

Investigation on Hepatic Insufficiency in Serum-Producing Horses and Prognostic Importance of Some Clinical and Biochemical Parameters

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Abstract: The objectives of this study were to evaluate possible hepatic insufficiency in serum-producing horses and to determine the possible prognostic value of clinical and biochemical parameters. Thirty horses of different species and of different sexualities were used. The control group comprised 10 horses that were not previously used for serum production. In order to compare the hepatic insufficiency, 20 horses, which were used for serum production, were divided into 2 groups according to their research time; Group I contained 12 horses, which were used for serum production for 1-2 years and Group II comprised 8 horses, which were used for serum production for 3-13 years. The management and feeding conditions were same for all groups. Clinical symptoms and 6 biochemical parameters that were related to hepatic insufficiency were evaluated. As a result, some clinical and biochemical findings, which might show different stages of hepatic insufficiency, were detected in a total of 15 horses (9 horses in Group I and 6 horses in Group II). Our findings showed that these hepatic insufficiency symptoms were not dependent on the usage duration for serum production. It was concluded that serum-producing horses must be monitored and precautions taken for prevention of hepatic insufficiency. Also, horses diagnosed for hepatic insufficiency must be removed from serum-producing process to avoid their poor prognosis.

Key words: Hepatic insufficiency, serum-producing horses, clinical signs, biochemical parameters, prognose

INTRODUCTION

Hepatic insufficiency or failure refers to the inability of the liver to perform its normal functions properly (Schall, 1976; Turgut and Ok, 1997). Serum hepatitis, aflatoxicosis, chloral hydrocarbons, pyrolizidine alcoholic toxicosis and hepatic metastases were reported as factors contributing to hepatic insufficiency in horses (Mendel *et al.*, 1988; Guglick *et al.*, 1995; West, 1989). Serum hepatitis in the horse is characterized by acute hepatic central lobular necrosis and it has been associated with the administration of biological products of equine origin (Messer and Johnson, 1994; Guglick *et al.*, 1995). Guglick *et al.* (1995) reported that development of subclinical or clinical serum hepatitis associated with tetanus antitoxin administration in adult horses and foals. Serum producing horses may die suddenly as a result of hepatic insufficiency without any accompanying clinical manifestations depend on used in serum production for several years (Abdelkader *et al.*, 1991).

The clinical signs of hepatic insufficiency may include hepatic encephalopathy, icterus, weight loss, hepatogenic photosensitization, diarrhoea, abdominal pain, hemorrhagic diathesis, ascites, steatorrhea, pruritis, endotoxic shock, polydipsia and haemolysis. The appearance of specific clinical signs of hepatic disease often reflects the type of altered function (Milne, 1993; Barton and Morris, 1998).

Clinical findings and laboratory analysis are used for the diagnosis. Bilirubin, bile acid, total serum protein, blood ammonia, blood glucose concentration for evaluation of carbohydrate metabolism and blood triglyceride concentration for evaluation of lipid metabolism and liver enzymes are often investigated for hepatic insufficiency (Schall, 1976; Barton and Morris, 1998).

The prognosis for hepatic insufficiency in horses depends upon the severity and type of the underlying disease. Patients with severe hepatic fibrosis and chronic liver disease have a poor prognosis

(Barton and Morris, 1998). Indicators of poor diagnosis in liver disease include greatly elevated Gama-Glutamyl Transferase (GGT) and Alkaline Phosphates (ALP) with a normal or Decreased Sorbitol Dehydrogenase (SDH) or Lactate Dehydrogenase (LDH) (Pearson, 1996). Severe pyrolizidine alkaloid toxicosis causes a grave prognosis (Bauer *et al.*, 1988; Mendel *et al.*, 1988; Pearson, 1996). Also, prognosis is poor in viral hepatitis (equine herpes virus infections, equine infection anaemia and equine viral arteritis) (Studdert, 1996). Serum hepatitis should be considered an uncommon but potentially fatal risk factor (Messer and Johnson, 1994). Prognosis of cholelithiasis depends on the extent of periportal fibrosis, severity of clinical signs and number and location of choleliths (Roussel *et al.*, 1984; Johnston *et al.*, 1989). Prognosis of the horses is found to be generally poor in toxicosis. If the patient has good appetite and do not produce any more toxic material, it may recover (Mendel *et al.*, 1988; Pearson, 1996).

The aim of this study was to evaluate hepatic insufficiency related to serum-producing applications and duration in hyperimmunized horses.

