NJBAS

Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Sciences (December, 2022), 30(2):173-177
DOI: http://dx.doi.org/10.4314/njbas.v30i2.22

ISSN 0794-5698

Full Length Research Article

C-Reactive Protein and Serum Amyloid-A Response to N-butyl Cyanoacrylate-based Sutures in Sahel Goats

***[1]A. A. Mutah, [1]H. P. Mana, [1]A. A. Haruna, [1]D. Laku, [1]P. B. Bokko and [2]G. Bilbonga**
[1]Department of Veterinary Surgery and Radiology, University of Maiduguri PMB 1069 Maiduguri, Borno
[2]Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, Kaduna
[*Corresponding Author: E-mail: mutahaliabba@gmail.com]

ABSTRACT
The use of biomaterials as suture materials triggering less acute phase response to tissue damage for cutanuous wounds closure has been the goal of numerous researchers. The introduction of Cyanoacrylate into clinical practice is of keen interest which could be an alternative to conventional suture materials. This study focused on monitoring responses to tissue damage elicited by Cyanoacrylate and Nylon based suture materials for wound closure. Ten apparently healthy male goats (Bucks) with mean age of 9.7±1.33 months and mean weight 13.2±1.31 kg were randomly separated into two groups A & B of five (5) bucks each. Incisions made in Group A were closed using N-butyl Cyanoacrylate while nylon was used in group B. Blood samples were taken before the procedures and after at 0, 5, 8, 24, 48 and 72 h for estimation of serum C-Reactive Protein (CRP) and Serum Amyloid-A (SAA). Results indicated that Nylon based suture material triggered early and significant tissue damages based on serum CRP elevated levels while serum SAA levels were comparable when nylon and N-butyl cyanocarylate were used as suture materials for wound closures.
Keywords: Wound, C-Reactive protein, Serum amyloid-A, N-butyl cyanoacrylate, Nylon.

INTRODUCTION
The acute phase response (APR) or acute phase reaction is a complex early, antigen non-specific, defense systemic reaction appearing before a specific immune response becomes activated (Petersen, 2004). The main purpose of APR is to restore the homeostasis by isolating and destroying the harmful agent thereby activating the repair process (Janeway *et al*., 2001; Ceciliani *et al*., 2002). The damaged tissue is the primary source that initiates APR (Murata *et al*., 2004). APR is activated by trauma, neoplastic growth, bacterial, parasitic and viral infection, burns, surgery, immunological disorders etc. (Gruys *et al*., 2005; Cray *et al*., 2009; Eckersall and Bell, 2010). The Acute Phase Proteins (APPs) are a large group of plasma proteins originating mainly from the liver in response to pro-inflammatory cytokines (Petersen *et al*., 2004; Jain *et al*., 2011). C-reactive protein (CRP) being one of the common test parameters explored in clinical practice for the assessment, diagnosis and prognosis of inflammation which also shows a 1000-fold or more increase in concentration during the occurrence of an injury, inflammation or tissue death (Pepys and Hirschfield, 2003). Measurement of CRP is a direct determination of an APR and, in the presence of inflammatory conditions, its serum levels change rapidly (Mitaka, 2005).

Serum amyloid A is a highly conserved acute phase protein, released in response to tissue damage or infection. Within 5-6 hours, the concentration of A-SAA increases exponentially during acute inflammation and injury to levels that are 1000- fold above the normal value. Serum amyloid A (SAA) is one of the major acute phase proteins in small ruminants. In wound healing, increased levels of SSA suggest acute inflammation and tissue injury; persistent levels may lead to delayed wound healing processes (Targońska-Stępniak *et al*., 2010; Nakamura, 2011; Migita *et al*., 2011). Cyanoacrylate (also known as liquid stitch, tissue adhesive or surgical glue) was first discovered in 1949 by Ardis (Bruns and Worthington, 2000). Cyanoacrylate adhesives have been used as tissue adhesives in combination with, or as an alternative to sutures in wound closure. Cyanoacrylate is a strong, biodegradable tissue adhesive that binds tissues together or creates a barrier against extravasation (Reece *et al*., 2001). It can thus be used as a hemostatic agent or “glue” tissues together in a surgical wound.

