Evaluation of Hemogram, Haptoglobine and Clotting Factors Indices in Cattle Affected with Acute and Chronic Peritonitis

Kamal M. Alsaad
Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract: The objective of the present research was to evaluate hemogram, haptoglobin and clotting factors indices in cattle affected with acute and chronic peritonitis. About (88) local cattle breed, 2-8 years old of both sexes were used in this study among these animals (23) were affected with Traumatic Retropertionitis (TRP), (18) animals suffering from acute ruminitis due to ruminal overload (21) animals were suffering from sever tympany that needed trocarization and (16) show sings of chronic peritonitis as a sequel of ruminatomy. Ten clinically normal local cattle breed were served as control group. Results indicated that clinically diseased cattle exhibited different clinical sings which were varying in degree with the severity and extent of the peritonitis. Statistically significant increase (p<0.05) were encountered in PCV and TLC in cattle exhibited acute peritonitis. Cattle affected with acute peritonitis show neutrophilia and lymphocytopenia. Eosinophilia were indicated in cattle affected with acute ruminitis and monocytes were increased significantly in animals affected with chronic peritonitis. Results were also showed significant decrease (p<0.05) in the mean values of total platelets count in cattle affected with acute peritonitis compared with normal control and animals affected with chronic peritonitis. Values of mean platelets volume, platelets distribution width, prothrombin time and partial thromboplastine time were increased significantly (p<0.05) in animals affected with acute peritonitis. Cloting time was only increased significantly (p<0.05) in cattle affected with TRP. Serum values of fibrinogen and haptoglobin in control and diseased cattle indicated significant increase of fibrinogen (p<0.05) in all diseased cattle whereas haptoglobin increased significantly (p<0.05) in animals affected with acute peritonitis.

Key words: Cattle, peritonitis, hemogram, haptoglobine, clotting factors indices, lymphocytopenia

INTRODUCTION

Inflammation of the peritoneum is accompanied by abdominal pain, fever, toxemia and a reduction in the amount of feces (Radostits et al., 2007). Peritonitis may occur as a primary disease or secondarily as part of an etiologically specific disease and symptoms are varying in degree with the severity and extent of the peritonitis (Ward and Ducharme, 1994). As a primary disease it results most commonly from injury of the serosal surfaces of the alimentary tract within the abdomen allowing gastro-intestinal contents to enter the peritoneal cavity (Smith, 1996). Less commonly there is perforation of the abdominal wall from the exterior from traumatic injury, perforation of the reproductive tract or the introduction of pathogens or irritating substances as result of injections into the peritoneal sac (Laakakos et al., 2001). Moreover, the effect of grain-induced acute ruminal acidosis in dairy cows promotes lysis of gram-negative bacteria that stimulate the inflammatory response (Gozho et al., 2005). Peritonitis in cattle were mentioned by Trent and Bailey (1986) and Holmdah and Ivarsson (1999) which were mostly concerning with perforated foreign bodies, ruminitis due to carbohydrate engorgement (Gozho et al., 2005) trocarization for tympanic rumen and as a sequel of ruminatomy (Alsaad et al., 2006).

Hemostasis is a complex process with positive and negative regulators for formation and degradation of fibrin (Bick, 2003). Imbalanced regulation may lead to hyper coagulation (Thrombosis), hypo-coagulation (Hemorrhage) or to both of them (Relar et al., 2005). Haemostatic abnormalities such as thrombocytopenia, prolonged clotting times, prothrombin time, activated partial thromboplastine time, increased fibrinogen degradation products activity have been reported in cattle with peritonitis (Gokee et al., 2005). Primary hemostasis can be eva-luated by determination of platelet numbers, platelet volume and platelet distribution width, more over secondary hemostasis can be evaluated by the activated partial thrombo-plastin for intrinsic and common pathway abnormalities, fibrinogen quantification (common pathway abnormalities and proth-rombin time for extrinsic and common pathway abnormalities (Smith, 1996). The acute phase proteins is a series of complex physiological events
occurring in the host after a tissue injury, inflammation or an infection (Petersen et al., 2004), it is a serum protein which increases in concentration during the acute phase response and play a role in the defense response of the host and can provide information on the progression of the inflammatory reaction (Horadagoda et al., 1999). The response occurs in all animals but in different species the response of individual proteins can be significantly different (Skinner et al., 1991).

