Cytokine 113 (2019) 128–138

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

# Evaluation of levels of interleukin-6, interleukin-8 and some haematologic parameters of dogs with cutaneous wounds

Daniel Onimisi Avazi[a,*], Augustine Cheh Awasum[a], Adamu Zoaka Hassan[a], Joseph Olusegun Ayo[b], Tagang Aluwong[b], Sa'idu Tanko Muhammed[c], Ayo Yila Simon[c], Mohammad Hadi Suleiman[d], Ayuba Caleb Kudi[e]

[a] Department of Veterinary Surgery and Radiology, ABU, Zaria, Nigeria
[b] Department of Veterinary Physiology, ABU, Zaria, Nigeria
[c] Veterinary Teaching Hospital, ABU, Zaria, Nigeria
[d] Department of Veterinary Anatomy, ABU, Zaria, Nigeria
[e] Department of Veterinary Medicine, ABU, Zaria, Nigeria

ARTICLE INFO

*Keywords:*
Wound
Inflammation
Interleukin-6
Interleukin-8
Haematology
Dog

ABSTRACT

The aim of the study was to investigate the time-course of serum and wound fluids interleukin (IL)-6 and IL-8 levels in dogs with cutaneous wounds and their relationship with some haematologic parameters. The experimental group comprised of six adult dogs that underwent surgery with wounds (n = 6) on the mid lateral aspect of the right antebrachium; and control group of six, apparently, healthy intact (free from cutaneous wounds) adult dogs, comprising equal number of both sexes. Vital signs evaluated were within normal limits. Samples of blood, serum and wound fluids harvested pre- and at 12 h, 36 h, 60 h, 156 h and 324 h post-injury, were utilised for IL-6 and IL-8 assay and haematology. Peak concentrations of IL-6 in wound fluid (1.33. ± 0.33 ng/mL) and serum (0.82 ± 0.24 ng/mL) of the experimental group at 12 h post-operation were higher ($P < 0.01$) than the control (0.30 ± 0.05 ng/mL). Concentrations of IL-8 at 12 h and 60 h in wound fluid (0.21 ± 0.05 ng/mL and 0.22 ± 0.11 ng/mL) respectively were lower ($P < 0.05$) than serum (0.71 ± 0.21 ng/mL and 0.73 ± 0.24 ng/mL) respectively in the experimental group and corresponding values recorded in controls (0.34 ± 0.09 ng/mL and 0.36 ± 0.14 ng/mL). The haematological and biochemical parameters exhibited minimum fluctuations, but values were within normal ranges. Significant correlations were obtained between serum and wound fluid IL-6 ($r = 0.827$, $P < 0.05$); wound fluid IL-6 and monocyte count ($r = 0.818$, $P < 0.04$); wound fluid IL-6 and haematocrit ($r = -0.894$, $P < 0.05$). There was a positive correlation between serum IL-8 and serum IL-6 ($r = 0.622$, $P > 0.05$) and serum IL-8 and wound fluid IL-8 ($r = 0.718$, $P > 0.05$) in the experimental group. In conclusion, IL-6 and IL-8 exerted modulated inflammatory processes following cutaneous wounds in dogs. Further studies are required to investigate the expression patterns of IL-6 and IL-8 in cutaneous wounds in order to improve the quality of management of cutaneous wounds.

## 1. Introduction

Interleukins (IL)-6 are members of the low-molecular-weight cytokine proteins (5–20 kDa) encoded by the IL-6 genes and involved in cell signalling, regulating lymphocyte function [1–3]. IL-8 are members of the chemokine family, also known as CXCL8 encoded by the IL-8 gene and are involved in acute phase response induced by chemotaxis in target cells. The granulocytes primarily neutrophils, are mobilized to the site of injury by these cytokines [4–7]. They are produced by macrophages, epithelial cells, airway smooth muscle cells, endothelial cells and other cell types [4,5]. They mediate the physiologic responses to injury and infection by binding to specific receptors enabling communication, differentiation, proliferation and other activity of the immune cells. This triggers inflammatory migration of the cells to the sites of injury and infection, thereby enhancing wound healing [1,2]. Wounds are inescapable events in life following physical, chemical, thermal, microbial or immunological insult to body tissues, resulting in disruptions, breakage and loss of cellular and anatomic or functional continuity of living tissues or organs [8,9]. Wounds may extend into subcutaneous tissues inflicting injury to tendons, muscles, vessels,

* Corresponding author.
*E-mail address:* danooavaz@yahoo.com (D.O. Avazi).

https://doi.org/10.1016/j.cyto.2018.06.024
Received 23 February 2018; Received in revised form 8 June 2018; Accepted 18 June 2018
Available online 28 June 2018

**Table 1**
Vital parameters (rectal temperature, pulse rates and respiratory rates) of the study dogs.

| Parameter | Experimental group | | | Control group | | |
|---|---|---|---|---|---|---|
| | Males + Females (n = 6) | Males (n = 3) | Females (n = 3) | Males + Females (n = 6) | Males (n = 3) | Females (n = 3) |
| Rectal temperature (°C) | 38.05 ± 0.05 (37.95–38.21) | 38.05 ± 0.04 (37.95–38.22) | 38.04 ± 0.04 (37.94–38.22) | 38.02 ± 0.02 (37.96–38.05) | 38.01 ± 0.03 (37.93–38.03) | 38.03 ± 0.06 (37.89–38.08) |
| Pulse rate (beats/minute) | 72.11 ± 0.64 (70.43–74.72) | 72.16 ± 0.51 (70.76–75.14) | 72.05 ± 0.45 (70.00–74.29) | 72.13 ± 0.27 (70.86–72.53) | 71.89 ± 0.85 (70.86–72.57) | 72.37 ± 0.75 (70.86–73.05) |
| Respiratory rate (cycles/minute) | 23.23 ± 0.176 (22.71–23.76) | 23.06 ± 0.31 (22.66–23.52) | 23.40 ± 0.35 (22.76–24.00) | 22.47 ± 0.07 (22.29–22.72) | 22.56 ± 0.44 (22.38–23.05) | 22.37 ± 0.38 (22.19–22.57) |

Mean = mean ± SEM; n = total number of individual in a group.

nerves, parenchymal organs and even bones [8,9].

Interleukin (IL)-6 activates its cell-surface type cytokine receptor from a complex, consisting of the ligand-binding with interleukin-6 receptor (IL-6R) via the IL-6Rα signal chain (cluster of differentiation 126; CD126) and the signal-transducing component, which is the glycoprotein 130 (gp-130, also called CD130) [10,11]. The common signal transducer for several cytokines is CD130, as it is almost ubiquitously expressed in most tissues [12]. The complexes bring together the intracellular regions of CD130 to initiate a signal transduction cascade through certain transcription factors, Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) [10,11]. The IL-8 is also secreted by cells with toll-like receptors such as macrophages, which are the first cells to release IL-8 to recruit other cells [13]. Numerous receptors are capable of binding IL-8 on surface membranes with the most frequently studied types being the G protein-coupled serpentine receptors CXCR1 and CXCR2. The serpentine receptors affinity of CXCR1 for IL-8 that are greater than those of CXCR2 [13]. IL-8 expression is regulated by a transcription factor; NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), which represents a novel anti-IL-8 therapy for use in inflammatory diseases [14].

Although the roles of IL-6 and IL-8 have been established to some extent in immune and inflammatory diseases, there is paucity of information on the role of these cytokines in normal physiological processes, such as cutaneous wound healing. Cutaneous injuries are among the most frequent reasons dogs are presented to veterinary clinics often due to accidents associated with their common hunting habits and recently, increased use as guard dogs and pets in the urban areas [15–18]. Therefore, there is the need to explore the potential of IL-6 and IL-8 levels in dogs, which may provide new insights into the biochemical factors involved in the regulation of wound healing.

The aim of the study was to determine the wound fluid and serum IL-6 and IL-8 levels post-injury and the associated fluctuations in leucocyte counts, erythrocyte and serum biochemical parameters in dogs with cutaneous wounds.

## 2. Material and methods

### 2.1. Animal model

After obtaining ethical approval from the Ahmadu Bello University Committee on Animal Use and Care with reference number ABUCAUC/2016/026, twelve (12) apparently healthy dogs commonly called the Nigerian indigenous dogs (NIDs) (age: 9–13 months old, Live weight: 8–12 kg) were enrolled for this study. The dogs were housed and maintained throughout the period of the research in standard dog cubicles in the Small Animal Clinic of the Ahmadu Bello University Veterinary Teaching Hospital which had been cleaned and disinfected prior to the arrival of the dogs. The dogs were provided with adequate amount of nutritive food in clean bowls and water also supplied ad libitum.

### 2.2. Study design and collection of samples

The dogs were apparently healthy as demonstrated clinically by their normal vital parameters (rectal temperature, pulse rate, respiratory rate) as presented in Table 1 and cooperation with no behavioural changes observed after successful screening, acclimatization and conditioning [19]. They were assigned into two groups of six dogs each: experimental group and control group, with a male to female ratio of 1:1. Pre-surgical considerations included aseptic surgical preparations of patients and anaesthesia were achieved by intravenous (cephalic veins) administration of pre-anaesthetics; atropine sulphate (Atropine® – Shanxi Shuguang Pharmaceutical Co., Ltd, Qixian, China) at 0.05 mg/kg, pentazocine (Pentalab® – Laborate Pharmaceuticals Ltd, India) at 1 mg/kg and midazolam (Roche Pharmaceuticals, Switzerland) at 0.25 mg/kg, while the anaesthetic agent was thiopental sodium 1 g (Pauco Pharmaceuticals, Nigeria) at 12 mg/kg. Cutaneous excisions (4 $cm^2$) were then created on the mid-lateral aspect of the fully draped right antebrachium of the dogs. Post-anaesthesia, the dogs were monitored until recovery.

