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Original Paper

Oxidative stress markers and hematological parameters associated with canine parvoviral enteritis in dogs

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Canine parvoviral enteritis (CPVE) is a global contagious disease of dogs caused by three

variants of canine parvovirus type 2 (CPV2). This present study aims to assess the values of

hematological parameters and oxidative stress as confirmation markers for CPV2 infection in household dogs. A total of ten suspected diseased dogs (less than 6 months) by Parvovirus were

introduced to the Awsim Veterinary Hospital in Giza governorate. Dogs were diagnosed primarily according to clinical signs (fever, bloody diarrhea, vomiting, depression, and

anorexia) and fecal SNAP test (Ag test kits for parvo). The result of SNAP test revealed that five dogs were negative and five were positive for CPV2 which was further examined by

hematology and oxidative markers. Hematological examination in CPV2-infected dogs showed macrocytic hypochromic non-regenerative anemia, leukocytosis, and thrombocytopenia. The

differential leucocytic count revealed relative lymphocytosis and monocytosis with nonsignificant change in eosinophil and basophil counts in CPV2 infected compared to apparently healthy control dogs. On the other hand, oxidative stress assessment revealed a decline in the

mean values of MDA, GPx, and CAT and an increase in the level of SOD in CPV2-infected

dogs compared to an apparently healthy control. These findings shed light on the potential

diagnostic utility of hematological parameters and oxidative stress markers in confirming

ARTICLE INFO

ABSTRACT

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1. INTRODUCTION

Canine parvovirus-2 (*CPV2*) is a highly fatal viral disease affecting the intestinal tract with signs of anorexia, vomiting, depression, lethargy, foul-smelling diarrhea, dehydration, and fever (Mylonakis et al., 2016; Khinchi et al., 2019). Although *CPV2* can affect dogs of all breeds, ages, and sexes, puppies are predominantly susceptible (Goddard and Leisewitz, 2010; Kumar et al., 2017). Like other viral diseases, vaccination programs are the only effective strategy for controlling *CPV2* infection (Carmichael, 2005); therefore, failure of vaccination is considered a critical cause of *CPV2* infection in the dog population (Sayed-Ahmed et al., 2020). *CPV2* belongs to the genus *Parvovirus* of the family *Parvoviridae* and it is closely related to the *mink enteritis virus* and *feline panleukopenia virus* (Sara et al., 2006).

Several enteric diseases causing signs similar to those of CPV2 (diarrhea, vomiting, and dehydration) render its diagnosis and differential diagnosis difficult (Abdel-Rhman et al., 2019). CPV2 can be diagnosed by several laboratory tests with prominent variations in their sensitivity and specificity, including rapid CPV test kits, Hematological analyses, biochemical analyses, and polymerase chain reaction (PCR); the most accurate method for the diagnosis of CPV infection (El-Zahar et al., 2019). Furthermore, CPV2 infection can be diagnosed by assessing the hematological parameters and oxidative stress markers because it is known that changes in both inflammatory and antioxidant status may be observed in CPV2 (Kocaturk et al., 2014; Ogbu et al., 2022).

CPV2 infection in household dogs. Hematological parameters may be important to establish the response of the case to treatment and to detect the expected prognosis (Terzungwe 2018). The changes in hematological parameters in the case of *CPV2* infection are due to the destruction of hematopoietic cells in the bone marrow and the lymph proliferative cells of the thymus, lymph nodes, and spleen (Amaravathi et al., 2016). However, after animal recovery from parvovirus infection, hyperplasia of the lymphoid, erythroid, and myeloid cells is compensated for (Shah et al., 2013).

On the other side, the oxidative stress assessment may be enzymatic through the detection of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (DiMascio et al., 1991) or nonenzymatic through the detection of some minerals and vitamins, including copper, zinc, and vitamin E (Irshad and Chaudhuri 2002).

In *CPV2* infection, oxidative stress occurs due to an imbalance in the production of reactive oxygen and nitrogen species (ROS) and neutralizing antioxidant enzymes (Khinchi et al., 2019). ROS is inhibited by antioxidant enzymes such as GPx, catalase, and SOD (Gaykwadet et al., 2016). There is an alteration in the activities of the antioxidant enzymes catalase and SOD, the first line of antioxidant defense against the damaging effects of free radicals, this alteration is clearer in cases of *CPV2* compared to healthy ones (Panda et al. 2009).

Accordingly, this study aims to underscore the alteration in both hematological parameters and oxidative stress markers

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as indicators for confirmation of the diagnosis of *CPV*2 infection in dogs.

2.MATERIAL AND METHODS

2.1. Ethical statement

The Benha University Ethical Committee for Animal Studies (BUFVTM11-04-23) approved all protocols, including the handling and acquisition of blood samples. The dog owners were properly notified and gave full consent for the practice of collecting blood samples.

2.2. Animals and sampling

A total of ten suspected diseased dogs by CPV2. These animals showed fever, bloody diarrhea, vomiting, depression, and anorexia. Rectal swabs were collected using specialized swab sticks. The examined animals were less than six months old. Various breeds, such as German Shepherds, Golden Retrievers, Pit Bulls, and Rottweilers, were admitted to Awsim Veterinary Hospital.

