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Circulatory level of interleukin-1 in periparturient cows with or without postpartum reproductive diseases

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ABSTRACT

Objective: To determine the concentration of interleukin-1, a pro-inflammatory cytokine in periparturient cows and their association with the clinical outcome of postpartum reproductive diseases (PRD). **Methods:** Blood sampling was done from advanced pregnant cows on 15 days prepartum (-15 d), calving day (0 d), 15 days (15 d) and 30 days (30 d) post partum and thorough gynaecological examination was performed on 0 d, 15 d, 30 d and 45 d for diagnosis of PRD like retained placenta (ROP), clinical metritis (CM), clinical endometritis (CE), cervicitis (CT) and delayed involution of uterus (DIU). The blood serum was used for estimation of pro-inflammatory cytokine Interleukin 1 (IL-1) in different groups of cows using an enzyme immunoassay technique. **Results:** The IL-1 concentration was significantly higher for normal cows than ROP, CM, CE and DIU cows at 15 d prepartum (-15 d) and on the day of calving (0 d). However, IL-1 level was significantly ($P<0.01$) lower for normal (574.86±71.52) than ROP (869.10±66.29), CM (859.58±110.49) and CE (902.33±54.02) cows at 30 d (postpartum). The concentration of IL-1 increased significantly from -15 d through 30 d for cows suffering from reproductive diseases. However, the normal cows showed a decreasing trend from the day of calving till 30 d. **Conclusions:** Decreased level of IL-1 at 15 d prior or at calving may be used to identify the cows susceptible for development of PRD. Further, increased serum level of IL-1 may be considered as a diagnostic tool for screening of endometritic cows around 4 wk postpartum in a large herd, where individual monitoring is difficult.

1. Introduction

The management and supervision of the dairy animal regularly during the transition period is very much important for early diagnosis and treatment of periparturient diseases to maintain effective reproductive health during the postpartum period. The improper balance between uterine infection and the intrauterine antimicrobial self-defence mechanisms often lead to the main post partum reproductive diseases such as puerperal metritis, clinical endometritis, pyometra and subclinical endometritis[1,2]. These diseases may delay the complete regeneration of endometrium, and disrupt the resumption of cyclic ovarian function resulting in postponement of the first insemination (AI), increasing the number of AIs per conception, and thus prolonging the calving interval and decreasing the calving rate[3,4]. Therefore, early diagnosis of the problems during advanced pregnancy may help the timely management of the problem to prevent animals suffering from post partum complications.

Interleukin-1 (IL-1) is a pleiotropic cytokine that plays a critical role in the generation of inflammatory response and the initiation of many normal biological events[5]. After stimulation by various factors (e.g. endotoxins), IL1β is mainly secreted by mononuclear cells, including monocytes and macrophages, in response to infections[6] and acts as

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a potent stimulator of T- and B-lymphocytes[7]. Increase in the serum concentration of IL-1 during parturition and subsequent enhancement of contraction and evacuation of the debris from the uterus has been reported[8]. Expression level of mRNA of various cytokine genes in the endometrial tissue[9-11] and peripheral blood monocytes[12] has been correlated with the postpartum reproductive diseases. However, perusal of available literature revealed no information regarding the prepartum level of the cytokines in relation to the exhibition of postpartum reproductive disease in cattle in general and concentration of IL-1 in peripheral blood in particular. It is expected and hypothesised that being a pro-inflammatory cytokine, IL- 1 may be increased in the cows suffering from postpartum reproductive diseases. Hence the present study was planned with the objective to determine the concentration of IL-1 in periparturient cows for prediction of the postpartum reproductive diseases at least two weeks before or on the day of calving, which may help in timely management of the cows for the reproductive problems.

2. Materials and methods

2.1. Animals

The experimental animals used in this study were selected from Vrindavani Cattle herd maintained at Cattle and Buffalo Farm, Livestock production and Management section, Indian Veterinary Research Institute, Izatnagar, India. A total of 41 healthy advanced pregnant cows between 2nd to 4th parity that had no reproductive disorder during the previous pregnancy/ calving were selected randomly at 240 days of pregnancy.

