Evaluation of serum and milk haptoglobin in some inflammatory diseases of cattle

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Abstract: **This study was designed to evaluate serum and milk haptoglobin (Hp) as an inflammatory indicators for the diagnosis of diseases in cattle in Fars province of Iran. Blood samples were collected from the coccygeal vein of 20 clinically healthy cattle and 100 cows with various inflammatory diseases. There were significant differences in serum and milk Hp between clinically healthy cattle and diseased cows ($p<0.05$). The results of the present study reveal that serum and milk Hp are sensitive factors for diagnosis of inflammatory conditions in cattle.**

Key words: haptoglobin, milk, serum, inflammatory diseases.

Introduction

Haptoglobin (Hp) is an α_2-globulin and is synthesized in the liver. It is one of the acute phase proteins, which increases in serum in acute inflammatory diseases. The primary function of Hp is to prevent the loss of iron by the formation of very stable complexes with free hemoglobin in the blood (Burtis and Ashwood 1999). In cattle, Hp has been proposed to be involved in the regulation of lipid metabolism (Nakagawa *et al*., 1997) and acts as an immunomodulator (Morimatsu *et al*., 1992, Murata and Miyamoto 1993). The reference value for serum Hp was determined in apparently healthy animals (mixed breed and ages) and has been determined to be <0.35 g/l (Horadagoda *et al*., 1994). Skinner *et al.,* (1991) evaluated serum Hp in inflammations in cattle and introduced Hp as an inflammatory indicator. Determination and evaluation of serum Hp showed that this protein could a be valuable factor in the diagnosis of inflammatory diseases (Alsemgeest *et al*., 1994). Hirvonen *et al.,* (1996) reported an increase in Hp as an inflammatory factor in heifers with mastitis. Gronlund *et al.,* (2003) reported that in cows infected with staphylococcal mastitis, Hp elevated rapidly in milk and serum in acute phase. The stage of the disease can be better evaluated by monitoring more than one acute phase protein so chronic as well as acute conditions should be evaluated and characterized by acute phase protein profiling (Eckersall 2004). Measurement of acute phase proteins could be a useful tool for evaluation of health in calf herds (Ganheim *et al.,* 2007). There are no published reports about the concentration of serum and milk Hp in theileriosis due to *Theileria annulata*. In addition, the comparative measurement of serum and milk haptoglobin in post abdominal surgical infection, acute metritis and acute local traumatic reticuloperitonitis (TRP) has been carried out for the first time in this research.Therefore, the present study was conducted to evaluate serum and milk Hp as an inflammatory indicator in various inflammatory diseases of cattle in Fars province, a tropical area in the South of Iran.

Materials and Methods

Data were obtained from six dairy farms in Fars

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province. Twenty clinically healthy adult cattle were selected for this study. Barely, corn and concentrates were used in the diet of dairy cows. All cattle were vaccinated against foot and mouth disease, brucellosis and anthrax. One hundred cows with various inflammatory diseases were examined for common infectious diseases such as acute local traumatic reticuloperitonitis (TRP), that their jugular veins were swollen and after blood and milk collection, they underwent surgery and the traumatic agent was observed (20 cases), subclinical mastitis did not show any mastitis clinical signs but the somatic cell count was more than 100000 cell per microliter and the bacterial culture showed *Staphylococcus aureus* and *Streptococcus agalactea* (20 cases), in cows with theileriosis due to Theileria annulata, lymph nodes were found to be swollen and their hematocrit indicated anemia and in their blood smear *Theileria annulata* was observed (20 cases), post abdominal surgical infection caused fever and the surgical site was painful and swollen (20 cases) and in acute metritis there was vaginal discharge, fever, early parturition, retained placenta and the bacterial culture exhibited the presence of *Actinomyces pyogenes* (20 cases). Diseased cows were thoroughly examined and appropriate samples were collected for hematology, clinical biochemistry and other relevant analysis like ELISA. Blood samples were collected from the coccygeal vein of both clinically healthy and diseased groups. For determination of serum Hp, blood samples were collected into plain vacutainers and the serum was immediatly separated following centrifugation for 15 min at 750 g. Serum samples were stored at -20 oC until analyzed. Milk samples were collected from the mammary gland's quarters. Serum and milk Hp were measured using a solid phase enzyme linked immunosorbent assay (ELISA) (Tridelta Development Plc,Co.Wicklow, Ireland). The analytical sensitivities of these tests were determined as 312.5 ng/ml and 0.0156 mg/ml Hp for milk and serum, respectively. The data were analyzed by one-way ANOVA, Duncan's multiple range test and receiver operating characteristics (ROC) using SPSS software (version 11.5). All values were expressed as mean and standard error (SE), and $p<0.05$ was seen as statistically significant.

