Survey on relationship between acute phase proteins (serum amyloid A, milk amyloid A and serum haptoglobin) in inflammatory diseases of dairy cattle

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Received: November 21, 2012 Accepted: February 12, 2013

Abstract

The objective of this study was to evaluate the concentration of some acute phase proteins (serum amyloid A, milk amyloid A and serum haptoglobin) and alteration in hematological values in some inflammatory diseases in dairy cattle. Fifty-six Holstein dairy cows with inflammatory diseases were used in this study including: subclinical mastitis, hematological infections (theileriosis and anaplasmosis), metritis, pneumonia and displacement of abomasum (n= 8 each). Clinically healthy cows were selected as control group (n= 8). Blood samples were collected from jugular vein of both groups. Milk samples were taken from four quarters of each cow and mixed together and then one sample of pooled milk from both groups was taken. There were significant differences in mean concentrations of serum and milk amyloid A between clinically healthy cows and diseased cows and also, between abomasal displacement and other infectious diseases (p<0.05). There were also significant differences in haptoglobin concentrations between cattle suffering from subclinical mastitis, theileriosis and pneumonia compared to those with anaplasmosis, abomasal displacement and metritis (p<0.05). Our results indicated the application of serum haptoglobin, serum amyloid A and milk amyloid A measurements as indicators of inflammatory diseases in dairy cattle. In conclusion, the current study shows that haptoglobin, serum amyloid A, and milk amyloid A measurements, could be successfully applied in cattle as valuable indicators of inflammatory diseases.

Keywords: Acute phase protein, serum amyloid A, haptoglobin, inflammatory diseases

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Introduction

Detailed functions of the acute phase proteins (APPs) are not fully understood, however, it is suggested that they are mainly engaged in processes like opsonization and trapping of microorganisms and their products, binding of cellular remnants (Whicher and Westacott, 1992), complement activation, neutralization of enzymes, and scavenging of free radicals and hemoglobin (Niewold et al., 2003). In the recent studies concerning new indicators of mastitis, acute phase proteins are strongly implicated (Eckssall et al., 2001 Grönlund et al., 2003, Nielsen et al., 2004, Grönlund et al., 2005, O’Mahony et al., 2006, Ihnat et al., 2007, Akerstedt et al., 2007, Hiss et al., 2007). APPs are often determined in serum of animals to monitor their health (Kostro et al., 2001; Murata et al., 2004, Petersen et al., 2004). APPs can be valuable to the veterinarian in identification of cows with inflammatory diseases and the stages of disease can be better evaluated by monitoring more than one APP like serum amyloid A (SAA) and serum haptoglobin (Hp), so chronic conditions as well as acute ones should be evaluated and characterized by APP profiling (Karreman et al., 2000, Eckssall, 2004). Hp is an α2-globulin and it is one of the APPs, which increases in serum in acute inflammatory diseases. Measurement of APPs could be a useful tool for evaluation of health in calf herds (Ganheim et al., 2007). SAA is an apolipoprotein of high-density lipoprotein (Nakayama et al., 1993, Gruys et al., 1994; Husby et al., 1994). It is described to bind lipopolysaccharide, comparable to lipopolysaccharide binding protein (Schroedl et al., 2001). There are no published reports about the comparison of serum Hp, SAA and MAA concentrations in subclinical mastitis, theileriosis, anaplasmosis, pneumonia, metritis, and abomasal displacement. Therefore, the aim of this study was to evaluate these APPs as inflammatory indicators in various inflammatory diseases of cows.

Materials and methods

Fifty-six Holstein cows with various inflammatory diseases from three dairy farms located in Tabriz suburb were included in this research. Eight clinically healthy adult cattle were also selected randomly as control. The diseased cows used in this study included subclinical mastitis, parasitic infections (theileriosis and anaplasmosis), pneumonia, metritis and abomasal displacement (n=8 each). The presence of disease was assessed on the basis of clinical examination and laboratory findings. Diseased cows were thoroughly examined and blood samples were collected from jugular vein of both groups for hematology, clinical biochemistry and other relevant analysis. To determine serum Hp, SAA and MAA serum was separated by centrifugation at 750 g for 15 min. Serum samples were stored at -20 °C until analysis. SAA and MAA concentrations were measured by using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (Tridelta Development Pic, Co.Wicklow, Ireland) and Hp was measured on the basis of the preservation of the peroxidase activity of hemoglobin which is directly proportional to the amount of haptoglobin using a colorimetric commercial kit (Tridelta Development Plc, Co., Wicklow, Ireland). Statistical analysis was performed using SPSS software (Ver. 16, IBM Corporation, USA).