Blood sample (5 mL) was collected from each dog via cephalic venipuncture at 12 h, 36 h, 60 h, 156 h and 324 h post-operation. Three milliliters (3 mL) of blood was dispensed into plain (for harvesting of

**Table 2**
Interleukin-6 levels (ng/mL) in dogs of non-specific sex with cutaneous wounds (n = 6).

| Time (h) | Exp. WF. Response (ng/mL) (n = 6) | Exp. Serum response (ng/mL) (n = 6) | Cont. Serum response (ng/mL) (n = 6) |
|---|---|---|---|
| Pre-operative (Start) | – | 0.29 ± 0.04 | 0.28 ± 0.05 |
| | – | (0.21–0.49) | (0.18–0.49) |
| Post-operative | | | |
| 12 | 1.33. ± 0.33[a] (0.52–2.74) | 0.82 ± 0.24[a] (0.41–1.84) | 0.30 ± 0.05[b] (0.19–0.54) |
| 36 | 0.51 ± 0.26 (0.14–1.77) | 0.46 ± 0.04 (0.37–0.65) | 0.29 ± 0.10 (0.10–0.70) |
| 60 | 0.18 ± 0.02 (0.12–0.22) | 0.47 ± 0.18 (0.23–1.38) | 0.17 ± 0.05 (0.06–0.35) |
| 156 | 0.14 ± 0.02[a] (0.10–0.20) | 0.79 ± 0.32[b] (0.24–2.24) | 0.16 ± 0.03[a] (0.10–0.26) |
| 324 | – | 0.17 ± 0.03 (0.10–0.29) | 0.21 ± 0.03 (0.10–0.28) |
| | – | | |
| *Mean ± SEM | 0.54 ± 0.18 | 0.50 ± 0.16 | 0.24 ± 0.04 |

[a,b] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. All assays and analyses were made in duplicates. Values in parenthesis are the ranges (minimum–maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, ng/mL = nano-gram per millilitre, n = total number of individuals in a group and (*) = Overall mean values.

**Table 3**
Interleukin-6 levels (ng/mL) in Male and Female dogs with cutaneous wounds (n = 3).

| Time (h) | Experimental Group (Wound Fluids) | | Experimental Group (Serum) | | Control Group (Serum) | |
|---|---|---|---|---|---|---|
| | Males (n = 3) | Females (n = 3) | Males (n = 3) | Females (n = 3) | Males (n = 3) | Females (n = 3) |
| Pre-operative (Start) | – | – | 0.22 ± 0.01 | 0.35 ± 0.08 | 0.33 ± 0.08 | 0.22 ± 0.02 |
| | – | – | (0.21–0.25) | (0.24–0.49) | (0.22–0.49) | (0.18–0.25) |
| Post-operative | | | | | | |
| 12 | 1.26 ± 0.37[a] | 1.40 ± 0.67[a] | 1.01 ± 0.43[a] | 0.63 ± 0.20[a,b] | 0.36 ± 0.09[b,c] | 0.23 ± 0.04[c] |
| | (0.52–1.71) | (0.86–2.74) | (0.41–1.84) | (0.47–1.04) | (0.25–0.54) | (0.19–0.30) |
| 36 | 0.19 ± 0.03 | 0.84 ± 0.49 | 0.44 ± 0.04 | 0.49 ± 0.08 | 0.13 ± 0.03 | 0.44 ± 0.16 |
| | (0.14–0.24) | (0.13–1.77) | (0.37–0.49) | (0.38–0.65) | (0.10–0.18) | (0.14–0.70) |
| 60 | 0.21 ± 0.01 | 0.15 ± 0.02 | 0.64 ± 0.37 | 0.29 ± 0.02 | 0.18 ± 0.08 | 0.26 ± 0.06 |
| | (0.18–0.22) | (0.12–0.18) | (0.23–1.38) | (0.26–0.34) | (0.10–0.09) | (0.16–0.35) |
| 156 | 0.11 ± 0.01[a] | 0.18 ± 0.02[a] | 0.96 ± 0.64[b] | 0.61 ± 0.23[b] | 0.10 ± 0.01[a] | 0.22 ± 0.02[a] |
| | (0.10–0.13) | (0.15–0.20) | (0.24–2.24) | (0.37–1.08) | (0.09–0.12) | (0.20–0.26) |
| 324 | – | – | 0.14 ± 0.04 | 0.20 ± 0.05 | 0.19 ± 0.05 | 0.23 ± 0.03 |
| | – | – | (0.10–0.21) | (0.12–0.29) | (0.10–0.28) | (0.19–0.27) |
| *Mean ± SEM | 0.44 ± 0.16 | 0.64 ± 0.41 | 0.57 ± 0.31 | 0.43 ± 0.16 | 0.20 ± 0.05 | 0.27 ± 0.04 |

[a,b,c] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. All assays and analyses were made in duplicates. Values in parenthesis are the ranges (minimum–maximum) from the values. ng/mL = nano-gram per millilitre, n = total number of individuals in a group and (*) = Overrall mean values.

serum) and 2 mL into sodium ethylenediamintetraacetate-containing (for blood cell counts) sample bottles. Wound fluids were also collected at 12 h, 36 h, 60 h and 156 h. post-operation by the wound washouts method [20]. The serum harvested from blood sample and wound fluid collected was stored at – 20 °C until used for serology including IL-6 and IL-8 determination.

### 2.3. Enzyme-linked immunosorbent assay

Enzyme Linked Immunosorbent Assay Kits used were Interleukin (IL)-6 Canine ELISA kit (ab193686, Lot: GR252142) and Interleukin (IL)-8 Canine ELISA kit (ab155465, Lot: GR252140) supplied by Abcam Ltd, United Kingdom. The assays were carried out in duplicate in strict adherence to the manufacturer's protocols and the absorbances at 450 nm were determined spectrophotometrically using Thermo Multiskan Ascent Photometer (Thermo Scientific, USA).

### 2.4. Haematology and serum biochemical tests

Haemogram of anticoagulated whole blood was obtained using the automated haematologic analyser (Mindray® BC-3600, Shenzhen, China). Serum was obtained from whole blood stored at room temperature without anticoagulant for 1 h and centrifuged at 4500*g* for 15 min. using automated centrifuge (BHG Hermle Z364, Gosheim, Germany). The Serum samples were analysed for creatinine, urea, total protein, albumin, glucose, $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, P, $HCO_3^-$ by enzyme immunoassay method and read using digital ultraviolet spectrophotometer (Digital System S. R. L, Italy) at 450 nm [21–25].

### 2.5. Statistical analysis

The numerical data were expressed as means and standard error of the means (means ± SEM). The statistical analysis was performed using Graphpad prism Version 5.03 (San Diego, California, USA) for windows. Data were subjected to analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Values of $P < 0.05$ were considered significant [26,27].

## 3. Results and discussion

### 3.1. Overview

Measureable levels of IL-6 and IL-8 protein was detected in all samples of both the experimental and control group similar to the observations in exotic dogs such as German Shepherds, French Bulldog, Dachshund, Pugs, Doberman and Beagles [28–31]. Tables 2–5 indicate the respective levels of IL-6 and IL-8 proteins irrespective of sex and in male and female injured and intact group of dogs as determined by the Enzyme-linked immunosorbent assay. The difference in the values of IL-

**Table 4**
Interleukin-8 levels (ng/mL) in dogs of non-specific sex with cutaneous wounds (n = 6).

| Time (h) | Exp. WF. Response (ng/mL) (n = 6) | Exp. Serum response (ng/mL) (n = 6) | Cont. Serum response (ng/mL) (n = 6) |
|---|---|---|---|
| Pre-operative (Start) | – | 0.35 ± 0.10 | 0.35 ± 0.10 |
| | | (0.12–0.70) | (0.12–0.71) |
| Post-operative | | | |
| 12 | 0.21 ± 0.05[a] | 0.71 ± 0.21[b] | 0.34 ± 0.09[a,b] |
| | (0.06–0.36) | (0.04–1.41) | (0.11–0.73) |
| 36 | 0.13 ± 0.02 | 0.47 ± 0.17 | 0.30 ± 0.10 |
| | (0.06–0.23) | (0.10–1.06) | (0.03–0.59) |
| 60 | 0.22 ± 0.11[a] | 0.73 ± 0.24[b] | 0.36 ± 0.14[a,b] |
| | (0.09–0.74) | (0.08–1.45) | (0.07–0.86) |
| 156 | 0.25 ± 0.08 | 0.46 ± 0.14 | 0.38 ± 0.16 |
| | (0.07–0.54) | (0.12–1.02) | (0.05–0.92) |
| 324 | – | 0.39 ± 0.05 | 0.22 ± 0.01 |
| | | (0.26–0.59) | (0.21–0.23) |
| *Mean ± SEM | 0.20 ± 0.03 | 0.52 ± 0.07 | 0.33 ± 0.02 |

[a,b] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. All assays and analyses were made in duplicates. Values in parenthesis are the ranges (minimum–maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, ng/mL = nano-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

