

Hematological and gas analyzer with acid base balance of pneumoperitoneum in dogs exposed to Laparoscopy

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Abstract: This study was created to investigate the effects of CO₂ insufflation- induced pneumoperitoneum on indices of acid- base status and blood picture in dogs. Twelve adult male dogs were subjected to pneumoperitoneum after general anesthesia, pneumoperitoneum was induced throughout the 60 minutes and blood samples were collected at intervals, 0, 5, 15, 30 and 60 minutes. Results of statistical analysis revealed that pneumoperitoneum disturb the acid- base homeostasis by increasing ($p \leq 0.05$) both pCO₂, tCO₂ and HCO₃⁻ whereas pO₂ was reduced with no significance on blood pH. Electrolytes including k⁺ and Ca²⁺ were significantly increased as a result of pneumoperitoneum yet Na⁺ was relatively stable. Total WBC count, granulocytes and platelets were reduced ($p \leq 0.05$) whereas no significance observed related to RBCs, Hb, MCV, MCHC, MCH and Hct. In conclusion, CO₂- mediated pneumoperitoneum alters acid- base status predisposing to respiratory and metabolic acidosis as well as disturbing immune response.

Keywords: pneumoperitoneum, acid- base, hematology, dogs.

Introduction

Laparoscopic surgery (LS) is a newer surgical technique that can be used for therapeutic or diagnostic purposes. Additionally, it is far less invasive than traditional laparotomy (Nesek-Adam et al., 2009). There are several advantages to choosing LS, the most important being characterized by minimal preoperative bleeding from the site, minimal pain, and lower cost. Laparoscopic surgery is much more cosmetic (Salihoglu et al., 2008). The small incision reduces hospital stay. There are far less complications associated with surgical site, infection at the site, and less complications related to the respiratory system (Uzunkoy et al., 2006). The abdomen is insufflated with CO₂ to create sufficient spaces (Ahn et al., 2011) for surgery. The additional space in the abdomen allows for excellent visualization. There are several choices of gases (helium, argon, and nitrous oxide) used to maintain and create pneumoperitoneum, however carbon dioxide is the best choice among these gases (Karapolat et al., 2011). Carbon Dioxide is water soluble and highly exchangeable within the blood. Carbon dioxide is an inert, economical, nonflammable and stable when compared to the other gases (Kuntz et al., 2000; Mynbaev et al., 2002a).

Despite of the many benefits of carbon dioxide, there are some associated complications, these complications can be observed throughout the insufflation process. These hemodynamic alterations include elevated blood pressure, increased vascular resistance, and decreased cardiac output (Mynbaev et al., 2002b). Absorption of CO₂ yields an increment in tidal CO₂ and pCO₂. Elevated intraperitoneal pressure can reduce vascular blood flow to the hepatic parenchyma, kidneys, heart and the gastrointestinal system (Mynbaev et al., 2002b). Hypercapnia may adversely affect acid- base homeostasis leading to respiratory and metabolic acidosis (Mynbaev et al., 2002a). A high pool of free radicals can be created during reperfusion which leads to oxidative tissue damage and consequential ischemia (Karapolat et al., 2011). Carbon dioxide induced pneumoperitoneum has a local impacts including reduced peritoneal pH and disruption in morphological integrity and visceral circulation of the peritoneum (Koster et al., 1999). This study was aimed to investigate the progressive adverse effects of experimental CO₂ induced pneumoperitoneum.

Materials and methods

Twelve intact male dogs who weights ranged from 12-18 kg. All animals were under general anesthesia and the abdominal region was prepared using aseptic technique and surgery. An approximately 0.5 cm incision was created in the umbilical region. Pneumoperitoneum was then induced by introducing a veress needle at the incision site. The electronic CO₂ insufflator was connected (karl storz). The intra abdominal pressure was maintained at 12 mm Hg and flow rate was 3 L/ minute. Pneumoperitoneum was maintained throughout the 60 minutes. Blood was collected using aseptic technique. A 16 gauge cannula were used to collect at time 0, 5, 15, 30 & 60 minutes after pneumoperitoneum application. Blood samples were accessed using 1 ml heparinized disposable syringes with rubber plug for blood gas