The data were checked for errors and compared with written reports; Outliers were rechecked during data processing to ensure that values were accurate. All variables were screened for normality by visual assessment of the distributions. All values were expressed as mean and standard error (mean±SE). Differences between groups were analyzed using one way ANOVA and Duncan's multiple comparison tests and considered as statistically significant when the p value was less than 0.05.

Results

The mean concentrations of SAA and MAA and their correlation in cows with
inflammatory diseases and clinically healthy cows are presented in Table 1. Statistical evaluations showed that there was no significant correlation between SAA and MAA in each of inflammatory diseases ($p<0.05$). There were significant differences in mean concentrations of SAA and MAA between clinically healthy cows and diseased cows and also, between abomasal displacement and other infectious diseases ($p<0.05$). Significant difference in Serum Hp concentrations was observed between clinically healthy cows and those with inflammatory diseases ($p<0.05$). There were also significant differences in Hp concentrations between cattle suffering from subclinical mastitis, theileriosis and pneumonia compared to those with anaplasmosis, abomasal displacement and metritis ($p<0.05$).

### Table 1. SAA and MAA concentration (mean ± SE) in cows with inflammatory diseases and clinically healthy cows.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Number</th>
<th>SAA (µg/ml)</th>
<th>MAA (µg/ml)</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subclinical mastitis</td>
<td></td>
<td>8</td>
<td>29± 2.1$^a$</td>
<td>15 ± 0.9$^a$</td>
<td>-0.03</td>
</tr>
<tr>
<td>Theileriosis</td>
<td></td>
<td>8</td>
<td>33± 3.9$^b$</td>
<td>29 ± 1.2$^b$</td>
<td>-0.0033</td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td></td>
<td>8</td>
<td>30± 4.3$^c$</td>
<td>17 ± 2.6$^c$</td>
<td>-0.003</td>
</tr>
<tr>
<td>Pneumonia</td>
<td></td>
<td>8</td>
<td>36± 2.6$^d$</td>
<td>28± 3.8$^d$</td>
<td>-0.0039</td>
</tr>
<tr>
<td>Metritis</td>
<td></td>
<td>8</td>
<td>41± 0.09$^e$</td>
<td>61 ± 1.1$^e$</td>
<td>0.008</td>
</tr>
<tr>
<td>Abomasal displacement</td>
<td></td>
<td>8</td>
<td>19± 4.9$^f$</td>
<td>8± 0.8$^f$</td>
<td>0.038</td>
</tr>
<tr>
<td>Clinically healthy cows</td>
<td></td>
<td>8</td>
<td>6± 0.5$^g$</td>
<td>0.5± 0.08$^g$</td>
<td>0.04$^*$</td>
</tr>
</tbody>
</table>

Means within a column with different superscript letters (a, b, c, d, e) significantly differ ($p<0.05$).