**Table 5**
Interleukin-8 levels (ng/mL) in Male and Female dogs with cutaneous wounds (n = 3).

| Time (h) | Exp. WF. Response (ng/mL) | | Exp. Serum response (ng/mL) | | Cont. Serum response (ng/mL) | |
|---|---|---|---|---|---|---|
| | Males (n = 3) | Females (n = 3) | Males (n = 3) | Females (n = 3) | Males (n = 3) | Females (n = 3) |
| Pre-operative (Start) | – | – | 0.33 ± 0.15 (0.13–0.62) | 0.38 ± 0.17 (0.12–0.70) | 0.38 ± 0.18 (0.12–0.71) | 0.33 ± 0.15 (0.13–0.61) |
| Post-operative | | | | | | |
| 12 | 0.17 ± 0.04[a] (0.08–0.22) | 0.25 ± 0.09[a,b] (0.06–0.36) | 0.77 ± 0.33[c] (0.33–1.41) | 0.66 ± 0.32[b,c] (0.04–1.07) | 0.36 ± 0.19[a,c] (0.11–0.73) | 0.31 ± 0.05[a,c] (0.22–0.38) |
| 36 | 0.12 ± 0.03 (0.10–0.14) | 0.13 ± 0.05 (0.06–0.23) | 0.52 ± 0.19 (0.20–0.86) | 0.43 ± 0.31 (0.10–1.06) | 0.39 ± 0.14 (0.11–0.59) | 0.21 ± 0.16 (0.03–0.52) |
| 60 | 0.32 ± 0.21 (0.09–0.74) | 0.11 ± 0.01 (0.09–0.14) | 0.83 ± 0.32 (0.21–1.28) | 0.63 ± 0.42 (0.08–1.45) | 0.35 ± 0.18 (0.08–0.69) | 0.36 ± 0.25 (0.07–0.86) |
| 156 | 0.28 ± 0.13 (0.12–0.54) | 0.22 ± 0.10 (0.07–0.41) | 0.56 ± 0.26 (0.12–1.02) | 0.36 ± 0.13 (0.13–0.58) | 0.38 ± 0.23 (0.15–0.84) | 0.39 ± 0.27 (0.05–0.92) |
| 324 | – | – | 0.42 ± 0.09 (0.31–0.59) | 0.36 ± 0.07 (0.26–0.51) | 0.22 ± 0.01 (0.21–0.23) | 0.22 ± 0.01 (0.21–0.23) |
| *Mean ± SEM | 0.22 ± 0.05 | 0.18 ± 0.03 | 0.57 ± 0.08 | 0.47 ± 0.06 | 0.35 ± 0.03 | 0.30 ± 0.03 |

[a,b,c] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly (P < 0.05) different. All assays and analyses were made in duplicates. Values in parenthesis are the ranges (minimum–maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, ng/mL = nanogram per millilitre, n = total number of individuals in group and (*) = Overall values.

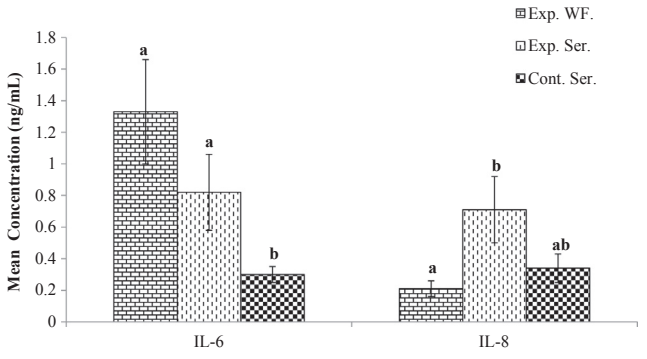


**Fig. 1.** Twelve (12) hours post-operative Interleukin (IL)-6 and -8 levels (ng/mL) in dogs with cutaneous wounds irrespective of sex (n = 6). [a,b] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly (P < 0.05) different separately for IL-6 and IL-8. All assays and analyses were made in duplicates. n = total number of individuals in a group.

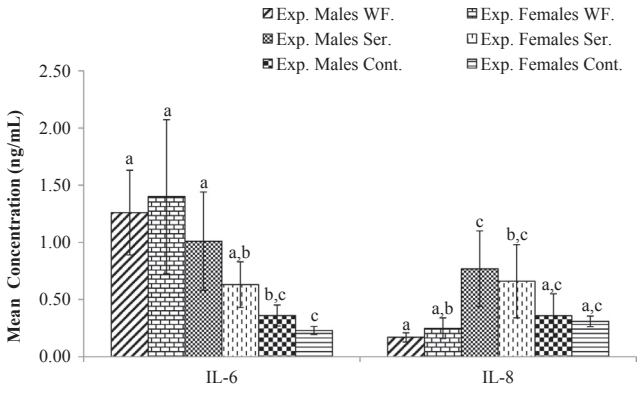


**Fig. 2.** Twelve (12) hours post-operative Interleukin (IL) -6 and -8 levels (ng/mL) in Male and Female dogs with cutaneous wounds (n = 3). [a,b,c] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly (P < 0.05) different. All assays and analyses were made in duplicates. n = total number of individuals in a group.

6 between the experimental and control group was significant (P < 0.05) at 12 h, while the difference in wound fluid and experimental serum IL-8 levels were significant (P < 0.05) at both 12 h and 60 h. Figs. 1 and 2 show the plot of IL-6 and IL-8 levels at 12 h irrespective of sex, and in males and females, respectively.

The haematology and serum biochemistry had minor fluctuations, although the values fell within the normal clinical limits [32–35] as shown in Figs. 3–5 and Tables 6–11, respectively. Fig. 6 indicate the various significant associations that existed between IL and 6, IL-8 and haematological parameters.

The urine specific gravity range was 1.015–1.030, while the pH range was 5–8. The urine pH, specific gravity and negative tests for ascorbic acid, leucocyte, nitrite, protein, ketone, bilirubin, glucose and urobilinogen in both groups pre- and post-operation were normal for healthy dogs [36].

### 3.2. Result and discussion

As shown in Tables 2 and 3, concentrations of IL-6 in wound fluid and serum of the experimental group attained its highest level at 12 h which was significantly different from the levels of the control group. This could be attributed to the local and systemic manifestation of the acute phase of inflammatory responses following injury in the experimental group. The higher IL-6 levels in wound fluids compared with those in the serum of the experimental group within the first 36 h could be ascribed to its dominating early local production, similar to earlier reports by Zuhoor et al. [23] stating that, higher levels of IL-6 were detected at operation site (seromas), compared with those in the serum in the early hours following breast surgery in humans. The finding emphasises the importance of early cytokine production from endothelial cells, with subsequent recruitment and activation of

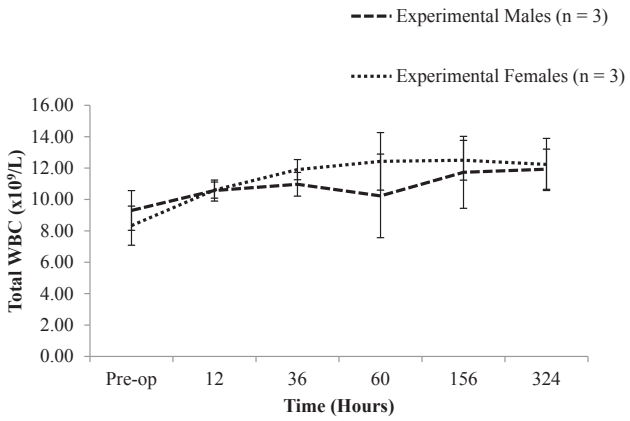


**Fig. 3.** Total White Blood Cells Count of the Experimental Group with minimal fluctuations. Values were increased post operation with higher values in females than males though both were maintained within normal range limits for dogs. Mean (Means ± SEM), n = total number of individuals in a group.

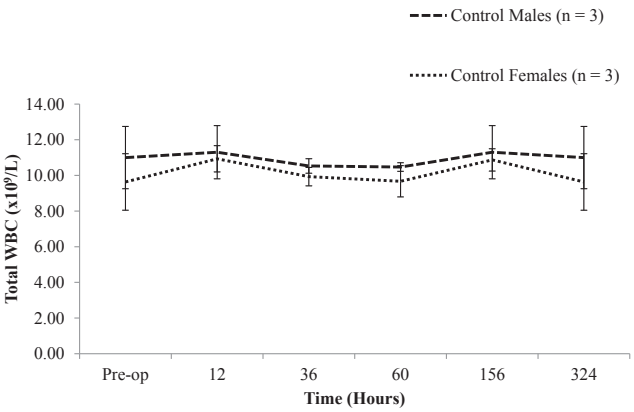


**Fig. 4.** Total White Blood Cells Count of the Control Group with minimal fluctuations. All values were maintained within normal range limits for dogs. Mean (Means ± SEM), n = Total number of individual in a group.

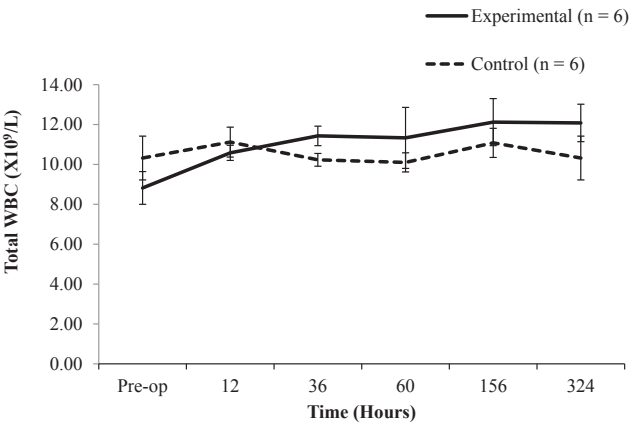


**Fig. 5.** Total White Blood Cells Count of the Experimental and Control Groups of dogs irrespective of sex. Mean (Means ± SEM), n = Total number of individual in a group.

fibroblasts and phagocytic cells, neutrophils, monocytes and macrophages [37,38]. Similarly, reports by Kondo and Ohshima [39] and Gallucci et al. [40] showed the observed induction of IL-6 after wounding in normal rats, attaining maximal levels between 12 h and 16 h before gradually returning to basal levels after 24 h.

In contrast, a second serum IL-6 spike was observed at 156 h in the experimental group which correspond with peak phagocytic, lymphocytic and fibroblastic activities at the interphase between the inflammatory and migratory/proliferative phases of wound healing [41]. The wound fluid IL-6 level at 156 h in the experimental group was within control group's limit which is necessary for the regulation of normal biological processes including metabolism, hematopoiesis, immune regulation and other physiologic functions [15,42,43].