In cattle and other ruminants, Haptoglobin (α-2 globulin produced in the liver) has been one of the acute phase proteins most commonly monitored as a marker of inflammation and has been found to be effective in detecting serious inflammatory conditions in cows such as traumatic reticuloenteritis (Hirvonen and Pyorala, 1998), bacterial infections (Ganheim et al., 2007) and increased concentration has been detected in bovine serum during the peripartum period (Humblet et al., 2006) in cows with fatty livers (Nakagawa et al., 1997).

Haptoglobin, binds free hemoglobin released from erythrocytes with high affinity, thereby inhibits its oxidative activity (Wassell, 2000). The main function of haptoglobin is binding free haemoglobin and the hemoglobin binding property has a bacteriostatic effect as it limits free iron available for bacteria (Eaton et al., 1982). The haptoglobin–hemoglobin complex will then be removed by the reticuloendothelial system (mostly the spleen) (Rossbacher et al., 1999).

In clinical settings, the haptoglobin assay is used to screen for and monitor intravascular hemolytic anemia and inflammatory disease behavior in intra-vascular hemolysis free hemoglobin will be released into circulation and hence haptoglobin will bind the hemoglobin and this will causes a decline in haptoglobin levels and conversely in extravascular hemolysis (Makimura and Suzuki, 1982). The reticuloendothelial system especially splenic monocytes, phagocytose the erythrocytes and hemoglobin is not released into circulation and hence haptoglobin levels are normal (Nazifi et al., 2008).

The haptoglobin concentration in the blood plasma of cattle tends to rise to more than 10 mg/100 mL in the presence of acute inflammation anywhere and might become 40 mg/100 mL and above in the presence of traumatic reticuloenteritis (Skinner et al., 1991; Hirvonen and Pyorala, 1998).

Studies of peritonitis related with evaluation of clotting factors indices and haptoglobin in cattle are very scarce and little information had been provided, therefore the study were amide to evaluation of hematological picture, haptoglobin and clotting factors indices in acute and chronic peritonitis in cattle in Mosul (Mosul-Iraq).

MATERIALS AND METHODS

Animals and clinical examination: The study was carried out in Mosul province (Mosul-Iraq) on (88) local cattle breed, 2-8 years old of both sexes. Diseased animals were brought and examined at the Consultant veterinary hospital/College of Veterinary Medicine/University of Mosul.

Careful clinical examination had been carried out in all animals. A complete history was obtained upon presentation in the clinic and emphasis was placed on clinical signs observed, cause and duration of the presenting complaint. Clinical diagnosis of acute and chronic peritonitis were included diseased cattle suffering from acute Traumatic Reticulomenteritis (TRP) (23), acute ruminitis due to ruminal overload (18), sever tympany that needed trocarization (21) and chronic peritonitis as a sequel of ruminiotomy (Post-surgical adhesions) (16). Ten clinically normal local cattle breed were served as control group.

Metallic foreign bodies were detected using metal detector in all the cases of TRP, those animals were treated surgically through ruminiotomy using Weingarts apparatus. Ruminatomy was also done in cattle suffering from ruminitis due to acute ruminal overload and animals with trocarization.

Blood samples and hematology: By jugular vein-puncture, 10 mL of blood were drained from each animal from these (2.5) mL of blood mixed with EDTA used to determine Total erythrocyte count (TEC), Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Platelets count (Plt), Mean Platelets Volume (MPV), Platelets Distribution Width (PDW), total and differential leukocytes count, (Automatic Full Digital cell Counter, Beckman, USA) (Meyer and Harvey, 1998). Another (2.5) mL of blood mixed with Trisodium citrate (used plasma) were used to determine Fibrinogen time (Ft) and Activated partial thromboplastine time (Aptt) (Coles, 1986). Clotting Time (CT) was also estimated according to (Bush, 1975).