analysis. Complete blood count was accessed with 1 ml non-heparinized disposable syringes. The blood sample was immediately transferred to EDTA coated containers. Blood gases were analyzed with Ca²⁺ cassette immediately after blood sampling of each via an automated blood gas analyzer (OPTI CCA, USA). Blood cells were detected after sufficient mixing time on electric mixer (50 rpm) using veterinary automatic hematology coulter (Abacus Junieur, Radim Ltd. Co. Italy). The analyzer being set to canine as the animal species. Data were subjected to one-way ANOVA (Steel and Torrie, 1980). The significance was observed using Duncan Multiple Test (Duncan, 1955) with $p \leq 0.05$ interval. Level of significance was determined using software, computer software, statistical package for social sciences SPSS. Results were presented as mean \pm SEM.

Results

The partial pressure of carbon dioxide (pCO₂) elevated significantly ($p \leq 0.05$) After 15 minutes of pneumoperitoneum induction when compared with both times zero and 5 minutes (figure 1). Total CO₂ (tCO₂) elevation was observed just after 5 minutes at the same level to the end of treatment (60 minutes) (figure 2).

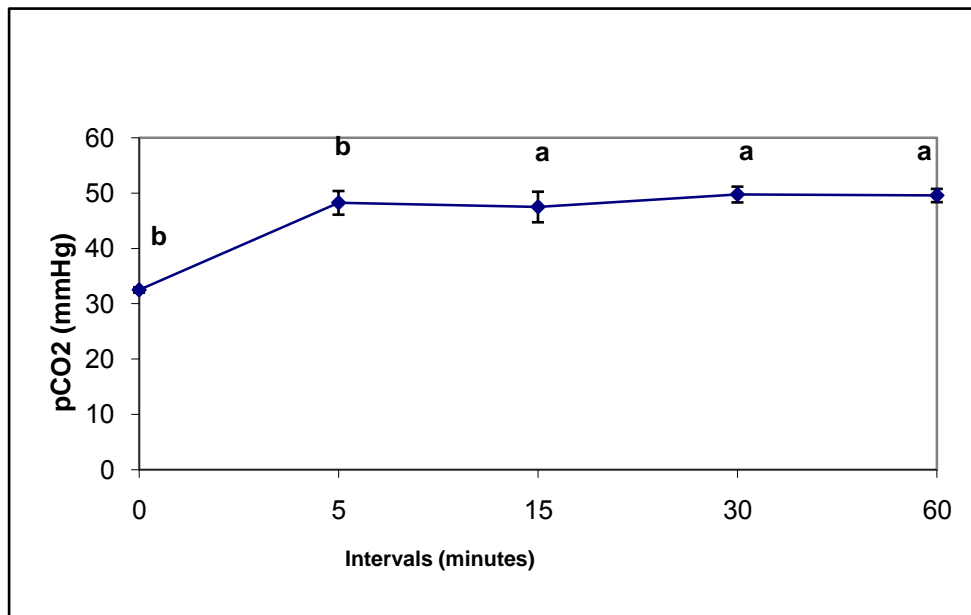


Figure (1): Effect of pneumoperitoneum on pCO₂ in dogs (Mean \pm SEM).

