

Evaluation of Interleukin-1 β , Interleukin-6 and Interleukin-10 Profiles in Serum and Surgically Created Wound Exudates of Alloxan-Induced Diabetic Rabbits

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Abstract

The knowledge of immune-modulatory actions of interleukins could be of immense value in the management of diabetic wounds. This study evaluated the interleukin profiles of diabetic wounded rabbits. Sixteen New Zealand White rabbits of mean weight 2.5 \pm 0.7 kg used for this study were divided into four groups; A, B, C and D, of four rabbits each; A (non-diabetic and non-wounded) as control, B (diabetic and non-wounded), C (wounded and non-diabetic) and D (wounded and diabetic). Three (3) cm² skin wounds were created in a standard aseptic procedure while diabetes was induced by administration of alloxan monohydrate. Serum and wound exudate were harvested on days 0, 3, 7, 14, 21 and 28 for Interleukin-1 β , Interleukine-6 and Interleukine-10 assays. The increases in the serum concentration of Interleukin-1 β and Interleukine-6 in the C rabbit group between days 3 and 7 (Interleukin-1 β) and between days 3 and 28 (Interleukine-6) were relatively higher than the corresponding day 0 and control values. The decrease in the serum concentration of IL-10 (ng /mL) in B rabbit group from days 7 (36.96 \pm 8.7) to 14 (128.12 \pm 12.05) were significantly lower ($P < 0.05$) than the corresponding values in groups A and C rabbits. The values of Interleukine-10 in wound exudate of C rabbit group from days 7 (160.57 \pm 23.76 ng /mL) to 21 (177.30 \pm 8.60 ng /mL) were significantly higher ($P < 0.05$) than the corresponding group D values. The evidence shows that, the values of Interleukin-1 β and Interleukine-6 are up regulated in the inflammatory phase of wound healing in normoglycaemic and diabetic rabbits while the values of Interleukine-10 is down regulated during wound healing in diabetic rabbits. Therefore, the knowledge of interleukin markers shall be of significant roles in proffering solution for management of normoglycaemic and diabetic wounds.

Keywords: Diabetic Wounds, Interleukin-1 β , Interleukine-6, Interleukine-10

Introduction

Cytokines are classes of biologically active low molecular weight proteins with hormone-like and or enzyme-substrate actions that play important roles of modulating the intensity, actions, production and duration of immune and physiologic cells of the body for effective response to the environment [1]. Among various classes of these cytokine is the most abundant class that stimulates immune cell proliferation known as the interleukin family.

Interleukins are subsets of cytokines secreted by leukocyte and other non-haematopoietic cells including: helper CD4⁺, T lymphocytes and monocytes, and acts on another type of leukocyte to produce numerous effects [2, 3]. They activate T cells by stimulating the proliferation of antigen-activated T and B cells and by stimulating proliferation and differentiation of B cells and Interferon-gamma (IFN- γ) which activates macrophages, Granulocyte Monocyte Colony-Stimulating Factor (GM-CSF) and consequently stimulate

hematopoiesis [4]. Interleukins play important roles in the complex signalling network of activities involved in wound healing [1]. The multiple factors that influence macrophage phenotype and other cells expressed in wounds are partly determined by the relative balance between the pro-inflammatory and anti-inflammatory stimuli present in the wound environment [5].

Wound healing is a fundamental, dynamic, coordinated, interdependent and overlapping cellular and immunologic response of tissue to injury [6-8]. It is essential to prevent pathogenic invasion of damaged tissues and to partially or completely reform the affected tissues [7,8].

Diabetes is a metabolic and heterogeneous disorder characterised primarily by hyperglycaemia due to deficiency of insulin or insensitivity of insulin receptors for normal processes of glucose metabolism in the body [9,10]. The hyperglycaemia is manifested as glycosuria, hyperlipidaemia, polyuria and polydipsia [10,11]. Diabetes has remained one of the leading causes of death, illness and economic losses in the world [12]. It is associated with delayed and chronic wound complication that has continued to demand medical attention and poses great challenges to clinicians [13].

Interleukin-1 β (IL-1 β) is one of the pro-inflammatory cytokines of the interleukin family that is regularly detected in wounds [14]. It is produced by the monocytes, macrophages and the B-cells, and it exerts its effect on various cells of the body [15]. IL-1 β stimulates inflammation by increasing mobilization of leukocytes from the bone marrow and secretion of acute-phase proteins from the liver while facilitating proliferation and maturation of other cells [4].

