The effect of anaesthetic techniques on neutrophil to lymphocyte ratio in patients undergoing infraumbilical surgeries.

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**Running Title:** NLR ratio and type of Anesthesia
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Abstract

Background: Neutrophil/lymphocyte ratio (NLR) is a simple marker in peripheral blood and is used as a parameter to assess inflammatory response and physiological stress during perioperative period. Anesthetic technique may influence NLR, thereby modulating inflammatory response and surgical outcome. The aim of this study was to evaluate the relationship between blood NLR and anesthetic techniques in patients undergoing infraumbilical surgeries.

Methods: Institutional Ethical Committee clearance and patient consent was taken. Prospective randomized double-blinded study was conducted from July 2017–November 2017, involving 80 ASA 1 and 2 patients, aged 18–60 years scheduled for elective infraumbilical surgeries. Patients refusal, those with ongoing acute infections, electrolyte abnormalities were excluded from study. The patients were randomized into 2 groups, Group G receiving general anesthesia and Group S receiving spinal anesthesia as per standardized protocol. In both groups, differential counts of leukocytes with NLR of peripheral blood were obtained preoperatively on the morning of surgery, 2 hours after surgery and 24 hours after surgery. The data was noted and analysed using appropriate statistical tests.

Results: Demographic parameters and basal Total Leukocyte Count (TLC) and NLR were comparable. TLC and NLR were significantly higher in Group G compared to Group S postoperatively. The post-operative increase in TLC and NLR from basal values was significantly higher in Group G compared to Group S.
Conclusions: General anesthesia is associated with greater increases in TLC and NLR compared to sub arachnoid block.

Keywords: General; Lymphocytes; Neutrophils; Spinal anesthesia.

Introduction

The neuroendocrine system is activated during anesthesia and surgery resulting in release of neuroendocrine hormones and cytokines [1–3]. Systemic leukocytic alterations, including leukocytosis, neutrophilia, and lymphopenia, may occur in response to surgery by various hormones, cytokines, and acute – phase reactants; apoptosis of lymphocytes; or inhibition of apoptosis of neutrophils [4,5]. During anesthesia and surgery there are changes in every stage of the immune system secondary to general physiological response, which are linked to the extent of surgery, age of the patient, general health, medications used and any blood transfusions performed. There are various tests for measuring neuroendocrine responses such as interleukin measurements (IL-6, TNF-α), cortisol levels, CRP, leptin levels, but the tests are expensive.

Neutrophil/lymphocyte ratio (NLR) is a simple and inexpensive marker for inflammatory response and provides relationship between inflammatory environment and physiological stress [6]. NLR has been found to be a simple predictor for outcomes in patients with cancers and also in patients with stable coronary artery disease [7,8]. NLR is not only affected by surgical trauma but also by anesthetic method [6,9] and normal value is found to vary in different populations viz 1.65 ± 0.79 - 2.8 ± 1.6 [10,11]. The effect of neuroendocrine changes induced by anesthetic techniques could influence NLR, but the effect remains largely unknown. Very few studies have been done to evaluate the effect of general anesthesia and spinal anesthesia on NLR. Therefore this study was conducted to evaluate effect of anesthetic techniques on leukocytic alterations.

Materials and Methods
After obtaining ethical committee clearance and informed written consent from patients, prospective, randomized controlled single blind study was conducted involving 80 patients aged 18–60 years, belonging to American Society of Anesthesiologists (ASA) grade I & II who were scheduled for elective open infraumbilical surgeries during July 2017–November 2017. The study was enrolled in clinical trials registry (CTRI/2018/05/013826).

Patients with ongoing acute infections, morbid obesity, chronic use of steroids or immunosuppressant or recent chemotherapy, history of endocrine disease, electrolyte abnormalities, hepatic and renal dysfunction, chronic alcohol abuse and surgeries lasting for more than 90 min were excluded from the study. Patients were randomly allocated to one of the two groups using numbers generated from www.random.org. Group G - received general anesthesia for infraumbilical surgery whereas Group S - received spinal anesthesia for infraumbilical surgery. Allocation concealment was ensured using sequentially numbered sealed envelopes, which was opened after shifting patient on operation table.

