

Serum Sialic Acid, Total Protein And Albumin Levels In Cows With Metritis Treated With Intrauterine Oxytetracycline

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Summary: Serum total sialic acid, total protein and albumin concentrations in healthy cows and cows with metritis treated with local intrauterine therapy were measured. Twenty multiparous cows with metritis from a dairy herd were treated with a daily dose of 25 ml intrauterine injectable oxytetracycline solution (100 mg/ml) for three consecutive days. A significant reduction ($p<0.001$) of vaginal discharge following therapy were observed while this reduction could not be correlated with mean serum sialic acid concentrations which were measured as 896 µg/ml in healthy cows and 1063,19 µg/ml before and 1053,40 µg/ml following the treatment. No difference were observed in serum total protein and albumin concentrations of healthy and sick cows.

Key Words: Cow, metritis, intrauterine therapy, sialic acid.

İntrauterin Oksitetrasiklin Sağaltımı Uygulanan Metritisli İneklerde Serum Siyalik Asit, Total Protein Ve Albümin Düzeyleri

Özet: Bu çalışmada sağlıklı ve lokal intrauterin tedavi ile sağaltımı yapılan metritisli ineklerde serum total siyalik asit, total protein ve albümin düzeyleri ölçüldü. Bir sütçü işletmedeki 20 metritisli ineğe enjektabl oksitetrasiklin solusyonu (100 mg/ml) günlük 25 ml dozda arka arkaya üç gün süreyle intrauterin olarak uygulandı. Sağaltımı takiben klinik belirtilerde belirgin düzeyde azalma izlenirken ($p<0.001$) bu azalma serum siyalik asit düzeyleri ile korelasyon göstermedi. Ortalama serum siyalik asit konsantrasyonu sağlıklı ineklerde 896 µg/ml, metritisli ineklerde sağaltım öncesi 1063,19 µg/ml ve sağaltım sonrası 1053,40 µg/ml olarak belirlendi. Sağlıklı ve hasta ineklerde serum total protein ve albümin konsantrasyonlarında da farklılık görülmedi.

Anahtar Kelimeler: İnek, metritis, intrauterin sağaltım, siyalik asit.

Introduction

Uterine infections are among the most frequent disorders to affect dairy cows in the postpartum period. Uterine infections known in general as "metritis" in cows are observed in a wide range of severity, varying from a slight vaginal discharge with normal general condition to purulent or acute septic form. Following birth with the temporary deficiency of local immunity of mechanical barriers (cervix, vagina) and inactivity of ovaries plays the major role in the pathogenesis

and disorders like retentio secundinarum, dystocia and hypocalcemia reducing the involution period hastens the occurrence of these infections. In general subacute and postpartum metritis' occurring in 8-14 days and 2-8 weeks causes infertility and prolonged open periods¹⁶.

In postpartum uterine infections various intrauterine antibiotics and antibiotic-hormone combinations were studied and while some of the workers have reported good therapy response and improved fertility results others stated no significant benefit of intrauterine therapy¹⁶. Frequently

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used antibiotics in field trials are tetracyclines, penicilins, cephalosporins, gentamycin, neomycine, rifampicine and sulphadiazine-trimethoprim. Besides some antiseptic solutions like lugol, iotagen and hydrogen peroxide have been used also. Tetracyclines especially oxytetracycline (OXY) which is widely used in ruminants have a broad antibacterial spectrum for both Gram negative and positive microorganisms and some chlamydia and rickettsia species with seldom antibacterial resistance problems. Tetracyclines following administration into the uterine lumen reaches therapeutic concentrations rapidly and as a result of poor disturbance into the bloodstream their presence in the uterine lumen and endometrium is prolonged. In addition their antibacterial activity is not reduced in anaerobic conditions with tissue debris and blood⁴. Drug formulations of antibiotics also play an important role in local antibiotic therapy. Most of the OXY preparations in veterinary practice are dissolved in propylene glycol as vehicle substance. Thus drugs dissolved in polyvinyl pyrrolidone may be preferred for local therapy⁹. Intrauterine daily dosage of oxytetracycline is 1 to 3 gr/day, may be raised up to 6 grs. Therapy should be continued at least 3 consecutive days.