Interleukin-6 (IL-6) has both pro and anti-inflammatory effects and has also been detected at a higher quantity in a chronic wound [16,17]. It plays an active role in the production of acute phase protein and stimulates the production of cellular and humoral immune responses such as B-cell differentiation, T-cell and immunoglobulin G secretion. In acute phase, IL-6 plays the dual roles of suppressing the level of pro-inflammatory cytokines while potentiating the anti-inflammatory cytokines [18,19].

Interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokines in wound healing. It is produced and expressed in wound sites by keratinocytes of wound epidermis and infiltrating monocyte cells [20]. IL-10 inhibits the infiltration of macrophages and terminates other inflammatory responses while regulating the differentiation of various immune cells, keratinocytes and other endothelial cells. It is considered anti-fibrotic because of its anti-inflammatory activity and as an inhibitor of Tumour Necrotic Factor (TNF- α).

Researchers have focused on understanding the functions of interleukin family of cytokine in wound healing for effective management of wounds [21]. The interleukin profile of animals may vary not only according to species, sex, age, breeds but also based on the health status of the animals and environment where they are located, and this has made it a subject of interest in

different fields of studies [16,17,22].

Few reports are available on the interleukin profile of wounded diabetic rabbits [23]. Understanding the immune-modulatory actions of interleukins and their variation in diabetic wound complications, no doubt shall give insight towards the study and management of the wounds [24,25]. It is expected that interleukin markers can be of significant roles in the study of normoglycaemic and diabetic wounds and in proffering solution to the management of the wound [1]. This study therefore, aimed at establishing the IL-1 β , IL-6 and IL-10 profiles in serum and surgically created wound exudates of alloxan-induced diabetic rabbits

Materials and Methods

Ethical clearance was obtained for this study from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), (Reference number: ABUCAUC/2019/028). This study was carried out in the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Sixteen adult New Zealand White (NZW) rabbits of both sexes, aged 12 0 ± 0.4 months and weighing 2.5 ± 0.7 kg were used for this study. They were procured from the National Animal Production and Research Institute (NAPRI), Zaria, Kaduna State, Nigeria.

The rabbits were kept in intensive management system in separate cages and fed morning and evening daily with combined poultry grower feeds, finisher feeds (Grand Cereals Limited (R), Jos, Plateau State) and miller's bran that were mixed in equal proportion in order to regulate the blood glucose level of the hyperglycaemic groups. *Daucus carota subsp. sativus* leaves was given to the rabbits twice weekly to avoid gastro-intestinal impaction and constipation while water was given *ad-libitum* throughout the experimental period. The rabbits were conditioned to the laboratory for two weeks before the commencement of the experiment. During this period, they were administered with anthelmintic Ivermectin (Hebei, Kexing pharmaceutical, China) prophylaxis at dosage of 0.4mg/kg start, and repeated after two weeks to prevent mites and other internal and external parasites. The rabbits were preliminarily clinically evaluated using body condition scores, vital and haematological parameters and adjudged apparently healthy before commencement of the study.

Experimental Protocols

The rabbits were divided into 4 groups (A, B, C and D) of 4 rabbits each comprising of two males and two females in each group. Group A (control): No Diabetes was induced and No Wound was created (NDNW); Group B: Diabetes was induced in this group, but No Wound was created (DNW); Group C: Wound was created, but Diabetes was Not induced (WND) and Group D: Wound was created and Diabetes was induced (WD), respectively.

Induction and Confirmation of Diabetes

Diabetes was induced by intravenous administration of 200 mg/kg of Alloxan monohydrate (SIGMA-aldrich, UK) through the marginal ear vein at two occasions (100 mg/kg each) at 72 hours

intervals [12,26]. Using a glucometer (ACCU-CHEK (R), Roche, Mannheim, Germany), diabetes was confirmed by rise in blood glucose level above normal value of NZW rabbits (150g/dl), to constant values of 250 - 350 mg/dl between day 3 and 28 post alloxan induction [12].

Wound Creation

The dorsum of each of the four rabbits in the NDW (group C) and WD (group D) were prepared aseptically following standard operating procedure. The rabbits were anaesthetized by intramuscular injection of xylazine hydrochloride ((Bioveta a. s(R), Czech Republic) at 7 mg/kg and Ketamine Hydrochloride (Laborate pharmaceutical, India at 50 mg/kg [27,28]. A full-thickness skin wounds of 3 cm² was created at the dorsum of each of the rabbits using a template designed from x-ray film [29]. The wounds were bandaged with sterile gauze and re-dressed only on the days of sample collections (days 0, 3, 7, 14, 21 and 28) [30].