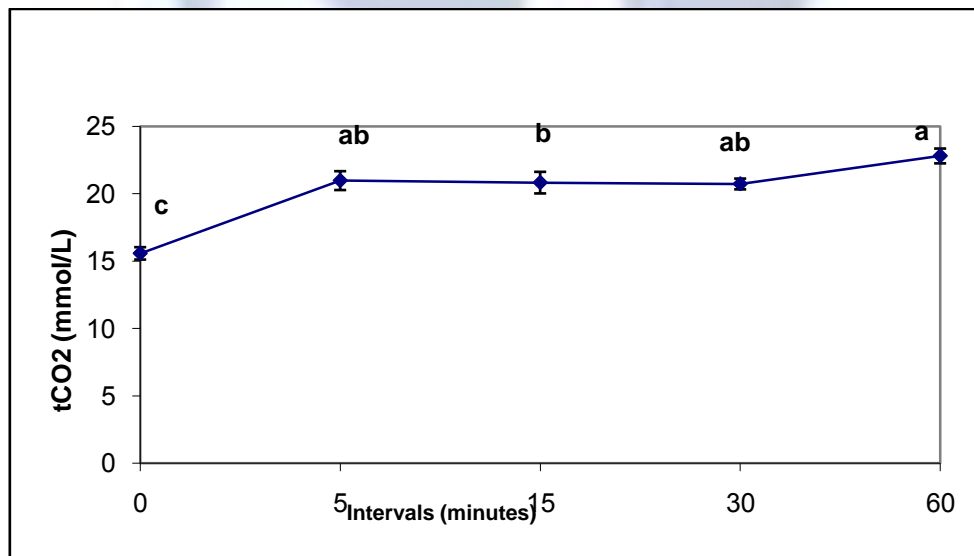


Figure (2): Effect of pneumoperitoneum on tCO₂ concentration in dogs (Mean \pm SEM).

The partial pressure of oxygen (pO₂) showed a significant fluctuation ($p \leq 0.05$) From 5 minutes till 60 minutes of the treatment with no significance among intervals (figure 3).

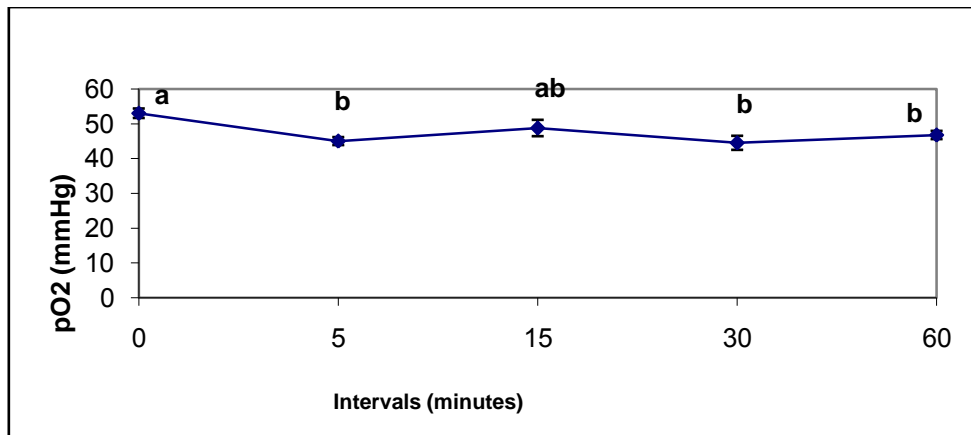


Figure (3): Effect of pneumoperitoneum on pO2 in dogs (Mean±SEM).

Bicarbonate (HCO_3^-) increased significantly at a time 5 minutes of induced peritoneum when compared with that of zero time. HCO_3^- trended to the same extent till the end of the experiment (figure 4). There was no significance observed in the value of blood pH throughout the time intervals of trial (figure 5).

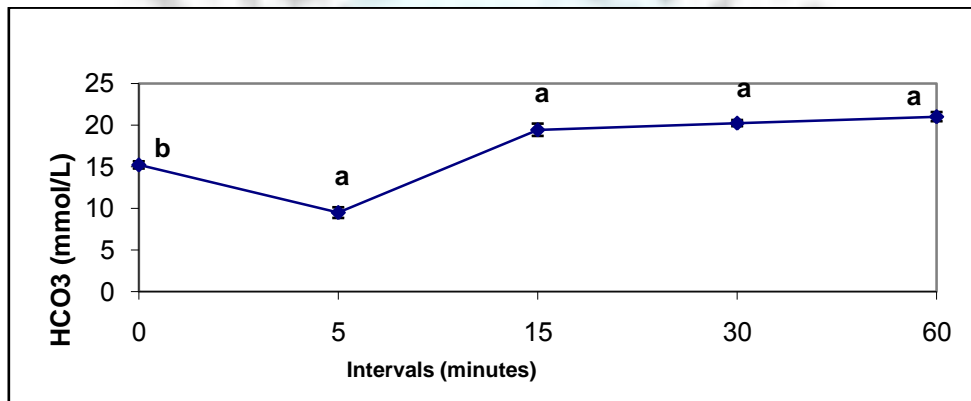


Figure (4): Effect of pneumoperitoneum on HCO3- concentration in dogs (Mean±SEM).

