>12,13</sup>. This pilot study showed that serum  $\beta$ -CTX levels at 12<sup>th</sup> week can be predict by 4<sup>th</sup> weeks  $\beta$ -CTX levels (R2: 0.944) and urine  $\beta$ -CTX levels at 12<sup>th</sup> week can be predict by first week level.

Biochemical BTMs have long been used with radiological assessment for treatment to fracture<sup>14,15</sup>.

Kumar et al investigated prognostic potential of BTM in delayed-healing tibial diaphyseal fractures and showed that serial monitoring of the biochemical markers of bone turnover can be used with radiological and clinical observation for predict delayed union formation<sup>16</sup>. They also observed that both bone formation and resorption markers peaked at the eighth week following the fracture.

All turnover markers elevates at fracture healing process. During the fracture healing process, resorption markers increase at earlier stage of the fracture, due to the osteoclastic removal of the necrotic tissues. Formation markers increase with osteoblast proliferation at the subsequent sequence<sup>17</sup>.

The bone resorption marker of  $\beta$ -CTX shows an earlier rise (week 2,  $139 \pm 33\%$ ) than the bone formation markers<sup>18</sup>. Ivaska et al showed that a few hours after fracture, BTM's were not altered from preinjury levels. But, both bone formation and resorption markers were significantly increased 4 month after fracture. The elevation was most pronounced after hip fracture. Bone turnover can be remained higher up to 12 month after fracture<sup>19</sup>. Serum alkaline phosphatase concentration can rising to a peak at 4<sup>th</sup> week<sup>20</sup>.

Nicholson G et al investigated effect of different anesthesia techniques such as propofol, propofol plus 'three-in-one block' or etomidate as part of a general anesthetic technique on plasma OC and serum BAP, interleukine-6, plasma epinephrine, norepinephrine and cystatin C concentrations for up to 3 days after major orthopedic surgery<sup>21</sup>. They showed that major orthopedic surgery results in significant decrease in OC and inhibition of cortisol response to surgery could not prevent a decrease in plasma OC concentration. This study represented short time effect of type of anesthesia on OC and hormonal, metabolic, inflammatory process at the major orthopedic surgery. However, the long term effects of anesthetic technique did not investigated. Regional anesthesia technique has several physiological advantages such as optimal pain relief, attenuation of proinflammatory and endocrine stress response, improved tissue and coronary perfusion, less inhibition of diaphragm activity<sup>22</sup>. This technique looks like superior than general anesthesia in early postoperative time<sup>23,24</sup>. However long-term effects of regional anesthesia are still investigating<sup>25</sup>.

In this study, the authors prohibit to the drugs which can affect to bone metabolism such as NSAID. Because, some animal models showed that NSAID's and cyclooxygenase-2 inhibitors can impair fracture healing in rats<sup>26-28</sup>. However, the effect of NSAID's and cyclooxygenase-2 inhibitors on fracture healing in human is controversy<sup>29</sup>. Tramadol did not significantly inhibit human osteoblast activity in vitro<sup>30</sup>. Therefore, we preferred to use tramadol for postoperative analgesia.

This pilot study showed that long term effect of general and regional anesthesia on BTM and fracture healing is similar. But it has several limitations such as small sample size, lack of cortisol level detection and personal physiological differences. Further

studies need to clarify effect of anesthesia on BTM and fracture healing.

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**Author Contributions:** Concept/Design : GÖ; Data acquisition: GÖ; Data analysis and interpretation: GÖ; Drafting manuscript: GÖ; Critical revision of manuscript: GÖ; Final approval and accountability: GÖ; Technical or material support: GÖ; Supervision: GÖ; Securing funding (if available): n/a.

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