

Serum amyloid A and haptoglobin levels in crossbred cows with endometritis following different therapy

S. S. Biswal^{1,2}, S. Das², S. Balasubramanian¹, D. N. Mohanty², K. Sethy² and M. Dasgupta²

1. Department of Animal Reproduction, Gynaecology and Obstetrics, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India; 2. Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India.

Corresponding author: S. S. Biswal, e-mail: shuvranshu06@gmail.com, SD: dassrinibas@gmail.com, SB: bala2716@yahoo.com, DNM: mohantydn@gmail.com, KS: babuivri@gmail.com, MD: mohuadgupta@gmail.com

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Abstract

Aim: To determine the serum variations of two major acute phase proteins, serum amyloid A (SAA) and haptoglobin (Hp) levels in crossbred endometritis cows following pre and post immunomodulation therapy.

Materials and Methods: 21 endometritis cows were randomly assigned to three groups (n=7) and treated with three different immunomodulators while seven healthy cows served as control. Uterine flushing collected from all animals was subjected to bacteriological study and serum samples were analyzed for SAA and Hp by sandwich ELISA method.

Results: *Escherichia coli* was most prevalent Gram-negative bacteria (6.02×10^6 CFU/ml) while *Staphylococcus* (0.86×10^6 CFU/ml) and *Streptococcus* (0.52×10^6 CFU/ml) were most predominant Gram-positive species isolated from uterine flushing. The pre-treatment SAA values ($\mu\text{g/ml}$) varied significantly ($p < 0.01$) between the treatment groups whereas no difference was observed in post-treatment groups. No significant difference ($p < 0.01$) was observed for Hp values between the treatment groups, but the mean SAA ($\mu\text{g/ml}$) and Hp ($\mu\text{g/ml}$) levels were significantly ($p < 0.01$) higher in pre-treatment when compared to post-treatment within the groups.

Conclusion: In the diagnosis and monitoring of bovine endometritis, both SAA and Hp might serve as reliable biomarkers.

Keywords: crossbred cows, endometritis, haptoglobin, serum amyloid A.

Introduction

Acute phase proteins (APPs) are large and varied group of glycoproteins in the serum that are unrelated to immunoglobulin. They are mainly synthesized by liver parenchyma cells as a part of acute phase response and released into the bloodstream in response to several stimuli like infection, inflammation, stress and trauma or tissue damage [1]. The APPs, whose concentrations increase due to inflammatory factors, are termed as positively reacting (e.g., haptoglobin [Hp], serum amyloid A [SAA], fibrinogen) and those whose concentrations decrease are negatively reacting (e.g., albumin, transferrin) [2]. The response pattern of individual APPs to various stressors and disease may differ in their serum concentration, which is a main disadvantage of APPs assay but still they serve as a nonspecific marker of clinical infection and used for prognostic purposes [3]. The main function of APPs is to defend the host against pathological damage and assist in the restoration of the homeostasis.

In bovine, to diagnose an inflammatory process is very difficult as the clinical symptoms are quite poor, and the classical parameters of the inflammatory

response are relatively slight and not very specific. Hematological tests that are used for diagnosis purpose sometimes fail because in bovine inflammation is not always followed by an increase in the leucocyte population [4]. So presently APPs are sensitive factors that enable very early and precise detection of inflammation in ruminants [5]. In cattle, mainly the concentration of two major APPs i.e. Hp and SAA in serum was elevated in association with several infection and inflammation [6].

In dairy cows, endometritis signifying inflammation of superficial layer of endometrium is the most common reproductive disorder which results in huge economic loss to farmers by decreasing milk production and increasing in calving to conception interval [7]. Its long-term consequences can cause irreversible changes of the genital tract leading to metritis and sterility [8]. Measuring the concentration of APPs has been applied to various viral diseases, mastitis, metritis and monitoring post-operational complications but there are very limited reports on APPs concentration in cows affected with endometritis.

Keeping the above facts in view, present study was undertaken to determine, whether an evaluation of APPs at selected time points during the treatment of endometritis with different immunomodulators could be used to monitor the course of treatment. We attempted to establish a relationship between the

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changes in concentration of investigated proteins and the clinical course of treatment.

