WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 6, 1008-1018. Research Article ISSN 2277–7105

INFLAMMATORY CYTOKINES LEVELS IN PATIENT' SERUM AND ITS RELATION TO THE DURATION OF WOUND HEALING IN ORTHOPAEDIC HOSPITAL ENUGU, NIGERIA

Lilian N. Chukwuma[1]*, Nneka R. Agbakoba[1], Ifeoma B. Enweani[1], Chidozie V. Udeogu[1], Chukwuemeka O. Arua[2] and Chidera L. Dilibe[3]

[1]Department of Medical Laboratory Science, Medical Microbiology unit, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria.

[2]Department of Medical Laboratory Science, Enugu State University Teaching Hospital, Parklane, Enugu State, Nigeria.

[3]Faculty of Pharmaceutical Sciences Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria.

Article Received on
03 February 2024,

Revised on 24 Feb. 2024,
Accepted on 14 March 2024

DOI: 10.20959/wjpr20246-31761

***Corresponding Author**
Lilian N. Chukwuma
Department of Medical Laboratory Science, Medical Microbiology unit, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria.

ABSTRACT

Background: Inflammatory cytokines play a role in health by regulating immune cells, production of blood cells, wound healing, and combating infections. The level of cytokines in the system needs to be evaluated to help checkmate imbalance in the release of both pro-inflammatory and anti-inflammatory cytokines that can affect wound healing and repair. The purpose of this study is to determine the levels of inflammatory cytokines in patients' serum and their relation to the duration of wound healing in National Orthopaedic Hospital Enugu, Nigeria. **Methodology:** A cross-sectional study of 230 blood samples was collected from patients with wounds of different age groups. 46 (Forty-six) samples were selected from the 230 samples using a convenient sampling technique and tested for pro-inflammatory and anti–inflammatory cytokines (IL-1β and IL-10) respectively. The cytokine evaluation of IL-1β and IL-10 of human serum samples was done by extracting the serum from clotted and retracted blood in plain tubes. This was analyzed using Human Interleukin1L-β and 1L-10 enzyme-linked immunosorbent assay (ELISA) kit respectively. The patient's records were taken from their folders to capture the duration of the wound stage at that particular period and also the Socio-

demographic data such as age, gender, duration of the wound, and stage from voluntary patients who met the criteria. **Results:** There was no significant difference in the regulation of the cytokine levels using the standard error of the mean. IL-1β (pg/ml) had 18.59±0.35 for the acute stage and 19.53±0.56 for the chronic stage, p-value-0.342 while IL-10 (pg/ml) had 689.31±246.90 for the acute stage, 445.72±15.13 for chronic stage, p-value-0.346 respectively. Significant value at $p \leq 0.05$. Percentage age duration of wound stage were 1-20yrs (11.3%), 21-40yrs (40.8%),41-60 (31.74%),61-80yrs (13.91%),>80(2.174%) The highest age duration of wound falls within the range of 21-40yrs and the least was >80yrs Distribution of wound stage for male in acute stage was 12(39%) and chronic stage (34)61% respectively, while the female in acute stage was 12(31.5%) and chronic stage 34(68.6%) respectively. The highest percentage distribution of wounds according to gender was 61.3% for males and female 38.7% respectively. The female gender was prevalent in the chronic phase of wound healing while the male gender was dominant in the acute phase. **Conclusion:** It is important to treat health issues that can lead to chronic inflammation to avoid complications, reduce the length of hospital stay and high medical costs, propagate and initiate high protein diets, and anti-inflammatory food diets, and also improve personal hygiene in order to uphold a healthy living and reduce mortality.

KEYWORDS: Pro-inflammatory, anti -Inflammatory, cytokine levels, evaluation, wound duration, healing.