2.2. Management of animals

The animals were managed under intensive system in the cattle sheds. During the day time they were allowed to remain in the paddock connected to the cattle shed. The feeding and watering of the cows were done in the paddock. All the animals were maintained under uniform feeding and managemental conditions.

2.3. Gynaecological examination of the animals

2.3.1. Prepartum

The pregnancy diagnosis of the cows was performed routinely in the farm at 60-70 days post insemination. On the basis of AI data supported with the record of pregnancy diagnosis, the expected date of calving was fixed by adding 280 days from the day of insemination. The parity, previous details of parturitions and reproductive problems during periparturient period were ruled out in the experimental animals by thorough vigil over the AI record, calving register and treatment record maintained by the AI section. Before start of the experiment, the animals were examined per rectally to see the status of the gravid uterus, fetal viability and development etc to include the healthy animals with normal pregnancy around 240 days. The cows having viable fetus in their uterus without any complicacy were selected for this experiment. The selected cows were also observed visually daily once at morning and if required twice daily from 240 days of pregnancy till the day of calving. The various external signs observed visually were abdominal enlargement, udder development, milk vein, teat enlargement/secretion, relaxation of sacro-sciatic ligament, tail head and sacrum, vulva, nature of vulvar discharge, colour of discharge, and consistency of discharge. The day of prepartum sample collection was determined considering both the expected date of parturition as well as the external signs of pregnancy. On the basis of the progression of the animals in a particular aspect recorded with a numerical scale, the exact date of first sampling (-15 d) were preponed/ postponed from the expected date of calving. The ranges of the prepartum collection day fell between 10 (- 10 d) to 22 days (- 22 d) prior to calving.

2.3.2. Calving

The cow on the day of calving was monitored /observed for nature of parturition, expulsion of placenta and lochia.

(1)Normal/ abnormal calving: The parturition was critically observed for any abnormality like dystocia, abortion or stillbirth *etc*. The cows were also observed for any production disease like ketosis/milk fever or parturient paresis.

(2)Expulsion/retention of fetal membranes: The placenta (fetal membranes) was considered to be retained, if it was not dropped naturally within 24 h after fetal expulsion.

2.3.3. Postpartum

After parturition, the individual cow was critically monitored for the diagnosis of post partum disorders like retained placenta, clinical metritis, clinical endometritis etc[2]. Uterine involution and ovarian activity was monitored at 15, 30 and 45 days post-partum by trans-rectal palpation[13].

2.4. Sampling

Blood was collected from jugular vein at-15 d (15 days before calving), 0 d (day of calving), 15 d (15 days after calving) and 30 d (30 days after calving) during peripartum period and allowed to clot and kept at +4 °C till separation of serum.

Clotted blood was centrifuged at 3 000 rpm for 20 minutes in a swing out rotor type of centrifuge (Remi, Mumbai). The separated serum was aspirated out from the supernatant gradually without disturbing the sediment. The separated serum was transferred to a sterile 2 mL plastic microfuge tube (Tarsons products, Kolkata, India) and stored at -20 ℃ till analysis for assay of Interleukin 1.

2.5. Assay of interleukin-1 (IL-1)

Out of the 41 cows included in the experiment, 17 were having different post partum reproductive diseases (PRD). Of the 17 cows with PRD, one cow suffering from cervicitis was not included for analysis. To study the IL-1 profile, serum samples of six normal representative animals was selected

randomly from the 24 normal experimental cows. The cytokine profile of these six normal cows was considered as control to compare the profile in the cows suffered from PRD (n=16).

2.5.1. Procedure

The interleukin -1 concentration in serum (ELISA) was estimated with commercially available bovine specific kit (Cusabio) from 15 days prior to calving (-15) until 30 days post calving (30 d). This assay employs the competitive inhibition enzyme immunoassay technique.

2.5.1.1. Reagent preparation

All the kit components and samples were brought to room temperature 30 min before use (18-25 ℃). Fifteen ml of wash buffer concentrate (20×) was diluted with distilled water to prepare 300 mL of wash buffer (1×).