Materials and Methods

Ethical approval

The approval for collecting blood samples and examination of cows was taken from Institutional Animal Ethics Committee.

Study area

The present study was carried out in the Department of Gynecology and Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar from September 2012 to July 2013.

Experimental design

About 21 Jersey crossbred cows (first-fifth parity) at 40-90 days post-partum presented to the Teaching Veterinary Clinical Complex, with the history of cloudy discharge were selected for the study. All the cows were subjected to rectal examination and cows with clinical signs of endometritis (mucopurulent discharge) were included in the study. After the diagnosis of endometritis, the cows were randomly and equally assigned to three treatment groups (n=7) and treated with three different drugs *viz.*: Oyster glycogen (OG) which enhances uterine neutrophilic influx and eliminates bacterial infection, prostaglandin F₂α (PGF₂α), which considered to be pro-inflammatory molecule that stimulates production of pro-inflammatory cytokines and enhances phagocytosis and lymphocytic function and levamisole (LE), which increases phagocytosis by increasing the number of polymorph nuclear (PMN) cells and intracellular C-GMP level. Seven clinically healthy cows in estrus (40-90 days post-partum) served as control.

Group I (OG): 5 g of OG (Hi Media Laboratories Pvt. Ltd.) was reconstituted with 50 ml of phosphate-buffered saline and given as intrauterine infusion; single dose.

Group II (PG): 500 µg of PGF₂α (Pragma, Intas Pharmaceuticals Ltd.) was administered through intramuscular route; single dose.

Group III (LE): 10 ml of LE hydrochloride (Kalmisol, KAPL) was administered subcutaneous route on alternate days on three occasions.

Group IV (Control): Normal post-partum cows at 40-90 days at estrus presented for artificial insemination without institution of any therapy.

Sample collection

Uterine samples were collected aseptically from all three treatment groups before treatment and post treatment estrus and in control group at the time of estrus as per method described by Gilbert *et al.*, [9] for bacterial isolation, identification and count. Briefly, the vulval lips and perineal area was thoroughly scrubbed with non-irritant soap solution followed by disinfection of the perineal area using 5% povidone

iodine. Then a sterile plastic sheath was introduced into the cranial vagina and manipulated through the cervix into the uterus. 20 ml sterile saline solution was infused into uterus; the uterus was massaged gently and uterine fluid sample was aspirated. The predominant bacterial count was made by agar plate count method. Blood was collected by jugular venipuncture prior to the institution of therapy and in post-therapy estrus. The collected blood was left for 2 h and then centrifuged at 1000 rpm for 10 min at room temperature for serum collection. The collected serum was stored in cryovial at -40°C until it was assayed. The SAA and Hp assay was done by solid phase sandwich ELISA kit (Life diagnostic, Inc.).

Statistical analysis

Mean values (± standard error) for cows of four groups were computed. In order to monitor the magnitude of variation before and after treatment within the group Student's *t*-test was performed and to observe variations between the groups, the data were analyzed statistically using analysis of variance as per method suggested by Snedecor and Cochran [10].

Results

Various predominant bacterial species isolated from uterine samples and their counts are presented in Table-1. Of the total 28 uterine samples collected, bacterial species isolated were *Escherichia coli* (21.43%), *Streptococcus* (17.86%), *Klebsiella* (17.86%), *Staphylococcus* (14.29%), *Micrococcus* (10.71%), *Proteus* (10.71%) and *Bacillus* (7.14%). The bacterial count (10⁶ CFU/ml) for different bacterial species ranged between 0.52 (*Streptococcus*) and 8.60 (*Micrococcus*). In OG group, the concentration of *Micrococcus* was very high (8.60) followed by *E. coli* (6.20), *Bacillus* (4.50), *Proteus* (3.14), *Klebsiella* (0.93) *Staphylococcus* (0.86) and *Streptococcus* (0.52). In the post-treatment period, the *E. coli* and *Micrococcus* were only identified in OG group with negligible count.

