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Serological, Clinical, Hematological and Biochemical Study of Canine Infectious Hepatitis in Adult Dogs in Basrah Province, Iraq

TARIQE HADI ABDULLHUSSAIN AND HUSSEIN ALI NAJI*

Department of Internal and Preventive Medicine, College of Veterinary Medicine, Basrah University, Iraq
**(e-mail: Hussein.naji@uobasrah.edu.iq; Mobile: 00964 77075 17175)*

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ABSTRACT

The current study was conducted on 84 dogs (64 unvaccinated dogs of both sexes of different breeds showing the clinical signs of canine infectious hepatitis and 20 clinically heathy dogs). The study was conducted during September 2020 to April 2021 in Basrah province, Iraq. 36/64 (56.25%) adult dogs (males and females) were positive for antibodies against CAV 1. In which male 25/38 (65.78%) were higher than female 11/26 (42.3%). There was significant increase in body temperature, respiratory and heart rates, when compared with control group. Contrarily, there was decrease in the TRBCs, Hb, PCV and MCHC with increase in the MCV. Even clotting, prothrombin and activated thromboplastine time was affected. The biochemical analysis indicated significant increase in AST, ALT, ALP, GGT and total bilirubin. However, significant decrease was encountered in total protein values. Further, kidney functions showed increase in BUN, creatinine level and BUN/creatinine ratio in diseased adult dogs.

Key words: Canine adenovirus, CAV 1, CAV 2, infectious canine hepatitis

INTRODUCTION

Canine adenovirus type 1 is the agent that causes the disease known as infectious canine hepatitis (ICH), which is a kind of viral hepatitis that affects dogs and many species of animals (CAV 1 and CAV 2; Walker *et al.* 2016). ICH is a severe and frequently fatal systemic disorder (Walker *et al.*, 2016). The CAV 1 disease is pretty well documented (Chen *et al.*, 2018). It is uncertain when the initial outbreak was originally observed because it might have occurred at the same time as canine distemper outbreaks in the same populations (Beineke *et al.*, 2015; Loots *et al.*, 2017).

Infectious canine hepatitis (ICH) is a severe contagious virus (Canis lupus familiaris), caused by the non-enveloped icosahedral canine adenovirus type 1 (canine adenovirus type 1, CAV 1 double stranded DNA, which is a member of the genus Mastadenovirus and family Adenoviridae. Similarly, in human adenoviruses immunologically is more frequently seen in puppies than in older dogs (Pereira *et al.,* 2021). The disease is typically found in puppies between the ages of eight weeks and a year. In addition to dogs, other animals affected by this disease include wolves, ferrets, otters, foxes, coyotes, raccoons, pinnipeds and bears.

Symptoms of canine infectious hepatitis include fever (up to 41°C), loss of appetite, abdominal pain, severe conjunctivitis, enlarged lymph nodes, jaundice, reddened tonsils, pain when the liver is palpated, lethargy, disorientation, hyperventilation, convulsions, increased, occasionally bloody diarrhea, and other symptoms (Shah *et al.*, 2020). The disease is divided into three overlapping types or syndromes per acute, acute and chronic form (Pintore *et al.*, 2016).

In Basrah, Iraq there were little information and studies about the diseases therefore this study were designed for investigating canine adino virus in adult dogs along with clinical, hematological and biochemical effect on infected dogs.

MATERIALS AND METHODS

The current study was conducted on 84 dogs (64 unvaccinated dogs of both sexes of different breeds showing the clinical signs of canine infectious hepatitis, in addition to 20 dogs apparently clinically healthy dogs as a control group). The study was conducted during September 2020 to April 2021 in Basra province, Iraq. Standard clinical cards were used for data collection, including, the history of diseased animals, symptoms, and the routine standard clinical investigation.

Dog blood samples, 3 ml were drawn into EDTA tubes for determination of complete blood picture, 3 ml were drawn into tri sodium tubes for measurement of clotting factors and 5-8 ml of blood were drawn into tubes without anticoagulant for the serum for the measurement the liver enzymes, kidney functions and determination of CAV antibodies/antigens (Elisa test). Blood samples taken into normal tubes were centrifuged at 2000 rpm for 15 min. The serums obtained were kept in a deep freezer under –20°C. Serum samples were inactivated for 30 min at 56°C before being used.

Analysis of hematological and serum biochemical parameters was performed in Al-Bayan Laboratory, Basrah. Hematological parameters were analyzed by Beckman Coulter/USA. The liver function and kidney function tests were measured by using IMMULITE 2000 (Siemens), Canine adenovirus antibody (ADV-Ab) by sandwich ELISA kit (Sunlong Biotech Co., Ltd/China) was used for detecting CAV antibodies in dogs. The test was performed as per the manufacturer's instructions. The plates were then read on an automatic plate reader at 450 nm.