Following cutaneous injury, an increased level of serum IL-8 was recorded, which peaked at 12 h and 60 h post-injury before normalising to control limits (Tables 4 and 5). The peaks encompassed the period of peak activities of the neutrophils and macrophages as well as the early stages of lymphocytic involvement in injuries, which is usually between 12 h and 72 h [41]. This finding may serve as evidence of IL-8 involvement in chemotaxis in target cells that initiated the inflammatory processes required for migration, with the accompanying phagocytosis by neutrophils and granulocytes at the site of injury [42–45].

As shown in Fig. 1, irrespective of sex, IL-6 levels in experimental dogs were higher ($P < 0.05$) than in the control dogs. The IL-8 level was lower in wound fluid and higher in experimental serum in comparison with that of the control serum. This finding serves as evidence that IL-8 is poorly secreted in wound fluids. The IL-6 level in wound fluid was higher ($P < 0.05$) than in experimental and control serum, except that between the experimental female wound fluid and male serum ($P > 0.05$) (Fig. 2). The IL-8 level was lower in wound fluid and higher in experimental serum, compared with the value recorded in control serum in both male and female dogs ($P > 0.05$). Although the trend of IL-8 level in wound fluid was similar to that obtained in the serum samples of injured dogs, it fell below the control circulatory level making its assay in serum in conjunction with IL-6 in wound fluid and serum samples a key parameter in the diagnosis, management and prognostic assessment of injuries and inflammation [23,46].

The results of this study demonstrate the effect of cytokine release (IL-6 and IL-8) in injury on white blood cell counts. As shown in Figs. 3–5, insignificant ($P > 0.05$) rises in total WBC count was observed in the experimental group following injury compared with those of the control group. The observed slight increase in the presence of injury is probably an effect of sufficient immune cell regulation under the signalling of the local and circulatory cytokines (IL-6 and IL-8), produced by epithelial cells, endothelial cells and circulatory leucocytes. This may be because the wounds were not extensive enough to trigger massive recruitment of immune cells into circulation. The higher values observed in females than in males with less fluctuations in the males than females for both injured and intact Dogs may be apparently due to hormonal effects, similar to observations by Ariyibi et al. [44] in apparently healthy dogs and Takeshi et al. [45] in dogs that underwent cardiopulmonary by-pass. The immune cells are vital to the regulation of the wound-healing process through the secretion of signaling molecules, such as cytokines, lymphokines and growth factors [47].

As shown in Tables 6 and 7, the gradual rise in neutrophil counts in the experimental group 12 h post injury compared with those in the control group was similar to finding reported by Robson et al. [48] that neutrophils counts rise in injury due to increased production of cytokines such as IL-6, IL-8, TGF-β, complement components such as C3a, C5a and formylmethionyl peptides by bacteria and platelet products. Increased need of wound debridement and phagocytosis, which constitute integral component of neutrophil primary function may also account for the rise [47,49]. Lymphocyte counts rose gradually in dogs with cutaneous wounds, with significant ($P < 0.01$) increase at 156 h, although within the reference limits together with those of the control group [34,35]. The finding was in agreement with the reports by Park and Barbul [47] that T-lymphocytes migrate into the wound after inflammatory cells and macrophages on the fifth day following injury during the proliferative phase, and peak at day 7. Significant ($P < 0.05$) increase in monocyte count was obtained 12 h post-injury

**Table 6**
Changes in neutrophil, lymphocyte, monocyte and eosinophil counts in dogs with cutaneous wounds irrespective of sex. (n = 6).

| Parameters/Groups | Pre-wounding | Hours Post-wounding | | | | |
|---|---|---|---|---|---|---|
| | 0 | 12 | 36 | 60 | 156 | 324 |
| Neutrophils *(3–12 × $10^9$/L) | | | | | | |
| Experimental (n = 6) | 6.27 ± 0.54 (4.59–7.84) | 7.57 ± 0.35 (6.37–8.93) | 8.08 ± 0.38 (6.91–9.29) | 8.45 ± 1.43 (4.55–13.11) | 8.05 ± 1.15 (5.19–11.74) | 8.75 ± 0.82 (5.67–11.02) |
| Control (n = 6) | 6.94 ± 0.76 (5.02–9.79) | 7.66 ± 0.46 (5.85–9.31) | 6.93 ± 0.36 (6.08–8.18) | 7.25 ± 0.38 (6.17–8.40) | 7.66 ± 0.46 (5.85–9.31) | 6.94 ± 0.76 (5.02–9.79) |
| Lymphocytes *(1–5 × $10^9$/L) | | | | | | |
| Experimental (n = 6) | 2.40 ± 0.26 (1.58–3.14) | 2.71 ± 0.16 (2.28–3.30) | 3.05 ± 0.23 (2.30–3.78) | 2.41 ± 0.20 (2.07–3.41) | 3.96 ± 0.20 (3.17–4.42) | 3.23 ± 0.14 (2.90–3.90) |
| Control (n = 6) | 3.15 ± 0.31 (2.24–4.18) | 3.23 ± 0.34 (2.42–4.51) | 2.98 ± 0.30 (1.84–3.70) | 2.99 ± 0.29 (1.88–3.70) | 3.45 ± 0.35 (2.41–4.51) | 3.15 ± 0.31 (2.24–4.18) |
| Monocytes*(0–1.4 × $10^9$/L) | | | | | | |
| Experimental (n = 6) | 0.07 ± 0.03 (0.00–0.21) | 0.25 ± 0.06 (0.00–0.35) | 0.22 ± 0.06 (0.00–0.38) | 0.18 ± 0.05 (0.00–0.31) | 0.06 ± 0.03 (0.00–0.14) | 0.08 ± 0.05 (0.00–0.29) |
| Control (n = 6) | 0.05 ± 0.02 (0.00–0.14) | 0.07 ± 0.02 (0.00–0.12) | 0.07 ± 0.05 (0.00–0.32) | 0.08 ± 0.04 (0.00–0.21) | 0.08 ± 0.03 (0.00–0.14) | 0.05 ± 0.02 (0.00–0.14) |
| Eosinophil *(0–1.3 × $10^9$/L) | | | | | | |
| Experimental (n = 6) | 0.09 ± 0.03 (0.00–0.22) | 0.05 ± 0.02 (0.00–0.12) | 0.08 ± 0.03 (0.00–0.13) | 0.09 ± 0.05 (0.00–0.32) | 0.05 ± 0.03 (0.00–0.16) | 0.04 ± 0.03 (0.00–0.15) |
| Control (n = 6) | 0.18 ± 0.05 (0.00–0.30) | 0.14 ± 0.04 (0.00–0.28) | 0.11 ± 0.04 (0.00–0.22) | 0.13 ± 0.03 (0.00–0.22) | 0.13 ± 0.03 (0.00–0.22) | 0.18 ± 0.05 (0.00–0.30) |

Reference values are in Parenthesis. All values obtained were within normal range limits for dogs. Mean (Means ± SEM), n = Total number of individual in a group, (*) = Reference values.

**Table 7**
Changes in neutrophil, lymphocyte, monocyte and eosinophil counts in dogs with cutaneous wounds.

| Parameters/Groups | Pre-wounding | Hours Post-wounding | | | | |
|---|---|---|---|---|---|---|
| | 0 | 12 | 36 | 60 | 156 | 324 |
| Neutrophils *(3–12 × $10^9$/L) | | | | | | |
| Experimental Males (n = 3) | 6.52 ± 0.84 | 7.22 ± 0.48 | 7.47 ± 0.37 | 7.30 ± 2.26 | 7.50 ± 2.12 | 9.21 ± 0.87 |
| Experimental Females (n = 3) | 6.01 ± 0.85 | 7.96 ± 0.51 | 8.68 ± 0.45 | 9.61 ± 1.93 | 8.60 ± 1.35 | 8.95 ± 1.66 |
| Control Males (n = 3) | 7.45 ± 1.20 | 7.64 ± 1.00 | 7.29 ± 0.59 | 7.29 ± 0.59 | 7.64 ± 1.00 | 7.45 ± 1.20 |
| Control Females (n = 3) | 6.43 ± 1.09 | 7.68 ± 0.28 | 5.89 ± 0.25 | 5.90 ± 0.25 | 7.68 ± 0.28 | 6.43 ± 1.09 |
| Lymphocytes *(1–5 × $10^9$/L) | | | | | | |
| Experimental Males (n = 3) | 2.61 ± 0.38 | 2.99 ± 0.16 | 3.21 ± 0.46 | 2.63 ± 0.40 | 4.15 ± 0.17 | 3.31 ± 0.30 |
| Experimental Females (n = 3) | 2.18 ± 0.37 | 2.43 ± 13.00 | 2.89 ± 0.17 | 2.60 ± 0.21 | 3.78 ± 0.36 | 3.16 ± 0.06 |
| Control Males (n = 3) | 3.28 ± 0.46 | 3.40 ± 0.56 | 2.63 ± 0.40 | 2.63 ± 0.40 | 3.40 ± 0.56 | 3.28 ± 0.46 |
| Control Females (n = 3) | 3.01 ± 0.51 | 4.09 ± 0.07 | 2.99 ± 0.58 | 2.99 ± 0.58 | 4.09 ± 0.07 | 3.01 ± 0.51 |
| Monocytes *(0–1.4 × $10^9$/L) | | | | | | |
| Experimental Males (n = 3) | 0.03 ± 0.03 | 0.29 ± 0.05 | 0.24 ± 0.08 | 0.28 ± 0.02 | 0.03 ± 0.03 | 0.06 ± 0.06 |
| Experimental Females (n = 3) | 0.10 ± 0.06 | 0.22 ± 0.11 | 0.21 ± 0.11 | 0.08 ± 0.05 | 0.08 ± 0.04 | 0.10 ± 0.09 |
| Control Males (n = 3) | 0.05 ± 0.05 | 0.07 ± 0.03 | 0.11 ± 0.01 | 0.12 ± 0.06 | 0.08 ± 0.04 | 0.05 ± 0.05 |
| Control Females (n = 3) | 0.05 ± 0.03 | 0.08 ± 0.04 | 0.03 ± 0.03 | 0.03 ± 0.03 | 0.08 ± 0.04 | 0.05 ± 0.03 |
| Eosinophils *(0–1.3 × $10^9$/L) | | | | | | |
| Experimental Males (n = 3) | 0.13 ± 0.05 | 0.07 ± 0.04 | 0.12 ± 0.01 | 0.15 ± 0.09 | 0.05 ± 0.05 | 0.05 ± 0.05 |
| Experimental Females (n = 3) | 0.04 ± 0.04 | 0.03 ± 0.03 | 0.04 ± 0.04 | 0.03 ± 0.03 | 0.05 ± 0.05 | 0.03 ± 0.03 |
| Control Males (n = 3) | 0.22 ± 0.07 | 0.19 ± 0.05 | 0.14 ± 0.07 | 0.18 ± 0.04 | 0.18 ± 0.02 | 0.22 ± 0.07 |
| Control Females (n = 3) | 0.14 ± 0.07 | 0.08 ± 0.04 | 0.09 ± 0.05 | 0.09 ± 0.05 | 0.08 ± 0.04 | 0.14 ± 0.07 |