In PG group, *Micrococcus* showed highest concentration (6.57) followed by *E. coli* (5.85), *Bacillus* (4.28), *Proteus* (3.14), and *Streptococcus* (1.46) whereas other microorganism had concentration <1 × 10⁶ CFU. In the post-treatment samples, only *Streptococcus* and *Bacillus* were eliminated, and enumeration of other bacteria showed a very low concentration. In LE group, predominant bacterial concentrations were observed to be *Micrococcus* (6.10) followed by *E. coli* (4.94), *Proteus* (4.24), *Bacillus* (3.70), *Klebsiella* (2.56) and *Staphylococcus* (1.40) whereas other microbes showed a very low concentration. The post-treatment bacterial counts were negative for *Streptococcus*, *Klebsiella* and *Micrococcus* whereas *Bacillus*, *Proteus* and *E. coli* showed a bacterial load of 2.12, 1.41 and 1.20, respectively with *Staphylococcus* as 0.02 concentrations. In control group, the bacterial load for *E. coli*, *Bacillus*

Table-1: Predominant bacteria isolated and their count (10⁶ CFU/ml) before and after treatment.

Microorganism	Frequency (%)	Treatment groups					
		Group I (OG)		Group II (PG)		Group III (LE)	
		Pre	Post	Pre	Post	Pre	Post
<i>E. coli</i>	6 (21.43)	6.20	0.63	5.85	0.02	4.94	1.20
<i>Staphylococcus</i>	4 (14.29)	0.86	-	0.78	0.04	1.40	0.02
<i>Streptococcus</i> (β hemolyticus)	5 (17.86)	0.52	-	1.46	-	0.98	-
<i>Proteus</i>	3 (10.71)	3.20	-	3.14	0.91	4.24	1.41
<i>Bacillus</i>	2 (7.14)	4.50	-	4.28	-	3.70	2.12
<i>Klebsiella</i>	5 (17.86)	0.93	-	0.87	0.20	2.56	-
<i>Micrococcus</i>	3 (10.71)	8.60	0.24	6.57	1.40	6.10	-

E. coli=*Escherichia coli*, OG=Oyster glycogen, LE=Levamisole, PG: Prostaglandin

and *Micrococcus* were 2.10, 1.30 and 1.15 with the absence of other contaminants.

The concentration of SAA and Hp before and after treatment with immunomodulators in endometritis cows and normal cyclic cows are presented in Table-2. The pre-treatment SAA values ranged from 33.97±2.14 to 35.42±0.58 µg/ml while the post-treatment values ranged from 13.29±1.62 to 18.92±1.48 µg/ml. For the control group, the SAA value was 16.80±1.62 µg/ml. The analysis of variance in the pre-treatment period revealed a significant difference ($p < 0.01$) among various groups while no significant difference could be observed among post-treatment values. The post-treatment values of SAA were significantly lower ($p < 0.01$) from pre-treatment values for Group I, II, III.

The pre-treatment Hp values ranged from 80.19±7.29 to 81.56±4.22 µg/ml while the post-treatment values ranged from 21.05±1.63 to 24.57±3.65 µg/ml. For control group, the Hp value was 22.39±1.93 µg/ml. The analysis of variance did not reveal any significant difference either in pre and post treatment values among the experimental groups. There was a significant reduction ($p < 0.01$) of Hp values between pre and post treatment period during post-treatment estrus for each treatment groups.

Discussion

The present study revealed that bacteria *E. coli* followed by *Streptococcus* and *Klebsiella* spp. were found to be accountable for endometritis in cows. The type of bacterial isolates observed in the present study was comparable to Kusum *et al.* [11]. Shweta [12] also reported the isolation of these organisms from uterine flushing of cows suffering from endometritis. But Bonnett *et al.* [13] reported that *Actinomyces pyogenes* was more prevalent followed by *E. coli* and *Streptococcus* in endometritis cows. Bacterial elimination was highest in OG group justifying its immunomodulatory effect to eliminate the bacterial infection by the chemotactic action that increased the PMN cell migration to uterus [14]. In PG group, there was a marked decrease in the bacterial load and bacterial species like *Streptococcus* and *Bacillus* were completely absent, which was due to the systemic

immunomodulation. In post-treatment sampling for LE group, *Proteus*, *Klebsiella* and *Micrococcus* were negative whereas after treatment bacterial count was moderately low. This decrease in bacterial count in LE group was due to its potent thymomimetic action which resulted in the systemic immunomodulation.