Results

The mean (± SE) concentration of serum and milk Hp and their correlation in cows with some inflammatory diseases and clinically healthy cows are presented in Table 1. There are significant differences in serum and milk Hp between clinically healthy cattle and diseased cows ($p<0.05$). ROC analysis of serum and milk haptoglobin of cases with different inflammatory diseases are shown in Table 2. The optimal cut-off point was set, by the ROC method, to >0.185 mg/ml for serum haptoglobin and >0.00 (ng/ml) for milk Hp with corresponding 98% sensitivity and 100.00 % specificity for serum Hp and 95.00 % sensitivity and 100 % specificity for milk haptoglobin.

Discussion

The concentration of serum Hp in clinically healthy cattle was 0.108±0.017 mg/ml, which is in agreement with the findings of others (Lipperheide *et al*., 1997; Nakagawa *et al*., 1997). Serum and milk Hp are sensitive indicators in inflammatory conditions in cattle. Alsemgeest *et al.,* (1994) observed an increase in serum Hp in acute, subacute and chronic inflammations such as pneumonia, peritonitis and abscess and introduced Hp as a valuable factor for diagnosing the healthy cows from the diseased one. Hirvonen and Pyorala (1998) suggested that plasma Hp concentration was the most accurate parameter for differentiating traumatic reticuloperitonitis from other non-infectious gastrointestinal disorders. Bacteriological sampling is not feasible as a routine test to identify subclinical mastitis because this approch is time consuming as well as non-economic, and indirect tests of mastitis are more suitable for selecting cows with intramammary infections for subsequent bacteriological sampling; in addition, CMT is a cow side test. Mastitis affects the composition of milk, and the degree of changes depends on the infecting agent and the inflammatory response. Acute phase proteins, Hp and serum amyloid A, are potential candidates for mastitis

Table 1: Concentrations of serum and milk haptoglobin and their correlation in cows with common infectious diseases and clinically healthy cows. Means within a column with different superscript letters (a,b,c,d) denote significant differences (p<0.05).

Group	Number	X± SE of SerumHp (mg/ml)	X± SE of Milk Hp (ng/ml)	Correlation coefficient between serum and milk Hp
Clinically healthy cows	20	$0.108^{a} \pm 0.017$	$0.00^{a} \pm 0.00$	0.164 (p>0.05)
Acute local traumatic reticuloperitonitis(TRP)	20	$0.870^{b} \pm 0.065$	$231.77^{b} \pm 58.89$	-0.024 (p>0.05)
Subclinical mastitis	20	$0.570^{c} \pm 0.063$	$107.14^{c} \pm 26.96$	-0.340 (p>0.05)
Post-surgical abdominal infection	20	$0.510^{c} \pm 0.073$	$219.89^{b} \pm 57.83$	-0.167 (p>0.05)
Theileriosis due to *Theileria annulata*	20	$0.910^{b} \pm 0.084$	$359.26^{d} \pm 55.10$	0.360 (p>0.05)
Acute metritis	20	$0.890^{b} \pm 0.071$	$112.12^{c} \pm 20.57$	0.102 (p>0.05)

Table 2: Shows ROC analysis of serum and milk haptoglobin of cases with different inflammatory diseases (n= 100). + LR : Positive likelihood ratio. -LR : Negative likelihood ratio.

Parameter	Cut- off point	Sensitivity (%)	95%CI	Specificity (%)	95%CI	+LR	-LR
Serum haptoglobin (mg/ml)	>0.185	98.00	92.9-99.7	100.00	83.00-100	-	0.02
Milk haptoglobin (ng/ml)	>0.00	95.00	88.7-98.3	100.00	83.00-100	-	0.05

monitoring (Pyorala 2003). Hirvonen *et al.,* (1996) found serum Hp as the most effective factor in the diagnosis of severity and prognosis of mastitis. Salonen *et al*., (1996) observed an increase in serum Hp in cows suffering from *E. coli* mastitis. Eckersall *et al.,* (2001) reported elevation of Hp in serum and milk in mild mastitis. Hp in milk and serum increases rapidly in acute phase of staphylococcal mastitis (Gronlund, *et al.,* 2003). Nielsen *et al.,* (2004) reported that the concentration of Hp in the serum and milk of the cows with clinical mastitis was higher than those with extra mammary infection. Hp concentration in milk increased significantly with increasing somatic cell count, suggesting that they may be indicators of the severity of an infection. Gronlund *et al.,* (2005) reported that a substantial variation in Hp and SAA concentrations in milk was observed in udder quarters with chronic sub-clinical mastitis.