Two blood samples (1 mL) from each dog were aseptically drawn from the recurrent tarsal vein and transferred into tubes containing EDTA to obtain whole blood samples for hematological examination and tubes without anticoagulant for assessment of oxidative stress. The blood samples were subjected to centrifuging at 3000 rpm for 5 minutes, then the sera were separated and stored at -20 °C until subsequent analysis.

2.3. CPV2 diagnosis by commercial SNAP test

All collected samples (10) underwent initial screening for CPV-2 infection using the SNAP test (Ag test kits for parvo) from IDEXX Laboratories GmbH, Ludwigsburg, Germany.

2.4. Evaluation of hematological parameters

The positive samples (five) with the SNAP test were analyzed using the Mindray BC 500; Veterinary Auto Analyzer Device (China) for measuring hematological parameters including red blood cells (RBCs) count, white blood cells (WBCs)count, differential leucocytic count, PCV%, hemoglobin (Hb) concentration, and blood indices (MCHC, MCH, and MCV).

2.5.Assessment of oxidative stress markers

The levels of MDA, SOD, CAT, and GPx were measured in the positive samples (5) with SNAP test using commercial kits (Biodiagnostic) Cat. No. (MD 2529, SD 2521, CA 2517, and GPx 2524), respectively, by using BMG lABTECH (Germany).

2.6.Statistical analysis

Chi-square test was used to compare the levels of oxidative stress and hematological categories in dogs infected with CPV and healthy control dogs using Microsoft Office Excel 2019. The results were expressed as the mean \pm standard deviation (SD). The following tests were used to analyze the significance, difference, and association of qualitative variables: the chi-square test (c2) for differences. The p-value was established at 0.05 for significant results and 0.001 for highly significant results.

3. RESULTS

3.1. SNAP test results:

Five samples out of ten (n = 10) were positive for SNAP Parvo test.

3.2. Hematological parameter findings :

The hematological analysis of *CPV2-infected* dogs revealed notable alterations in erythrocyte and leukocyte parameters compared to non-infected dogs. The *CPV2-infected* dog samples revealed significant reductions in RBCs count, PCV% and Hb concentrations. Moreover, CPV-infected dogs showed a noticeable increase in MCV values and a significant decrease in MCHC, which meant that the infected dog suffered from macrocytic hypochromic anemia.

On the other hand, the leukogram of CPV-infected dogs showed a marked elevation in WBCs count, lymphocyte count, and monocyte count compared to healthy dogs. Furthermore, platelet levels were significantly diminished (thrombocytopenia) in diseased dogs compared to healthy ones. However, basophil and eosinophil counts exhibited negligible differences between CPV-infected and healthy dogs, as shown in Table 1.

Table 1 Mean values of hematological parameters (mean ± SE) in sera of infected and non-infected dogs by CPV

| Parameters | Control | Infected | P value | Reference range |
|-------------|------------------|------------------|--------------|--------------------------|
| RBCs | 7.67 ± 0.80 | 4.52 ± 0.45 | 0.023* | 5.5-8.5 million/c.mm |
| HGB | 14.89 ± 0.87 | 10.74 ± 2.08 | 0.026^{*} | 12-18 gm |
| Platelets | 349.46±134.31 | 308.4±241.88 | 0.000^{**} | (200-500) *103 /c.mm |
| PCV | 39.77 ± 5.70 | 32.9 ± 4.36 | 0.025^{*} | 39 % - 55 % |
| WBCs | 9.4 ± 3.26 | 17.14 ± 9.72 | 0.000^{**} | $(6 - 15) * 10^3 / c.mm$ |
| MCV | 61.6 ± 10.9 | 64.32 ± 9.19 | 0.005** | $60 - 77 \mathrm{fl}$ |
| MCH | 22.80 ± 1.96 | 22.74 ± 3.93 | 0.061 | 19.1 – 26.2 pg |
| MCHC | 35.74 ± 5.95 | 31.44 ± 4.37 | 0.001** | 32 – 36 g % |
| Basophil | 0.00 ± 0.00 | 0.26 ± 0.58 | 0.860 | 0 - 1 % |
| Eosinophil | 4.4 ± 0.55 | 4.73 ± 0.72 | 0.975 | 2 - 10 % |
| Lymphocytes | 24.79 ± 6.63 | 41 ± 19.57 | 0.001** | 10-36 % |
| Monocytes | 9.10 ± 3.57 | 14.8 ± 6.61 | 0.002^{**} | 0 - 13% |

* The result is considered Significant if P value < 0.05

3.3. Oxidative stress parameters:

In the infected group, a significant reduction in the levels of malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GPx) were observed compared to the control group. Conversely, there was a significant elevation in the level of superoxide dismutase (SOD) in the infected group relative to the control group as shown in table 2