*Indicates $p<0.05$

### Discussion

Numerous studies have described the serum concentration of APPs in animals with various diseases, but only a few are devoted to their levels in milk from cows with inflammatory disease. Therefore, the present study was undertaken to determine the concentration of SAA, MAA and serum hptoglobin and assess their usefulness for the detection of common inflammatory diseases in cows. Quantification of APP concentration in serum can provide valuable diagnostic information in the detection, prognosis, and monitoring of diseases in several animal species. The inclusion of APPs measurements in health monitoring programs on a herd basis in livestock has been suggested, not only for the identification of individual animals with disease, but also as a mean of identifying animals with subclinical disease. Measurement of APPs, especially Hp and SAA, as indicators of health in herds has gained an increasing interest among researchers (Murata et al., 2004, Ganheim et al., 2007). Hp and SAA have been found to increase in the serum of cattle affected by many different diseases (Asemgeest et al.,1994, Horadagoda et al.,1994, Godson et al.,1996, Hirvonen et al.,1996, Smith et al.,1998, Heegard et al., 2000, Sheldon et al.,2001, Nielsen et al., 2004, Murata et al.2004, Grounland et al., 2005, Félix et al., 2008). SAA could be a useful diagnostic marker of early post-operative complications and also for evaluation of health in calves (Dabrowski et al.,2007, Ganheim et al., 2007). There is only one published report about the comparison of SAA and MAA concentrations in some infectious diseases (Nazifi et al., 2008a). In the present study the comparative measurement of SAA and MAA as well as serum Hp was conducted as inflammatory indicators in various inflammatory diseases of cattle in East Azerbaijan province located in northwest of Iran. The previous study has revealed that the concentration of SAA was higher in serum and milk of the cows with mastitis than in the cows with extramammary inflammatory conditions (Nielsen et al., 2004). However, in the present study the mean concentrations of SAA and
MAA were lower in serum and milk of those with subclinical mastitis and abomasal displacement than in the cows with other inflammatory diseases. The reason may be that the severity of these two diseases is lower than other inflammatory diseases. The mean concentration of serum Hp in our study was also at the minimum value in cows with abomasal displacement than the others. SAA concentrations below the detection limit were considered as a good indicator of healthy udder quarters (Gronlund et al., 2005). The results of the present investigation showed that there is no correlation between concentration of SAA and MAA in each of the inflammatory diseases under study. These results can be due to intramammary synthesis and secretion of SAA3 to colostrum and mastitis milk and has beneficial functions for the gut mucosa of the offspring (Eckersall et al., 2001, McDonald et al., 2001, Mack et al., 2003, Larson et al., 2003a,b, Larson et al., 2005). In diseased cattle, the lowest concentrations of Hp (0.075±0.018 mg/mL) were observed in cows with abomasal displacement (Table 2). In the present study, the concentration of SAA and Hp in cows infected with *Theileria annulata* was significantly higher than the clinically healthy cows (p<0.05). An effect of free hemoglobin in serum samples towards reduction of measured concentration of Hp has been found (Eckersall et al., 1999, Petersen et al., 2001). These data are different from our results in cattle with anaplasmosis and theileriosis, in which we observed increased serum Hp concentrations. In one study reported by Glass et al. (2003), following experimental infection with *Theileria annulata* in cattle, Hp appeared only in some of the animals, and generally at a low level. This shows that Hp should be used with caution as a marker of inflammation in Theileriosis. In the present study, the concentration of SAA and serum Hp in cows with pneumonia and metritis was significantly higher than clinically healthy cows (p<0.05). Alsemgeest et al. (1994) introduced Hp as a valuable marker for differentiating healthy cows from pneumonic ones. Godson et al. (1996) evaluated APPs in cows with respiratory infections and introduced Hp as a diagnostic factor. Berry et al. (2004) found that Hp concentrations were higher in calves treated by respiratory disease for multiple times compared with those never treated, or treated on a single occasion. Increasing in Hp and SAA amounts has been reported after intratracheal inoculation with *Manheimia hemolytica* (Horadagoda et al., 1994, Cheryk et al., 1998). In diseased cows subjected to our study (n=48), Hp levels were significantly higher (0.132±0.069 mg/mL) than those in healthy ones (0.02±0.14 mg/mL; p<0.05). Increased serum Hp concentration in cattle was found in various acute infections under field conditions (Skinner et al., 1991, Alsemgeest et al., 1994), mastitis (Hirvonen et al., 1999, Ohtsuka et al., 2001, Gronlund et al., 2003, Nielsen et al., 2004, Gronlund et al., 2005), and metritis (Smith et al., 1998). Our study showed that acute respiratory infections resulted in a significant increase of serum Hp levels. These results are in agreement with one previous study carried out by Nazifi et al. (2008b). Similar findings were demonstrated in cattle with experimental infection with *Pasteurella haemolytica* (Katoh & Nakagawa, 1999, Ganheim et al., 2003). In our study, subclinical mastitis, metritis, and abomasal displacement were also found to provoke significantly elevated Hp values.