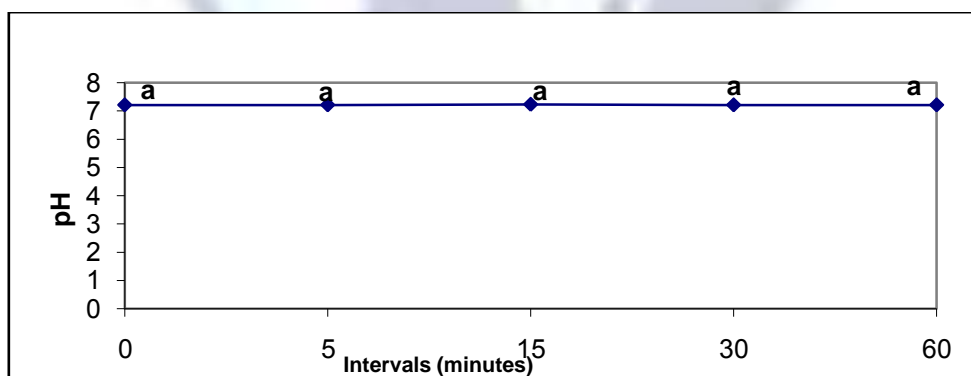


Figure (5): Effect of pneumoperitoneum on blood pH in dogs (Mean±SEM).

Sodium ion (Na^+) concentration did not have a significant alteration, except at time 30 minutes, which was considered less than the value of zero time (figure 6). The concentration of potassium ion (K^+) elevated after 5 minutes compared with the value of zero time with no significance among the intervals of treatment, except the value of 60 minutes which was significantly elevated ($p \leq 0.05$) especially when compared with the value of 15 minutes (figure 7). Related to the concentration of calcium ion (Ca^{+2}), a significant elevation was recorded at times 5, 15 and 30 minutes when compared with time zero. The three values were significantly paralleled yet they were significantly ($p \leq 0.05$) Less than that of 60 minutes which represents the highest concentration throughout the trial (figure 8).

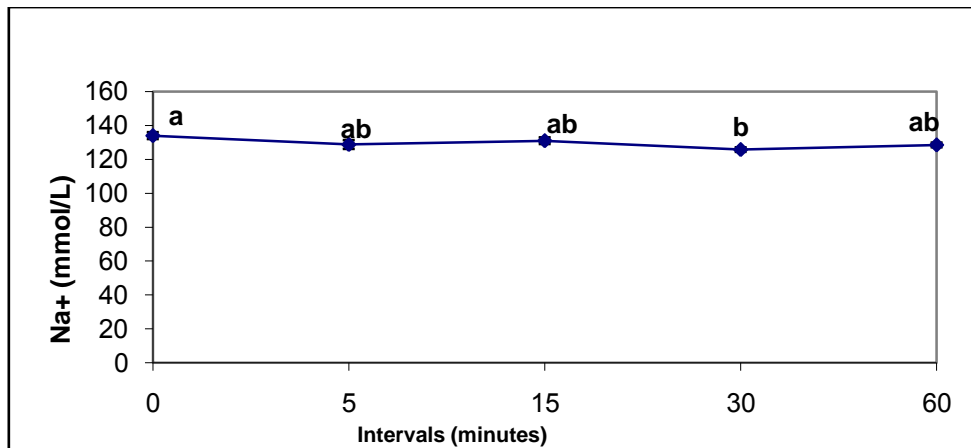


Figure (6): Effect of pneumoperitoneum on Na⁺ concentration in dogs (Mean±SEM).