Serum samples: Blood serum samples were tested spectrophoto-metrically for fibrinogen using available kits (Biolabo, France).

Estimation of haptoglobin: Haptoglobin (Bovine-Haptoglobin-ELISA) were estimated according to (Hiss et al., 2004) microtiter plates were coated with purified hHp (5 ng in100 mL of 50 mM NaHCO3, pH 9.6) at 4°C for 20 h. After blocking with 300 μL of 2.5% casein in 0.05 M NaCl, pH 7.4 at room temperature for 1.5 h, the plates were stored at -20°C. Prior to use, the plates were
washed 5 times. To each well, 50 µL of test sera (dilution 1/100 in healthy cows or 1/1000 in diseased cows) was added in duplicate. Calibration curves were created using 50 µL of purified Hp at dilutions from 0-0.10 µg mL⁻¹ in duplicate. An amount of 50 µL of the antisera (dilution 1/50,000) was then added and incubated for 2 h at room temperature. After 3 washes, 100 µL of the second antibody conjugated to peroxidase (1/20,000 dilution) was added and incubated for 30 min. After 5 washes, the wells were filled with 150 µL of a freshly prepared substrate solution containing 0.05 M citric acid, 0.055 M Na₂HPO₄, 0.05% urea hydrogen peroxide, 2% ProClin 150 and 2% of a tetramethylbenzidine solution (12.5 mg mL⁻¹ dimethylsulfoxide). The reaction was stopped after 30 min with 50 µL of 1 M Oxalic acid and the Optical Density (OD) was determined at 450 nm with a microtiter plate reader. The Hp concentrations in unknown samples were then calculated from the calibration curve.

**Statistical analysis:** The significance of variations in the various values of diseased cattle and those of normal control animals were analyzed statistically using SPSS (Leech et al., 2007).

**RESULTS AND DISCUSSION**

Clinically diseased cattle exhibited different clinical signs which were varying in degree with the severity and extent of the peritonitis. Statistically significant increase (p<0.05) were encountered in PCV and TLC in cattle exhibited acute peritonitis. Cattle affected with acute peritonitis show neutrophia and lymphocytopenia. Moreover eosinophilia were indicated in cattle affected with acute ruminitis and monocytes were increase significantly in animals affected with chronic peritonitis Table 1.

Changes of blood clotting indices were also noticed in diseased cattle compared with normal control animals and the results showed significant decrease (p<0.05) in the mean values of total platelets count in cattle affected with acute peritonitis compared with normal control and animals affected with chronic peritonitis on the other hand values of mean platelets volume, platelets distribution width, prothrombin time and partial thromboplastin time were increased significantly (p<0.05) in animals affected with acute peritonitis. Values of clotting time was only increased significantly (p<0.05) in cattle affected with TRP Table 2.

Serum values of fibrinogen and habto-globin in control and diseased cattle are shown in Table 3. Fibrinogen were significantly increase (p<0.05) in both animals suffering the acute and chronic peritonitis whereas haptoglobin were increased significantly (p<0.05) only in cattle affected with acute peritonitis (Table 3).

Inflammation of the peritoneum in cattle may be acute or chronic, local or diffuse and most commonly is secondary to contamination of the peritoneal cavity. It is often accompanied by different clinical signs, abdominal pain, fever, toxinemia and reduced fecal output are the most prominent sings (Radostis et al., 2007) moreover peritonitis is a dynamic process that started as acute reaction and some cases can evolve into amoce chronic