All patients were assessed preoperatively and informed thoroughly about both the techniques of anesthesia, their risks and benefits. Patients were kept fasting for 8 hours before surgery. Alprazolam 0.5 mg and Ranitidine 150 mg was given orally the previous night of surgery. Preoperative haemoglobin, total count, differential count and NLR were calculated apart from other investigations as required. On the day of surgery, intravenous (IV) access was established and an IV infusion of Ringer lactate was started. Once the envelope was opened, patients were informed about the technique they were about to receive and they were given choice to opt out of the study if they were not happy with the technique of anesthesia and routine standard of care was followed.

In group G, monitoring included electrocardiography (ECG), peripheral oxygen saturation (SpO₂), non-invasive blood pressure (NIBP), end tidal carbon dioxide, train of four (TOF). Monitors were connected to patients and baseline hemodynamic parameters were recorded. Patients were
premedicated with Glycopyrrolate 10 µg/kg, Midazolam 0.03 mg/kg, Fentanyl 2 µg/kg and preoxygenated for 3 min. Patients were induced with Propofol (2 mg/kg) IV followed by Vecuronium 0.1 mg/kg IV. After 3 minutes, intubation was done with appropriate sized endotracheal tube. Anesthesia was maintained with oxygen 33% in Nitrous Oxide 66% and Isoflurane 1–2%, which was titrated to maintain hemodynamic parameters within 20% of basal readings. Adequate muscle relaxation was ensured by maintaining TOF count < 2 with intermittent injections of vecuronium 0.02 mg/kg. At the end of surgery, muscle relaxation was reversed with Glycopyrrolate 10 µg/kg and Neostigmine 0.05 mg/kg IV and patients extubated when TOF ratio > 0.9.

In group S, ECG, NIBP and SpO₂ monitors were connected and baseline hemodynamic parameters were recorded. Intravenous line was started using 18 G IV cannula, 500 ml of Ringers Lactate solution was infused and Midazolam 0.03 mg/kg IV was administered before administration of spinal anesthesia. Using 25 G spinal needle lumbar puncture was done in L2-L3 or L3-L4 intervertebral space, after confirming free flow of cerebrospinal fluid, 0.5% hyperbaric bupivacaine (12.5 mg) was injected into the space.

In both groups, hemodynamic parameters (heart rate, systolic blood pressure, diastolic blood pressure, mean arterial pressure, SpO₂) were continuously monitored and recorded every 5 min till the end of surgery. Intraoperative fluid management was guided by hemodynamic changes and intraoperative blood loss. Post operatively, intravenous fluids, antibiotics and other medications were administered as per standard institutional protocol. Pain was treated with Paracetamol 1 gm intravenous infusion every 6 hours. Tramadol 50 mg IV was administered as rescue analgesic if pain persisted even after paracetamol administration. Post-operative nausea and vomiting was managed by administration of ondansetron 4 mg IV. Patient’s blood was drawn from ante cubital vein in the preoperatively on the morning of surgery, 2 hours after surgery and 24 hours after
surgery. Neutrophil and lymphocyte counts were derived from differential percentages of leukocytes measured by automatic cell counters. The calculation of NLR was entrusted to one of the anesthesiologists who were blinded to the group.

The sample size was calculated based on a previous study [14], we hypothesized that NLR would be lower in patients receiving spinal anesthesia compared to general anesthesia. Keeping two tailed type I ($\alpha$) error at 0.05, atleast 36 patients in each group would be required to detect a minimum difference of 1 in NLR between the two groups, at a power of 80%, assuming a standard deviation of 1.5 and NLR under General anesthesia to be 10 (based on previous study). We enrolled 40 patients in each group to compensate for drop outs.