INTRODUCTION

Cytokines are small proteins that are secreted by many cell populations, especially the helper T cells (Th) and macrophages.[2] Cytokines are triggered by pathogens, genetic disorders, malignant and autoimmune diseases produced in and by peripheral nerve tissue during physiological and pathological processes by resident and recruited macrophages, mast cells, endothelial cells, and Schwann cells.[2] They stimulate the production of blood cells, and aid in the development, maintenance, and repair of tissue.[1,2] The cytokines released during pathological and physiological processes are evaluated in order to maintain homeostasis and promote normal wound healing. The level of cytokines is vital in wound healing and needs to be controlled to prevent dysregulation in its expression and blocking of inappropriate production of the pro-inflammatory cytokines.[4] Moderate immune response promotes wound healing.[4] Inflammation is a natural process that helps to fight infections and heal injury.[4] IL-1β plays a role in the initial stage of wound healing (inflammation) by recruiting

growth factors and cells that remove excess debris.[13,4] IL-1β is part of the pro-inflammatory response that maintains effectively a pro-inflammatory wound.[13,4] IL-10 is an anti-inflammatory marker that plays a role in health, maintenance of host immune response to pathogens, and prevention of excessive release of pro-inflammatory cytokines so that normal wound healing will not be hindered.[17,4] IL-10 has made a positive impact in regenerative wound healing especially in fetus and adult wounds because of its role in the regulation of the inflammatory response.[3] IL-10 also promotes wound remodeling and the formation of new blood vessels.[12] Some studies have reported the upregulation or overactivity of cytokines in venous hypertension patients as a contributory factor to poor wound healing.[4] Excess cytokines can be reduced with food that acts as anti-inflammatory diets and also enhanced by taking high protein diets.[19]

Inflammatory markers such as IL-6, 1L-10, IL-1, and TNF-α (Tumor necrotic factor alpha) have shown a significant increase in chronic venous ulcers than in acute healing wounds.[3] Cytokine levels evaluated were higher in non-healing wounds than in healing wounds.[14] Harris et al.[5], investigated the relationship between the healing state of venous leg ulcers and cytokine levels collected from eighteen[18] patients with either non-healing or healing venous leg ulcer (VLU), and no significant differences were reported in the level of the examined cytokines. Quantitative analysis of interleukin 1 beta (IL-1β) with age, showed a mean age of participants, (n=50) 30.30 ± 19.98, median, 26.50, minimum, 0.04 while IL1 β had a mean of (n=50) 15.20±7.92, median, 16.50, minimum, 1.60.[12] However, studies have been conducted by many researchers to detect the role of cytokines in venous leg ulcers but with variable results.[4,5] Wound can be classified according to healing duration; acute and chronic stages.[9,8] Acute wound infection includes cuts or open wounds, abrasions, and surgical wounds and chronic wounds include pressure wounds such as ulcers and bedsores.[9] In the acute stage, normal wound healing takes place such as hemostasis, inflammation, proliferation, and remodeling, and occurs within 6-12weeks (3 months), while chronic wound healing does not heal within 3 months, and normal healing processes are hindered and can become complicated.[9,8,15] Evidence-based information on hospitalized patients without wound infection recorded 72% for 1-2 days and 10% for more than 5 days.[6,7] Wound healing differs significantly between males and females.[18] Dermal wounds heal faster in females than in males while the mucosal wound heals faster in males and lower inflammatory responses have been associated with quick wound healing in mucosal wounds.[18] A dermal wound takes place within 4-6 weeks while a mucosal wound takes more than 6 weeks.[18] Age

delays wound healing and sex modulates healing irrespective of age.[16] Comparative studies by different authors have shown that wounds of younger men heals faster than older women and older women, appear to be at a higher risk especially in wound closure.[16,18,15]

The objective of the study is to evaluate the cytokine levels (IL10, IL-1β) in patient's serum and its relationship with wound healing.

MATERIALS AND METHOD

Study setting and design

Enugu state is heavily populated and estimated at 460 /km² and Orthopaedic Hospital Enugu is situated at Abakpa junction Abakaliki road, Enugu. It is one of the National Orthopaedic Hospitals in Nigeria that serves the Eastern and Southern path of the country. A cross-sectional study was employed for this research. A convenient sampling method was deployed for the collection of blood samples from patients from March 2023 to September 2023.

Study Population, sample size calculation, and subject selection and criteria

The study population comprised males and females of different age groups recruited in National Orthopaedic Specialist Hospital Enugu, Nigeria.