A one-way analyses of variance and Tukey's post hoc test for pair-wise comparisons between control and samples was used (SYSTAT for Windows version 11.00). All of the results are displayed as the mean ± SEM, and the data were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of ELSIA in the dogs with a clinical symptoms of canine infectious 36/64 (56.25%) in adult dogs (males and females) were positive for antibodies against CAV. In adult dogs, 25/38 (65.78%) were positive to antibodies against CAV in male than female 11/26 (42.3%; Table 1). According the serological tests, the canine adenovirus (CAV) was classified into two types (virulent strain, CAV 1) and (attenuated strain, CAV 2). CAV 1 induced infectious canine hepatitis (ICH), while CAV 2 caused infectious tracheobronchitis (ITB).

The results of ELISA test in adult dogs according to the gender showed the per cent of samples were positive to antibodies against CAV 1 in male than female, as the number of male in the present study was more than female.

Table 1. The result of CAV antibody according to the gender

Result / Gender	Positive and %	Negative and %	Total and %
Male	25 (65.78%)	13 (34.22%)	38 (100%)
Female	11 (42.3%)	15 (57.7%)	26 (100%)
Total	36 (56.25%)	28 (53.75%)	64 (100%)

Though, the males have more stress than females.

The ELISA tests is a sensitive, reliable and fast and reliable way for investigation of anti-adenovirus antibody (De Jonge *et al.*, 2020; Pereira *et al.*, 2021). Some kits used to detect the antibody could not distinguish between CAV types 1 and 2; the discrimination of the test may be considered redundant as both natural infection and vaccination against either of the 2 types provided cross-protection. McRee *et al.* (2014) reported higher percentage (100) of the prevalence of CAV antibody, while in our study the prevalence of CAV 1 antibody was 61.5% because present study used specific ELISA kit (sandwich) for CAV 1 antibodies which differentiated between vaccinated or naturally infected dogs (Naji *et al.*, 2021).

The results of the clinical signs observed in adult infected dogs with CAV 1 included anorexia, congestion of mucous membrane, lethargy, conjunctivitis, inappetence, weakness, polydipsia, vomiting, hematemesis, diarrhea, cough, tonsillitis, tachypnea, and icterus of mucous membrane, diarrhea with frank blood or melena, ecchymotic and petechial hemorrhages, hematuria, abdominal palpation reveal abdominal pain, hepatomegaly or splenomegaly, some neurologic signs such as circling, head pressing, ataxia, seizures, unilateral or, less commonly, bilateral corneal edema, blepharospasm, photophobia, and a serious ocular discharge nystagmus and apparent blindness (Table 2). Coagulopathies was manifested as cutaneous or mucosal petechial hemorrhages; gingival hemorrhages; epistaxis; or prolonged bleeding from venipuncture sites.

The results of the clinical signs observed in infected dogs with CAV 1 included anorexia, congestion of mucous membrane, lethargy, conjunctivitis, inappetence, weakness, polydipsia, vomiting, hematemesis, diarrhea, cough, tonsillitis, tachypnea, and icterus of mucous membrane, diarrhea which may be

due to frank blood or melena, ecchymotic and petechial hemorrhages, hematuria, abdominal palpation revealing abdominal pain, hepatomegaly or splenomegaly. This result is in agreement with Sykes (2014), Hornsey *et al.* (2019) and De Jonge *et al.* (2020), who reported that ICH had three forms per acute, acute and chronic form. The per acute form was more common in puppies characterized with circulatory failure, coma and death during 24 to 48 h, while the acute from occurred in adult dogs characterized with high morbidity. The results of vital signs showed significant increase in body temperature, respiratory and heart rates, when compared with control group (Table 3). The results of vital signs showed significant increase in body temperature, respiratory and heart rates, which occurred due to the viremia which led to septic fever and caused increase in body temperature due to the inflammation, besides that the damage of hepatocyte and kidney cell led to increase phospholipases in the cells membrane leading to formation the Arachidonic acid (ARA). The archidonic acid broke down cycalooxygenase 1 and 2 (cox 1 and cox 2) leading to formation of the thromboplastin and prostaglandin. Prostaglandin products modulated the hypothalamic thermoregulatory mechanism, resulting in an increase. This result is in agreement with Pintore *et al.* (2016), Balboni *et al.* (2017) and Hornsey *et al.* (2019).