In medical and veterinary applications, nbutyl cyanoarylate, isobutyl cyanoacrylate, and octyl cyanoacrylate are commonly used (Singer *et al*., 2004).The *n*-Butyl cyanoacrylate (n-BCA, NBCA), a cyanoacrylate ester (longer chain derivatives) and a butylester of 2-cyano-2-propenoic acid developed in the 1970s, was the first medical grade tissue adhesive to have negligible tissue toxicity, good bonding strength and acceptable wound cosmesis (Bruns and Worthington, 2000). Surgical incisions are sutured to oppose wound edges to facilitate healing and minimize scar formation (Ogawa *et al*., 2011; Dennis *et al*., 2016). The main purpose of surgical wound closure is to achieve quick wound healing and a better cosmetic appearance, and hence reducing the risks of complications including tissue responses, dehiscence and infection. These require the need for surgeons to choose the best available method for wound closure (Hochberg *et al*., 2009). In this study, the extent of tissue damages elicited by Cyanoacrylate and Nylon based suture materials were assessed.

MATERIALS AND METHODS

Experimental Animals (Sahel Goats)

Ten apparently healthy bucks with mean age and weight 9.7±1.33 and 13.2±1.31 kg, respectively were procured from Maiduguri livestock market and housed under prevailing ambient conditions in the large animal pens of the Veterinary Teaching Hospital, University of Maiduguri, Borno State, Nigeria. All the bucks were handled humanly according to the faculty animal research guidelines. The bucks were randomly separated into two (2) groups of five (5) bucks each (groups A and B) and allowed to acclimatize for a period of 4 weeks. Feed and water were provided *ad libitum* throughout the study period.

Pre-surgical Preparation/Anaesthesia

Following standard preoperative evaluation, the bucks were placed on a right lateral recumbent position; the left paralumbar fossa of each buck was saved and aseptically prepared using 0.2% chlorhexidine gluconate (SAVLON ® Johnson & Johnson, London). The bucks were sedated with diazepam (Jarzepam® SWISS parenterals India) at a dose rate of 0.3 mg/kg intravenously. Inverted L block with 2% lidocaine (XYLOCAINE® NAMAN PHARMA DRUGS, India) was infiltrated to induce local anesthesia at a dose rate of 5 mg/kg following sedation. Five minutes after induction, 3 mL of blood samples were collected using 21 guage needle from the left jugular vein into plain vacutainer tubes and kept at room temperature for 2 hours before centrifugation at 4000 g for 5 min (Hettich Universal II, Kirchlengern, Germany) then stored at 20 °C. Sera obtained served as baseline data for estimated CRP and SAA.

Surgery

A 5 cm full thickness skin incision was made on the upper paralumbar fossa of the left flank of each goat in a dorso-ventral fashion to expose fascia and muscles. Immediately, subcuticular closure of the skin incisions was carried out using polyglycolic acid (HINCRYL® HLL Lifecare Ltd, India) size 2 for the two groups (A and B) using simple continuous suture pattern. The skin incisions on group (A) were opposed by gentle placing the thumb and index finger to bring the incised edges together and sutured by topical application of N-butyl cyanoacrylate liquid adhesive (3M Vetbond® Animal Care Products U.S.A). The skin incisions on group (B) were sutured with Nylon (TOPECARE® Huaian Angel Medical Instruments Co, Ltd. China) size 2 using simple interrupted suture pattern.

Post-surgical Blood Sample collection

Exactly 3 mL of blood sample were collected at 0, 5, 8-, 24-, 48- and 72-hours post-surgery from the left jugular vein of all bucks in both groups. The samples were allowed to stand for 2 hours under room temperature then centrifuged at 4000 g for 5 min and stored as previously described.