The sample size was obtained using Naing's sample size determination formula Naing et al.[11] Calculation formula: $N=z^2\times p\ (1-p)/d^2$ N=minimum sample size P=prevalence of pathogenic bacteria isolates in the wound (82%), Aisha et al.[10] D=desired level of significance=0.05 (5%), Z= the level of statistical significance of the expected result, in this case, 1.96 for a 95% confidence interval using the prevalence of a previous study Aisha et al.[10], the sample size was calculated as 227 and approximated to 230 participants. The participants that passed the selection criteria were captured. Written informed consent was collected from each participant.

Ethical Considerations

Ethical approval was obtained from the Ethical Committee National Orthopaedic Specialist Hospital, Enugu, Nigeria.

Sampling Technique

A convenient sampling method was used in the collection of blood samples from patients voluntarily during clinical hours. The subjects were properly informed of the study before sample collection.

Sample collection and demographic

The Venipuncture method was employed with the consent of the subjects, using a 5ml sterile syringe which was transferred into clean dry plain tubes. Serum samples were extracted after each blood clotting and retraction prior to cytokine examination.

Blood samples were collected from the participants attending National Orthopaedic Specialist Hospital, Enugu, Nigeria. Questionnaires were extracted from the literature review and given to all the participants before the collection of their samples in order to educate them on the study. The patient's record was taken from their folders to capture the duration of the wound stage at that particular period and also the Socio-demographic data such as age, gender, duration of wound and stage.

Ethical approval

Ethical approval was sought and obtained from the Ethical Committee National Orthopaedic Specialist Hospital, Enugu, Nigeria.

Laboratory analysis of specimen

Serum samples: The cytokine evaluation of interleukin 1 beta and interleukin 10 (IL-1β and IL-10) respectively of human serum samples was done by extracting the serum from clotted and retracted blood in plain tubes after which it was sent to the laboratory for preservation and freezing prior to cytokine evaluation. The samples were thawed at room temperature for 10-20mins, centrifuged for 20mins, and analyzed for pro-inflammatory and anti-inflammatory cytokines; 1L- β and 1L-10 respectively using Human Interleukin 1L- β and 1L-10 enzyme-linked immunosorbent assay (ELISA) kit respectively. Fourty six (46) serum samples were selected for cytokine analysis in 230 investigations of patients' serum from different age groups and tested for pro-inflammatory (IL-1β) and anti-inflammatory (IL-10) cytokines respectively. Standards were added to the standard well, the blank well was set with standard and samples were diluted at 40µl for testing of sample well. 10 µl of testing samples was added to make a final dilution of 5 folds. 100 µl of HRP-conjugate reagent was also added to each well, except the blank well. The plates were closed with closure plate membrane and incubated for 60 minutes at 37^{o}C. Washing was done 20 folds, diluted with distilled water, and reserved. The closure plate membrane was uncovered, dried by swinging and the liquid discarded. Wash buffer was added to every well for 30 seconds and then drained five (5) times. 50µl of chromogen solution A and chromogen B solution was added to each well and incubated for 15 minutes at 37^{o}C after which a 50µl stop solution was added to

each well which changed from the blue colour to the yellow colour of the wells. The blank well was set to zero and the absorbance read at 450nm within 15 minutes using a microtiter plate reader to obtain values. A reference chart was used to calculate the concentration by comparing the optical density (OD) of the samples to the standard curves respectively.

Principle of Assay

The assay uses purified Human IL-1 β and IL-10 antibodies respectively to coat microtiter plate wells and make solid-phase antibodies. Standard cytokines are added to the wells, combination of antibodies with the HRP-labeled chromogen enzyme initiates a reaction which is washed and a substrate added. The reaction is terminated by adding a stop solution and the colour change is directly proportional to the concentration of cytokine present in the sample which is measured using a spectrophotometer. It is determined by comparing the OD of the samples with the standard curves.

Data analysis

Data obtained was analyzed using Statistical Package for Social Science (SPSS) version 23 software, M-S Excel. Descriptive statistical analysis was employed such as Frequency, Percentage, Bar chart, Mean (±SEM).