2.5.1.2. Assay procedure

A blank well was set without any solution. Fifty μL of standards or samples were added to the assigned wells of the antibody pre-coated microtitre plate except in the blank well. Fifty μL of conjugate was added to each well excepting the blank well and mixed the content properly. The plate was covered with the sheet provided with kit to close all the wells and kept for incubation at 37 ℃ for 1 h. After incubation, the content of the well was decanted. All the wells were filled with the wash buffer one by one with help of a squirt bottle, given mild jerk to wash the well properly and then decanted. The procedure of washing was repeated for five times. After final wash, the plate was inverted and dried by hitting onto an absorbent paper, until no moisture appeared. Fifty μL of HRP-Avidin (not to blank well) was added to each well. The plate was sealed and incubated for 30 min at 37 ℃. The washing of the wells for five times as mentioned above was done. Fifty μL of Substrate A was added to each well. Fifty μL of Substrate B was added to each well, mixed well and the plate was again covered as described and kept for incubation for 15 minutes at 37 ℃ in dark environment. Finally fifty μL of stop solution was added to each well and gently tapped to ensure thorough mixing. The optical density (OD) was determined at 450 nm using a microplate reader (Biorad, USA) within 10 min. Standards of known concentrations of IL-1 (pg/mL) 250, 500, 1 000, 2 000, and 4 000 were run concurrently with the sample being assayed. Average of OD was calculated out for each standard and blank. The average OD of blank was subtracted from the average OD of Standard and samples and a standard curve was plotted relating the resultant OD of the standards to the concentration of IL-1 in the standard. The unknown IL-1 concentration in each sample was interpolated from the curve.

2.6. Statistical analysis

The data on IL-1 concentration in serum of periparturient animals was analyzed by Analysis of Variance Test (ANOVA) using statistical software SPSS version 16. Data were skewed and were transformed to their natural logarithm and then back-transformed for data presentation. The effects of postpartum reproductive diseases (Control, Retained placenta, Clinical metritis, Clinical endometritis and delayed involution), and periods (days of sample collection -15 d, 0 d, 15 d and 30 d) were observed. When an effect between groups and time was observed, post-hoc multiple comparisons were performed using Duncan's multiple range test and cross checked with LSD. All the data are expressed as Mean ±S.E.M. The level of significance was set at P<0.05. When P<0.10 indicates tendency to be significant.

3. Results

The IL-1 level (pg/ml) in cows suffering from various reproductive diseases during periparturient period from -15 d (prepartum) to + 30 d (post partum) is presented in Table 1. The level of IL-1 differed significantly between the groups at -15 d, 0 d (P<0.05) and 30 d (P<0.01). The IL-1 concentration was significantly higher for Normal cows (664.88±64.79) than ROP (441.62±71.47), CM (435.00±103.23), CE (484.89±49.00) and DIU (437.04±83.17) at -15 d prepartum. The level was also significantly higher for normal cows on the day of calving (0 d) than the cows suffering from various reproductive diseases. However, IL-1 level was significantly (P<0.01) lower for normal (574.86±71.52) than

Table 1

IL-1 concentration (pg/mL) in the peripheral blood of cows with or without postpartum reproductive diseases.

Groups	Periparturient period (days)				Overall
	-15 d	0 d	15 d	30 d	
ROP (9)	441.62 ± 71.47^{aA}	461.36 ± 62.79^{abA}	618.88 ± 54.61^{B}	869.10 ± 66.30^{bcB}	606.92 ± 42.55^{q}
CM (5)	435.00 ± 103.23^{aA}	459.95 ± 104.14^{abA}	679.09 ± 81.22^{AB}	859.58 ± 110.49^{bcB}	608.41 ± 60.85^{q}
CE (6)	484.89 ± 49.00^{abA}	520.46 ± 51.16^{abcA}	637.32 ± 63.81^{A}	902.33 ± 54.02^{cB}	642.83 ± 43.44^{q}
DIU (3)	437.04 ± 83.17^{aAB}	286.49 ± 42.95^{aA}	471.56 ± 24.16^{BC}	606.14 ± 18.09^{abC}	450.31 ± 40.20^{p}
NM (6)	664.88 ± 64.79^{b}	737.45 ± 95.52^{c}	701.81 ± 69.51	574.86 ± 71.52^{a}	669.75 ± 37.94^{q}
Overall (29)	530.64 ± 37.91^{P}	550.96 ± 46.21^{PQ}	646.45 ± 32.43^{Q}	750.01 ± 41.83^{R}	–