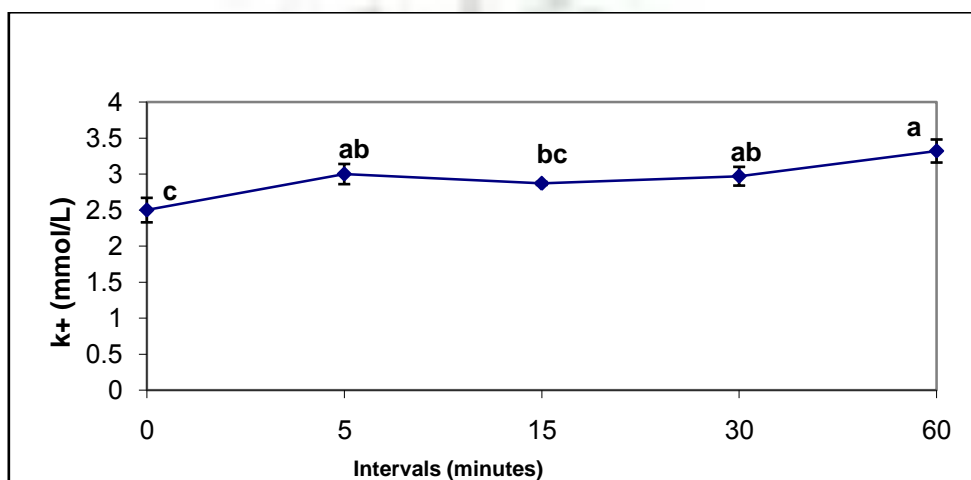


Figure (7): Effect of pneumoperitoneum on k⁺ concentration in dogs (Mean±SEM).

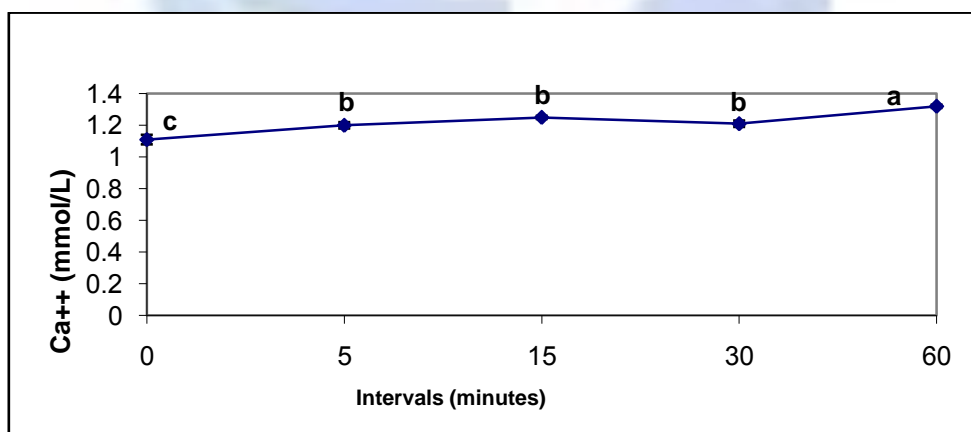


Figure (8): Effect of pneumoperitoneum on Ca⁺⁺ concentration in dogs (Mean±SEM)

Table 1 confirms that there was no significant change related to red blood corpuscles (RBCs), hemoglobin concentration (Hb) in addition to the values of mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and packed cell volume (PCV) had no significant changes when compared with time zero (table 1). White blood cells (WBCs) and granulocytes started to decrease after 15 minutes. They maintained the same level since 15 minutes till the end of the procedure. Monocytes count dropped to a further level at time intervals 15 and 30 minutes throughout the period of treatment. No distinct significant alterations were observed in lymphocytes (table 2). Platelets significantly ($p \leq 0.05$) decreased after 5 minutes relative to zero time. At time 15 minutes, there was a significant elevation of platelets. Platelets elevation persisted beyond the value of time zero, then decreased again at times 30 and 60 minutes when compared that of time 5 minutes.