Determination of C-Reactive Protein (CRP) and Serum Amyloid-A (SAA)

CRP and SAA were determined using ELISA kits (Abbkine Inc, China). Briefly, 50 µL of manufacturer standard was added in into microplate wells while 40 µL of sample diluents were added into separate wells after which 10 µL of serum was added. Each treatment was carried out in duplicate. The microplate was covered and incubated for 45 minutes at 37 ºC. Each well was the aspirated and washed using 250 µL of wash buffer (pH 7.2) using a squirt bottle. A total of five wash was performed at an interval of 1-3 minutes per wash. The microplate was then inverted and blotted against a clean paper towel after which 50 µL of HRP-Conjugated detection antibody was added to each well with the exception of blank wells. The plate was covered and incubated again for 30 minutes at 37 ºC. The washing process was repeated as above after decantation of the HRP-Conjugated antibody. Subsequently, 50 µL each of Chromagen solutions A and B were added to each well and incubated for 15 minutes at 37 ºC. Finally, 50 µL of stop solution was added to each well and absorbance was read at 450 nm (Acurex Plate Reader, 415 central Ave Toledo Ohio USA). A standard curve was plotted using the formula with the aid of computer software and this was used for estimation of CRP and SAA

Statistical Analysis

All data obtained were expressed as mean ± standard deviation and analyzed using ANOVA within the groups (SPSS version 23). The Turkey's post hoc test was used to indicate level of significance. P-value < 0.05 was considered statistically significant.

RESULTS

The C-Reactive Protein baseline levels for groups A and B were 244.00 ± 66.27 µg/L and 279.50 ± 50.21 µg/L respectively (Table 1). This study revealed a significant difference within both groups at varying time intervals ($p<0.05$). There was a significant difference observed between group A and B at 24 and 48 hours with group B animals having higher values of 386.58 ± 57.44 µg/L and 393.46 ± 46.89 µg/L. Mean serum CRP levels in both groups were seen to increase steadily from baseline to 48 hours before rising to peak level of 415.96 ± 48.15 and 431.54 ± 120.00 for group A and B respectively at 72 hours. Serum CRP levels increase steadily from baseline in group B reaching a peak of 431.54 ± 120.00 µg/L at 72 hours showing a significant difference between the baseline to 8 hours and 24-72 hours ($p<0.05$). CRP levels in group A showed a significant difference only at 72 hours compared to baseline value ($p<0.05$).

Table 1: C-Reactive protein levels following wound closure with n-butyl cyanoacrylate and nylon suture in Sahel bucks

		TIME (HOURS)					
SUTURES	**BASELINE**	**0**	**5**	**8**	**24**	**48**	**72**
Group A (µg/L)	244.00 ± 66.27[a]	288.54 ± 68.35[a]	288.54 ± 68.35[a]	244.84 ± 71.29[a]	270.04 ± 57.84[a]	287.38 ± 41.07[a]	415.96 ± 48.15[b]
Group B (µg/L)	279.50 ± 50.21[a]	292.18 ± 44.83[a]	294.62 ± 46.82[a]	357.60 ± 95.74[a]	386.58 ± 57.44[b]	393.46 ± 46.89[b]	431.54 ± 120.00[b]

Values are Mean ± standard deviations of duplicate determinations, n=10. Values with different superscripts across the rows are significantly different at ($p<0.05$). Group A = 0.023; Group B = 0.035

The baseline SAA levels for groups A and B were 155.22 ± 40.10 µg/L and 150.68 ± 34.43 µg/L respectively (Table 2) However, no significant difference ($p> 0.05$) was observed in SAA levels in response to n-butyl cyanoacrylate and nylon sutures for 72 hours post operation when compared with baseline. Interestingly, the mean serum SAA levels of group A dropped to 131.80 ± 26.30 at 8 hours post-surgery but increased steadily to 184.66 ± 45.03 µg/L after 72 hours. A similar trend was observed for SAA levels in group B

Table 2: Serum amyloid-A levels following wound closure with n-butyl cyanoacrylate and nylon suture in Sahel bucks

		TIME (HOURS)					
SUTURES	**BASELINE**	**0**	**5**	**8**	**24**	**48**	**72**
Cyanoacrylate (µg/L)	155.22 ± 40.10[a]	132.92 ± 13.78[a]	132.92 ± 13.78[a]	131.80 ± 26.30[a]	149.08 ± 52.91[a]	183.16 ± 63.73[a]	184.66 ± 45.03[a]
Nylon (µg/L)	150.68 ± 34.43[a]	143.98 ± 21.86[a]	143.92 ± 21.96[a]	163.08 ± 43.85[a]	217.80 ± 100.51[a]	154.70 ± 20.88[a]	198.58 ± 72.32[a]

Values are Mean±standard deviations of duplicate determinations, n=5.