SAA is an apolipoprotein (9-14KDa) that helped in uptake and removal of cholesterol from inflammatory site. It has different isoforms that are expressed constitutively at different levels in response to inflammatory stimuli [15]. SAA1 and SAA2 are expressed in liver while inflammation whereas SAA3 is produced from distinct tissues like adipose tissue, mammary gland and intestinal cells [16]. SAA4 does not respond to external stimuli [17]. SAA mainly scavenged the cholesterol from dying cells and prevented the accumulation of atherosclerotic plaques. It also directly bound to Gram-negative bacteria leading to opsonization of the target pathogens, inhibition of phagocyte burst and platelet activation [18]. SAA is a rapidly reacting APPs which showed a higher increase in concentration in case of acute as compared to chronic inflammation [19].

In this study, the pre-treatment SAA values in endometritis cows significantly decreased after treatment with immunomodulators. The present observations corroborated with the study of Ceciliani *et al.* [20] who reported that the concentration of SAA in healthy cows was 1.3±0.4 mg/L. Chan *et al.* [21] showed the SAA concentration decreased to <51.9 mg/L during 1st week after parturition. The decrease in concentration of SAA after treatment to a value, which was nearly equal to normal animals, indicated substantial remission of infective process irrespective of immunomodulators employed.

Hp is a glycoprotein composed of two α and two β chains which are connected by disulfide bridges [22]. Hp binds to free hemoglobin released from erythrocytes with high affinity and reduced the oxidative damage to itself and albumin [23]. Hp - hemoglobin complex reduced the availability of the iron residue for bacterial growth and therefore it had an indirect antibacterial activity [24]. It has also effect on angiogenesis, chaperon activity and also act as a proinflammatory mediator. Various studies have indicated the

Table-2: SAA ($\mu\text{g/ml}$) and HP ($\mu\text{g/ml}$) values in endometritis cows before and after treatment (mean \pm SE).

Groups	APPs					
	SAA			Hp		
	Pre-treatment	Post treatment	t-value	Pre-treatment	Post treatment	t-value
Group I (OG)	35.40 \pm 1.56	13.29 \pm 1.62	9.56**	81.30 \pm 3.98	24.57 \pm 3.65	10.51**
Group II (PG)	35.42 \pm 0.58	16.62 \pm 2.17	8.96**	80.19 \pm 7.29	24.19 \pm 2.79	7.17**
Group III (LE)	33.97 \pm 2.14	18.92 \pm 1.48	5.77**	81.56 \pm 4.22	21.05 \pm 1.63	13.37**
Group IV (control)	16.80 \pm 1.62	-	-	22.39 \pm 1.93	-	-
F value	33.19**	1.37 ^{NS}	-	0.018 ^{NS}	0.390 ^{NS}	-

**p<0.01, ^{NS}Not-significant, APPs=Acute phase proteins, SAA=Serum amyloid A, Hp=Haptoglobin, OG=Oyster glycogen, LE=Levamisole, PG=Prostaglandin, SE=Standard error

significance of Hp in many inflammatory conditions like mastitis, pneumonia, enteritis, peritonitis, endocarditis, abscess and lameness. It is also induced in cows with fatty liver syndrome and starvation.

In our study, Hp ($\mu\text{g/ml}$) level in cows affected with endometritis significantly decreased after treatment with immunomodulators. It indicated that after treatment with immunomodulators irrespective of types the cows were reviving from infection. Hp acts synergistically along with immunomodulators for elimination of infection. The present study corroborated with the study of Ceciliani *et al.* [20] who reported a value of <0.1 g/L for normal healthy cows. Dubuc *et al.* [25] reported Hp concentration >0.8 g/L in 1st week of parturition not only prone to metritis but also to endometritis.

Conclusion

The present study investigated SAA and Hp concentrations in crossbred cows suffering from clinical endometritis following treatment with different drug regimen. Based on the results it could be inferred that assessment of both SAA and Hp concentrations at different time points during treatment could serve as reliable biomarkers for both diagnosis and monitoring of clinical endometritis in cows.

Authors' Contributions

SD and DNM designed the study. SSB and MDG collected samples for study and carried out the work. SB and KS assisted in manuscript writing and cross scrutiny. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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