Concentration of Hp in serum increases following abscess formation, endotoxin administration and post-operation (Alsemgeest 1994). Hp is a prominant acute phase protein in most species studied, but the serum concentration can be influenced by other factors besides the acute phase response. Increased levels of free Hb in the serum are followed by decreased serum concentration of free Hp. In cattle, during an acute hemolytic crisis due to babesiosis (Bremner 1964), Hp disappeared from the circulation. In addition to the acute phase response, non-acute renal disease and obstractive jaundice may cause hyperhaptoglobulinemia (Feldman *et al.,* 2000). Increased serum or plasma Hp concentration in cattle was found after trauma (Earley and Crowe 2002; Fisher *et al*., 2001), experimental local aseptic inflammation (Conner and Eckersall 1988), various acute infections under field conditions (Alsemgeest *et al*., 1994; Skinner *et al*., 1991), acute inflammation (Lipperheide *et al*., 1997), mastitis (Gronlund *et al.,* 2003; Gronlund *et al*., 2005; Hirvonen *et al*., 1999; Nielsen *et al*., 2004; Ohtsuka *et al*.,2001), castration (Earley and Crowe 2002; Fisher *et al*., 2001), metritis

(Smith *et al.,* 1998), severe uterine bacterial post partum infection (Sheldon *et al*., 2001), off feeding for 3 days (Katoh *et al*., 2002; Lipperheide *et al*., 1997), transportation for 2 days (Murata and Miyamoto 1993) and after major injuries at slaughter/culling (Hirvonen *et al*., 1997; Saini *et al*., 1998).

Hp has also been associated with bacterial infection of the uterus and delayed uterine involution (Sheldon *et al*., 2001). However, others have reported that Hp concentration remains low in acute postpartum metritis and high concentrations are seen only in cases of severe metritis (Smith *et al*., 1998). In dairy cows with toxic puerperal metritis, antimicrobial therapy is associated with a decrease in serum Hp concentration (Smith *et al.,* 1998).

Following surgical castration of 14 month old bulls, a higher plasma Hp concentration has been observed when compared to banded and intact bulls (Fisher *et al*., 2001). A higher Hp serum concentration has also been reported following ruminotomy (Hirvonen and Pyorala 1998). Hirvonen and Pyorala (1998) reported an increase in serum Hp in 55% of cows prior to and 2-3 days after operation of TRP or abomasal displacement. Schonfelder *et al.,* (2006) reported that no correlations existed between plasma Hp concentration and wound healing in cattle with surgically corrected torsio uteri intrapartum. A postoperative increase of Hp indicated a higher risk of secondary illnesses and complications (Schonfelder *et al*., 2006).

This investigation showed that there was no correlation between the concentration of serum and milk haptoglobin in each of the inflammatory diseases that had been studied. These results can be due to intramammary synthesis of Hp (Gronlund *et al*., 2003).

Statistically investigation of our data showed that most sensitivity and specificity of serum haptoglobin for mentioned inflammatory diseases are 98% and 100%, respectively. False positive and negative of this test were zero and 2%, respectively. Milk Hp sensitivity and specificity were 95% and 100%, respectively. False positive and negative of this test were zero and 5%, respectively (Table 2).

The results of the present study reveal that serum and milk Hp are useful indicators of various inflammatory conditions in cattle. The sensitivity of haptoglobin is more than hematological and clinical tests for diagnosis of these conditions. Hematological parameters are very valuable in different stages of inflammatory diseases and clinical tests diagnose the disease when it has been developed. Acute phase proteins (e.g haptoglobin) increase when a disease develops and decrease in the recovery stage and can diagnose the disease in early stages.

In conclusion, measuring serum and milk Hp can be help in inflammatory conditions diagnosis in cattle.They have the highest sensitivity and specificity in comparison with routine diagnosing tests.

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ارزیابی هاپتوگلوبین سرم و شیر در برخی بیماریهای التهابی گاو

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چکیده

این مطالعه بر مبنای ارزیابی هاپتوگلوبین سرم و شیر بعنوان شاخص التهاب در بیماریهای التهابی گاو طراحی شد. نمونه های خون از ورید دمی ۲۰ راس گاو به ظاهر سالم و ۱۰۰ راس گاو مبتلا به بیماریهای التهابی مختلف جمع آوری شد. تفاوتهای معنی داری در غلظت هاپتوگلوبین سرم و شیر میان گاوهای به ظاهر سالم و بیمار دیده شد ($p<0/05$). نتایج مطالعه حاضر نشان داد که هاپتوگلوبین سرم و شیر فاکتورهای حساسی برای تشخیص حالات التهابی در گاو می باشند.

واژه‌های کلیدی: هاپتوگلوبین، شیر، سرم، بیماریهای التهابی گاو.

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