The term "sialic acid" represents a series of substances consisted of approximately 20 different molecules derived from neuraminic acid. Main localisation of sialic acids is the outer cell membrane where it is located as components of glycoproteins, gangliosides or polysaccharides. Local sialic acid contents of inflamed tissues rises and sialic acids are excreted from the body as glycoproteins (serum and mucus) or oligosaccharides (urine and milk). Sialic acids in free form were found in larger quantities in the urine of some patients compared with healthy persons¹⁰. The main role of sialic acid in bacterial or viral infections were determined as that it may enhance or decrease the infectivity of the pathogen¹⁰. High levels of sialic acid were determined in the bovine milk in colostral phase, during dry off and mastitic milk however its pathophysiological role in mastitis is unclear. Atroshi et al¹ further observed a slight positive correlation between sialic acid content of mastitic milk and serum. Also a correlation between serum sialic acid and total protein concentrations were found. Albumin levels also tend to rise in severe infectious diseases.

Monitoring the therapy response in the local treatment of metritis may be a useful tool for prediction of subsequent fertility. Thus sialic acid levels in sera of infected cows may be an indicator of therapy response. Our aim was to determine the total sialic acid, total protein and albumin levels in cows with post puerperal metritis before and following local intrauterine antibiotic therapy.

Material and Methods

Animals and Treatments

Cows used in this study were chosen from a commercial dairy farm. Age of the cows were between 2-6 years. All the cows were housed in tie stall barns and fed with corn silage and concentrate. The chronic problem in this herd was prolonged open periods due to postpartum genital infections. Infections were observed in the 2 –8 weeks postpartum and the general condition of these animals were not effected. A non odorous, mucoid vaginal discharge containing cream white, clotty particles which increases especially when the animal lies in the barn. The sexual cycles of the cows were not affected and the discharge gets even worse with the oestrous. Twenty of these infected cows were selected as the materials of this study. Seven healthy cows free from genital or other infections from the same barn were selected as controls to get the normal mean serum concentrations of sialic acid, total protein and albumin. Besides the retrospective fertility and examination data, all the treatment and control group cows were examined by vaginal speculum in order to confirm the uterine infections. Cows with no abnormal vaginal or cervical discharge were considered as normal and were excluded from the study. Severity of the clinical signs were graded as (+) for clotty particles near cervix and excessive mucoid discharge, and (++) for distinguishable cloudy cervical mucus with fibrin particles inside.

All of the animals in the treatment group starting from day 0 received a total daily dose of 25 ml of oxytetracycline hydrochloride solution (100 mg / ml) into the uterine lumen for three consecutive days. The vehicle solution of the antibiotic was non-irritant as indicated by the manufacturer. The antibiotic solution were administered by recto-vaginal procedure using a 43 cm long metal intrauterine catheter which is adaptable to a 50 ml syringe. The 25 ml solution

were divided to both uterine horns evenly. The vaginal examination was repeated on day 7 following this administration.

Sample Collection and Laboratory Analysis

Blood samples from all of the cows were collected on day 0 before the onset of the treatment. The local intrauterine therapy were administered until day 3 and the treated animals were examined again on day 7 and the severity of the vaginal discharge recorded as none (-), poor (+) or high (++). Finally, second blood samples were collected only from the treatment group. Samples were transferred daily to the laboratory where centrifugated at 3000 rpm for 10 minutes, the sera were harvested, frozen and kept under - 20° C until the day of analysis. For the analysis of total sialic acid in serum samples the method of¹⁴ was used. The levels of serum total protein and albumin were detected using the commercial available kits (Biomedical Systems, Barcelona, Spain). The analysis were carried out according to the manufacturer's instructions

Statistical analysis

Differences between control and treatment groups for serum total sialic acid, total protein and albumin concentrations were analysed with One Way Anova. The clinical status of the cows in the treatment group before and after the intrauterine therapy were compared with chi-square test.

Results

Mean total sialic acid levels in the treatment group before the treatment (1063,19±58,95 µg/ml) were higher than in the control group (896±125,47 µg/ml) while the difference wasn't statistically significant. There wasn't also any significant difference following the treatment (1053,40±52,98 µg/ml).

No difference for total protein and albumin concentrations were observed between the control and the treatment group before and after local intrauterine therapy. The mean concentrations were listed in Table I.

It was observed that the severity of the clinical signs (vaginal discharge, clotty particles) in general were reduced following the intrauterine therapy (Table II). In 12 of the animals the vaginal discharge have turned to normal while a total

of 8 animals had abnormal discharge. The reduction in the clinical signs of the infection were significant ($p < 0,001$, $\chi^2 = 22,020$).