Table 2: Mean values of oxidative stress markers (mean ± SE) in infected and non-infected dogs by CPV

| Animal | MDA | SOD | CAT | GPx |
|----------|-------------|-------------------|-------------|------------------|
| | (nmol/mL) | (U/mL) | (U/ml) | (U/l) |
| Infected | 11.442±7.47 | 268.2 ± 50.98 | 0.216±0.068 | 917.42±97.61 |
| Control | 24.3o±20.47 | 161.178±106.54 | 0.708±0.58 | 11154.57±4216.55 |
| P value | 0.001** | 0.001** | 0.03* | 0.001** |

* The result is considered Significant if P value < 0.05

4. DISCUSSION

CPV2 infection can be diagnosed by assessing the hematological parameters and oxidative stress markers because it is known that changes in both inflammatory and antioxidant status may be observed in CPV2 infection (Kocaturk et al., 2014). In this investigation, CPV2 infection revealed divergent trends in hematological parameters and oxidative stress markers, reflecting the complexity of the disease.

This study revealed a significant reduction in the mean values of Hb concentration, RBCs count, and PCV % in the *CPV2*-infected dogs (El-Zahar et al., 2019; Ogbu et al., 2022). This reduction could be attributed to the loss of blood due to the sloughing of the intestinal epithelium, leading to the destruction of intestinal capillaries (Baruah et al., 2007; Sulthana, 2015), along with inadequate compensation due to the destruction of progenitor cells in the bone marrow, thymus, spleen, and lymph nodes (Terzungwe, 2018). Moreover, the reduction in PCV% may also be due to blood and fluid loss resulting from vomiting and diarrhoea during the infection (Agnihotri et al., 2017, Khare et al., 2020; Minnat and Sadeq, 2023).

Macrocytic hypochromic anemia is also observed in this study because there is a significant decrease in mean MCHC (Amaravathi et al., 2016; Ogbu et al., 2022).

The results of WBCs count revealed a significant increase in *CPV-infected* dogs compared to healthy control dogs (Shah et al., 2013; Alves et al., 2019). This elevation might be due to monocytosis because of the secondary bacterial infection associated with parvoviral enteritis (Goddard et al., 2008). Contrarily, Yilmaz and Senturk (2007) and Behera et al. (2020) recorded leukopenia in *CPV2-infected* dogs and attributed this to bone marrow infection or consumption of neutrophils in the sloughed intestinal mucosa.

Moreover, the results recorded relative monocytosis and lymphocytosis in *CPV2-infected* cases. Also, the platelet count in *CPV2-infected* cases was lower than that of healthy cases (Castro et al., 2013; Shah et al., 2013). This finding may be attributed to loss of blood through hemorrhagic diarrhea, increased consumption of platelets in coagulation and increased destruction of platelets by macrophages (immune-mediated thrombocytopenia) (Agnihotri et al., 2017). Top of Form

Regarding the oxidative stress markers, the results confirmed that there is an increase in SOD activity in dogs with *CPV2* infection compared to healthy control dogs (Panda et al., 2009; Elsayed et al., 2020). Contrary to this, Ukwueze et al., (2020) and Kataria et al. (2020) reported that the mean value of SOD is lower in the *CPV2*-infected dogs. The higher level of SOD in *CPV2*-infected dogs may be due to the enhanced synthesis of antioxidant enzymes as a compensatory mechanism (Khinchi et al., 2019).

In the present study, GPx was observed to be diminished in CPV-infected dogs (Kocaturk et al., 2014; Kataria et al., 2020), although Elsayed et al. (2020) and Nafie et al. (2021) reported an elevation of GPx levels in diseased dogs compared to healthy dogs.

Regarding the results of catalase activity, it was found that a significant decrease was recorded in diseased dogs compared to healthy dogs (Beigh et al., 2014; Kataria et al., 2020). However, Panda et al. (2009) and Harizan et al. (2021) stated that the catalase activity of *CPV2*-infected dogs is higher than that of control dogs. The reduction could be attributed to the consumption of antioxidants that act as "scavengers" of free radicals during oxidative processes (Harizan et al., 2021).

Moreover, lowered activities of antioxidant enzymes such as catalase and glutathione peroxidase in dogs are indicative of a state of oxidative stress, which might be because of continual assault beyond the autoregulatory mechanism, which causes a decline in enzyme activity (Rautray et al., 2016).

Additionally, there was a significant reduction in MAD in CPV-infected dogs compared to controls (Panda et al., 2009). This may be attributed to the fact that MDA levels decline after the first 24 hours of life, with a half-life observed to be around 9.7 to 13.4 days (Mila et al., 2014).

5. CONCLUSIONS

Assessing hematological parameters and oxidative stress markers is a convenient, cost-effective, and efficient way to confirm and diagnose CPV2 infection in household dogs. This study proved that dogs infected with CPV-2 are likely to show a decrease in hematological parameters, such as RBC counts, PCV, and HB concentrations, resulting in anemia which needs effective therapy to reduce the death of CPV2-infected dogs. In addition, there is evidence suggesting that oxidative stress plays a role in the development of CPV2 infection in dogs. Therefore, the use of antioxidant supplementation may help strengthen the body's defense mechanisms and reduce stress levels.

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