Serum Sampling

Two millilitres of blood was collected from the ear vein of each of the rabbits on day 0 (before wound creation) and on days 3, 7, 14, 21 and 28 post-surgery [30]. The blood was dispensed into a sterile non-EDTA plastic test tube and allowed to clot at room temperature for 1 hr. The serum was then separated by centrifugation at 2000g for 10 min, after which the serum was harvested and preserved frozen at -20°C until needed for analysis [31]. The serum was used for (interleukin) assays; interleukin-1 beta (IL-1 β), interleukin- 6 (IL-6) and interleukin-10 (IL-10).

Wound Wash Sampling

Half (0.5) a millilitre of normal saline was used to moisten the wounds and the wound effluent collected using a sterile swab. The swab was washed in sample bottles containing 2 ml of normal saline and centrifuged at 4, 500 x g for 15 minutes and the sediment was collected in a sterile container [32,33]. The sediment was preserved at -200C in NAPRI Biotechnology Research Laboratory ABU Zaria, Nigeria until required for interleukin assay; interleukin 1 beta (IL-1 β), interleukin- 6 (IL-6) and interleukin-10 (IL-10) analysis.

Interleukins Evaluation

The inflammatory cytokines; interleukins-1 β (IL-1 β), interleukins-6 (IL-6) and interleukins-10 (IL-10) were evaluated in the serum and wound exudate using the Elabscience Quantitative Cytokine Rabbit Enzyme-Linked Immunosorbent Assay (ELISA) Kits with preset minimum detectable levels of the cytokines as described by the manufacturers [34]. Cytokine (interleukins (IL-1 β), interleukins-6 (IL-6) and interleukins-10 (IL-10)) rabbits ELISA Kit used was an in-vitro enzyme-linked immunosorbent assay for the quantitative measurement of rabbit cytokines (interleukins) in serum, plasma, exudates and cell culture supernatants. This assay employed a 96-well plate, tested and coated with an antibody specific for the rabbit cytokine. Standards and samples were pipetted into the wells and the cytokine (interleukins) present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated and an anti-rabbit cytokine

antibody was added. After washing away unbound biotinylated antibody, Horseradish Peroxidase (HRP) - conjugated streptavidin was pipetted to the wells. The wells were again washed in, 3, 3', 5', 5'-Tetramethylbenzidine (TMB) substrate solution and were then added to the wells where colours of different intensity developed in proportion to the amount of cytokine (interleukins) bound. The Stop Solution changed the colour from blue to yellow, and the intensity of the colour was measured at 450 nm.

Data Analysis

The data obtained from serum and wound exudate interleukin assay, were expressed as mean \pm Standard Error of Mean (SEM), and subjected to statistical analysis with Graph-Pad Prism version 5.03. The data of serum interleukins were analysed using repeated One-Way Analysis of Variance (ANOVA) while the data of wound exudate interleukins were analysed using Students' T. Test, and Bonferroni post hoc. Values of $P \leq 0.05$ were considered significant. The results obtained were presented in tables and figures.

Results

Interleukin -1 β , Interleukin - 6 and Interleukin -10 in the Serum

The mean \pm SEM peak serum IL-1 β concentration were 239.92 \pm 5.78 in group B; 232.11 \pm 11.18 in group C and 239.21 \pm 5.91 in group D; all of which were observed on psd 3. These peak values decreased to their lowest concentration of 229.21 \pm 7.81 in group B, 219.42 \pm 18.44 in group C and 228.56 \pm 12.73 in group D (psd 21, 28 and 28) respectively. These peak values were also relatively higher than their corresponding day 0 values (229.94 \pm 8.85, 229.2 \pm 6.71 and 215.67 \pm 4.83) for groups B, C and D respectively (Table 1).

The mean \pm SEM peak serum IL-6 concentration were 84.64 \pm 13.79 in group B; 81.88 \pm 12.49 in group C and 91.01 \pm 15.74 in group D; (psd 3, 7 and 3) respectively. These peak values decreased to their lowest concentration of 66.10 \pm 5.72 in group B, 62.51 \pm 8.68 in group C and 76.08 \pm 15.66 in group D (psd 21, 28 and 21) respectively. These peak values were also relatively higher than their corresponding day 0 values (Table 2). However, these changes in the values of IL-1 β (Figure 4.2.1) and IL-6 (Figure 4.2.2) throughout the post-surgery days were not statistically significant ($P > 0.05$) between and across the group.