MATERIALS AND METHODS

Animals: Twenty horses, which were from different species and different genders, 5-25 years old and used for serum-production for tetanus in The Ministry of Health of Turkey, Refik Saydam Hygiene Institute Serum Producing Farm for 1-13 years, were used in the study. The animals were feed hay, concentrated dry food and grass. The horses usually were turned out in summertime. Ten horses, which were not previously used for serum production, in the same farm, were used as the control group. The horses used in the experimental groups were divided into 2 groups according to usage duration; Group I comprised 12 horses, which were used for serum production for 1-3 years and Group II comprised 8 horses, which were used for serum production for 3-13 years. The horses were numbered 1-12 for Group I, 13-20 for Group II and 21-30 for the Control Group (Table 1). Clinical examination was performed and a single serum sample was collected from the animals. The study was performed between 2 hyperimmunization stages.

Serum-producing process: The horses, which were chosen for serum production, were placed in quarantine and investigations were made for zoonosis and other infections. The healthy horses were included in the

Table 1: Used animals in the study

Groups	n	Horse No.	Age (years)	Duration of serum producing (years)
I	12	1-12	5-22 (Mean: 13.4)	1-2
II	8	13-20	8-25 (Mean: 16.6)	3-13
Control	10	21-30	8-21 (Mean: 12.4)	-

serum-producing programme. The animals were subjected to serum production for tetanus in 2 stages: preimmunization and hyperimmunization.

Preimmunization stage: In this stage, inactivated tetanus toxin with 0.4% formalin (toxoid, main toxin) was administered and a total of 6 intramuscular injections were given. Mean toxin count was increased from 10-100 mL gradually. Aluminum potassium sulfide 0.4% was added into main toxin as the adjuvant before the injections. The horses were rested approximately one month.

Hyperimmunization stage: In this stage, preimmunized horses were rendered hyperimmunized by administering the tetanus toxin directly. The application constituted a total of 10 toxin injections and toxin count was gradually redounded from 10-100 mL.

Antibody titres in the horses' blood were determined after the hyperimmunization stage. Blood (approximately 3 L) was collected from the horses that had high antibody titer (min 400 IU) intervals for 3-4 days.

If these horses were later included in the serum-producing process, only hyperimmunization stage is exposed. These horses were hyperimmunized by directly injecting toxin and toxin count is gradually redounded from 50-100 mL. Aluminum potassium sulfide 0.4% was added to the toxin as the adjuvant before all the injections.

Clinical examinations: Individual information such as protocol number, race, age, sex, usage duration of all the horses and clinical findings: skin (photosensitization), mucous membranes (normal, hyperemic and icteric), findings on central nervous system (depression, sensitivity, ataxia, stretching in the face and lips, leaning the head on a barrier), appetite (+/-), weight loss (+/-), abdominal pain (+/-), characteristics of defecation (stool consistency, normal/moderate/ diarrhoea), body temperature (normal/increase/decrease), pulse (normal/increase/decrease) and respiratory (normal/increase/decrease) rates were recorded in the clinical examination protocols.

Biochemical analysis: Blood samples were collected from jugular vein into empty tubes for biochemical analysis. Serum was isolated by centrifugation (5 min, 3000 g). Serum GGT, ALP, Aspartate Aminotransferase (AST)

enzyme activities and total cholesterol, total bilirubin, Blood Urea Nitrogen (BUN) concentrations were analyzed using an autoanalyzer (Falcor 350, Menarini Diagnostics, United Kingdom). The analysis was performed using the commercial kits (Cypress-diagnostic, Belgium) according to the manufacturer's instructions.

Statistical analysis: One-way ANOVA was used for comparison of the obtained data. Differences were considered as significant when $p < 0.05$ (Sumbuloglu and Sumbuloglu, 1994).

RESULTS

Clinical findings: Mean body temperature, pulse rate and respiratory rate of control and experimental groups are shown in Table 2. Body temperature, pulse rate and respiratory rate of control groups were detected within physiological limits. No significant difference was found in the average pulse rates between the groups ($p > 0.05$). Average body temperature and respiratory rate of Group I were different from controls and Group II ($p < 0.05$).

Body temperatures were above the physiological limit in 11 horses (8 horses (66.6%) in Group I and 3 horses (37.5%) in Group II). Also, increased pulse rate was detected in 1 horse in Group I (8.3%) and increased respiratory rate was detected in 4 horses in Group II (33.3%). Photosensitization was not observed in any animals. Sclera was found to be slightly icteric in 4 horses in Group I (33.3%) and in 2 horses in Group II (25%) and moderately icteric in 3 horses in Group I (25%) and in 1 horse in Group II (12.5%). Symptoms of central nervous system based on hepatic encephalopathy were not found in any animals. Due to tooth problem, lack of appetite was detected in 1 horse in Group II (12.5%) and prominent loss in weight was observed in 2 animals in Group I (16.6%). Defecations of all horses were normal.