Table (1): Effect of pneumoperitoneum on some hematological parameters. (Mean± SEM)

Time (minutes) / Parameters	0	5	15	30	60
RBC (10 ¹² /L)	5.31± 0.23 a	5.19± 0.23 a	4.91± 0.35 a	5.04± 0.24 a	5.01± 0.32 a
Hb (g/dl)	10.62± 0.31 a	10.37± 0.35 a	9.60± 0.42 a	9.85± 0.23 a	9.75± 0.25 a
MCV (fL)	63.25± 2.52 a	61.0± 2.27 a	61.75±2.28 a	61.25±2.25 a	60.50±2.06 a
MCHC (g/dl)	31.60± 0.56 a	31.40± 0.62 a	31.02±0.62 a	32.2± 0.45 a	32.22± 0.52 a
MCH (pg)	19.95± 0.77a	19.65± 0.65a	18.85± 0.42a	19.57± 0.71a	19.50± 0.71a
HCt (%)	33.15± 1.12 a	32.63±1.09 ab	29.77±1.20 b	30.75±0.78 ab	30.37±0.93 ab

Table (2): Effect of pneumoperitoneum on on leukocyte count (Mean± SEM).

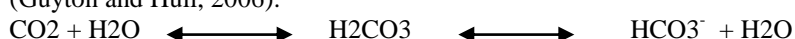
Time (minutes) / Parameters	0	5	15	30	60
WBC (10 ⁹ /L)	11.08± 0.75 ab	12.51± 1.18 a	7.53± 1.30 c	8.56± 0.87 bc	7.68± 0.87 c
Lymphocytes (10 ⁹ /L)	2.95± 0.38 ab	4.28± 0.77 a	2.94± 0.80 ab	2.89± 0.22 b	1.88± 0.31 b
Granulocytes (10 ⁹ /L)	7.56± 0.57 a	7.04± 0.77 ab	4.52± 0.82 c	5.78± 0.75 abc	5.21± 0.67 bc
Monocytes (10 ⁹ /L)	0.57± 0.05 ab	0.70± 0.06 a	0.46± 0.05 bc	0.28± 0.06 c	0.59± 0.08 ab
Plts	582.33±20.09 a	385.54±13.36 c	507.04±27.03 b	379.0±22.08 c	422.66±21.38 c

Discussion

Laparoscopy via insufflations with carbon dioxide is safe and carries minimum physiological disorders (Salihoglu et al., 2008). The process of pneumoperitoneum via CO₂ insufflation might affect the acid- base homeostasis in of patients through the excess amount of CO₂. Excess CO₂ can disturb the blood pH and subsequent enzyme activity, this can potentially negatively affect certain metabolic pathways within the body (Guyton and Hull, 2006). Due to the fact that pH is a critical factor relative to metabolic activities, buffer systems in the body have been dedicated to keeping blood pH in a controlled range thus preventing enzyme denaturation (Bishop et al., 2005). Bicarbonate and carbonic acid are deemed the most efficient buffer system in the body (Guyton and Hull, 2006).

The pneumoperitoneum induced elevation in pCO₂ with subsequent hypercarbia and increased tCO₂ was observed in the current study and was in agreement with Guzel et al., (2012) study that was done in pigs. Michael et al., (1999) observed that there was an exponential relationship between CO₂ and the pressure of CO₂ via insufflation into the abdominal cavity. He found that the transperitoneal absorption of CO₂ was the key factor for development of hypercapnia. He noted increased pCO₂ resulted from CO₂ tension gradient between pneumoperitoneum and the blood causing CO₂ to be readily absorbed into circulation. Furthermore, CO₂ has the potential to increase the functional absorptive surface and thus absorbed CO₂ must be eliminated by means of expiration. Interestingly, more than 120 L may persist in the body, especially within the bones (Koivusalo and Lindgren, 2000). It has been demonstrated in animal models that elimination of CO₂ within the lungs is characterized by early phase of fast CO₂ elimination followed by a slower phase of elimination due to peritoneal surface distention with compression of peritoneal vessels (Kjeld et al., 2012).

The progressive absorption of CO₂ and consequent increased pCO₂ is sufficient to increase carbonic acid. The latter event requires a buffer system to correct. The following equation is one of the primary buffer system within the body (Guyton and Hull, 2006).



