

Effect of low-dose ketamine on post-operative serum IL-6 production among elective surgical patients: a randomized clinical trial.

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Abstract

Background: Surgery and Anesthesia cause an excessive pro-inflammatory response. Mulago Hospital is faced with staff shortage making post-operative pain management difficult. Interleukin-6 (IL-6) drives inflammatory pain, endothelial cell dysfunction and fibrogenesis. Ketamine is cheap and, readily available. We hypothesized that its attenuation of serum IL-6 was a surrogate for clinical benefit.

Materials and methods: Institutional Review Board's approval was sought and RCT was registered at clinicaltrials.gov (identifier number: NCT01339065). Consenting patients were randomized to receive pre-incision intravenous ketamine - 0.5mg/kg or 0.9% saline placebo in weighted dosing. Blood samples were collected and laboratory analyzed at baseline, post-operatively in PACU, 24 and 48 hours respectively.

Results: We recruited 39 patients of whom 18 were randomized to the ketamine arm and 21 in the placebo arm with follow up at 24 and 48 hours. Serum IL-6 and IL-1 β levels were analyzed using ELISA assay of pre-coated micro wells. Ketamine suppressed serum IL-6 at PACU with reduced increase at 24 hours. There was no reaction in 98% of IL-1 β assayed.

Conclusion: Low-dose ketamine attenuated early serum IL-6 levels due to surgical response with reduced 24 hour increase, but the difference was not statistically significant and we recommend more studies.

Keywords: Ketamine, post-operative inflammation, interleukin 6, interleukin 1- β .

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Introduction

Anaesthesia and surgery cause immune system impairment expressed as excessive pro-inflammatory responses leading to exacerbated pain, poor wound healing, psychological stress coupled with complications such as sepsis or acute respiratory distress syndrome (ARDS)¹⁻⁴. Post-operative pain management remains a challenge even in advanced health systems like the USA, where 50-

70% patients experience post-operative pain⁵. Enhanced surgical inflammation and pain is mediated by hormonal and cytokine cascade responses^{6,7}. Interleukin 6 (IL-6) affects the immune system homeostatic processes with context-dependent pro- and anti-inflammatory properties that have become a prominent target for clinical intervention to improve disease outcome and patient well-being by focusing on how and when to block it⁸.

Mulago National Referral and Teaching Hospital (MN-RTH) is Uganda's only tertiary hospital with a bed capacity of 1500 beds and an accident and emergency department that receives about 48,000 patients per year⁹. There is poor staff motivation, overcrowding, limited quality assurance and a cumbersome procurement system¹⁰. This is compounded by a poorly funded health system due to meagre resources as Uganda is classified as a Low Income Country (LIC) and Heavily Indebted Poor Country (HIPC)¹¹.

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Ketamine is an N-Methyl-D-Aspartate (NMDA) receptor antagonist which in low sub-anaesthetic doses produces analgesia by desensitization of sensitized Central Nervous System (CNS) NMDA receptors thus inhibiting pain transmission¹²⁻¹⁴. Ketamine is used as a 'third line' treatment for chronic pain relief when conventional treatments have failed¹⁴. It is the favoured induction anaesthetic for non-contraindicated and hypovolemic patients due to its catecholamine release effects and sedation^{15,16}. It also attenuates post-operative delirium after major cardiac surgery¹⁷.

Surgical inflammation is consistently associated with IL-6 whose serum levels increase by 1–3 hours, peaking at 4–24 hours and remaining elevated for 48–72 hours¹⁸. The greater the surgical trauma, the greater the serum IL-6 response e.g. abdominal surgery produces a bigger response than hip replacement¹⁹. Comparatively IL-1 β and TNF are detectable in much lower serum levels than IL-6 after elective surgery although in both haemorrhagic and septic shock, results have shown significantly elevated levels of IL-1 β , TNF and IL-6 with increased risk of ARDS, multiple organ failure and death²⁰⁻²². Ketamine affects post-operative inflammation in cardiopulmonary bypass by cytokine production attenuation²³. We thus hypothesized that pre-incision low dose intravenous ketamine would attenuate post-operative IL-6 and IL-1 β production. This reduction would be a surrogate of good post-operative clinical course as studies showed good post-operative pain reduction when using ketamine as an adjuvant in multi-modal management strategy²⁴.

Materials and methods

This study was approved by the Makerere University's School of Medicine Research and Ethics Committee (SOMREC) and was registered at clinicaltrials.gov (Identifier: NCT01339065). We conducted a prospective, randomized, double-blind, placebo-controlled trial from January to March 2011 among patients due for elective abdominal or perineal surgery in MNRTH.