Reference values are in Parenthesis. All values obtained were within normal range limits for dogs. Mean (Means ± SEM), n = Total number of individual in a group, (*) = Reference values.

and was maintained up to 36 h and 60 h before declining to values within control ranges at 60 h and 156 h, respectively for females and males. This finding collaborated the report by Witte and Barbul [41] that macrophages migrate into the wound 48–96 h after injury and become the predominant cell population to participate in the inflammatory and debridement processes. The major contribution of the monocytes to wound healing as they differentiate to macrophages is the secretion of cytokines and growth factors which control the healing

**Table 8**
Changes in HCT (PCV), haemoglobin concentration and erythrocyte counts in dogs with cutaneous wounds irrespective of sex. (n = 6).

| Parameters/Time (h) | Pre-operative | Hours Post-operative | | | | |
|---|---|---|---|---|---|---|
| | 0 | 12 | 36 | 60 | 156 | 324 |
| HCT *(0.36–0.55) | | | | | | |
| Experimental (n = 6) | 0.367 ± 0.003 (0.351–0.374) | 0.354 ± 0.007 (0.326–0.370) | 0.358 ± 0.013 (0.309–0.388) | 0.368 ± 0.010 (0.326–0.392) | 0.370 ± 0.005 (0.360–0.392) | 0.374 ± 0.007 (0.345–0.391) |
| Control (n = 6) | 0.368 ± 0.004 (0.357–0.380) | 0.364 ± 0.003 (0.351–0.375) | 0.365 ± 0.003 (0.356–0.376) | 0.365 ± 0.003 (0.356–0.376) | 0.365 ± 0.002 (0.360–0.373) | 0.370 ± 0.003 (0.361–0.380) |
| HGB *(120–180 × g/L) | | | | | | |
| Experimental (n = 6) | 123.50 ± 8.35 (90.66–154.51) | 123.10 ± 1.28 (117.97–126.69) | 124.30 ± 3.98 (108.10–135.10) | 126.90 ± 4.14 (113.76–142.98) | 119.40 ± 2.62 (110.55–128.27) | 123.80 ± 4.16 (104.88–131.70) |
| Control (n = 6) | 123.80 ± 1.98 (117.08–130.90) | 119.30 ± 1.52 (115.45–124.59) | 132.40 ± 10.09 (117.52–172.60) | 124.20 ± 1.96 (115.37–128.20) | 125.70 ± 2.88 (115.45–135.70) | 124.30 ± 1.70 (118.39–128.85) |
| RBC *(5.5–8.5 × $10^{12}$/L) | | | | | | |
| Experimental (n = 6) | 6.19 ± 0.10 (5.87–6.48) | 5.54 ± 0.37 (3.82–6.31) | 6.05 ± 0.28 (4.78–6.59) | 5.96 ± 0.26 (4.99–6.60) | 6.01 ± 0.13 (5.64–6.54) | 6.08 ± 0.30 (4.96–6.97) |
| Control (n = 6) | 6.01 ± 0.14 (5.62–6.35) | 6.20 ± 0.08 (5.96–6.46) | 6.29 ± 0.07 (6.13–6.58) | 6.19 ± 0.14 (5.49–6.43) | 6.53 ± 0.17 (6.01–7.10) | 6.04 ± 0.15 (5.62–6.54) |

Reference values are in Parenthesis. All values obtained are within normal range limits for dogs. Mean (Means ± SEM), n = Total number of individual in a group, (*) = Reference values.

**Table 9**
Changes in HCT (PCV), haemoglobin concentration and erythrocyte counts in dogs with cutaneous wounds.

| Parameters/Time (h) | Pre-operative | Hours Post-operative | | | | |
|---|---|---|---|---|---|---|
| | 0 | 12 | 36 | 60 | 156 | 324 |
| HCT *(0.36–0.55) | | | | | | |
| Experimental Males (n = 3) | 0.371 ± 0.002 | 0.356 ± 0.010 | 0.363 ± 0.017 | 0.379 ± 0.006 | 0.376 ± 0.009 | 0.378 ± 0.007 |
| Experimental Females (n = 3) | 0.363 ± 0.006 | 0.353 ± 0.014 | 0.353 ± 0.023 | 0.356 ± 0.019 | 0.364 ± 0.003 | 0.369 ± 0.013 |
| Control Males (n = 3) | 0.374 ± 0.004 | 0.369 ± 0.003 | 0.366 ± 0.006 | 0.365 ± 0.060 | 0.366 ± 0.003 | 0.370 ± 0.002 |
| Control Females (n = 3) | 0.362 ± 0.004 | 0.359 ± 0.004 | 0.364 ± 0.003 | 0.366 ± 0.030 | 0.365 ± 0.004 | 0.369 ± 0.006 |
| HGB *(120–180 × g/L) | | | | | | |
| Experimental Males (n = 3) | 124.80 ± 18.56 | 121.87 ± 2.56 | 126.93 ± 5.11 | 128.43 ± 1.74 | 124.13 ± 2.60 | 127.98 ± 3.39 |
| Experimental Females (n = 3) | 122.24 ± 1.41 | 124.29 ± 0.39 | 121.46 ± 6.76 | 125.35 ± 8.96 | 114.71 ± 2.34 | 119.64 ± 7.58 |
| Control Males (n = 3) | 127.04 ± 1.94 | 119.08 ± 2.80 | 121.06 ± 1.88 | 123.32 ± 3.98 | 123.35 ± 3.99 | 125.68 ± 1.70 |
| Control Females (n = 3) | 120.66 ± 2.36 | 119.53 ± 1.93 | 143.81 ± 19.40 | 124.99 ± 1.62 | 127.97 ± 4.50 | 122.89 ± 3.11 |
| RBC *(5.5–8.5 × $10^{12}$/L) | | | | | | |
| Experimental Males (n = 3) | 5.65 ± 0.12 | 6.11 ± 0.13 | 6.32 ± 0.21 | 6.47 ± 0.08 | 6.24 ± 0.17 | 6.59 ± 0.22 |
| Experimental Females (n = 3) | 6.27 ± 0.15 | 5.44 ± 0.81 | 5.77 ± 0.53 | 5.45 ± 0.28 | 5.78 ± 0.08 | 5.56 ± 0.36 |
| Control Males (n = 3) | 6.11 ± 0.19 | 6.17 ± 0.10 | 6.35 ± 0.13 | 6.05 ± 0.29 | 6.40 ± 0.28 | 6.05 ± 0.22 |
| Control Females (n = 3) | 5.91 ± 0.22 | 6.23 ± 0.15 | 6.22 ± 0.05 | 6.32 ± 0.07 | 6.66 ± 0.22 | 6.02 ± 0.26 |

Reference values are in Parenthesis. All values obtained are within normal range limits for dogs. Mean (Means ± SEM), n = Total number of individual in a group, (*) = Reference values.

**Table 10**
Changes in serum biochemical parameters in dogs with cutaneous wounds irrespective of sex.

| Parameters | Pre-operative (n = 6) | Post-operative (n = 6) | Control (n = 6) | Reference range |
|---|---|---|---|---|
| Urea (mg/dL) | 13.82 ± 1.75 | 15.53 ± 2.48 | 13.73 ± 0.78 | 6–25 |
| Creatinine (mg/dL) | 1.27 ± 0.122 | 1.17 ± 0.07 | 1.26 ± 0.11 | 0.5–1.6 |
| Glucose (mg/dL) | 78.67 ± 1.98[a,c] | 95.67 ± 4.26[b] | 78.83 ± 1.91[c] | 70–138 |
| Total protein (g/dL) | 7.32 ± 0.26 | 7.50 ± 0.38 | 7.37 ± 0.30 | 5.0–7.4 |
| Albumin (g/L) | 2.57 ± 0.08 | 2.60 ± 0.09 | 2.55 ± 0.09 | 2.3–3.1 |
| $Na^+$(mmol/L) | 140.50 ± 1.59 | 137.00 ± 3.08 | 144.20 ± 2.15 | 139–154 |
| $K^+$(mmol/L) | 3.62 ± 0.13 | 3.92 ± 0.25 | 3.60 ± 0.13 | 3.6–5.5 |
| $HCO_3^-$(mmol/L) | 19.67 ± 0.42 | 20.33 ± 1.43 | 19.67 ± 0.84 | 18–25 |
| $Cl^-$ (mmol/L) | 112.30 ± 2.93 | 106.30 ± 3.52 | 113.50 ± 3.21 | 102–120 |
| $Ca^{2+}$ (mmol/L) | 2.40 ± 0.07 | 2.45 ± 0.04 | 2.40 ± 0.15 | 2.2–3.0 |
| $PO_4^{3-}$ (mmol/L) | 1.35 ± 0.15 | 1.48 ± 0.15 | 1.57 ± 0.19 | 0.81–2.42 |

[a,b,c] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. Values are Mean ± SEM, Mean values of parameters in rows for males and females separately did not differ significantly ($p \geq 0.05$), Values were observed to be within normal limits with a slight increases post operation. $Na^+$; Sodium ion, $K^+$; Potassium ion, $HCO_3^-$; Hydrogen bicarbonate, $Cl^-$; Chloride ion and $Ca^{2+}$; Calcium ion, $PO_4^{3-}$; Phosphate. Reference range [32,33,35]. n = Total number of individual in a group.