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**Table 1:** Hemogram, total and differential leukocytes count of normal control and diseased cattle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TRP</th>
<th>Acute peritonitis</th>
<th>Tocolarization</th>
<th>Chronic peritonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRBC×10⁶</td>
<td>7.66±1.25⁴</td>
<td>7.24±1.77⁴</td>
<td>6.97±1.92⁴</td>
<td>7.81±1.89⁴</td>
<td>6.88±2.12⁴</td>
</tr>
<tr>
<td>HB g/100 mL</td>
<td>12.21±1.11⁴</td>
<td>11.92±1.45⁴</td>
<td>12.15±2.46⁴</td>
<td>12.79±2.15⁴</td>
<td>12.89±1.67⁴</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.32±3.56⁴</td>
<td>40.12±5.60⁴</td>
<td>44.21±4.22⁴</td>
<td>41.30±2.89⁴</td>
<td>34.21±3.87⁴</td>
</tr>
<tr>
<td>Nutrophils (%)</td>
<td>44.02±1.22⁴</td>
<td>56.45±2.45⁴</td>
<td>51.21±1.42⁴</td>
<td>57.11±3.44⁴</td>
<td>43.15±2.78⁴</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>48.11±1.44⁴</td>
<td>35.89±2.70⁴</td>
<td>36.66±2.77⁴</td>
<td>36.14±3.55⁴</td>
<td>44.22±2.60⁴</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.11±1.0⁴</td>
<td>3.01±1.2⁴</td>
<td>7.12±2.45⁴</td>
<td>2.46±12.0⁴</td>
<td>2.42±24.0⁴</td>
</tr>
<tr>
<td>Monoctyes (%)</td>
<td>4.12±1.42⁴</td>
<td>3.65±1.54⁴</td>
<td>3.24±1.78⁴</td>
<td>2.77±82.0⁴</td>
<td>8.45±2.67⁴</td>
</tr>
<tr>
<td>Basoaltes (%)</td>
<td>1.65±0.22⁴</td>
<td>1.11±0.67⁴</td>
<td>1.77±1.55⁴</td>
<td>1.52±1.21⁴</td>
<td>1.76±0.42⁴</td>
</tr>
</tbody>
</table>

Values are mean±standard error of mean; Values with different letters mean the presence of significance differences (p<0.05)

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**Table 2:** Indices of clotting factors in diseased cattle and control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TRP</th>
<th>Acute peritonitis</th>
<th>Tocolarization</th>
<th>Chronic peritonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL×10⁹</td>
<td>411.22±4.21⁴</td>
<td>278.67±66.87⁴</td>
<td>307.54±71.65⁴</td>
<td>320.54±45.60⁴</td>
<td>399.76±90.21⁴</td>
</tr>
<tr>
<td>PDW/FL</td>
<td>17.42±3.42⁴</td>
<td>21.45±1.77⁴</td>
<td>19.22±2.81⁴</td>
<td>20.45±2.55⁴</td>
<td>17.34±3.29⁴</td>
</tr>
<tr>
<td>Cr/min</td>
<td>3.55±1.22⁴</td>
<td>6.89±2.56⁴</td>
<td>3.92±2.05⁴</td>
<td>3.83±1.52⁴</td>
<td>3.71±1.62⁴</td>
</tr>
<tr>
<td>Pct/sec</td>
<td>12.54±2.55⁴</td>
<td>20.44±4.76⁴</td>
<td>17.65±5.42⁴</td>
<td>19.78±4.17⁴</td>
<td>13.22±2.77⁴</td>
</tr>
<tr>
<td>Aplt/sec</td>
<td>52.54±3.29⁴</td>
<td>61.67±5.62⁴</td>
<td>50.88±4.76⁴</td>
<td>60.72±5.54⁴</td>
<td>51.76±3.76⁴</td>
</tr>
</tbody>
</table>

Values are mean±standard error of mean; Values with different letters mean the presence of significance differences (p<0.05)
peritonitis (Alsaad et al., 2006). In current study significant different were encountered in total and differential leukocytes in diseased cattle, different reports indicated leukocytosis in acute peritonitis especially those related to TRP (Yoshida, 1986; Ward and Ducharme, 1994; Karademire and Atalan, 2003). Karademire (2005) refer to the existence relationship between the interperitoneal exudates and the level of total and differential leukocytes count. Moreover, Radostitis et al. (2007) mention that in acute local peritonitis a neutrophilia and a regenerative left shift are common and both the neutrophilia and the left shift will be increased on the first day and will last for up to 3 days when in uncomplicated cases the count begins to return to normal. Furthermore, in chronic cases the levels do not return completely to normal for several days or longer periods and there is usually a moderate leukocytosis, neutrophilia and a monocytosis. On the other hand (Ismail et al., 2007) indicated leucopenia with degenerative left shift in acute diffuse peritonitis which suggests an unfavorable prognosis.