We enrolled consenting patients with inclusion criteria of American society of anaesthesiologist (ASA) class 1 and 2, adult Patients (18-70 years) scheduled for elective abdominal or perineal surgery. We excluded hypertensives or those having blood pressures above 140/100 for 3 consistent readings 15 minutes apart, septic or febrile patients ($T >38^{\circ}\text{C}$), pheochromocytoma patients, neuro-surgery patients, epileptics, emergencies' or spinal and local infiltration anaesthesia plus those that received intra-operative blood transfusion.

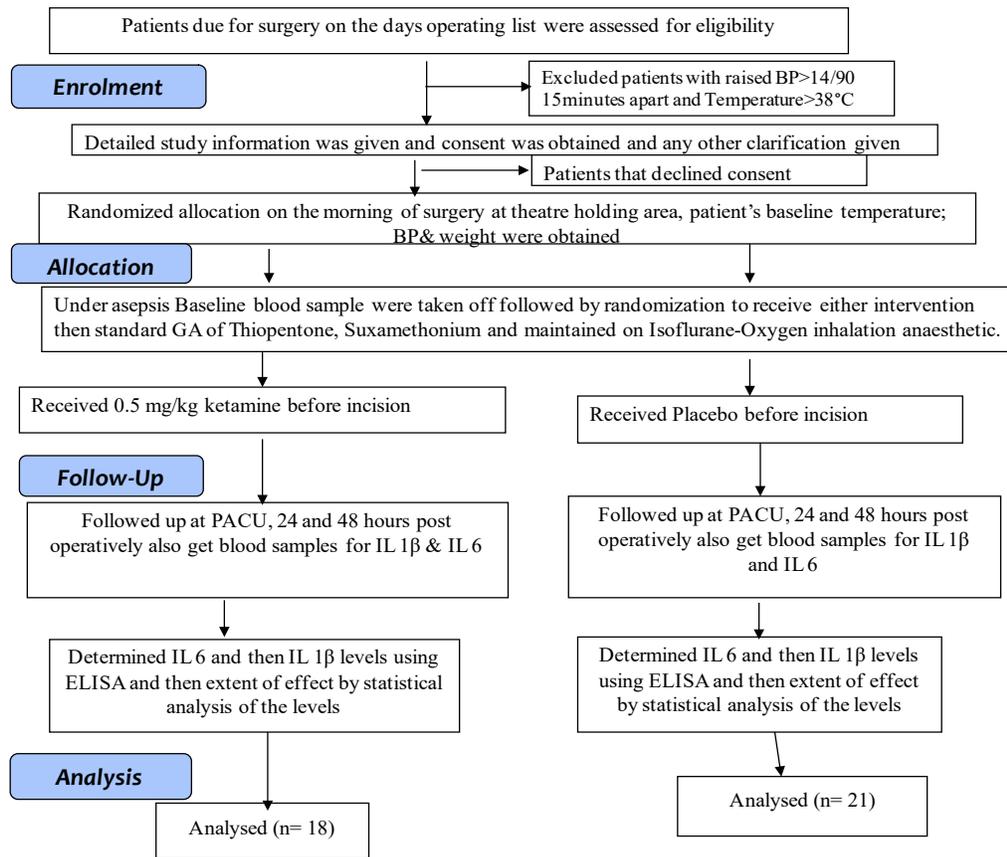
Sample size calculation was done using Stuart JP's formula basing on power of 90% with standard deviation of 75 of post-operative IL-6 level at 4 hours after surgery in the cardiopulmonary bypass study²³. This gave us a total patient estimate (N) of thirty (30) with fifteen (15) in each arm.

Randomization and concealment: Participants were randomly assigned according to body weight to receive either ketamine 0.5mg/kg or placebo using blocks of 4. A statistician independent of the study generated sequential random treatment codes using a computer program. To enable blinding, a pharmacist at a separate location pre-mixed the two interventions in similar brand and consistency sterile 10 mls syringes and these syringes had 10 mg/ml colourless solution that catered for a lowest possible weight of 20 kg and highest possible weight of 200 kg.

Concealment was achieved by labelling each syringe according to the sequential random treatment codes and placed in an opaque carrier bag that was brought to the main operating room (OR) on the morning of surgery.