RESULTS

The data from the research was collated and organized accordingly for statistical analysis.

Fourty six (46) serum samples were selected for serum cytokine analysis in 230 investigations of patients with wounds from different age groups and tested for pro-inflammatory (IL-1β) and anti-inflammatory (IL-10) cytokines respectively. There was no significant difference in the regulation of the cytokine levels using standard error of mean: IL-1β (pg/ml) had 18.59±0.35 for acute stage and 19.53±0.56 for chronic stage, p-value-0.342 while IL-10 (pg/ml) had 689.31±246.90 for acute stage, 445.72±15.13 for chronic stage, p-value-0.346 respectively, as seen in Table 1. Significant value is $p \leq 0.05$. Percentage age duration of wound stage was 1-20 years (11.3%),21-40years (40.8%),41-60 years (31.74%),61-80 years (13.91%), and >80 years (2.174%). The highest age duration of wound falls within the range of 21-40yrs and the least was >80yrs, as observed in Figure 3. In terms of wound stage, the acute stage for males had 12(39%) and (34)61% in the chronic stage respectively, while the females had 12(31.5%) in the acute stage and 34(68.6%) in chronic stage respectively as seen in figure 2. The highest percentage distribution of wounds according to gender was 61.3% for males and 38.7% for females respectively as shown in

Figure. The female gender was prevalent in the chronic phase of wound healing while the male gender was dominant in the acute phase as seen in Figure 1.

DISCUSSION

Cytokine levels with wound healing have been reviewed by many authors in relation to the duration or stage of healing.[4,5,12] The pro-inflammatory and anti-inflammatory cytokines analyzed in this study had no significant difference in the activities of IL-1β and IL10 respectively using standard error of the mean (SEM) which is in line with other researchers.[5] There was no significant difference in the acute and chronic stages of wound healing. This may indicate a balance in the regulation of the IL-10 and IL-1 β in the production of inflammatory cytokines. The percentage age duration of wound stage/healing was highest in the young between the ages of 21-40yrs and the least healing was observed in the elderly >80yrs which is similar to the findings of other authors.[9,16,18,15] A likely explanation may be due to low immunity, autoimmune diseases, arthritis, and diabetes in the elderly. Socio-demographic data according to wound duration showed that females are more prone to chronic infection than males and the wound heals faster in males than in females. This corresponds with other author's reports[8,18,16] that reported findings that sex hormones play a vital role in wound healing. The highest percentage distribution of wounds according to gender was 61.3% for males and female 38.7% respectively. An explanation for this may be due to the males' lifestyle, the nature of work, the risks and customs.[9]

Table 1: Levels of inflammatory cytokines present in the patient's serum (IL-1 β, IL-10) in relation to duration of wound healing.

	Duration 0-12weeks (acute) >12wks (chronic) Acute (n=12)	Chronic (n=34)	p-value
IL-1 β (pg/ml)	18.59±0.35	19.53±0.56	0.342
IL-10 (pg/ml)	689.31±246.90	445.72±15.13	0.346

Result reported as Mean (±SEM), Mean difference significant at p≤0.05, SEM – Standard Error of Mean.

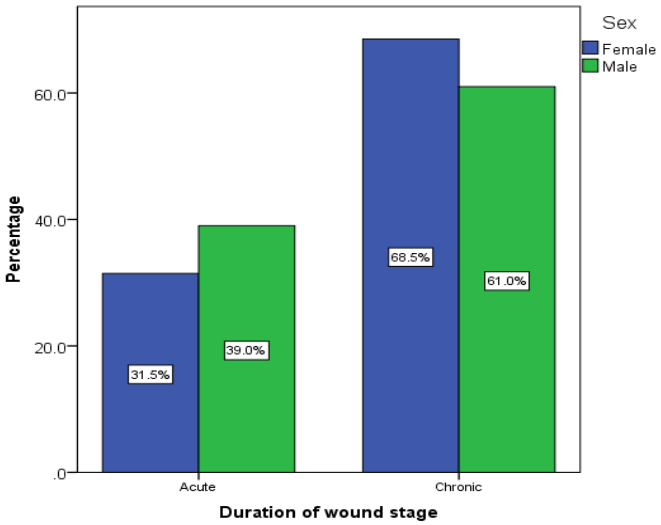

Figure 1: Duration of Wound Stage according to Sex.