Since the treatment of most cases of acute peritonitis is surgical especially TRP, the coagulation status of the cattle should be known before surgery to prevent the complication of possible haemostatic dysfunction (Holmdah and Ivanstrass, 1999).

Coagulation profiles change on the basis of severity of peritonitis in cattle were reported (Gokce et al., 2005). Peritonitis in cattle very often induces changes in the coagulation system which may lead to the development of disseminated intravascular coagulation (Bick, 2003). The most common coagulopathy in cattle with peritonitis is a hypercoagulable state associated with disseminated intra-vascular coagulation and the intensity of this coagulopathy depends on the severity and duration of the inflammatory process (Gokce et al., 2005). In current study results showed decrease values of total platelet count as well as increase platelet volume and its distribution width beside increase values of clotting time, prothrombin time and activated partial thromboplastine time specially in animals suffering the acute type, these results were also mentioned by Smith (1996) and Holmdah and Ivanstrass (1999) as thrombocytopenia and increase clotting time of the blood were reflected the petechial hemorrhages which might be seen on mucus membranes of diseased animals which may attributed to the release of endogenous mediators such as platelet activating factor in inflammatory disorders (Rebar et al., 2005). Any bleeding tendency were occur in the body regions must followed by the process of clotting and there are several factors play an important role in this process such as vascular factors (which is characterized by a rapid response is narrowing and contraction of the vessel causing decreased pumping mechanism of the blood) and are directly related to the integrity of blood vessels, more over the numbers of blood platelets and its activity have significant role in the process of coagulation in which the accumulation of blood platelets (Platelets aggregates) and then its adherence within the vessel wall of a blood causing Platelets thrombus (Temporary plug) (Smith, 1996). Depression of platelets number may also occur due to depression of bone marrow activity, spleenomegaly and platelets sequestration (Rebar et al., 2005). The clotting phase (Coagulation) considered as the final stages of the clotting mechanism which are activated by such factors as Hageman factor (XII), Plasma thromboplastin antecedent factor (XI) and Thromboplastin component (IX) in the form of cascades which is responsible for transform prothrombin to thrombin and the fibrinogen to fibrin, resulting in deposition of Fibrin clot within the blood vessels, (calcium plays a major role at this stage especially in the process of shrinkage of these clots and that might lead to a decrease of its level in the blood) (Smith, 1996) which will lead to disorders of haemostatic mechanism enhanced by Disseminating intra-vascular coagulopathy causing micro thrombosis and infarction of special organs (Bick, 2003). It has been reported that prolonged PT and PTT was the most frequently observed abnormality in the coagulation profile and was more likely to be prolonged in cattle with acute peritonitis that did not survive (Gokce et al., 2005).

Inflammatory response to tissue injury is a mechanism by which the host sets up defense against further injury and starts the healing process (Laakakos et al., 2001; Nazifi et al., 2008). The early and immediate set of reactions is known as Acute Phase Response (APR) (Ganheim et al., 2007). One of the predominant features of APR is changes in the concentrations of a number of plasma proteins associated with the host response (Skinner et al., 1991). These changes are mainly the result of alterations in acute phase proteins synthesis in the liver (Humblet et al., 2006). Haptoglobin has been one of the acute phase proteins