The mean \pm SEM peak serum IL-10 concentration were 228.47 \pm 56.03 in group B and 215.10 \pm 43.68 in group D all of which on day 0 while the mean \pm SEM peak serum IL-10 concentration in group C increased from psd 3 (196.93 \pm 24.96) and peaked on psd 14 (225.12 \pm 31.57). These peak values in these groups decreased to their lowest concentration of 128.12 \pm 12.05 in group B; 183.39 \pm 29.37 in group C and 172.63 \pm 19.74 in group D (psd 14, 28 and 21) respectively. The value of IL-10 in group C from psd 3 (196.93 \pm 24.96 to 28 (183.39 \pm 29.37) were significantly higher ($P < 0.05$) than groups A and B (158.30 \pm 18.28 to 154.93 \pm 11.81 and 143.22 \pm 8.68 to 156.89 \pm 15.17) respectively (Table 3). Also, the mean IL-10 value in group C from psd 7 (206.64 \pm 47.79) to 14 (174.01 \pm 14.39) were significantly higher ($P < 0.05$) than the corresponding values in group D (140.44 \pm 10.29 to 174.01 \pm 14.39).

Post-surgery days	Groups			
	Control (NDNW) Group A	DNW Group B	WND Group C	WD Group D
0	243.04 ± 10.51	229.94 ± 8.85	229.2 ± 6.71	215.67 ± 4.83
3	236.41 ± 11.68	239.92 ± 5.78	232.11 ± 11.18	239.21 ± 5.91
7	234.34 ± 15.89	234.2 ± 6.43	229.9 ± 3.57	233.5 ± 7.77
14	227.80 ± 9.37	237.94 ± 15.93	224.94 ± 13.71	250.89 ± 16.76
21	224.97 ± 10.85	229.21 ± 7.81	224.47 ± 10.88	249.34 ± 11.56
28	229.99 ± 13.10	234.22 ± 8.19	219.42 ± 18.44	228.56 ± 12.73

Values are significantly different at P<0.05. Group A=Non-Diabetic and Non-Wounded (NDNW); Group B=Diabetic and Non-Wounded (DNW); Group C=Wounded and Non-Diabetic (WND); Group D=Wounded and Diabetic (WD).

Table 1: The Mean Serum Interleukin-1β (ng /mL) recorded in the Different Experimental Rabbit Groups Post-Operatively.

Post-surgery days	Groups			
	Control (NDNW) Group A	DNW Group B	WND Group C	WD Group D
0	71.40 ± 3.76	75.45 ± 10.16	64.03 ± 2.91	77.52 ± 10.64
3	66.23 ± 2.67	84.64 ± 13.79	72.91 ± 11.35	91.01 ± 15.74
7	62.75 ± 2.25	72.22 ± 7.68	81.88 ± 12.49	88.77 ± 18.00
14	63.33 ± 4.67	76.83 ± 6.74	73.70 ± 2.23	81.01 ± 15.03
21	58.01 ± 2.08	66.10 ± 5.72	75.20 ± 12.96	76.08 ± 15.66
28	57.46 ± 1.55	73.27 ± 11.12	62.51 ± 8.68	77.83 ± 9.08

Values are significantly different at P<0.05. Group A=Non-Diabetic and Non-Wounded (NDNW); Group B=Diabetic and Non-Wounded (DNW); Group C=Wounded and Non-Diabetic (WND); Group D =Wounded and Diabetic (WD).

Table 2: The Mean Serum Interleukin-6 (ng /mL) recorded in the Different Experimental Rabbit Groups Post-Operatively.

Post-surgery days	Groups			
	Control (NDNW) Group A	DNW Group B	WND Group C	WD Group D
0	170.65 ± 7.14	228.47 ± 56.03	184.37 ± 46.31	215.10 ± 43.68
3	158.30 ± 18.28 ^{ab}	143.22 ± 8.68 ^a	196.93 ± 24.96 ^b	161.48 ± 13.77 ^{ab}
7	158.90 ± 9.36 ^a	136.96 ± 8.70 ^a	206.64 ± 47.79 ^b	140.44 ± 10.29 ^a
14	158.93 ± 10.07 ^a	128.12 ± 12.05 ^a	225.12 ± 31.57 ^b	174.01 ± 14.39 ^a
21	159.37 ± 6.25 ^a	150.32 ± 12.58 ^a	210.72 ± 36.28 ^b	172.63 ± 19.74 ^{ab}
28	154.93 ± 11.81 ^a	156.89 ± 15.17 ^a	183.39 ± 29.37 ^b	177.23 ± 6.67 ^{ab}

Values with the different superscript alphabets ^{a,b,c} are significantly different at P<0.05. Group A=Non-Diabetic and Non-Wounded (NDNW); Group B=Diabetic and Non-Wounded (DNW); Group C=Wounded and Non-Diabetic (WND); Group D = Wounded and Diabetic (WD).