Table I. Serum total sialic acid, protein and albumin levels of normal cows and cows with metritis before and after intrauterine therapy

Parameter	Group	N	Mean
Sialic acid (µg/ml)	Control	7	896 ± 125,47
	Before treatment	20	1063,64 ± 58,95
	After treatment	20	1053,40 ± 52,98
Total protein (g/dl)	Control	7	6,51 ± 0,55
	Before treatment	20	5,30 ± 0,30
	After treatment	20	5,60 ± 0,24
Albumin (g/dl)	Control	7	3,17 ± 0,15
	Before treatment	20	3,34 ± 0,12
	After treatment	20	3,23 ± 0,07

Table II. Clinical status of cows before and following intrauterine therapy

Group	-	+	++	Total
Before treatment		14 (70)	6 (30)	20
After treatment	12 (60)	6 (30)	2 (10)	20
Total	12	20	8	40

($\chi^2 = 22,020$) $P < 0,001$

Discussion

Confirming the presence and the effects of subclinical, chronic or non-specific uterine infections with laboratory diagnosis is essential. Besides biopsies and cervical bacteriological cultures, serum concentrations of some electrolytes⁷, some immunological tests² were studied for this purpose. The diagnostic significance of serum acute phase proteins in bovine endometritis were studied by some of the workers⁵. One of them was serum haptoglobulin concentrations which have shown no significant alterations in chronic, subclinical metritis as well as acute puerperal metritis cases while there was considerable high values in perimetritis cases^{6,8,12,13}.

Oxytetracycline has been widely studied in metritis therapy via the intrauterine route^{3,11}. The drug penetrates well in the uterine wall and endometrium after intrauterine infusion of routine dosages. Sufficient therapeutic levels in the uterine layers are reached at two hours and kept until 24 hours after local administration. In our study we used a daily dose of 5 mg/kg for three days

and this caused a significant reduction in clinical signs in 7 days following the initialization of treatment ($p < 0.001$). This time of sampling was chosen considering the withdrawal period after the last infusion so any effect of local irritation could be excluded. Normal serum sialic acid concentrations in healthy cows were reported in a study as $517 \mu\text{g}/\text{ml}$ ¹, while in a study carried out by Turkey, Seyrek et al. have observed mean concentration as $1141,38 \pm 68,14 \mu\text{g}/\text{ml}$ (Unpublished data). In this study in the control group it was $896 \pm 125,47 \mu\text{g}/\text{ml}$ and this level was similar to the previously observed data. Before the local intrauterine therapy sialic acid levels in cows with metritis were $1063,19 \pm 58,95 \mu\text{g}/\text{ml}$. Intrauterine oxytetracycline treatment caused a significant reduction in the number of animals with abnormal vaginal discharge and a reduction in the severity of the signs, however this improvement did not have any effect on serum sialic acid levels as it was $1053,40 \pm 52,98 \mu\text{g}/\text{ml}$ 7 days after beginning the therapy. Mean total sialic acid levels in the treatment group before therapy were higher than normal cows but this did not show a significance and no difference were observed following therapy.

It has been reported that immunoglobulin concentrations are higher in the uterine fluids of mares less resistant to endometritis than the ones that are more resistant⁸. Tunon et al.¹⁵ have observed that in gynecologically healthy mares protein content of serum and immunoglobulins shows a similar profile while this similarity could not be confirmed by the correlation between serum albumin and protein concentrations. It was observed that immunoglobulin concentrations, especially IgG and IgA in uterine fluids of cows with abnormal puerperium rises⁵. In cows IgA is synthesised locally in the bovine uterus at the mucosal surface, while IgG's are both produced locally or derived from the peripheral circulation. In this study blood total protein and albumin concentrations were also not affected by the treatment and there was no significant difference between the control and the treatment group. This result may indicate that quantitatively protein content of serum in cows may not be positively correlated with that of endometrium while immunoglobulin and protein profiles of healthy cows and cows with metritis needs to be further studied.

Clinically it was observed that measuring serum total protein and albumin cannot have a benefit for monitoring therapy response of intrauterine therapy in cows. Slight increase in serum total sialic acid concentrations are encountered in metritis cases in this study and this may be an indicator of subclinical inflammation but accordance of these levels with the therapeutic course after local intrauterine therapy were not observed.

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