Procedure: patients were taken through the consent process during the pre-operative visits and at the OR a syringe was blindly picked from the opaque carrier bag in presence of a theatre nurse with its code becoming the patient's study number. The patients' baseline weight, blood pressure and temperature were recorded as per flow chart.

APPENDIX II: FLOW CHART



In theatre a 5 mls baseline blood sample was collected under asepsis from an accessible vein and was stored at room temperature to allow some clotting before lab centrifuge for analysis. General anaesthesia was induced by slow thiopentone (2mg/kg) bolus, followed by opiate analgesia of intravenous morphine (0.1 mg/kg) and suxamethonium 100 mg for intubation then Isoflurane-oxygen mixture was used for anaesthesia maintenance. At pre-incision, we administered the clear colourless syringe contents to patients according to randomization. Fluid and volume replacement and monitoring were done as per MNRTH OR protocols. Surgery duration was noted and patients were extubated awake at the end of surgery. Post-operatively, patients were monitored in the post-anaesthesia care unit (PACU) where a second 5 mls of blood was collected before transfer back to ward. Patients were followed up at 24 and 48 hours for third and fourth sample collection respectively with clinical patient assessment. Tramadol was our post-operative pain analgesic for the study duration after which they reverted back to MNRTH standard of care i.e. intramuscular diclofenac or pethidine. The collected samples were centrifuged then stored at sub-zero temperatures for standard ELISA analysis at Makerere University's licenced and internationally

certified immunology laboratory as per procured ELIZA kits (Biolegend- San Diego, CA, USA). This was done under strict protocols as per international Quality Assurance (QA) standards under the laboratory manager's supervision. The incubated samples were read by ELIZA readers using optical densities, first for blanks then standard dilutions of pre-packed for IL-1beta or IL-6 ELIZA antibody reagents from Bio legend- San Diego, CA, USA. The blanks and standard optical densities were used as the Y-axis with corresponding concentrations in the X-axis and a standard curve was calculated using a computer generated 4-PL curve-fit to get a best fit curve through these points and the interleukin concentrations were read off according to their corresponding Optical density from ELIZA reader (See Bar graph1) .

Data collection and management: Interviewer-administered and pre-tested questionnaires were used for data collection. The data was cleaned, coded, and double-entered into Epidata version 3.1 using Epi-Info 6.04® and analysed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). The participants' characteristics were summarized using means, medians and standard deviations that were presented using tables and histograms. Categorical vari-

ables were summarized using proportions, percentages and presented using pie charts or bar charts.

Our primary specific objective was to determine change in levels of IL- 6 inflammatory marker after surgery from baseline, immediately after surgery in PACU, at 24 hours and 48 hours. Our secondary objective was to assess the IL- 1 β levels at similar time points.

Data was analysed using ANOVA or the Kruskal–Wallis tests as appropriate for association between changes of levels of IL-6 and IL-1 β with each predictor, proportions compared using chi-square and odds ratios. Data was assessed for normal distribution of variance using nor-

mality plots and the Kolmogorov–Smirnov test. Categorical data was analysed using Fisher’s exact test and naive pooled analysis was performed for each subject providing one data point for the fit. A p-value of ≤ 0.05 was considered statistically significant

Results

We enrolled 39 patients that were randomized to receive 0.5mg/kg of ketamine (18) or 0.9% normal saline placebo (21) respectively. The baseline characteristics were similar in both groups i.e. age, sex, ASA score and type of surgery. The majority of patients were aged 18-28 years (Table 1).

Table 1: Age characteristics of enrolled patients

Age group (years)	Ketamine n[%]	Placebo n[%]
18-28	4 (25)	10 (43.5%)
29-38	5 (31.3)	3 (13%)
39-48	2 (12.5)	4 (17.4)
49-58	3 (18.8)	2 (8.7)
58-70	2 (12.5)	4 (17.4)

Mean weight was 60 kgs with mean surgery duration of 1 hour (shortest procedure being 20 minutes for examination under anaesthesia and longest being 4.5 hours for ureteroplasty). Statistically no relevant change was no-

ticed in clinical variables of our study participants as vital signs did not deviate from normal values during and after surgery (table 2 and table 3). Gender had no statistically significant bearing on the outcome ($p= 0.522$).