**Table 11**
Changes in serum biochemical parameters in dogs with cutaneous wounds.

| Parameters | Pre-operative | | Control | | Post-operative | | Reference range |
|---|---|---|---|---|---|---|---|
| | Males (n = 3) | Females (n = 3) | Males (n = 3) | Females (n = 3) | Males (n = 3) | Females (n = 3) | |
| Urea (mg/dL) | 12.43 ± 1.45 | 15.20 ± 3.36 | 13.73 ± 0.78 | 13.87 ± 3.94 | 13.53 ± 4.01 | 17.53 ± 3.28 | 6–25 |
| Creatinine (mg/dL) | 1.20 ± 0.21 | 1.33 ± 0.17 | 1.20 ± 0.15 | 1.33 ± 0.17 | 1.17 ± 0.09 | 1.17 ± 0.12 | 0.5–1.6 |
| Glucose (mg/dL) | 78.33 ± 1.76 | 79.00 ± 4.04 | 79.00 ± 2.00 | 78.67 ± 3.76 | 93.00 ± 6.08 | 98.33 ± 6.84 | 70–138 |
| Total protein (g/dL) | 7.10 ± 0.45 | 7.53 ± 0.29 | 7.13 ± 0.55 | 7.60 ± 0.31 | 7.13 ± 0.47 | 7.87 ± 0.59 | 5.0–7.4 |
| Albumin (g/L) | 2.50 ± 0.15 | 2.63 ± 0.09 | 2.43 ± 0.13 | 2.67 ± 0.09 | 2.50 ± 0.15 | 2.70 ± 0.10 | 2.3–3.1 |
| $Na^+$(mmol/L) | 142.00 ± 3.06 | 139.00 ± 1.00 | 144.70 ± 3.93 | 143.70 ± 2.73 | 135.30 ± 6.36 | 138.70 ± 2.03 | 139–154 |
| $K^+$(mmol/L) | 3.50 ± 0.27 | 3.73 ± 0.08 | 3.53 ± 0.23 | 3.67 ± 0.15 | 3.67 ± 0.09 | 4.17 ± 0.50 | 3.6–5.5 |
| $HCO_3^-$(mmol/L) | 19.33 ± 0.67 | 20.00 ± 0.58 | 20.00 ± 0.00 | 19.33 ± 1.86 | 20.00 ± 2.65 | 20.67 ± 1.76 | 18–25 |
| $Cl^-$ (mmol/L) | 107.70 ± 2.96 | 117.00 ± 3.51 | 110.30 ± 5.78 | 116.70 ± 2.85 | 101.00 ± 2.52 | 111.70 ± 5.21 | 102–120 |
| $Ca^{2+}$ (mmol/L) | 2.37 ± 0.09 | 2.43 ± 0.12 | 2.40 ± 0.10 | 2.40 ± 0.06 | 2.40 ± 0.06 | 2.50 ± 0.06 | 2.2–3.0 |
| $PO_4^{3-}$ (mmol/L) | 1.30 ± 0.21 | 1.40 ± 0.26 | 1.70 ± 0.29 | 1.43 ± 0.27 | 1.43 ± 0.28 | 1.53 ± 0.19 | 0.81–2.42 |

Values are Mean ± SEM, Mean values of parameters in rows for males and females separately did not differ significantly ($p \geq 0.05$), Values were observed to be within normal limits with a slight increases post operation. $Na^+$; Sodium ion, $K^+$; Potassium ion, $HCO_3^-$; Hydrogen bicarbonate, $Cl^-$; Chloride ion and $Ca^{2+}$; Calcium ion, $PO_4^{3-}$; Phosphate. Reference range [32,33,35]. n = Total number of individual in a group.

process [15,50,51]. Eosinophil counts remained within reference ranges, with minimal fluctuations occurring during the period of experiment apparently due to cytokines balancing effect in cell recruitment.

Tables 8 and 9 demonstrated the erythrocyte response to injury with obvious drop in haematocrit, haemoglobin concentration and red blood cell counts 12 h post injury before normalising to values within control limits at 36 h. This finding could be attributed to surgical stress and losses associated with extravasation of blood and fluids due to increased vascular permeability resulting from the vasodilatory effects exerted by vasoactive amines released by platelets into the circulation [52,53]. The finding is similar to those by Simon and Ali [51] and Coutin et al. [54], who showed that trauma associated with surgery creates a unique extravascular environment with increased capillary permeability in dogs. The mean erythrocyte parameters were largely higher in males than females. In this study, the values for the parameters obtained were consistent with the report by Olayemi et al. [17], for apparently healthy, dogs Nigerian indigenous dogs, but lower than values earlier reported for clinically-healthy tropical dogs [32,44,55], although differences in the values between the groups were insignificant.

Serum biochemical parameters are of significance as sensitive changes in health status of an individual can be picked up very early, reflected as alterations in these values [19]. As shown in Tables 10 and 11, all the serum biochemical values were within reference range limits, although the slight increase (upward shift) in the experimental group post-operative were insignificant [32,33,35]. As documented by earlier researchers, the relative increases could be attributed to changes in feeding habit and nutrition due to surgical stress [32].

Correlations between the IL-6, IL-8 and the haematologic parameters showed some significant relationships in the experimental group. Fig. 6(A–F) demonstrate the correlations between the cytokine levels and some haematologic parameters in injured dogs irrespective of sex. Statistically significant ($P < 0.05$) positive correlations was obtained between serum and wound fluid IL-6 ($r = 0.827$, $P = 0.04$) in experimental group, suggesting the suitability of both wound fluid and serum samples for IL-6 evaluations. The strong negative relationship between IL and 6 and erythrocyte parameters as shown in Fig. 6B, D and IL-6 with HCT ($r = -0.894$, $P = 0.02$) could be ascribed to the effect of the released IL-6 on haematopoesis in response to losses following surgical trauma and microvascular injuries. As shown in Fig. 6C, E and F, the positive correlations between wound fluid levels of IL-6 and monocyte count ($r = 0.818$, $P = 0.04$), serum IL-8 with serum IL-6 ($r = 0.622$; $P = 0.19$) and wound fluid IL-8 ($r = 0.718$; $P = 0.12$) respectively suggest their modulatory (up-regulatory and down-regulatory) effects on the wound healing processes. The observation showed that IL-6 and IL-8 may be prospective predictive tool of outcomes of wound healing that would further influence decisions regarding adoption of specific management modalities as healing progresses. This finding was similar to observations reported by earlier researchers [56–59].

The result of this study showed that wounds influence changes in IL-6 and IL-8 levels, evidenced by fluctuations in the levels of the cytokines released by leucocytes, epithelial and endothelial cells. The changes resulted in controlled recruitment and activation of other cells of the immune system, such as monocytes, dendritic cells, and T-cells, which provided evidences for the key role the cells play in the regulation of specific host immune responses [60,61].

It is worthy of note that all the dogs used for the experiment were humanely handled from transportation, accommodation, feeding and through the entire study stages. All the dogs survived with complete closure of the entire wounds surfaces by day 42 and have been fostered to suitable families.

The limitations of the present study includes: Firstly, we did not examine IL-6 and IL-8 levels following injury on other frequently predisposed body sites, e.g., the head and neck, back and thigh regions. Secondly, we did not study these cytokines levels expressed in relation to the various stages of healing. Therefore, we could not establish the relationship between changing levels of IL-6 and IL-8 in the individual stages of wound healing. Thirdly, we did not evaluate for the IL-6 and IL-8 gene expressed in the Dogs. However, to our knowledge, this is the first study to evaluate IL-6 and IL-8 levels in cutaneous wounds of these dogs in Nigeria.

## 4. Conclusion

The results showed a significant early increase in wound fluid and serum IL-6 as well as serum IL-8 in dogs with cutaneous wounds, compared to the serum of non-wounded dogs with modulations of haematological parameters. It provided evidence that validation and investigation into the expression patterns and functionality of IL-6 and IL-8 in cutaneous wounds may be warranted.