ROP: Retained placenta; CM: Clinical metritis; CE: Clinical endometritis; DIU: Delayed involution of uterus; NM: Normal; Values are shown as mean±SEM; Means with different superscripts in a column (a, b, c) and row (A, B, C) differ significantly; Overall means with different superscripts in a column (p, q, r) and row (P, Q, R) differ significantly. Figures within parenthesis indicate number of observations. All five cows from CM group of cows and two cows from CE group had suffered from ROP.

ROP (869.10±66.29), CM (859.58±110.49) and CE (902.33±54.02) cows at 30 d (postpartum).

The concentration of IL-1 increased significantly from 15 days before calving through 30 days post partum for all groups' of cows suffering from reproductive diseases. However, the normal cows showed a decreasing trend from the day of calving (737.45±95.52) to 15 d (701.81±69.51) and 30 d (574.86±71.52), when the value became significantly (P<0.01) lower than ROP, CM and CE.

4. Discussion

The significantly higher level of IL-1 observed for normal than cows with PRD during -15 d to the day of calving might be attributed to the natural conditioning of the animal necessary for the labor as reported earlier in preterm labor of women[14]. The increased IL-1 in the late gestation followed by a decrease during postpartum period in murine[15] is in concurrence with the present finding. A decreased IL-1 level in cows with PRD during -15 d to 15 d pp indicates disturbed immune mechanism in these animals around calving day leading to development of ROP, CM and CE. In line with the present study a decreased IL-1 expression in monocytes from peripheral circulation of metritic cows and endometrial cells from endometritic cows during 7 to 14 d postpartum has also been reported [11,12]. However, the source and nature of determination of IL-1 was real time expression in monocyte in the previous study and being estimation of protein in the blood serum in the present study. The proinflammatory cytokines (TNFα and IL-1) enhance phagocytosis and bacterial killing by professional phagocytes, neutrophils and monocytes[16,17]. Impaired monocyte function at various days of parturition from calving to 42 days post calving was reported to be associated with the development of metritis [12].

Further, a linear increasing trend was observed from -15d through 30 d postpartum in cows suffering from ROP, CM and CE. The values could not be compared prepartum due to the non-availability of literature in respect of IL-1 concentration in the blood circulation. The higher IL-1 level in cows with PRD on day 30 is in agreement with the finding of increased expression of IL-1 on day 35 and 49 pp in endometrial cells from endometritic cows[11] and on the day 0 to 7 and 21 to 28 by *Escherichia coli* stimulated circulating monocytes[12]. They further speculated that decreased expression of pro-inflammatory cytokines (TNFα and IL-1) could lead to poor chemotaxis and activation of neutrophils and monocytes, which would impair bacterial clearance and predispose cows to development of endometritis. However, they reported decreased expression of IL-1 in monocyte on day 14 and 35 pp. The cellular expression of mRNA transcript and protein level in the blood may vary. Since, synthesis of the primary RNA transcript (mRNA) is the first step in protein production and secretion, several factors can affect the process and ultimately the amount of protein that can be measured in the circulation, namely post-transcriptional modification of mRNA, mRNA degradation, post-translational modification of proteins, protein targeting and transport, and protein degradation[11,18].