Table 3: The Mean Serum Interleukin-10 (ng /mL) recorded in the Different Experimental Rabbit Groups Post-Operatively.

Interleukin -1β, Interleukin - 6 and Interleukin -10 in Wound Exudates

The values of Interleukin-1β observed in wound exudate (WE) in group C was low on psd 3 (217.82 ± 7.06), increased slightly on psd 7 (219.95 ± 7.02) and subsequently declined on psd 14 (215.86 ± 9.26) and 21 (212.89 ± 14.46). On the other hands the values of IL-1β observed in WE in group D was highest on psd 3 (227.85 ± 12.91), declined slightly on psd 7 (224.22 ± 7.67) and 14 (222.92 ± 16.18) but subsequently rose again on psd 21 (225.60

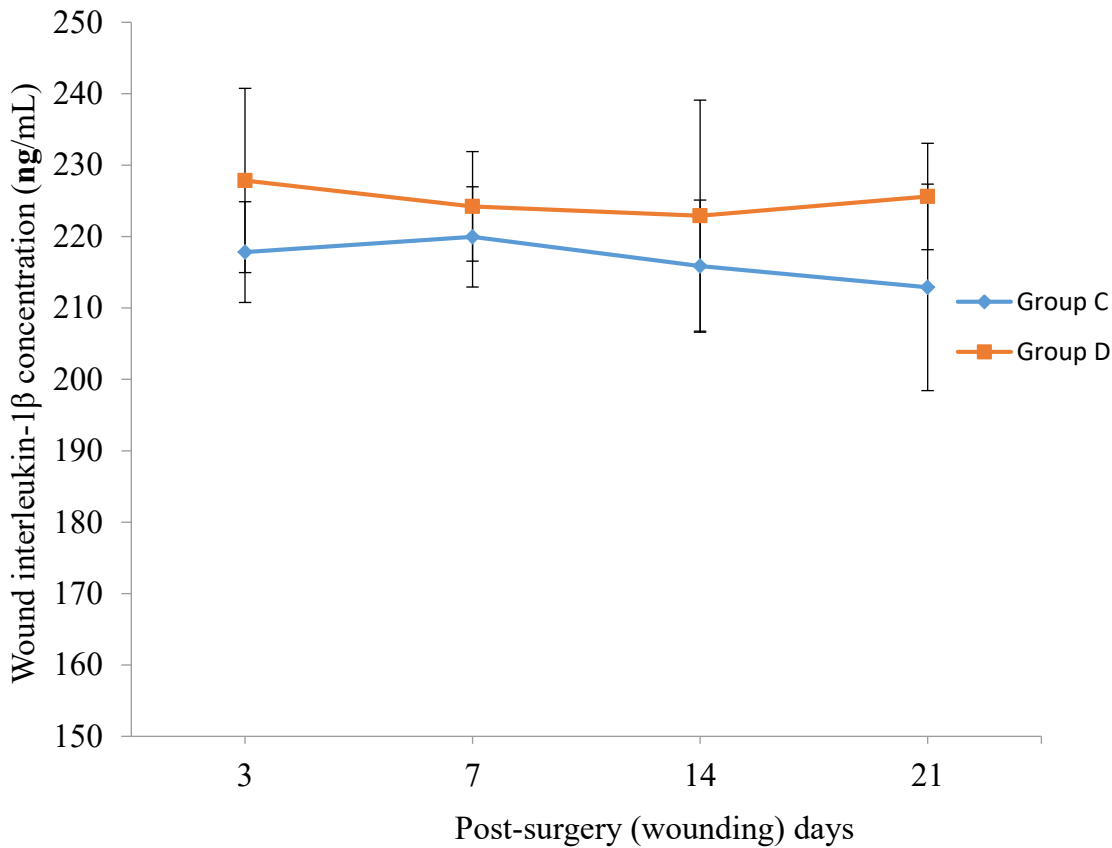
± 7.45) (Figure 1). The values of IL-1β observed in WE in group D appeared relatively higher than the value observed in group C throughout the study. However, there was no significant difference (P>0.05) between the values across the groups.