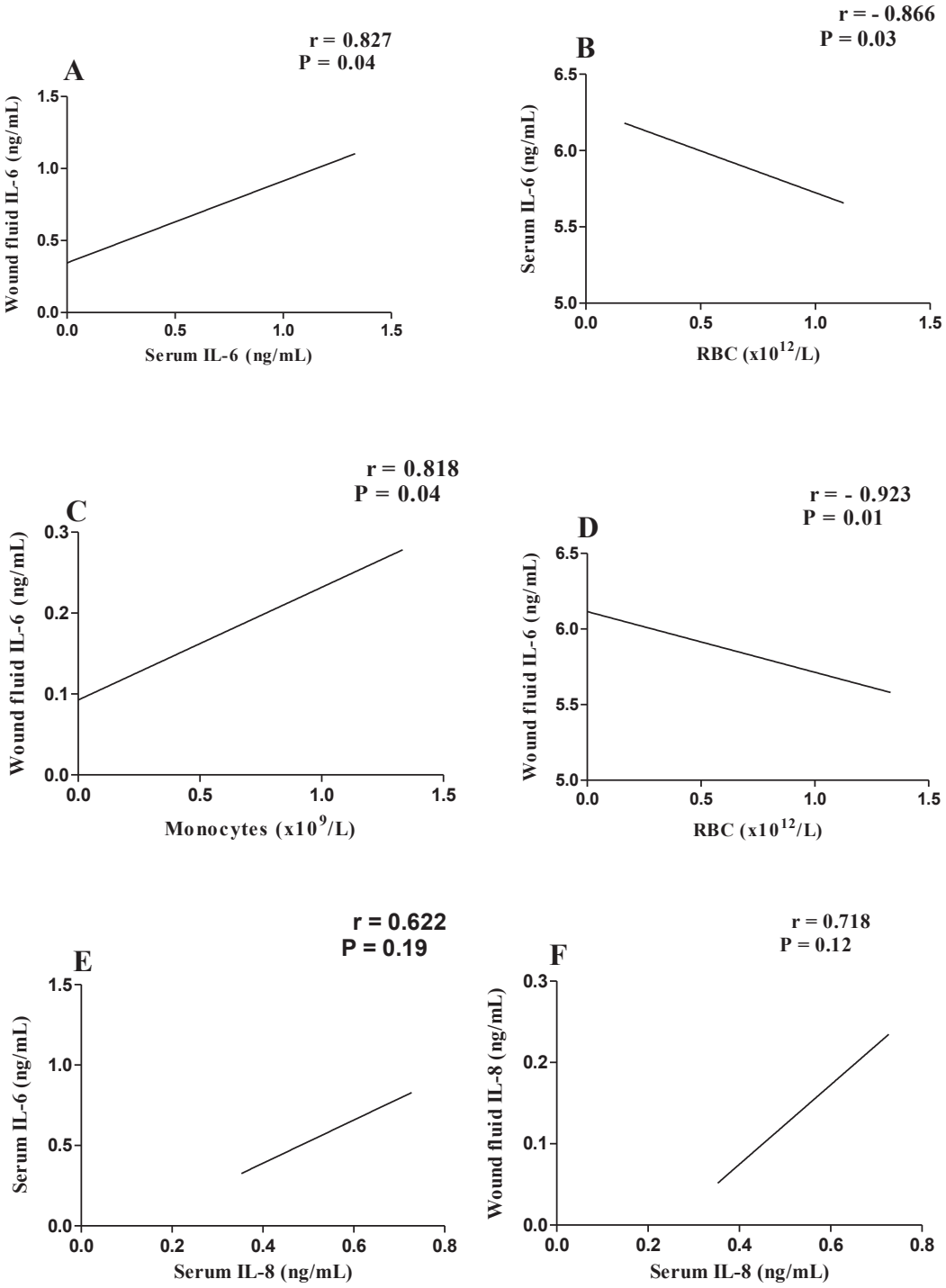


**Fig. 6.** Relationship between the levels of IL-6 with some haematologic parameters in the experimental group: (A) Serum IL-6 and wound fluid IL-6; (B) Serum IL-6 and red blood cell counts; (C) Wound fluid IL-6 and Monocyte counts; (D) Wound fluid IL-6 and red blood cell counts; (E) Serum IL-6 and Serum IL-8; (F) Wound fluid IL-8 and Serum IL-8. r = Pearson's correlation coefficient, $P < 0.05$ are considered significant. n = 6 (n = Total number of individual in a group).

## Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. AVAZI, Daniel Onimisi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception, design: Awasum Cheh Augustine, Avazi Daniel Onimisi, Hassan Adamu Zoaka, Ayo Joseph Olusegun, Muhammed Saidu Tanko, Yila Ayo Simon, Aluwong Tagang, Suleiman Mohammad Hadi, Kudi Ayuba Caleb.

Acquisition of data: Avazi Daniel Onimisi, Awasum Cheh Augustine, Hassan Adamu Zoaka, Muhammad Sa'idu Tanko.

Analysis and interpretation of data: Avazi Daniel Onimisi, Awasum Cheh Augustine, Hassan Adamu Zoaka, Ayo Joseph Olusegun, Aluwong Tagang, Suleiman Mohammad Hadi Kudi, Ayuba Caleb.

## Competing interest

None.

## Research funding

None declared.

## Acknowledgments

Many thanks to Dr. Y. Yunusa (Veterinary Teaching Hospital Molecular Laboratory, Ahmadu Bello University, Zaria), Mallam Sanni and Mr. I. Mohammed (Chemical Pathology Laboratory, University Medical Centre, Ahmadu Bello University, Zaria) for the assistance and guidance they provided.