Biochemical findings: Biochemical findings are shown in Table 3. BUN, total cholesterol, ALP, AST, GGT and total bilirubin concentrations of all animals of the control group were determined within physiological limits.

Serum urea nitrogen and total bilirubin values were determined within physiological limits in the experiment groups. Decreased total cholesterol levels were detected in 2 horses in Group II (25%); increased ALP levels were detected in 8 horses in Group I (66.6%) and in 5 horses in Group II (62.5%); decreased AST levels were detected in 3 horses in Group I (25%) and in 5 horses in Group II (62.5%); increased GGT levels were detected in 5 horses in Group I (41.6%) and in 4 horses in Group II (50%).

BUN levels of the experiment groups were found to be lower than the control group. Average BUN levels of Group I were found to be lower than Group II. A statistically important difference in BUN levels was determined between Group I and the control group ($p < 0.05$), but statistical importance was not found between Group II and the control group. No statistical significance was found in mean total cholesterol values between the 3 groups ($p > 0.05$). But, serum ALP levels showed a prominent increase in the experiment groups and a statistically significant difference was noticed between each group ($p < 0.01$).

AST levels of Group I and II and a difference was observed between these 2 groups ($p < 0.001$). No statistically significant difference was found in the average serum GGT values between all the groups ($p > 0.05$). A statistically significant difference was found in total bilirubin values between the control group and the other 2 groups ($p < 0.05$).

Hepatic insufficiency findings detected animals: In this study, some prominent findings showed that different stages of hepatic insufficiency were detected in some horses in Groups I and II (Table 4).

Table 2: Body temperature, pulse rate and respiratory rate of the groups (Mean±standard deviation and minimum and maximum values)

Parameters	Control (n = 10)	Group I (n = 12)	Group II (n = 8)
Body temperature (°C)	37.6±0.08 ^a (37.2-38.0)	38.2±0.15 ^b (37.0-38.8)	37.7±0.2 ^a (37.0-38.6)
Pulse rate (min)	32.8±1.31 (29.0-40.0)	35.0±2.45 (28.0-60.0)	31.5±1.89 (28.0-43.0)
Respiratory rate (min)	12.7±0.96 ^a (8.0-18.0)	15.9±1.13 ^b (12.0-24.0)	12.5±0.9 ^a (8.0-16.0)

^{a,b}Means with different superscripts in the same line differ significantly ($p < 0.05$)

Table 3: Biochemical findings of the groups (Mean±standard deviation and minimum and maximum values)

Parameters	Control (n = 10)	Group I (n = 12)	Group II (n = 8)	Reference range*
BUN (mg dL ⁻¹)	18.9±2.32 ^a (9.7-29.20)	11.31±0.53 ^b (9.7-16.30)	15.37±1.35 ^a (10.2-20.6)	10-24
T. Cholesterol (mg dL ⁻¹)	88.6±4.16 (76.0-110.0)	94.83±3.87 (76.0-117.0)	90.12±6.26 (72.0-120.0)	75-150
ALP (IU L ⁻¹)	261.5±17.4 ^a (163.0-344.0)	521.0±66.69 ^b (305.0-1160.0)	398.62±21.9 ^a (306.0-520.0)	143-395
AST (IU L ⁻¹)	270.4±10.6 ^a (213.0-318.0)	136.0±15.70 ^b (86.0-227.0)	91.75±5.49 ^a (68.0-120.0)	98-278
GGT (IU L ⁻¹)	17.0±1.96 (3.0-24.0)	42.9±16.0 (12.0-200.0)	26.25±6.11 (10.0-53.0)	<30
T. Bilirubin (mg dL ⁻¹)	1.18±0.16 ^a (0.62-2.030)	0.73±0.13 ^b (0.22-1.89)	0.58±0.7 ^b (0.26-0.86)	0-2.0

^{b,c}Means with different superscript in the same line differ significant ($p < 0.05$); *Barton and Morris (1998) and Turgut (2000)

Table 4: Different stages of hepatic insufficiency findings detected animals in Group I and II