IL-1 is produced as a procytokine upon initial stimulation of immune cells via toll like receptors. Its release is required for the subsequent release of other inflammatory and anti inflammatory cytokines to remove and resolve the infection. IL-1 level declined in normal cows from 0 d to 30 d and became significantly lower than ROP, CM and CE at 30 d postpartum. Due to the linear decrease in normal cows and increase in cows with PRD, the values did not differ significantly at 15 d postpartum. The decline in IL-1 concentration in normal cows from the day of calving might be due to the intrinsic mechanism of animal under natural involution process. The higher IL-1 level at -15 d and calving day in normal cows might be due to a greater active innate immunity during calving or before calving and therefore, they remained healthy during the puerperal period. Elevated plasma levels of proinflammatory cytokines, TNF-α, IL-1 and IL-6 during late gestation are not necessarily detrimental to the course and outcome of pregnancy but may be a part of a regulatory network in normal pregnancy and parturition in murine [15,19] and cows[20] supports the present study.

The significantly lower IL-1 in the normal cows seen at 30 d postpartum might indicate the healthy status of the uterus and become free from infection as also revealed in clinico-gynaecological examination. It is also interesting to note that some of the animals in the present study that did not suffer from any kind of reproductive diseases had nearly involuted uterus by 15 d postpartum. The greatest reduction in uterine size in cows with an undisturbed puerperium was reported to occur 10-14 days after calving[21]. In most of normal animals, the uterus was found to be grossly involuted or near to involution by 30 d postpartum. However, cows with uterine diseases had suffered from delayed involution of uterus and some cows showed purulent discharge of varying degrees indicated heavy load of infection. Of the five metritic cows, one cow showed persistent severe purulent uterine discharge till 50 days pp. The higher level of IL-1 at 30 d in cows with PRD might be associated with infection and inflammation of the uterus.

Due to paucity of literature, the IL-1 protein level in the peripheral circulation at various days of periparturient period could not be compared in cattle. The concentrations of cytokines IL-1, TNF-α and IL-6 increased most significantly in lochia of women patients with postpartum endometritis and active viral infection by 3.4, 4.2, and 1.6 times, respectively[22] was in accordance with the IL-1 concentration in cows suffering from PRD on day 30 postpartum in the current study. IL-1 is a pleiotropic cytokine that plays a critical role in the generation of inflammatory response and the initiation of many normal biological events[5]. After stimulation by various factors (e.g. endotoxins), IL1 is mainly secreted by mononuclear cells, including monocytes and macrophages, in response to infections[6] and acts as a potent stimulator of T- and B-lymphocytes[7].

The lower IL-1 concentration in cows 15 days prepartum to the day of calving might lead to the development of uterine disease due to the immunological imbalance in terms of disturbance in PMN number, function and migration to the uterus. Role of rise in the serum concentration of IL1 during parturition for increased leucocyte trafficking in the uterus has been reported[8]. The main pro-inflammatory cytokines (IL-1 and TNFα) stimulate the expression of IL-8 and adhesion molecules on vascular endothelial cells,

leading to neutrophil and monocyte chemo-attraction, and activate neutrophils and monocytes, promoting increased phagocytosis and bacterial killing[17,23]. The increased concentration of IL-1 during parturition has resulted increase in leucocyte trafficking in the endometrial tissue by enhancing vasodilatation. IL-1 increases the plasma calcium concentration, which stimulates myometrial contractions and the removal of debris from the uterus. The cytokine also stimulates prostaglandin synthesis, which enhances contraction and evacuation of the uterus[8,24]. Therefore, higher level of IL-1 before calving and around calving day in the present study indicated higher immunity against reproductive diseases in healthy cows and thereby protected them from the development of PRD. The decreased IL-1 concentration prepartum may be used for prediction of cows at prepartum for development of postpartum reproductive diseases and increased concentration at 30d postpartum may be used as a diagnostic marker for the reproductive diseases.

Measurement of IL-1 prepartum has the potential to show immune status of the animals against postpartum uterine diseases. Decreased circulatory level of IL-1 at 15 d prior or at calving may be used to identify the cows susceptible for development of post partum reproductive diseases (PRD). Increased serum level of IL-1 may be considered as a diagnostic tool for screening of endometritic cows around 4 week postpartum in a large herd, where individual monitoring is difficult.

Declare of interest statement

We declare that we have no conflict of interest.

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