## References

[1] H. Wang, J.M. Vishnubhakat, O. Bloom, M. Zhang, M. Ombrellino, A. Sama, et al., Pro-inflammatory cytokines (tumour necrosis factor and interleukin-1) stimulate release of high mobility group protein-1 by pituicytes, Surgery 126 (2) (1999) 389–392.
[2] A. Orzechowski, Cytokines in skeletal muscle growth and decay, in: L. Sakuma (Ed.), The Plasticity of Skeletal Muscle, Springer, Singapore, 2017, pp. 113–139, , http://dx.doi.org/10.1007/978-981-10-3292-9_5.
[3] T. Charlotte, D. Ruth, C. Ernest, Anti cytokine therapy in chronic inflammatory artheritis, Cytokine 86 (2016) 92–99.
[4] B. Wolff, A.R. Burns, J. Middleton, A. Rot, Endothelial cell "memory" of inflammatory stimulation: human venular endothelial cells store interleukin 8 in Weibel-Palade bodies, J. Exp. Med. 188 (9) (1998) 1757–1762, http://dx.doi.org/10.1084/jem.188.9.1757.
[5] J.O. Utgaard, F.L. Jahnsen, A. Bakka, P. Brandtzaeg, G. Haraldsen, Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells, J. Exp. Med. 188 (9) (1998) 1751–1756, http://dx.doi.org/10.1084/jem.188.9.1751.
[6] J.C. Hedges, C.A. Singer, W.T. Gerthoffer, Mitogen-activated protein kinases regulate cytokine gene expression in human airway myocytes, Am. J. Respir. Cell Mol. Bio. 23 (1) (2000) 86–94, http://dx.doi.org/10.1165/ajrcmb.23.1.4014.
[7] K. Bacon, M. Baggiolini, H. Broxmeyer, R. Horuk, I. Lindley, A. Mantovani, IUIS/World Health Organisation Subcommittee on chemokine nomenclature, Chemokine/chemokine receptor nomenclature, J. Interferon Cytokine Res. 22 (10) (2002) 1067–1068, http://dx.doi.org/10.1089/107999002760624305.
[8] M.T. Karen, A.J. Spencer (Eds.), Veterinary Surgery: Small Animal Surgery, first ed., vol. 2, Elsevier Saunders, Philadelphia, 2012.
[9] B.M. Borena, A. Martens, S.Y. Broeckx, E. Meyer, K. Chiers, L. Duchateau, J.H. Spaas, Regenerative skin wound healing in mammals: state-of-the-art on growth factor and stem cell based treatments, Cell Physiol. Biochem. 36 (1) (2015) 1–23.
[10] M. Hibi, M. Murukami, M. Saito, T. Hirano, T. Taga, T. Kishimoto, Molecular cloning and expression of an IL-6 signal tranducer, gp 130, Cell 63 (6) (1990) 1149–1157.
[11] A. Schwantner, A.J. Dingley, S. Ozbek, S. Rose-John, J. Grötzinger, Direct determination of the interleukin-6 binding epitope of the interleukin-6 receptor by NMR spectroscopy, J. Biol. Chem. 279 (1) (2004) 571–576, http://dx.doi.org/10.1074/jbc.M311019200.
[12] P.C. Heinrich, I. Behrmann, G. Müller-Newen, F. Schaper, L. Graeve, Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway, Biochem. J. 334 (Pt 2) (1998) 297–314.
[13] D.J. Brat, A.C. Bellail, E.G. Van Meir, The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis, Neuro Oncol. 7 (2) (2005) 122–133.
[14] M. Rottner, J.M. Freyssinet, M.C. Martínez, Mechanisms of the noxious inflammatory cycle in cystic fibrosis, Respir. Res. 10 (1) (2009) 23, http://dx.doi.org/10.1186/1465-9921-10-23.
[15] J.A. Owen, J. Punt, S.A. Stranford, P.P. Jones, Receptors and signalling: cytokines and chemokine, in: Kuby (Ed.), Immunology, W.H. Freeman and Company, California, 2013, pp. 105–140.
[16] A.Z. Hassan, B.D. Remi-Adewumi, D.A.Y. Adawa, F.B. Hassan, A.A. Adeiza, Wound management – a review, SJVS 2 (2003) 1–9.
[17] F.O. Olayemi, I.O. Azeez, A. Ogunyemi, F.O. Ighagbon, Study on erythrocyte values on the Nigerian indigenous dog, Folia Vet. 53 (2) (2009) 65–67.
[18] O.I. Olumayowa, Gross morphometric study of the eyeball and tongue of the Nigerian local dog, Ital. J. Anat. Embryol. 116 (1) (2011) 104–110.
[19] J.I. Ihedioha, J.I. Ugwuja, O.A. Noel-Uneke, I.J. Udeani, G. Daniel-Igwe, Reference values for the haematology profile of conventional grade outbred albino mice (*Mus musculus*) in Nsukka, Eastern Nigeria, Anim. Res. Int. 9 (2) (2012) 1601–1612.
[20] A. Ambrosch, R. Lobmann, A. Pott, J. Preissler, Interleukin-6 concentrations in wound fluids rather than serological markers are useful in assessing bacterial triggers of ulcer inflammation, Int. Wound J. 5 (1) (2008) 99–106.
[21] M.G. Kerr, Veterinary Laboratory Medicine, Clinical Biochemistry and Haematology, Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Melbourne, 1989, pp. 1–386.
[22] V. Ramnath, P.S. Rekha, K.S. Sujatha, Amelioration of heat stress-induced disturbances of antioxidant defence system in chickens by Brahma rasayana, Evid. Based Complement. Alternat. Med. 5 (1) (2008) 77–84.
[23] K.A. Zuhoor, Proinflammatory cytokines dynamics at operation site and serum after breast surgery, Arch. Clin. Exp. Surg. 2 (3) (2012) 161–169.
[24] A. Alessandra, D.G. Comelia, D. Nadia, B. Davide, S. Sabina, K. Vittorio, M. Giuseppe, Anticoagulants used in plasma collection affects adipokine multiplexed measurement (IL6, TNF-α), Cytokine 85 (2016) 5–10 https://dio.org/10.1016/j.cyto.2016.03.001.
[25] A. Najib, D. Roger, J.Q. Joshua, L. Qian, G. David, W.B. Anthony, Stability of cytokines and soluble activation markers in unprocessed blood stored under different conditions, Cytokine 84 (2016) 17–24 https://dio.org/10.1016/j.cyto.2016.05.010.
[26] G.W. Snedecor, W.G. Cochran, Statistical Methods, Oxford and IBH Publishing Corporation, Calcutta, India, 1994, p. 509.
[27] S.K. Das, S. Varadham, G. Gupta, S. Mukherjee, L. Dhanya, D.N. Rao, D.M. Vasudevan, Time-dependent effect of ethanol in blood oxidative stress parameters and cytokines, Indian J. Biochem. Biophys. 46 (2009) 116–121.
[28] J.M. Schmidt, C. Rummel, J. Hauer, M. Kolecka, N. Ondreka, V. McClure, et al., Increased CSF aquaporin-4, and interleukin-6 levels in dogs with idiopathic communicating internal hydrocephalus and a decrease after ventriculo-peritoneal shunting, Fluids Barriers CNS 13 (2016) 12.
[29] N. Stephan, K. Franz-Josef, S. Sonja, Interleukin-6 (IL-6) serum concentrations in dogs with hepatitis and hepatic tumours compared with those with extra-hepatic inflammation and tumours, Comp. Clin. Pathol. 21 (2002) 539–544.
[30] H. Melanie, K. Franz-Josef, N. Stephan, Evaluation of serum interleukin-8 (CXCL8) concentrations in tumor bearing dogs, Am. J. Anim. Vet. Sci. 10 (4) (2015) 202–211, http://dx.doi.org/10.3844/ajavsp.2015.202.211.
[31] D.A.P.C. Zuccari, R. Castro, G.B. Gelaleti, U.M. Mancini, Interleukin-8 expression associated with canine mammary tumors, Genet. Mol. Res. 10 (3) (2011) 1522–1532, http://dx.doi.org/10.4238/vol10-3gmr1145.
[32] J.N. Awah, H.O. Nottidge, Serum biomedical parameters in clinically healthy dogs in Ibadan, Trop. Vet. 16 (1998) 123–129.
[33] L.C. Pause, G.F. Grauer, Association of gastrointestinal haemorrhage with increased blood urea nitrogen and BUN/Creatinie ratio in dogs: literature review and retrospective study, Vet. Clin. Pathol. 27 (4) (1998) 107–111.
[34] A.Z. Hassan, F.B. Hassan, An Introduction to Veterinary Practice, Ahmadu Bello University Press Limited, Zaria, Nigeria, 2003, pp. 47–65.
[35] J.J. Ihedioha, V.O. Anosa, K.A.N. Esievo, Prevelence of and clinicopathologic findings associated with ascites in dogs in Enugu state, Nigeria, Comp. Clin. Pathol. 22 (2) (2013) 185–193.
[36] J. Archer, Urine analysis, in: E. Villiers, L. Blackwood (Eds.), Manual of Canine and Feline Clinical Pathology, second ed. BSAVA, Gloucester, UK, 2005, pp. 149–168 (Chapter 10).
[37] O.J. Warren, A.J. Smith, C. Alexiou, P.L. Rogers, N. Jawad, C. Vincent, et al., The inflammatory response to cardiopulmonary bypass: part 1–mechanisms of pathogenesis, J. Cardiothorac. Vasc. Anesth. 23 (2009) 223–231.
[38] T. John, S. Petter, E. Joel, T. Victoria, H.B.W. Allan, Reference range and short-and-long-term biological variation of IL-6, IL-17A and tissue necrosis factor-alpha using high sensitivity assays, Cytokine 64 (3) (2013) 660–665 https://dio.org/10.1016/j.cyto.2013.09.018.
[39] T. Kondo, T. Ohshima, The dynamics of inflammatory cytokines in the healing process of mouse skin wound: a preliminary study for possible wound age determination, Int. J. Legal Med. 108 (5) (1996) 231–236.
[40] R.M. Gallucci, P.P. Simeonova, J.M. Matheson, C. Kommineni, J.L. Guriel, T. Sugawara, M.I. Luster, Impaired cutaneous wound healing in interleukin-6–deficient and immunosuppressed mice, FASEB J. 14 (15) (2000) 2525–2531.
[41] M.B. Witte, A. Barbul, General principles of wound healing (In: Park, J. E. and Barbul, A. (2004). Understanding the role of immune regulation in wound healing. Am. J. Surg. 1997; 187(Suppl to May 2004): 11S–16S), Surg. Clin. North Am. 77 (3) (1997) 509–528.
[42] P. Sheeran, G.M. Hall, Cytokines in anaesthesia, Br. J. Anaesth. 78 (2) (1997) 201–202.
[43] G. Gueret, F. Lion, N. Guriec, J. Arvieux, A. Dovergne, C. Guennegan, et al., Acute renal dysfunction after cardiac surgery with cardiopulmonary bypass is associated with plasmatic IL6 increase, Cytokine 45 (2) (2009) 92–98, http://dx.doi.org/10.1016/j.cyto.2008.11.001.
[44] A.A. Ariyibi, M.O. Oyeyemi, R.A. Ajadi, Comparative study of some haematological and biochemical parameters of clinically healthy Alsatian and local dogs, Afr. J. Biomed. Res. 5 (3) (2002) 145–147.
[45] M. Takeshi, K. Hiroshi, M. Masashi, M. Takahiro, S. Asako, H. Kayoko, U. Masami, Plasma cytokine levels in dogs undergoing cardiopulmonary bypass, Res. Vet. Sci. 101 (2015) 99–105.
[46] M.T. Sharabiani, R. Vermeulen, C. Scoccianti, F.S. Hosnijeh, L. Minelli, C. Sacerdote, P. Vineis, Immunologic profile of excessive body weight, Biomarkers 16 (3) (2011) 243–251, http://dx.doi.org/10.3109/1354750X.2010.547948.
[47] J.E. Park, A. Barbul, Understanding the role of immune regulation in wound healing, Am. J. Surg. 187 (Suppl to May 2004) (2004) 11S–16S.
[48] M.C. Robson, D.L. Steed, M.G. Franz, Wound healing: biologic features and approaches to maximize healing trajectories, Curr. Probl. Surg. 38 (2) (2001) 72–140.
[49] N.Z. Canturk, N. Esen, B. Vural, Z. Canturk, G. Kirkali, G. Oktay, S. Solakoglu, The relationship between neutrophils and incisional wound healing, Skin Pharmacol. Appl. Skin Physiol. 14 (2) (2001) 108–116.
[50] G. Broughton, J.E. Janis, C.E. Attinger, The basic science of wound healing, Plast. Reconstr. Surg. 117 (7 Suppl.) (2006) 12S–34S.
[51] R.M. Simon, M.G. Ali, Wound healing and scar formation, in: D.F. Ross, W.B. Neil, C. Sabrina (Eds.), Plastic and Reconstructive Surgery: Approaches and Techniques, John Wiley & Sons Ltd., 2015.
[52] W.T. Lawrence, Physiology of the acute wound, Clin. Plast. Surg. 25 (3) (1998) 321–340.
[53] M. Richardson, Acute wounds: an overview of the physiological healing process,

Nurs. Times 100 (4) (2004) 50–53.
[54] J.V. Coutin, I.L. Otto, C.M. Geraldine, F.E. Marion, I.M. Emily, R.W. Stephen, O.R. Thomas, Cefazolin concentration in surgically created wounds treated with negative pressure wound therapy compared to surgically created wounds treated with nonadherent wound dressings, Vet. Surg. 2015 (44) (2015) 10, http://dx.doi.org/10.1111/j.1532-950X.2014.12218.x.
[55] D.I. Saror, T.W. Schillhorn van Veen, J.B. Adeyanju, The haemogram of dogs with intestinal parasites in Zaria, Nigeria, J. Small Anim. Pract. 20 (4) (1979) 243–247.
[56] F. Carlstedt, L. Lind, B. Lindahl, Pro-inflammatory cytokines, measured in a mixed population on arrival in the emergency department, are related to mortality and severity of disease, J. Intern. Med. 242 (5) (1997) 361–365.
[57] R. Moseley, J.E. Stewart, P. Stephens, R.J. Waddington, D.W. Thomas, Extracellular matrix metabolites as potential biomarkers of disease activity in wound fluid: lessons learned from other inflammatory diseases? Br. J. Dermatol. 150 (3) (2004) 401–413.
[58] S. Masih, Cytokines: Utility and Laboratory Measurement, Med. Lab. Obs. 2011, pp. 1–3.
[59] H.R. Ahmed, E. Mohamed, F.A. Adel, Tissue extract fluid cytokine levels as markers for wound vitality: an experimental comparative study, J. Am. Sci. 9 (1) (2013) 188–193.
[60] U. Auf-dem-Keller, A. Kümin, S. Braun, S. Werner, Reactive oxygen species and their detoxification in healing skin wounds, J. Investig. Dermatol. Symp. Proc. 11 (1) (2006) 106–111.
[61] O. Soehnlein, C. Weber, L. Lindbom, Neutrophil granule proteins tune monocytic cell function, Trends Immunol. 30 (2009) 538–546.