Horse No.	Age (year)	Serum producing duration	Clinical findings	Biochemical parameters*
Group I				
1	19	2	Weight loss, anorexia and moderate icterus	Severe increases in serum ALP (1160 IU L ⁻¹) and GGT levels (200 IU L ⁻¹)
2	10	2	Slightly icteric mucosal membranes	Increased serum ALP level (522 IU L ⁻¹)
5	10	2	Only moderate icterus in mucosal membranes, weight loss, anorexia and increased body temperature	Normal
6	17	1	Moderate icteric mucosal membranes, increased body temperature	Serum ALP level (426 IU L ⁻¹)
7	-	1	Increased body temperature	Increased serum ALP level (571 IU L ⁻¹)
9	20	1	Only slightly icteric mucosal membrane and slightly increased body temperature,	Normal
10	16	1	Slightly icterus in mucosal membranes, increased body temperature	Increased serum ALP (493 IU L ⁻¹) and GGT (40 IU L ⁻¹) levels
11	10	1	No clinical signs	Increased serum ALP (529 IU L ⁻¹) and GGT levels (30 IU L ⁻¹)
12	22	1	Weight loss, anorexia, moderate icteric mucosal membranes, increased body temperature	Increased serum ALP level (688 IU L ⁻¹)
Group II				
13	8	4	Moderately icteric mucosal membranes	Increased serum ALP level (415 IU L ⁻¹)
14	16	9	Elevated body temperature	Slightly increased serum GGT level (30 IU L ⁻¹)
15	25	3	Only weight loss	Increased serum ALP (520 IU L ⁻¹) and GGT levels (53 IU L ⁻¹)
17	16	9	No clinical signs	Increased serum ALP level (412 IU L ⁻¹)
19	12	8	Slightly icteric mucosal membranes	Increased serum ALP level (405 IU L ⁻¹)
20	22	13	No clinical signs	ALP (403 IU L ⁻¹) and GGT (50 IU L ⁻¹) levels

*Reference ranges of ALP and GGT are 143-395 and 1-25 IU L⁻¹, respectively

DISCUSSION

Some researchers reported that >80% of the liver mass must be lost before clinical signs of hepatic disease become apparent (Pearson, 1996; West, 1996; McGorum *et al.*, 1999; Zientara *et al.*, 1994). But, Fowler (1965) reported that even though hepatic diseases have different aetiological causes and duration, they showed similar clinical signs.

Barton and Morris (1998) and West (1996) reported that the most common clinical signs of hepatic insufficiency in horses were Hepatic Encephalopathy (HE), icterus and weight loss. In this study, no clinical or biochemical abnormalities were not detected in the control group. Also, HE symptoms, such as abdominal pain, abnormal intestinal motility and photosensitization, were not detected in any animals.

West (1996) and Pearson (1996) reported that icterus was a permanent finding in hepatic insufficiency of horses. Also, some researchers reported that 10-15% of the normal horses have a slightly yellow discoloration of the mucous membranes and sclera. This is due to the fact that the concentration of unconjugated bilirubin in normal equine serum exceeds that in most other species and plasma carotene levels of many grazing horses impart a yellowish tinge to the mucous membrane (McGorum *et al.*, 1999, Turgut and Ok, 1997). Similarly, slightly icteric sclera was detected in 4 horses and moderately icteric sclera was detected in 3 horses in Group I in this study. In addition, slightly icteric sclera was detected in 2 horses and moderate icterus was detected in one horse in Group II.

Body temperatures of the horses with hepatic insufficiency, are usually normal (Fowler, 1965; Pearson, 1996). However, it could increase in case of secondary infections (Fowler, 1965). In this study, differences in the body temperature were found to be statistically significant between Group I and other groups.

Average respiratory rate of horses were found to be statistically significant between the control group and Group I and also between Group I and II. These data could depend on excitement levels of horses during examination because they were rarely taken out from the shelter.

Abdelkader *et al.* (1991), serum activities of GGT, ALP, AST and concentrations of total bilirubin and total bile acids were screened in 27 horses used for producing hyperimmune serum for investigation of hepatic disease. Similar to the that study, serum activities of GGT, ALP, AST and concentrations of total bilirubin, total cholesterol and blood urea nitrogen were detected for evaluation of hepatic diseases in serum-producing horses in the present study.

McGorum *et al.* (1999) concluded that serum GGT test may be the most sensitive test for detecting hepatic diseases in horses. ALP and GGT enzymes are primarily found in the biliary ducts and their serum concentrations increase when the bile flow is obstructed by an intra-or extrahepatic causes (Milne, 1993). Abdelkader *et al.* (1991) have found that ALP and GGT levels increase in serum-producing horses and that these increases were directly proportional to the time of immunization. GGT is an important indicator of hepatic failure in serum-producing

horses. Increased GGT concentration has been observed in horses following acute hepatocellular necrosis and this increasing followed a persistent course during chronic illnesses, especially cholestasis (Barton and Morris, 1998). Hoffmann *et al.* (1987) reported that determination of GGT levels is the best indicator for equine cholestasis. GGT is present in the biliary ducts and its increase in blood signifies hepatobiliary disease or hyperplasia (Pearson, 1996).