Table 2: Baseline characteristics for study participants

Age in years	n (%)	Percent
18 - 28	14(35.9)	35.9
29 - 39	9 (23.08)	23.08
Over 40	16(41.03)	41.03
Gender		
Male	22(56.41)	
Female	17(43.59)	
	Mean \pm(SD)	
Weight (Kgs)	59.67 \pm 9.76	
Time taken for the surgery (hrs)	1.20 \pm 1.09	
MAP	88.82 \pm 8.82	
NIBP (mmHg)	127.38/74.08 \pm 11.25/12.52	
HR	86.77 \pm 14.76	
SPO2	99.41 \pm 0.94	

Table 3: Clinical characteristics before and after surgery

Clinical Parameter	Before (n +/- SD)	After(n +/- SD)
MAP (mmHg)	90 +/- 9	90 +/- 8
Systolic BP	130 +/- 11	126 +/- 11
Diastolic BP	75 +/- 13	71 +/- 13
HR	84 +/-15	95 +/- 15
SPO2	100 +/- 1	100 +/- 1

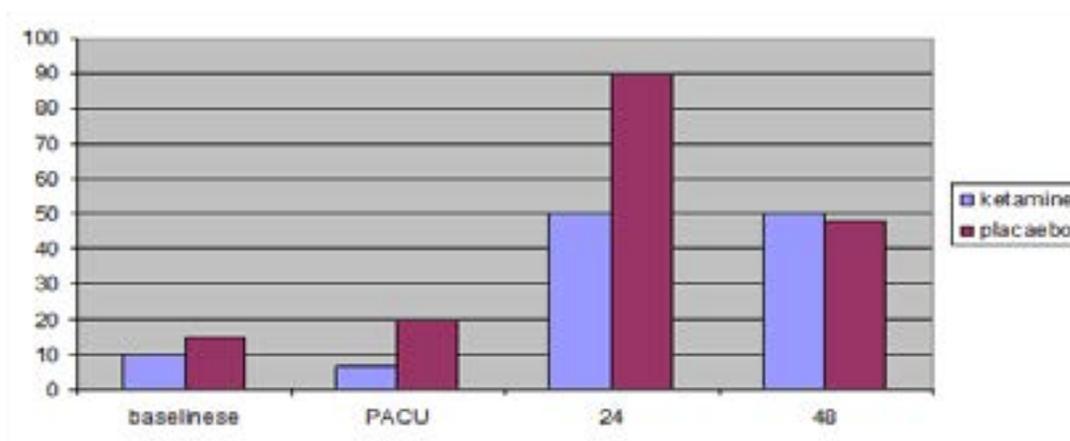
On sample analysis in the laboratory, we noted that IL-6 levels in the ketamine group dropped from baseline in the PACU while those in the placebo group started increasing. At 24 hours ELISA analysis showed a median IL-6 level increase in both groups with a much higher increase

in the placebo group (90 +/- 167 picogrammes/ml) than in the ketamine group (50 +/- 285 picogrammes/ml). At 48 hours IL-6 median concentrations showed a drop in the placebo group while the ketamine group remained stable (table 4 and figure 1).

Table 4: Changes in IL-6 levels in both groups from base line to 48 hours

COLLECTION TIME	KETAMINE GROUP Median IL-6 pg/ml (n± SD)	PLACEBO GROUP Median IL-6 pg/ml (n± SD)	p-value
Baseline	10 ±167	15 ±261	0.508
PACU	6.5 ± 232	20 ± 171	0.412
24 hours	50 ± 285	90 ± 167	0.402
48 hours	50 ± 316	48 ±54	0.373