The values of IL-6 observed in the wound exudate (WE) in group C was low on psd 3 (62.21 ± 3.28), increased slightly on psd 7 (71.8 ± 5.54) and remained higher on psd 21 (74.9 ± 10.36). While the values of IL-6 observed in WE in group D rabbits was high

on psd 3 (79.71 ± 13.85), peaked on psd 7 (82.81 ± 15.95) but declined progressively up to psd 21 (73.95 ± 9.66). No wound exudate was collected on psd 28 (Figure 2). The values of IL-6 observed in wound exudate (WE) in group D appeared relatively higher than the values observed in the group C rabbits from psd 3 to 14. However, there was no significant difference ($P>0.05$) between the values across the groups.

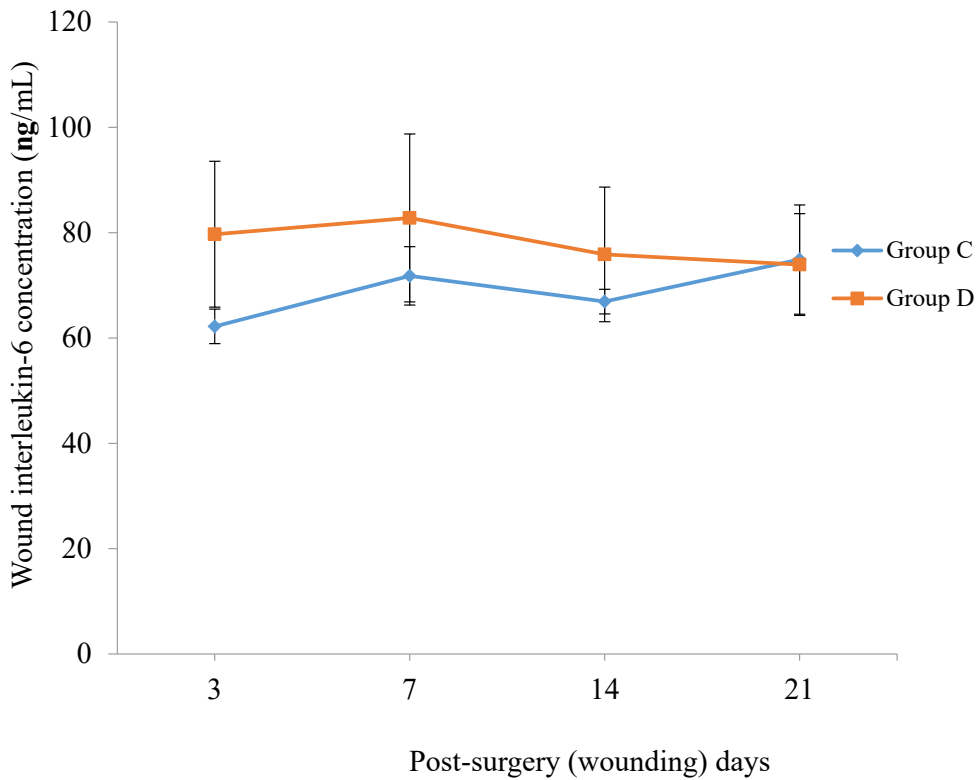
The values of IL-10 observed in WE in group C rabbits was low

on psd 3 (149.00 ± 9.34), peaked on psd 14 (188.79 ± 6.17) and started declining on psd 21 (177.30 ± 8.60). On the other hand, the values of IL-10 observed in WE in group D rabbits was high on psd 3 (153.33 ± 14.31), decreased constantly to the lowest level on psd 14 (129.64 ± 8.78) and subsequently peaked on psd 21 (155.82 ± 4.70) (Figure 3). The value of IL-10 observed in the wound fluid (WE) of the rabbits in group (C) was significantly higher ($P<0.05$) than the values observed in group D rabbits from psd 14 to 21.