DISCUSSION

Suturing wound is an essential aspect of modern operative surgery. With the advancement in technology and the search for a less invasive, ideal and suitable material for wound closure, the use of cyanoacrylate is being increasingly explored. Several studies on the use of cyanoacrylate as an alternative to conventional sutures have been carried out due to its future prospects in cosmetic surgery. In this study, cyanoacrylate (3M vetbond) and nylon-based suture materials were used for skin incision closure and acute phase responses were compared. CRP and SAA investigations have shown can identify the presence of infection, tissue damage or inflammatory lesions hence important as markers for acute phase response to tissue damage in animals. However, species have substantial differences in the relative changes in acute phase protein production following stimulation (Eckersell *et al*., 2010).

Serum CRP levels in this study showed no significant increase from baseline (244.00 ± 66.27 µg/L) to 0-48 hours in group A bucks ($p>0.05$) but differed significantly ($p<0.05$) at 72 hours (415.96 ± 48.15 µg/L) post incision closure. In group B, serum CRP levels were seen to differ significantly ($p=0.012$) from 24-72 hours (386.58 ± 57.44 µg/L, 393.46 ± 46.89 µg/L, 431.54 ± 120.00 µg/L) compared to baseline (150.68 ± 34.43 µg/L) rising earlier than in group A bucks. These rising values suggests early and more tissue damage responses to nylon than n-butyl cyanoacrylate. However, the mean serum CRP levels in both groups were observed to have risen significantly after 72 hours suggesting CRP as a late acute phase protein (APP) in this species. This finding agrees with the description of CRP by Cray *et al*. (2009) where CRP was classified in ruminants as a moderate APP with concentration changes between 2 - 10 folds in the first 3 – 4 days post infection and the acute inflammation became chronic leading to APR being induced. Although scanty information on CRP in goats exists, Haligur and Ozmen (2011) demonstrated notable changes in CRP concentrations in goats during lung inflammation. This effect as shown in this study is more pronounced in tissue damage elicited by nylon-based sutures when compared to N-butyl cyanoacrylate.

Mean SAA levels of 217.80 ± 100.51 µg/L in group B bucks were seen to rise compared to levels of 149.08 ± 52.91 µg/L in group A bucks at 24 hours post incision closure which correlates to the study by Konstandi *et al* (2019) where SAA levels become apparent during surgical stress and inflammation. This may be attributed to stress imparted by nylon suture and repeated stitches on group B bucks. SAA concentration changes in animals exposed to subjective internal or external challenges such as surgical stress, inflammation, infection and trauma and it belongs to a major group of APP in cattle and small ruminants (Cray *et al*., 2009). The production of SAA is triggered by proinflammatory cytokines such as interleukin-I and VI, tumour necrosis factor (TNF), transforming growth factor-β

(TGF-β) and interferon-γ (Nakamura, 2011). Although no significant difference in SAA levels was observed between the cyanoacrylate and nylon groups ($p>0.05$) possibly due to mild subcutaneous inflammation which may have altered the normal tissue response reactions to this acute phase protein (Shivamurthy *et al*., 2010). Apparently, there is no significant difference based on Serum Amyloid-A levels in tissue damage associated with Nylon and N-butyl Cyanoacrylate suture materials.

CONCLUSION

Nylon based suture material triggered early and significant tissue damages based on serum CRP elevated levels while serum SAA levels were comparable when nylon and N-butyl cyanocarylate were used as suture materials for wound closures.

ACKNOWLEDGEMENTS

This research component was funded by Tertiary Education Trust Fund (TETFund) as part of TETFUND/DESS/NRF-20/4/VOL1 Grant.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare

REFERENCES

Bruns, T. B. and Worthington, J. M. (2000). Using tissue adhesives for wound repair: A practical guide to dermabond. *American Family Physician*, **61**(5):1383-1388.

Ceciliani, F., Giordano, A. and Spagnolo, V. (2002). The systemic reaction during inflammation: the acute-phase proteins. *Protein and Peptide Letters*, **9**(3):211-23.

Cray, C., Zaias, J. and Altman, N. H. (2009). Acute phase response in animals: a review. *Comparative Medicine*, **59**(6):517–526.