Abdelkader *et al.* (1991) reported that response of ALP to the liver changes in serum-producing horses was more limited than that of the GGT. The authors have also reported that a 10-fold increase in serum GGT levels can be expected over a period of 6-7 years, whereas the increase would be 2-fold with ALP. However, in the present study, we found ALP level 3 times higher than the upper range of reference values in only one horse in Group I (horse no: 1), whereas serum GGT activity was 8-fold higher in the same animal. However, this horse was used for serum production for only 2 years. Also, another horse (horse no: 4) in group I which was used for 5 years for serum production, showed a 4-fold increase in serum GGT level. From these data, it might be interpreted that hepatic failure can develop sooner in hyperimmunized horses due to hepatic toxication in horses. In agreement with the findings of Abdelkader *et al.* (1991), the increase in serum ALP level did not exceed 2-fold in any horses except in 2 horses in Group I, despite the differences in the immunization duration.

In addition, we found that serum ALP levels were above the reference range in 66.6% of horses used for serum production for 1-2 years (Group I). The difference in mean GGT levels between Groups was not statistically significant. Serum GGT levels, especially of Group I, was above the mean serum GGT levels of the control Group and was also higher than the reference range. Increased ALP levels were found in 62.5% of the horses immunized for 3-13 years (Group II).

Increases in AST are most frequently associated with muscle damage, but the increase may be also seen following acute hepatic necrosis. AST value is most useful when analyzed with other tissue-specific enzymes in hepatic diseases (Barton and Morris, 1998).

Abdelkader *et al.* (1991) reported that serum AST level was not affected for 6-7 years of immunization in serum-producing horses. In the present study, a significant difference was detected between mean serum AST levels in Groups I and II, compared with the control group, but these values were within the normal reference range and similar to results of this study.

The lack of any clinical signs of hepatic encephalopathy may be related to the fact that BUN was within the reference range in all the horses. However,

mean BUN levels of the experimental groups showed a decrease compared with the control group. Also, a significant difference was detected between Group I and the control group. West (1996) reported that the amount of ammonia being converted to urea decreases as a result of hepatic dysfunction and this decrease was directly proportional to the decrease in the liver capacity, resulting in a decrease in blood urea nitrogen and increase in ammonia concentration.

In a study carried out on horses with primary hepatic disease, increased bilirubin was detected in only 8 out of 34 horses (McGorum *et al.*, 1999). In the same study, the authors found no difference between the total bilirubin levels among 26 dead and 8 live horses. Abdelkader *et al.* (1991) reported that considering the reserve capacity of secretion and excretion functions of the liver, the changes in serum total bilirubin concentration does not become evident as long as there is no major disruption in the bile flow. The some researchers also found that total bilirubin level was not altered in serum-producing horses during the whole serum production period of 5 years. In this study, even though there were statistically significant differences in the mean total bilirubin levels between control, Group I and II, bilirubin levels of all the animals were found to be in the reference range, which is in agreement with the findings by other researchers.

Cholesterol is one of the products that is excreted from the body by the liver and total cholesterol, as a biochemical parameter, can yield information on the changes in the hepatic function (Batmaz *et al.*, 1998; Karagul *et al.*, 2000). Increase in cholesterol is an indicator of liver diseases and obstruction of bile ducts, whereas its decrease is a sign of hepatic degeneration (Barton and Morris, 1998). Coles (1986) argued that the tests of lipid metabolism are neither sensitive nor specific in horses. This study agreed with the findings of Coles (1986), since significant differences were not observed in mean serum total cholesterol levels between the control and experiment groups. Total cholesterol levels were slightly below the reference limits in only 2 horses in Group II.

CONCLUSION

At the end of the evaluation of all of these results, some prominent clinical and biochemical findings, which might indicate that different stages of hepatic insufficiency were detected, alone or in combination in a total of 15 horses (9 horses, which were used for serum production for 1-2 years and 6 horses, which were used for serum production for 3-13 years). In addition, our findings showed that these hepatic insufficiency symptoms were not dependent on the usage duration for serum production. It was concluded that serum-producing horses must have been monitored for hepatic insufficiency and precautions must have been taken.

Also, the horses diagnosed with hepatic insufficiency must have been removed from serum-producing process for avoiding their poor prognosis.

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