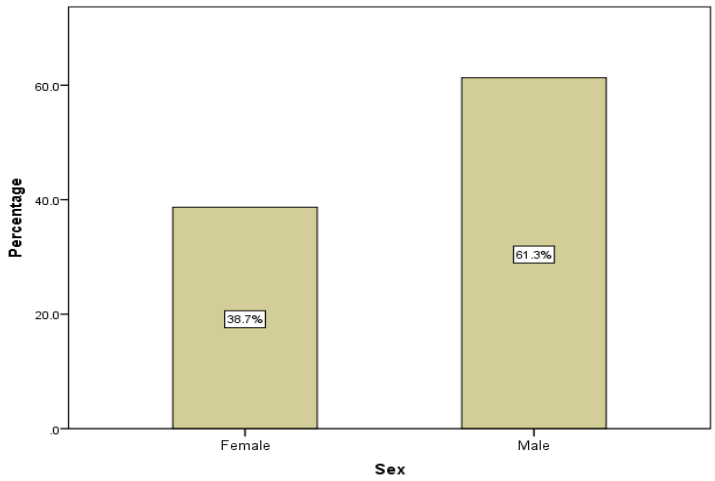

Figure 2: Patient Gender Distribution.

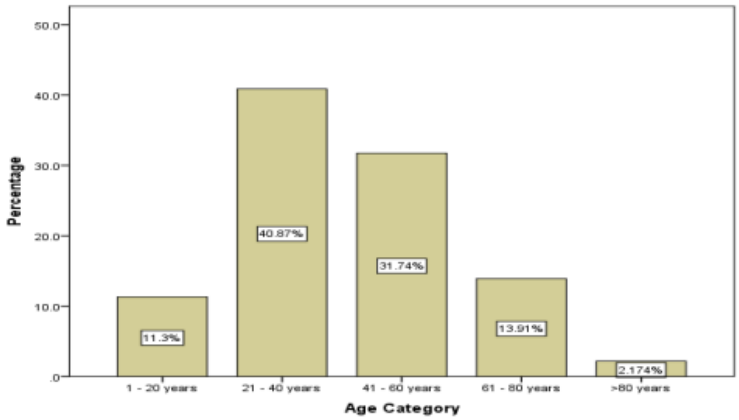

Figure 3: Socio-demographic data according to Patients' Age.

Limitations of the study

This study is only a cross-sectional study made at that particular period with the participants, no further feedback was obtained regarding the healing stage of the study participants.

CONCLUSION

This study on cytokine levels with wound duration has no significant difference in the regulation of IL10 and IL-1 β with the acute and chronic stages of wound healing. The only difference was in the percentage distribution of age groups and gender of the participants. It is vital to treat health issues that can lead to chronic inflammation on time to avoid complications and reduce the length of hospital stay and high medical costs. There is a need to enhance the intake of high protein diet and anti-inflammatory food diets in order to regulate and maintain homeostasis and also improve personal hygiene in order to uphold a healthy lifestyle and reduce mortality.

Compliance with ethical standards

No conflict of interest is to be disclosed.

Ethical approval: 24/5/2022.

Contributions of authors

LNC and NRA devised and planned the study. LNC wrote the first draft of the manuscript and reviewed the literature along with NRA, CVU, and EIB. COA and CLD collected clinical samples from subjects. LNC performed laboratory analysis and reviewed the manuscript. All authors read and approved the final manuscript.

Source of funding: No funding was received for the research work.