Figure 1: Median serum IL-6 levels for primary outcome

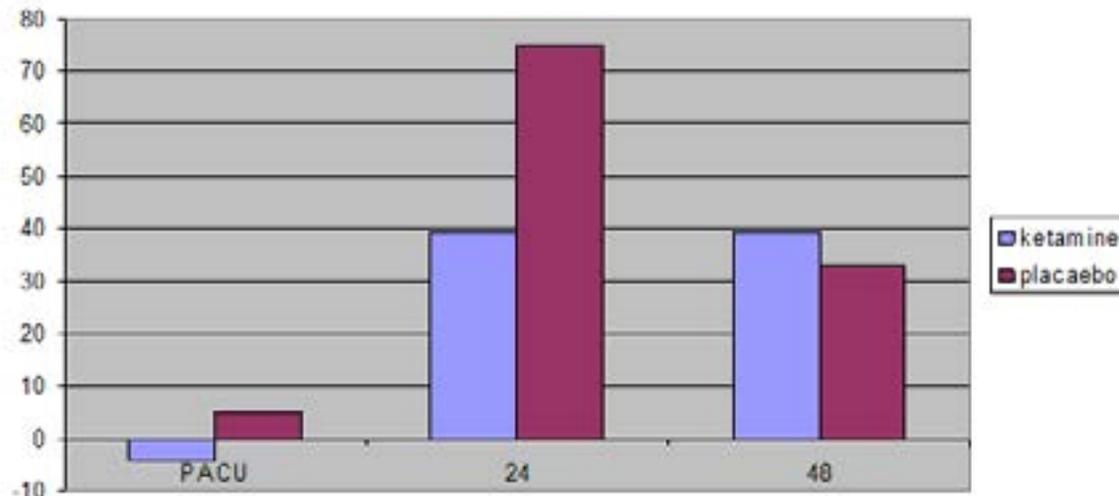


Generally, when compared to the ketamine group the placebo group showed IL-6 increase from baseline peaking at 24 hours as illustrated in figure 2. However the above findings had P-value of ≥ 0.05 which was statistically insignificant. There were no detectable ELISA readings

in 98% IL-1 β pre-coated antibodies micro wells analysed. We had no mortality at the end of the study.

Side effects: There was one case of excess salivation after induction which had 0.4mg of atropine administered and suctioning done. We also had a PACU case of awakening hallucination managed with diazepam 1mg.

Figure 2: Showing median changes from baseline readings for IL-6



Discussion

This study was done to determine and compare the effect of pre-incision 0.5mg/kg ketamine on the post-operative serum IL-6 and IL-1 β cytokine levels. We found that ketamine group had an early marked reduction of IL-6 serum levels in the PACU with reduced elevation at 24 and 48 hours when compared to the placebo group, a finding similar to cardiac bypass surgery studies that showed IL-6 serum level reduction over a 7 day post-operative period²³. We focused on IL-6, its serum concentration is a good indicator of inflammatory cascade activation and a predictor of subsequent morbidity and mortality^{21,24,25}. Also cytokine attenuation reduces acute neutrophil infiltration which causes surgical inflammation followed by tissue damage from accumulation of neutrophil-secreted proteases and reactive oxygen-species²⁶.

Ketamine was chosen because it is inexpensive, readily available in MNRTH and it has multiple beneficial properties with regard to modulating inflammation as shown by systematic reviews²⁷. Its other clinical benefits such as its bronchodilator effects are used in ICU to improve severe bronchospasm, asthma exacerbation or status asthmaticus²⁸, and its anti-inflammatory effects render it the drug of choice for use in septic surgical patients^{29,30}.

Surgical tissue damage causes glutamate release sensitizing the central pain pathway by activation of post-synaptic NMDA receptors in the spinal cord, manifesting clinically as heightened pain sensation³¹ and ketamine's NMDA blockade hence reduces post-operative pain sensation.

The IL-6 serum level reduction would reduce patients morbidity i.e. pain and infections as it is involved in the acute phase response to injury and infection³². This explains our study's increased IL-6 levels for both the intervention and control group after 24-48 hours. Our postulated improvement of post-operative clinical course was also strengthened because the NMDA receptor that ketamine antagonises has a role in CNS processing of pain^{12,31}. Cytokine attenuation is also clinically beneficial in the paediatric setting for *Neisseria meningitidis* management with erythropoietin³³.

Limitations

Our study limitations include: (a) a small sample size that gave statistically insignificant but remarkable IL-6 serum level reduction, (b) inability to follow up other clinical outcomes past the 48 hours study time.

Conclusion and recommendations

Low-dose ketamine attenuated early IL-6 response to surgery with reduced increase at 24 and 48 hours, but the difference did not reach statistical significance probably because of small sample size and wide variations in IL-6 values.

We recommend further and larger sample sized studies to explore ketamine's post-operative benefits as pain management remains a challenge in Uganda with inadequate supply of analgesics among anesthetic providers with only 45% having either pethidine or morphine available and 21% never having them available³⁴. In MNRTH this is further compounded by overwhelming patient numbers and glaring staff shortages of a nurse: patient ratio of 1:40 at a given time³⁵.

Competing interests

This was an academic thesis research for Dr. TS Luggya's Masters of Medicine in Anaesthesia and Critical Care³⁶ and the authors declare that they have no competing interests.

Co- author contribution:

- JMK and TR supervised, guided and reviewed the research.
- LS and A Kintu assisted with patient recruitment, data management, lab follow up and final manuscript proof reading.
- JVBT and A Kwizera helped with conceptualization, proposal development and literature search guidance.

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