Data entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Shapiro Wilk test was applied to assess for normal distribution of continuous variables. Categorical data are expressed as frequencies, continuous and discrete variables as mean (SD) or median (interquartile range). Chi square test and Fisher exact test was used for comparing categorical data. Independent sample $t$ test and Mann Whitney $U$ test were applied for continuous data with normal and skewed distribution respectively. Paired $t$ test was used for intragroup comparison of normally distributed data where as skewed data was compared using Wilcoxon sign rank test. P value of < 0.05 was considered significant.

**Results**

Eighty patients were considered for analysis as shown in CONSORT (Fig. 1). There were no drop outs. Demographic characteristics, duration of surgery and the type of surgeries in both groups were comparable. The maximum level of sensory block attained in Group S was between T6 and T8 in most of the patients (T4 in 7 patients, T6 in 17 patients, T8 in 12 patients and T10 in 4 patients). Baseline total leukocyte count (TLC) was comparable in both the groups and increased post operatively in both groups. Intergroup comparison showed significantly higher TLC in group G
compared to group S at 2 hours, but not at 24 hours (Table 2). The median (IQR-interquartile range) increase in TLC in group G was 600 (300–3325) cells/cu mm (P = 0.001) and 1100 (600–2875) cells/cu mm (P = 0.001) respectively at 2 and 24 hours respectively from baseline values, which was clinically and statistically significant. In group S, the median (IQR-interquartile range) increase in TLC was 300 (100–625) cells/cu mm (P = 0.68) at 2 hours and 535 (200–1250) cells/cu mm (P = 0.07) at 24 hours respectively from baseline, which was clinically and statistically not significant. The magnitude of change in TLC was significantly higher in Group G compared to group S at 2 hours (P = 0.002) (Fig. 2A) and 24 hours (P = 0.002) (Fig. 2B).

The baseline NLR was comparable in both the groups and increased in both the groups post-operatively. The NLR was significantly higher in group G compared to group S at 2 hours and 24 hours respectively (Table 2). The median [IQR] increase in NLR from baseline was 5 (2.73–7.16) (P < 0.001) at 2 hours and 4 (2.59–6.05) (P < 0.001) at 24 hours respectively in group G, which was clinically and statistically significant. The median [IQR] increase in NLR from baseline was 1 (0.24 –0.94) (P < 0.001) at 2 hours and 0 (0.13–1.01) (P = 0.002) at 24 hours respectively, which was clinically not significant. The magnitude of change in NLR was significantly higher in Group G compared to group S at 2 hours (P < 0.001) (Fig. 3A) and 24 hours (P < 0.001) (Fig. 3B).

There was no significant change in Haemoglobin concentration in preoperative and postoperative period in both the groups (Fig. 4). The median (IQR) change in haemoglobin was 0.4% (0.2– 0.625) (P = 0.001) at 2 hours and 0.45% (0.2–1.0) (P < 0.001) at 24 hours respectively in group G, where as in group S it was 0.2% (0.1%–0.85%) (P < 0.001) at 2 hours and 0.3% (0.2%–0.85%) (P < 0.001) at 24 hours respectively, which showed statistical significance but were not clinically significant. Intergroup comparison of magnitude of change in haemoglobin concentration did not show significant difference (at 2 hours P = 0.37, at 24 hours P = 0.36).
There was no significant difference in the heart rate and mean arterial pressure in between the two groups (Figs. 5A and 5B).

The mean volume of intravenous fluids administered was 940 ± 241.57 ml in group G and 1042.5 ± 227.45 ml in group S (p – 0.054).