REFERENCES

1. Xanthe, LS, Allison, JC. The Role of inflammatory response in burn injury. *Journal of Chapter Metrics Overview*, 2017; 1: 813.
2. Zihan, L, Yiyao, W, Yixiang, L, Jingyi, Z, Lanian, Y, Xiang, Yu, Yongqin, Z, Xinzhi, Li, Yinhong, S. Host defense against the infection of Klebsiella pneumonia: New strategy to kill the bacterium in the era of antibiotics. Front cell infectious Microbiology, 2022; 12. https/doi:10.3389/fcimb20221050396. 24 November 2022.
3. Alice, K, Swathi, B, Louis, DL, Timothy, M, Cromblehole, Sundeep, GK. Regenerative wound healing: The role of Interleukin -10.*Advance Wound Care*, 2014; 3(4): 315-323.

4. Xiao, T., Yan, Zhu., Xiao, S., Xia, Y Pro-inflammatory cytokines regulate epidermal stem cells in wound epithelization. *Stem Cell Research and Therapy*, 2020; 11: 232.
5. Harris, HE, Lundberg, IE, Shinwan, K, Jan, TV, Borma, R Cytokines inflammatory disease. *International Journal of Molecular Sciences*, 2019; 20(23): 6008.
6. Moro, DD, Bello, H O, Akano, SO. Incidence and Antibiotic Resistance Pattern of Bacteria Associated with wound infection in some Hospitals in Lagos, Nigeria. *Asian Journal of Applied Sciences*, 2018; (6)4.
7. Nestler, C, Evan, R, Pearsal, N, Deninese, G, Nestler, MJ. A human perspective Microbiology, *Asian Journal of Applied Sciences*, 2018; 6(4): 586-58.
8. Jahnavi, M, Smith, C, Rajesh, R. Chronic wounds and tissue engineering: Prospective and promise, 2022; 101-102. https/doi;10.1016/8978-0-12-824064-9-00001-0,28 January 2022.
9. Chukwuma, LN, Agbakoba, NR, Enweani, IB, Udeogu, CV, Dilibe, EA, Ekelozie, IS. Microbial population of wound isolates and socio-demographic characteristics in patients attending the clinic in National Orthopaedic Hospital, Enugu, Nigeria. *World Journal of Advance Research and Reviews*, 2024; 21(2): 1652-1659.
10. Aisha, M, Gbonjubola, OA, Yakubu, KI. Incidence and antibiotics susceptibility pattern of bacterial isolates from wound infections in a Tertiary hospital in Nigeria. *Journal of Pharmaceutical Research*, 2013; 12(4): 617-621.
11. Naing, L, Winn, T, Rusli, BN. Practical issues in calculating the sample size for prevalence studies. *Architecture Orofacial Science*, 2005; 1: 9-14.
12. Mahmoud, NN, Hamad, K, Shibitin, A, Juma, S., Gould, L, Sharma, S, Mahmoud, M. Investigating inflammatory markers. *ACS Pharmacology and Transitional Science*, 2024; 7(1): 18-25.
13. Omidera, RA, DVM, Mohammed, Farahpour, R, DVM, Dvsc. A brief look at the inflammatory stage and role of medicinal plants and derivations on modulating the inflammatory responses: A systematic Review in wound healing, understanding implications and identifying artifacts. *Journal Sage Pub.Com*, 2023. https//doiorg/10.1177/15347346.
14. Hahn, G, Glaser, JJ, Elster, EA. Biomarkers to predict wound healing: The future of complex war wound management. *Plastic Reconstruction Surgery*, 2011; 127(1): 21S-6S.
15. Hurrow, J, Bowler, PG. Wound infectious microbiological immunological, clinical, and therapeutic distinctions. *Journal of Wound Care*, 2022; 31(5): 436-445.

16. Farahpour, MR. Medicinal plants in wound healing. *Wound Healing Current Perspective*, 2019; 4: 33-47.
17. Emily, HS, Xinyi, W, Swathi, B, Monish, JB, Paul, L.B, Sundeep, GK. *Advances in Wound Care*, 2020; 9: 4.
18. Heather, AW, Brandon, M, Patrick, MZ. Wound healing phase. National Library of Medicine, Slate Pearls publishing Trasine Island, 2023. PMID:29262065 Basehore. Updated January, 2024.
19. Ling, V. and Ioannis, Z. The role of anti-inflammatory diet in conjunction with Covid-19. *Diseases*, 2021; 9(4): 76.