Dennis, C., Sethu, S., Nayak, S., Mohan, L., Morsi, Y. Y. and Manivasagam, G. (2016). Suture materials - Current and emerging trends. *Journal of Biomedical Materials Research*,**104**(6):1544-59.

Eckersall, P. D. and Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Veterinary Journal*, 185 (1):23-7.

Gruys, E., Toussaint, M. J. M., Niewold, T. A. and Koopmans, S.J. (2005). Acute phase reaction and acute phase proteins. *Journal of Zhejiang University Science B*, **6(**11):1045–1056.

Haligur, M. and Ozmen, O. (2011). Immunohistochemical detection of serum amyloid-a, serum amyloid-p, c-reactive protein, tumour necrosis factor-α and TNF-α receptor in sheep and goat pneumonias. *Révue de Médecine Vétérinaire*, **162**:475–481.

Hochberg, J., Meyer, K. M. and Marion, M. D. (2009). Suture choice and other methods of skin closure. *The Surgical Clinics of North America*, **89**(3):627-41.

Jain, S., Gautam, V. and Naseem, S. (2011). Acute-phase proteins: As diagnostic tool. *Journal of Pharmacy and Bioallied Sciences,* **3** (1): 118-27.

Janeway, C. A., Travers, P., Walport, M. and Schlomschik, M. J. (2001). Immunobiology*:* the immune system in health and disease (5th ed.). New York: Garland Science.

Konstandi, M., Sotiropoulos, I., Matsubara, T., Malliou, F., Katsogridaki, A., Andriopoulou, C.E. and Gonzalez, F. J. (2019). Adrenoceptor-stimulated inflammatory response in stress-induced serum amyloid A synthesis. *Psychopharmacology*, **236**(6):1687–1699.

Migita, K., Koga, T., Komori, A., Torigoshi, T., Maeda, Y., Izumi, Y., Sato, J., Jiuchi, Y., Miyashita, T., Yamasaki, S., Kawakami, A., Nakamura, M., Motokawa, S. and Ishibashi, H. (2011). Influence of Janus kinase inhibition on interleukin 6-mediated induction of acute-phase serum amyloid A in rheumatoid synovium. *The Journal of Rheumatology*, **38**(11):2309-17.

Mitaka, C. (2005). Clinical laboratory differentiation of infectious versus non-infectious systemic inflammatory response syndrome. *Clinica Chemica Acta*, **351**:17-29.

Murata, H., Shimada, N. and Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. *Veterinary Journal*, **168**(1):28-40.

Nakamura, T. (2011). Amyloid A amyloidosis secondary to rheumatoid arthritis: pathophysiology and treatments. *Clinical and Experimental Rheumatology*, **29**(5):850-7.

Ogawa, R., Akaishi, S., Huang, C., Dohi, T., Aoki, M., Omori, Y., Koike, S., Kobe, K., Akimoto, M. and Hyakusoku, H. (2011). Clinical applications of basic research that shows reducing skin tension could prevent and treat abnormal scarring: the importance of fascial/subcutaneous tensile reduction sutures and flap surgery for keloid and hypertrophic scar reconstruction. *Journal of Nippon Medical School*, **78**(2):68–76.

Pepys, M. B. and Hirschfield, G. M. (2003). C-reactive protein: a critical update. *The Journal of Clinical Investigation*, 1**11**(12):1805-12.

Petersen, H. H., Nielsen, J. P. and Heegaard, P. M. (2004). Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research*, **35**(2):163-87.

Reece, T. B., Maxey, T. S. and Kron, I. L. (2001). A prospectus on tissue adhesives. *American Journal of Surgery*, **182**(2):40S-4S.

Shivamurthy, D. M., Singh, S. and Reddy, S. (2010). Comparison of octyl-2-cyanoacrylate and

conventional sutures in facial skin closure. *National Journal of Maxillofacial Surgery*,**1**: 15-20.

Singer, A. J., McClain, S.A. and Katz, A. A. (2004). A porcine epistaxis model: hemostatic effects of octylcyanoacrylate. *Otolaryngol Head Neck Surgery*,**130**(5):553-7.

Targońska-Stępniak, B., Dryglewska, M. and Majdan, M. (2010). Influence of long-term leflunomide treatment on serum amyloid concentration in rheumatoid arthritis patients. *Pharmacological Reports*, **62**(4):719-25.