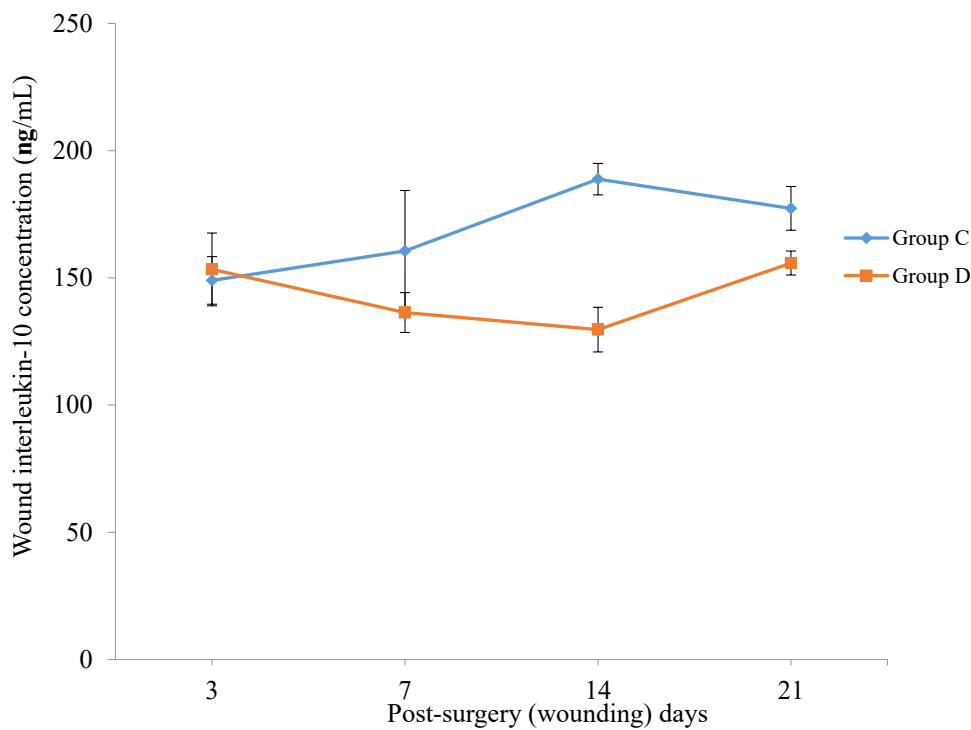
Key: Group C=Non-diabetic and Wounded (WND); Group D=Diabetic and Wounded (WD).

Figure 1: The Mean Wound Exudates Interleukin-1 β (ng /mL) recorded in the Wounded Experimental Rabbit Groups (C and D).



Key: Group C=Non-diabetic and Wounded (WND); Group D=Diabetic and Wounded (WD).

Figure 2: The Mean Wound Exudates Interleukin-6 (ng /mL) recorded in the Wounded Experimental Rabbit Groups (C and D).



Key: Group C=Non-diabetic and Wounded (WND); Group D=Diabetic and Wounded (WD).

Figure 3: The Mean Wound Exudates Interleukin-10 (ng /mL) recorded in the Wounded Experimental Rabbit Groups (C and D).

Discussion

The transient increase in the value of serum IL-1 β on psd 3 in groups B, C and D might have resulted from acute inflammation which released IL-1 β and other acute-phase protein from the liver. IL-1 β was better expressed in this early stage of wounds probably because, it is beneficial at the acute phase of wound healing, as it secretes and regulates other proinflammatory mediators, activates neutrophils and subsequently, contributes to local inflammatory response [35-37] reported a transient increase of serum IL-1 β following musculoskeletal trauma. Downregulation of this serum IL-1 β at the repair phase of the wound observed in group C (from psd 7) indicated progressive wound healing [36]. Observed downregulation of IL-1 β in progressive wound healing from post-wound day 14 to 21 in rats.

The prolonged upregulation of serum IL-1 β and IL-6 with higher value in the WD group (up to psd 28) when compared with the DNW group (up to psd 14) might have resulted from combined skin wound and hyperglycaemia of diabetes which have severe pathologic implications that might have prolonged the inflammation and other preparatory phases of wound healing in the WD group. There is a positive co-relation between prolonged duration of inflammation and upregulation of IL-1 β [36,38,39]. Also, Interleukin-6 is the principal regulator of most acute-phase protein genes and regulates local and systemic inflammatory responses, including the synthesis of hepatic acute-phase reactants like C-reactive protein in both hyperglycaemic and normoglycaemic wounds [38,39]. In addition, it has been documented that alloxan-induced diabetic rabbits have displayed obvious hyperglycaemia, delayed wound healing and significantly higher wound expression of IL-6 [40,41]. It has also been observed that regulation in circulating IL-6 level could be a key tool in the field of diabetic wound healing, and that diabetic insulin resistance β -cell inflammation correlated with upregulation of IL-6 [42,43].

The mean value of serum IL-6 was also relatively higher in non-alloxan group C from psd 3 to 21 when compared with the control. Such upregulation was possible because, in the acute phase of wound healing, IL-6 plays the dual roles of suppressing the level of pro-inflammatory cytokines while potentiating the anti-inflammatory cytokines [18]. IL-6 has a reputation for dictating the transition from inflammatory to repair phase in wound healing as it has stimulatory effects on T and B cells for cellular and humoral immune responses, while maintaining its inhibitory role on TNF and IL-1 β production by macrophages [19]. Transient increase in the level of IL-6 from post wound days 2 to 6 following surgery have been reported by earlier researchers [37,44-46].