A compensatory bicarbonate (HCO_3^-) elevation was observed secondary to increased in blood CO_2 (Fukushima et al., 2011) through the effect of $\text{H}_2\text{CO}_3/\text{HCO}_3^-$ buffer system, a result previously observed by Guzel et al., (2012). Papers concerned with acid- base balance illustrated that CO_2 insufflations resulted in a status of acidosis either of respiratory (Guzel et al., 2012b), metabolic (Lee et al., 2011) or mixed (Kapetanakis et al., 2011). However in this study the pH did not have any significance on the acid-base balance. This is not the observations of Guzel et al., (2012) who reported a decline in pH in pigs who were exposed to pneumoperitoneum. The results of the current study may be an extract of the activity of body buffer systems which readily neutralized the prospective acidosis (Tomita et al., 2001). In contrast pCO_2 , pneumoperitoneum resulted in a decrease in pO_2 , this was previously reported by Guzel et al., (2012), when venous blood was sampled. This finding is in partially attributed to the high pressure created in abdominal cavity when pneumoperitoneum is induced. This pressure reduces diaphragm and respiratory movements (Hashikura et al., 1994).

There was a propensity of Na^+ to drop yet it was insignificant in our study. This finding coincided with a report by both of Kwak et al., (2010), De Almeida and Ganem (2004). These were justified as a reflex of hemodynamic stability created by ventilation (De Almeida and Ganem, 2004). The significant hyperkalemia at the end of the trial was in agreement with Garg et al., (2011). Their study attributed this effect to prospective tissue trauma (even when limited incision from laparoscopy). Absorption of CO_2 from the peritoneal cavity was the key factor for the incidence of hyperkalemia (Demiroglu et al., 2007). A positive correlation was found between serum potassium and intra abdominal pressures. Potassium increased 0.4 mEq/L for each 10 mm Hg increment in pCO_2 (Edwards et al., 1977). This correlation was emphasized in figures 1 and 7.

Kidney's play a vital role to restrict the anticipated pCO_2 induced acidemia. The kidney's propel the net movement of potassium out of the cells as a direct result of hydrogen ions (H^+) entering the cells. The causative agent of acidosis is through an inverse correlation between H^+ and K^+ ions. These ions are closely regulated by aldosterone hormone (De Almeida and Ganem, 2004). This action is another key event to prevent acidemia and stabilize blood pH in this current study and in previous studies. Hypercalcemia observed in dogs subjected to pneumoperitoneum via CO_2 was inconsistent with Garg et al., (2011). There was no distinct interpretation of this impact. Concentration of parathyroid hormones will need assessed in future studies. Despite complete blood counts and consequent differential WBC count are of low value in our study. These parameters are highly considerable in clinical applications (Hanly et al., 2003), however neither RBC count nor Hb concentration was significantly altered throughout the period of pneumoperitoneum. Guyton and Hull, (2006) demonstrated that increased pCO_2 reduces Hb- O_2 affinity. Decreased pO_2 , saturation of O_2 and oxygen bound Hb is known as the Bohr effect.

The total WBC count was reduced in some of the time intervals, previously reported by Bostanci et al., (2010) in a rat model. Hanly et al., (2003) reported the results of CO_2 can suppress immunological status and consequent inflammatory response at the level of gene expression. It then attenuates the expression of α_2 - macroglobulin and β -fibrinogen. CO_2 insufflation may promote chemoattractants to macrophages within the peritoneal cavity potentially making leukocyte counts diminished in the blood. Nickholgh et al., (2008) affirmed this hypothesis through studying the increased adherence of leukocytes and platelets on the endothelium. Carbon dioxide induced ischemia will trigger the migration of leukocytes and platelets from peripheral blood in an attempt to repair the affected area (Karapolat et al., 2011). These events may explain the decline in both differential WBCs and platelets in the current study.

None of indices including MCV, MCHC and MCH had significant changes throughout the experiment. This reflects a logical outcome in regard to these indices which have been calculated from RBC, Hb and Hct. The latter had no significance observations from their means along 60 minutes insulfation. This study illustrated that CO_2 insufflation, in the abdominal cavity of dogs, at a pressure of 12 mm Hg was a causative agent of a pronounced change in acid- base status thus encouraging a shift to acidosis as well as diminishing WBCs counts possibly affecting the body immunity function.

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