Table 2. Clinical signs shown in diseased dogs (N=36)

S. No.	Signs	Adult dogs
1.	Anorexia	36 (100%)
2.	Lethargy	33 (91.6%)
3.	Polydipsia	29 (80.5%)
4.	Weakness	24 (66.6%)
5.	Diarrhea	22 (61.1%)
6.	Icterus of M. M	22 (61.1%)
7.	Congestion of mucous membrane	8 (22.2%)
8.	Petechial hemorrhages	6 (16.6%)
9.	Vomiting, hematemesis	12 (33.3%)
10.	Cough, tonsillitis, tachypnea	20 (55.5%)
11.	Bloody diarrhea or Melana	8 (22.2%)
12.	Hematuria	6 (16.6%)
13.	Abdominal pain, hepatomegaly or splenomegaly	25 (69.4%)
14.	Head pressing, ataxia	8 (22.2%)
15.	Prolonged bleeding from venipuncture sites	17 (47.2%)
16.	Nystagmus	5 (13.8 %)
17.	Seizures	11 (30.5%)
18.	Blepharospasm, photophobia	5 (13.8%)
19.	Corneal odema (blue eye)	4 (11.11%)
20.	Encephalitis signs	20 (55.5%)

Table 3. Body temperature, respiratory and heart rate in infected adult dogs compared with control group

Parameters	Control	Diseased dogs
Body temperature (°C)	38.4±0.856*	41.4±1.3**
Respiratory rate/min	18.44±3.48*	52±2.88**
Heart rate/min	112+9.24*	148±11.4**

*P<0.05 and **P<0.01.

In the current study, the results of hematological study showed significant decrease in the TRBCs, Hb, PCV and MCHC, while significant increase in the MCV in diseased adult dogs when compared with control groups (Table 4), the type of anemia macrocytic hypochromic. The hematological results of current study showed significant decrease in the TRBCs and Hb in dogs, this may be due to the hemolytic anemia, in addition to the blood loss with hematuria and bloody diarrhea. This result is in agreement with that of Duarte *et al.* (2014), Headley *et al.* (2018) and Hornsey *et al.* (2019). They referred to the hemolysis or blood loss result as to inflammation-associated production of pro-inflammatory cytokines resulting in suppression of erythrocytes, production and inhibition of iron absorption and utilization, leading to decrease in PCV in adult dogs.

Table 4. Blood parameters in diseased adult dogs

Groups / Parameters	Control Mean±S. E.	Infected Mean±S. E.
TRBCs × 10^6 µl	7.52±1.47*	4.6±0.4**
Hb g/100 ml	13.7±1.9*	8.9±1.7**
PCV %	46.55±3.72*	36.06±4.11**
MCV fl	68.72±4.28*	78.46±5.58**
MCHC g/dl	35.1±0.72*	28.84±1.62**

*P<0.05 and **P<0.01.

Also the results showed significant increase of MCV, while significant decrease MCHC, this referred to the type of anemia according morphological was macrocytic hypochromic that indicated regenerative anemia because the immature erythrocytes were frequently larger and contained less amount of hemoglobin than mature RBC. A regenerative anemia may have hypochromic (low MCHC) and macrocytic (high MCV). RBC indices with a high red blood cell distribution width (RDW)

supported our suggestion about the causes of hemolytic anemia (Jarad and Abed, 2020; Shah *et al.*, 2020; Tvedten, 2022).

The results showed a significant increase in total leukocytes count (leukocytosis) which was due to a significant increase in lymphocytes (lymphocytosis) in infected dogs, while the results showed significant decrease in neutrophils (neutropenia) in diseased dogs when compared with control groups (Table 5). This result is in agreement with that of Knowles *et al.* (2018). The neutropenia occurred due to defect of neutrophils production, rapid turnover or may be by inducing immune destruction of neutrophils, neutropenia that occurred with common viral diseases developed during the first 1 to 2 days of infection and may persist for some days. Leukopenia occurred early in the course of infection and may be profound (Naji, 2017). Initially there was a lymphopenia, after which neutropenia occurred and worsened progressively until death.

Table 5. Total and absolute differential leukocyte counts in infected dogs

Parameters \ Groups	Control Mean±S. E.	Infected Mean±S. E.
TLC $\times 10^3$ µl	9.2±1.43*	15.4±3.52**
Neutrophiles $\times 10^3$ µl	6.82±1.06*	3.82±0.74**
Lymphocytes $\times 10^3$ µl	3.56±0.92*	9.64±2.38**
Monocytes $\times 10^3$ µl	0.92±0.18	1.06±0.52
Eosinophiles $\times 10^3$ µl	1.1±0.72	1.4±0.66
Basophiles $\times 10^3$ µl	0.62±0.21	0.77±0.14

*P<0.05 and **P<0.01.