The mean value of serum IL-10 observed in group C was significantly higher ($P < 0.05$) than the control and B group (from psd 3 to 28) and D group (psd 7 and 14). The significant increase in the value of IL-10 in normal wound healing on psd 3 was in line with the report of previous researchers [47,48]. IL-10 is a known anti-inflammatory cytokine that should ideally be upregulated at the repair and maturation phase of a normal wound healing. IL-10

detection in abundance during the repair and late stage of wound healing in this study was probably due to its role in inhibiting the proinflammatory cytokines [49]. It was possible that Increased IL-10 reduced inflammation and created environment conducive for regenerative wound healing as alluded by [50]. The mean values of serum IL-10 detected in rabbits in groups B and D from post-surgery days 3 to 28 were relatively lower than the pre-surgery values. It might be argued that effects of diabetes in the body system has deleterious effect in the production and detection of IL-10 in the serum. [51] noted that there is association between obesity, type-2 diabetes and low circulating levels of IL-10. The low level or total absence of IL-10 have been reported in isolated macrophages and keratinocytes from diabetic mice, rats and endothelial cells at the wound margins of human diabetic foot ulcers [37, 43,52,53] also reported that IL-10 decreased less than the pre-surgery values following musculoskeletal surgery and, [54] observed the tendency of increase in IL-10 levels in type -2 diabetic patients after they obtained adequate metabolic control. In contrast, over expression of IL-10 is associated with intense inflammatory phase, delayed epithelialization, prolonged and disorganized granulation, impaired angiogenesis and delayed healing [55].

The mean values of IL-1 β and IL-6, expressed in diabetic wound exudate (WE) (group D) were relatively higher than the values expressed in non-diabetic WE (group C). This could be due to prolonged retention of inflammatory mediators in delayed wound healing of diabetic wound as seen in group D. [56] stated that wounds with a high degree of inflammation contained increased level of IL-1 β and IL-6, and that wound inflammation may be correlated with total protein leakage absorbed into the wound dressing. The decline in the mean values of IL-1 β in WE in the group C from psd 7 might be indicative of faster and progressive wound healing at a shorter duration of time in normoglycaemic wounds as against the hyperglycaemic wounds. In contrast, the high value of IL-1 β in the WE of rabbits in the WD group for the period of 21 days might be attributed to prolonged inflammatory response following delayed wound healing of these diabetic rabbits. The peak IL-1 β in this WE of WD group might also be associated with high serum values of IL-1 β in this group as against the values recorded in the normoglycaemic wound (group C) as observed in this study. The mean values of IL-6 in the WE of groups C and D rabbits reached their peak level on psd 7 and the values remained high in subsequent days in group C. It was probable that IL-6 continues its mediation role between the preparatory and maturation phases of wound healing of normoglycaemic wound, where it remain at high values. The cause of the slight decline in the values of IL-6 in the WD group from psd 7 is not clear and also not in consonance with the serum values of IL-6 observed in this study, and is therefore subject for further investigation.

The values of IL-10 in wound exudate of the WND group was significantly higher ($P < 0.05$) than the values in the WD group from psd 14 to 21 while values of IL-10 in wound exudate of the group D rabbits decreased progressively and was lowest on psd 14. Diabetes could have probably caused expression of lower values of

IL-10 from the wound exudate. The positive co-relation between diabetes and low circulating levels of IL-10 is also a factor in this finding. The low level or total absence of IL-10 have been reported in isolated macrophages and keratinocytes from diabetic mice, rat and humans [52,53]. This might also be correlated with the findings in serum IL-10 in group D which was lower than the serum interleukin in other groups [51]. However, this finding is contrary to the study of [57] who reported over expression of IL-10 associated with delayed wound healing, although his report did not specify the fluid (blood, serum or wound fluid) used for his studies.

Conclusion

It was concluded that the values of IL-1 β and IL-6 are upregulated in the inflammatory phase of wound healing in normoglycaemic and diabetic rabbits while the values of IL-10 is downregulated during wound healing in diabetic NZW rabbits. It is therefore, imperative that clinicians take cognisance of the variation in the interleukin levels in serum and wound exudate of patients as such knowledge will contribute immensely in the efficient management of wound and other clinical challenges.

Acknowledgment

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Disclosure of Interest

The authors declare that they have no conflict of interest.

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