Discussion

In the present study it was observed that spinal anesthesia was associated with significantly lower NLR ratios compared to general anesthesia and also the rise in total leucocyte count was lesser with spinal anesthesia compared to general anesthesia. Studies on stress caused by surgical trauma have reported a suppression of cellular resistance and susceptibility to inflammation. In addition the increase in postoperative leukocyte values and reduction in lymphocyte values increase the tendency for infection [12]. These leukocyte changes have been proposed to cause inflammatory cytokines similar to IL-6. Studies have shown that TLC and changes in leukocyte subtypes are important markers for morbidity and mortality in cancer patients, renal failure patients and cardiovascular patients [5,13,14].

Volatile agents have been found to modulate both proinflammatory and anti inflammatory mediators, thereby resulting in lower lymphocyte counts [15, 16]. The decreased neuroendocrine response to surgery with regional anesthesia [17] may explain the lesser increase in TLC and NLR in patients receiving spinal anesthesia.

Erbaş et al. [18] evaluated in a retrospective study, the relationship between blood Neutrophil /lymphocyte ratio and anesthetic techniques in patients undergoing caesarean section and concluded that postoperative neutrophil to lymphocyte ratio in patients undergoing caesarean section under spinal anesthesia was found to be significantly lower as compared to general anesthesia, but there are no studies reporting the effect of anesthetic technique on NLR ratio in patients undergoing non obstetric surgeries, till date, to the best of our knowledge. The observations of our study were
similar to that of Erbaş et al. TLC and NLR were significantly lower in Group S compared to Group G postoperatively. Another study found no increase in WBC following spinal anesthesia compared to General anesthesia [19]. In present study, though the increase in TLC was significant in general anesthesia compared to spinal anesthesia, the margin may not be high.

In a study investigating effect of two general anesthetic techniques on leukocytic alterations including neutrophil-to-lymphocyte (N/L) ratio, after gynecologic laparoscopy. Significant increase in total leukocytic count, neutrophil count, and decrease in lymphocytic count were observed at all time points after surgery in patients who received total intravenous anesthesia and in patients which received inhalational anesthesia. N/L ratio was significantly lower in total intravenous anesthesia group compared to inhalational anesthesia group [20].

A study assessing the correlation between NLR and post-operative complications after major abdominal surgeries found that NLR at day 7 correlated better with incidence of post-operative complications compared to other parameters including C reactive protein. The cut off for predicting post-operative complications was found to be 5.5 [21]. Where as another study found a preoperative NLR value of 2.3 or more to be associated with major postoperative complications in patients undergoing colorectal surgeries [22]. The baseline NLR in the present study was little higher than these values, which may be attributed to probable preoperative stress as the baseline samples were taken on the previous day of surgery before premedication. The type of anesthesia may have indirect effect on post-operative complications by influencing the NLR and hence appropriate choice of anesthesia may influence outcomes. However, a study assessing the effect of general and spinal anesthesia on stress response to hemorrhoidectomy found no significant difference related to the effects of general and spinal anesthesia except for increase in leptin levels [23]. This could be attributed to the magnitude of surgery. 8 patients included in the study had undergone open interval appendicectomy, they were included in the study as they were symptom
free (history of acute appendicitis 6 months back, managed conservatively) and had no features suggestive of acute infection at the time of surgery. The amount of intravenous fluids received by patients in spinal anesthesia group was marginally higher than in general anesthesia group. However, this might not have had significant diluent effect on cell counts.

There are limitations of the present study. Patients were not followed up beyond 24 hours to observe for wound site/other infections, as the post operative management was entirely based on surgical team protocol over which we did not have any control and hence the relationship between NLR ratio and incidence of post-operative infections and post-operative outcome could not be established. However, a retrospective analysis of records found 1 patient in general anesthesia group with urinary tract infection on post operative day 3, which improved with antibiotic treatment, and there was no delay in discharge from hospital. Other tests which could correlate with neuroendocrine response could not be done. Further studies are required to see if combination of a regional technique along with general anesthesia could influence the post-operative outcome by its effect on NLR, compared to general anesthesia alone.