Blood clotting indices were also changed in infected dogs compared with control groups as the mean values of total platelets count, whereas significant increase was encountered in clotting, prothrombin and activated thromboplastine time (Table 6). This result occurred due to consumption of platelets in petechial ecchymotic hemorrhages or in disseminated intravascular coagulation (DIC). This finding corresponded with Sykes (2014). The authors suggested the common probable mechanisms for the genesis of DIC in dogs infected with ICHV was endothelial tissue disruption, which was thought to allow release of tissue thromboplastin into the blood stream, platelet adherence at the site of injury, and activation of the intrinsic clotting mechanism as well as enhancing the activation of plasminogen to plasmin, It made APTT prolonged to 6-7 times normal value (Naji *et al.*, 2018).

Table 6. Clotting factors in infected dogs and control group

Parameters \ Groups	Control Mean±S. E.	Infected Mean±S. E.
Thrombocytes $\times 10^3$ µl	493±80.1*	274±40.64**
Clotting time/min	90.38±1.9*	143.19±4.27**
Prothrombin time/sec	8.56±0.75*	15.82±1.68**
Activated partial thromboplastin time/sec	17.62±2.08*	31.4±3.98**

*P<0.05 and **P<0.01.

The results of biochemical analysis indicated significant increase in AST, ALT, ALP, GGT and total bilirubin. However significant decrease was encountered in total protein values, in addition to no significant change in albumin level in infected dogs compared with control animals (Table 7). Elevated livers enzymes indicated the liver damage due to the replication of the adino virus in hepatocyte beside the degeneration in bile canaliculi. This finding is in agreement with that of Sykes (2014). In addition to the results showed elevated in total bilirubin in diseased dogs when compared with control group, this finding occurred due to the hematolytic anemia or hepatocyte damage as per agreement with Pereira *et al.* (2021), who referred to the total bilirubin considering as a marker for liver diseases and as suppurative evidence hemolytic anemia.

Table 7. The liver functions test in infected adult dogs and control group

Parameters \ Groups	Control Mean±S. E.	Infected Mean±S. E.
AST U/L	19.22±4.81*	52.72±11.4**
ALT U/L	105.04±13.62*	534.62±20.94**
ALP U/L	119.17±13.52*	207.02±22.82**
GGT U/L	17.5±3.18*	33.54±6.08**
Total bilirubin (mg/dl)	0.74±0.15*	6.18±0.46**
Total protein (g/dl)	5.9±0.88*	3.07±1.16**
Albumin (g/dl)	3.72±0.58*	4.2±0.48*

*P<0.05 and **P<0.01.

The present study showed significant decrease in total protein in deceased dogs due to the damage or degeneration in hepatocytes, representing the sources of protein synthesis as per agreement with Kasem *et al.* (2015) and Hornsey *et al.* (2019) who suggested the

hypoproteinmia occurred due to failure of passive transfer (in neonates), malnutrition/ starvation, over-hydration, severe liver or kidney disease, congestive heart failure (with oedema), intestinal malabsorption, blood loss and burns. While the albumin level did not alter in infected dogs when compared with control group, this result happened due to level of albumin with chronic protein loss in kidney and liver disease as corresponding with Mazzei *et al.* (2015) and Hasan and Al-Amery (2020).

The results of kidney functions test showed significant increase in BUN, creatinine level and BUN/creatinine ratio in diseased adult dogs when compared with control groups (Table 8). This indicated damage in interstitial tissue effecting on kidney functions leading to elevated BUN and creatinine levels. This finding is in agreement with that of Mehl *et al.* (2018) who referred BUN and creatinine were commonly used in clinical practice for evaluating the renal functions, the abnormal concentration of BUN and carnitine called azotemia. A serum creatinine concentration was the most used measure of severity of renal dysfunction and was the basis for staging chronic kidney disease (CKD). To optimize accurate staging of CKD, serum creatinine concentrations should be evaluated on two or more occasions when the dogs were well-hydrated. In addition the results showed significant increase of level and BUN/ creatinine ratio in adults diseased dogs, due to the pre-renal disorders, complicating the disease (Feldman *et al.*, 2014; Martiny and Goggs, 2019).

Table 8. The kidney functions test in infected dogs and control group

Groups / Parameters	Control Mean±S. E.	Infected Mean±S. E.
BUN (mg/dl)	25.05±2.58*	152.82±5.92**
Creatinine (mg/dl)	1.37±0.12*	7.08±1.07**
BUN/Creatinine ratio	18.3*	30**

*P<0.05 and **P<0.01.

CONCLUSION

The present study of the Elisa test indicated that the samples were positive to antibodies against CAV 1 in male higher than female in unvaccinated dogs. Also the clinical signs varied according the severity of infection. The virus infection caused some changes in hematological parameters, clotting, liver enzymes and kidney functions.

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