In conclusion, the current study shows that Hp, SAA, and MAA measurements could be successfully applied in cattle as valuable indicators of inflammatory diseases. The studied parameter could be influenced by other disorders and significant changes in hemoglobin levels; so it should not be used independently but together with other blood laboratory indices with regard to better elucidation of the systemic status. However, APPs increase during the development of the disease and decrease in the recovery stage could be used for diagnosis of the disease in early stages.
Table 2. Serum Hp concentrations (mean ± SE) in cows with inflammatory diseases and clinically healthy cows.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Number</th>
<th>Serum Hp (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subclinical mastitis</td>
<td></td>
<td>8</td>
<td>0.15± 0.09a</td>
</tr>
<tr>
<td>Theileriosis</td>
<td></td>
<td>8</td>
<td>0.18± 0.08a</td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td></td>
<td>8</td>
<td>0.08± 0.12b</td>
</tr>
<tr>
<td>Pneumonia</td>
<td></td>
<td>8</td>
<td>0.22± 0.03a</td>
</tr>
<tr>
<td>Metritis</td>
<td></td>
<td>8</td>
<td>0.09± 0.08b</td>
</tr>
<tr>
<td>Abomasal displacement</td>
<td></td>
<td>8</td>
<td>0.075 ± 0.018b</td>
</tr>
<tr>
<td>Clinically healthy cows</td>
<td></td>
<td>8</td>
<td>0.02 ± 0.14c</td>
</tr>
</tbody>
</table>

Means within a column with different superscript letters (a, b, c, d) significantly different (p<0.05).

Acknowledgements

This research was supported by a grant from University of Tabriz.

References


Katoh, N. and Nakagawa, H. (1999) Detection of haptoglobin in the high-density lipoprotein fractions from sera of calves with experimental pneumonia and cows with naturally occurring fatty liver. *Journal of Veterinary Medical
Survey on relationship between acute phase proteins


بررسی ارتباط بین پروتئین‌های فاز حاد انتخابی (آمیلولید سرمی و شیری A و هایپوگلوبرین) در بیماری‌های انتخابی گاو‌های شیری

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دریافت مقاله: 10/9/1391 نهایی پذیرش: 24/11/1391

چکیده

هدف از مطالعه حاضر بررسی علائم پروتئین‌های فاز حاد انتخابی (آمیلولید سرمی و شیری A و هایپوگلوبرین) و نیز تغییرات بوجود آمده در پارامترهای خون‌شناختی در بیماری‌های شیری در گاوگان شیری زنده‌هایی با گروه بیماری A می‌باشد. در آزمایشاتی که آزمایشگاهی، فیزیولوژی، تربیت و نمونه‌برداری و جابجایی بیماران به‌طور مداوم در بین مطالعه انتخاب شدند (از هر بیماری A راس گاو 1 راس قانونی)، تعداد 8 راس گاو نیز به عنوان گروه کنترل در نظر گرفته شدند. سپس اقدام به خونگیری از ویدر گردنه گاوگان شیری می‌گردید. نمونه‌های شیر از چهار کاربری پیشگیری از ویدر گردنه گاوگان شیری می‌گردید.

به‌طور جدایی با هم مخلوط شدن و پس از 10 دقیقه اتصال به انجمانی روز نمونه‌ای به‌طور دوباره، نتایج نشان داد که در رابطه با علائم پروتئین‌های فاز حاد انتخابی (آمیلولید سرمی و شیری A و هایپوگلوبرین) در بیماری‌های شیری در گاوگان شیری زنده‌هایی با گروه شیری B می‌باشد.

می‌تواند و پیشنهاد می‌کند که این نشان‌گرفته‌ها می‌تواند در شناسایی بیماری‌های شیری موثر و موثر شود.

واژگان کلیدی: پروتئین فاز حاد، سرم آمیلولید A، هایپوگلوبرین، بیماری‌های شیری