In conclusion, spinal anesthesia is associated with significantly lesser variations in neutrophil to lymphocyte ratio and total leucocyte count compared to general anesthesia.

References


Table 1. Demographic and Surgical Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group G (n=40)</th>
<th>Group S (n=40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>40.13 ± 9.8</td>
<td>41.68 ± 10.42</td>
<td></td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>19:21</td>
<td>25:15</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.0 ± 6.6</td>
<td>63.8 ± 7.3</td>
<td></td>
</tr>
<tr>
<td>Height (cms)</td>
<td>156.78 ± 12.24</td>
<td>160.24 ± 13.41</td>
<td></td>
</tr>
<tr>
<td>ASA grade (I:II)</td>
<td>22:18</td>
<td>21:19</td>
<td></td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>46.5 ± 13.87</td>
<td>41.75 ± 11.52</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Type of surgery*

- Unilateral inguinal hernia: 7 vs. 15, P = 0.25
- Bilateral inguinal hernia: 3 vs. 6
- Incisional hernia: 10 vs. 4
- Umbilical hernia: 12 vs. 7
- Open interval appendicectomy: 4 vs. 4
- Gynaecological surgeries: 4 vs. 4

Values are expressed as mean ± SD or number of patients.
Table 2. Table Showing Total Leucocyte Count and Neutrophil to Lymphocyte Ratio in Both Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group G</th>
<th>Group S</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative total leucocyte count (cells/cu mm)</td>
<td>9017.25 ± 2873.96 (8098.11–9936.38)</td>
<td>9048.25 ± 2450.62 (8264.5–9831.99)</td>
<td>0.959*</td>
</tr>
<tr>
<td>Post-operative total leucyte count at 2 hours (cells/cu mm)</td>
<td>10616.75 ± 3409.35 (9526.38–11707.11)</td>
<td>9172.25 ± 2314.8 (8431.93–9912.56)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Post-operative total leucocyte count at 24 hours (cells/cu mm)</td>
<td>10792 ± 2939.63 (9851.86–11732.13)</td>
<td>9652.75 ± 2408.09 (8882.6–10422.89)</td>
<td>0.062*</td>
</tr>
<tr>
<td>Preoperative neutrophil to lymphocyte ratio</td>
<td>2.71 ± 1.46 (2.24–3.17)</td>
<td>2.90 ± 0.99 (2.58–3.21)</td>
<td>0.501#</td>
</tr>
<tr>
<td>Neutrophil to lymphocyte ratio at 2 hours (median [IQR]) (95%CI)</td>
<td>6 [5.03–8.6] (6.15–7.99)</td>
<td>3 [2.72–3.9] (3.19 – 4.18)</td>
<td>&lt; 0.001$</td>
</tr>
<tr>
<td>Neutrophil to lymphocyte ratio at 24 hours (median [IQR]) (95%CI)</td>
<td>6 [5.51–10.75] (6.85–9.68)</td>
<td>3 [2.79–4.16] (3.24–3.91)</td>
<td>&lt; 0.001$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (95% CI) or median (IQR) (95% CI). ¥95% CI, *Independent sample t test, #Independent sample t test applied after log transformation, $Mann Whitney U test.
Legend for figures

Fig 1: CONSORT Flow Diagram

Fig. 1. CONSORT flow diagram
**Fig. 2.** (A) Figure showing distribution of difference in total leucocyte count at 2 hours from basal in Group G and Group S, (B) Figure showing distribution of difference in total leucocyte count at 24 hours in Group G and Group S.
**Fig. 3.** (A) Figure showing the difference in neutrophil/lymphocyte ratio (NLR) at 2 hours from baseline in group G and group S, (B) Figure showing difference in NLR at 24 hours from baseline in group G and Group S.
Fig. 4. Figure showing comparison of hemoglobin levels between two groups.
Fig. 5. (A) Figure showing comparison of intraoperative heart rate between two groups, (B) comparison of mean arterial pressure between the two groups.