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Acute peritonitis TRP</th>
<th>Acute peritonitis</th>
<th>Toxication</th>
<th>Chronic peritonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen mg/100 mL</td>
<td>416.30±28.59</td>
<td>590.14±42.12</td>
<td>598.17±22.67</td>
<td>-498.21±42.78</td>
<td>650.45±20.81</td>
</tr>
<tr>
<td>Haptoglobin mg/100 mL</td>
<td>0.22±4.700</td>
<td>1.28±0.420</td>
<td>0.71±0.250</td>
<td>0.86±31.09</td>
<td>0.31±0.620</td>
</tr>
</tbody>
</table>
most commonly monitored as a marker of inflammation in cattle (Makimura and Suzuki, 1982; Gray et al., 2009). The APR process is initiated at the site of injury leading to release of soluble mediators that mobilize the defense response of the whole organism, the cause of the injury can be infective, traumatic, immunological, neoplastic or other (Eckersall and Conner, 1988). The APR is thus part of the non-specific innate immune response and its components are relatively consistent despite the large variety of conditions that induce it (Petersen et al., 2004). The function of the APR is to prevent tissue damage and initiation of APR most commonly starts by the release of inflammatory mediators from tissue macrophages or blood monocyte cells that gather at the site of damage, these inflammatory mediators set off both the local and systemic inflammatory processes (Rossbacher et al., 1999). As a result, the APR is expressed by such clinical, systemic inflammatory signs as fever, inappetence and depression which are reflections of multiple endocrinalogical, haematological, immuno-logical, metabolic and neurological changes in the diseased animal (Eckersall and Conner, 1988).

The main function of haptoglobin is binding free haemoglobin and the haemoglobin binding property has a bacteriostatic effect as it limits free iron available for bacteria (Eaton et al., 1982; Wassell, 2000). Haptoglobin also has numerous other functions related to the host defense response in infection and inflammation for example stimulation of angiogenesis and modulation of granulocyte activity (Dobryszyc, 1997). The inhibitory effect of haptoglobin on granulocyte activity has been suggested to be beneficial in acute inflammation by reducing the late inflammatory response which can be harmful to the host (Rossbacher et al., 1999). In current study results showed increase values of haptoglobin in cattle affected with acute peritonitis, same results were also mentioned by Eckersall and Conner (1988), Hirvonen and Pyorala (1998) and Nazifi et al. (2008). The determination of haptoglobin in acute peritonitis in cattle are a very diagnostic tool and the levels in serum rise quickly following acute tissue damage within 24-48 h and also fall very rapidly once the stimulus is removed (Nazifi et al., 2008). Haptoglobin has been found to be effective in detecting serious inflammatory conditions in cows such as traumatic reticuloperitonitis (Panndorf et al., 1976; Hirvonen and Pyorala, 1998).

In current study, increase fibrinogen level were indicated. Fibrinogen is a constitutive plasma protein that conceducers as an acute phase protein in most species including cattle (Petersen et al., 2004). Determination of the fibrinogen concentration was found to be particularly useful in detecting inflammatory diseases (Van Wuijkhuise-Sjouke, 1984). It increases in various inflammatory diseases of cattle such as peritonitis, endocarditis, pericarditis, pneumonia, mastitis, enteritis and nephritis and the most consistent changes have been reported in peritonitis and pericarditis (McSherry et al., 1970; Sutton and Hobman, 1975; Jain, 1993). Hirvonen and Pyorala (1998) described fibrinogen to be a good marker of traumatic reticuloperitonitis. Fibrinogen, together with haptoglobin is probably the acute phase protein most commonly used as a marker of host inflammatory response in research of cattle (Hirvonen et al., 1999). Fibrinogen factor I of the coagulation system is the circulating precursor of fibrin, this plasma protein plays an important role in haemostasis and thrombosis by its interaction with thrombin, factor XIII (Fibrin stabilizing factor), plasminogen, glycoprotein and endothelial cells (Jain, 1993).

CONCLUSION

Peritonitis were affected cattle and exhibited different clinical signs, a significant changes were noticed between diseased and control animals in hematological and some biochemical values with differences indicated in blood clotting indices.

ACKNOWLEDGEMENT

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REFERENCES


