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Feline Panleukopenia - Effects of Treatment with Filgrastim on Mortality, Hematological and Biochemical Parameters

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ABSTRACT

***Background*:** Panleukopenia is a highly contagious and fatal disease of cats. Although infection may be prevented by intensive vaccination of animals in the home environment, infection is frequently seen in stray animals that are not vaccinated and are in contact with the virus. Treatment protocols in the disease consist of classical supportive therapies because there is no specific antiviral drug. The aim of this study is to evaluate the effects of Filgrastim administration on mortality, complete blood count, and serum biochemical parameters in the treatment of cats infected with Feline Panleukopenia Viruses (FPV).
***Materials, Methods & Results*:** The study population consisted of 2-6 months old cats of different breeds and sexes who were brought to our clinic with complaints of lethargy, anorexia, high fever, diarrhoea and vomiting. A complete blood count was taken from the symptomatic patients during physical examination and FPV Ag test was performed. Fourteen cats with positive FPV antigen test and 7 healthy cats with negative FPV Ag test without clinical symptoms were included in the study. The study included 7 Panleukopenia-infected cats treated with Filgrastim in addition to conventional supportive therapy, 7 Panleukopenia-infected cats treated with conventional supportive therapy, and 7 healthy cats. Complete blood count and serum biochemical parameters were analyzed from the samples taken from the patients on days 0, 3 and 7. As a result of the treatment, the mortality rate was 14.28% in both groups. In this study, it was observed that the administration of Filgrastim (Group 1) had statistically significant results on WBC ($P < 0.009$), lymphocytes ($P < 0.009$) and granulocytes ($P < 0.003$) between days 0 and 7 in cats with FPV. This suggests that the administration of Filgrastim in addition to supportive therapy increases the number of lymphocytes and granulocytes. The administration of Filgrastim as an adjunct to supportive therapy had no effect on the serum biochemistry values of the patients. In conclusion, the administration of Filgrastim in the treatment of panleukopenia did not result in a change in the mortality rate.
***Discussion*:** The incidence of FPV decreases as the age of the animal increases. In cats aged 3-5 months, the amount of antibody from the mother decreases. In this study, it was determined that the age of all FPV Ag positive cats included in group 1 and group 2 was 2-6 months. It has been reported that there may be a rapid increase in neutrophil counts in cats with FPV treated with filgrastim. In this study, it was observed that the use of Filgrastim (Group 1) in cats with FPV produced statistically significant results on WBC, lymphocytes and granulocytes between days 0 and 7. This shows that the use of Filgrastim in addition to supportive therapy increases the number of lymphocytes and granulocytes. The incidence of FPV decreases as the age of the animal increases. In cats aged 3-5 months, the amount of antibody from the mother decreases. In this study, it was determined that the age of all FPV Ag positive cats included in group 1 and group 2 was 2-6 months. Serum biochemistry parameters are not specific for FPV. Hypoalbuminaemia is the most common abnormality, probably due to decreased protein intake and leakage from mucosal lesions into the gastrointestinal tract. Similar to the literature, no significant change was observed in biochemical parameters. In addition, serum biochemical examinations have shown that Filgrastim can be administered to cats without any side effects. Before administering an immune modulator such as G-CSF, the stage of the disease and the status of the immune system should be taken into consideration.

Keywords: panleukopenia, filgrastim, granulocyte colony stimulating factor, feline parvovirus infection.

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INTRODUCTION

Panleukopenia caused by Feline Parvo Virus (FPV) is a highly contagious disease that affects the normal growth and development of young cats [22]. Viruses belonging to the parvovirus family are specific for a particular host species, but can also infect different mammalian hosts [6]. FPV can be transmitted from infected cats to susceptible cats by direct contact, transplacental (passing through the placenta during pregnancy) or contact with the virus on the surface [22]. The virus has a specific tropism for cells with high mitotic activity such as bone marrow, lymphoid tissue and intestinal crypt cells. FPV can cause a wide range of symptoms depending on the virulence of the viral strain, the health status of the host and the presence of coinfection [14]. In the peracute form of infection, the mortality rate can reach 100%. In the acute form, the mortality rate is around 90% [13]. Treatment protocols in the disease consist of classical supportive therapies because there is no specific antiviral drug [10]. Granulocyte Colony Stimulating Factor (G-CSF), a cytokine produced by the bone marrow, increases the release of granulocytes from the bone marrow, shortens their maturation time and promotes granulopoiesis [23]. In recent years, recombinant human Granulocyte Colony Stimulating Factor (rhG-CSF) has been used in the treatment of neutropenia due to panleukopenia. Increased treatment success has been reported due to this G-CSF [17].

The aim of this study is to evaluate the effects of Filgrastim administration on mortality, complete blood count, and serum biochemical parameters in the treatment of cats infected with FPV.

MATERIALS AND METHODS

Animals

The study population consisted of 2-6-month-old cats (n = 21) of different breeds and sexes who were brought to our clinic with complaints of lethargy, anorexia, high fever, diarrhoea and vomiting. A complete blood count was taken from the symptomatic patients during physical examination and FPV Ag test[1] was performed. Fourteen cats with positive FPV antigen (Ag) test and 7 healthy cats with negative FPV Ag test without clinical symptoms were included in the study.

Treatments and tests performed

The cats constituting the study were divided into 3 groups. Filgrastim[2] was administered to 7 cats in the 1st group with positive FPV Ag test at a dose of 5 mcg/kg/day, subcutan for 5 days in addition to classical supportive treatment for 7 days. The 7 cats in the 2nd group with positive FPV Ag test received classical supportive treatment for 7 days. Classical supportive treatment consisted of balanced crystalloid solutions[3] [44 mg/kg, i.v., SID], vitamin B complexes[4] [1 mL/cat, SC, SID], vitamin C[5] [3 mL/cat, i.v., SID], antibiotics Metronidazole[6] [15 mg/kg, i.v., BID], Ceftriaxone[7] [30 mg/kg, i.v., BID], antiemetic, Maropitant[8] [1 mg/kg, SC, SID], gastroprotection, Pantoprazole[9] [1 mg/kg, i.v., SID]. In the 3rd group, 7 cats with negative FPV Ag test did not receive any treatment. From the 1st and 2nd group cats, before starting the treatment (on day 0), on days 3 and 7, whole blood was collected from the vena saphenous vein. On the 0th, 3rd and 7th days, complete blood count samples from the vena saphenae were taken in Dipotassium Ethylenediaminetetraacetic Acid (K2EDTA) tubes[10] and White Blood Cell (WBC), Lymphocyte (Lymphocyte) and Lymphocyte (LYM) counts were determined in an automatic whole blood device[11] immediately after being turned upside down 6 times, Granulocyte (GRAN), MID, Erythrocyte (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Procalcitonin (PCT) and Platelet (PLT) values were measured. From the cats in groups 1 and 2, blood samples were collected in gel serum separation tubes[12] before starting the treatment (Day 0) and on the 3rd and 7th days. Albumin (ALB), Total Protein (TP), Globulin (GLO), Albumin-Globulin Ratio (A/G), Calcium (Ca), Glucose (GLU), Blood Urea Nitrogen (BUN), Phosphorus (P) were determined with a biochemistry device[13], Amylase (AMY), Cholosterol (CHOL), ALT, Total Bilurubin (TBIL), Alkaline Phosphatase (ALP), Creatine (CRE), Blood Urea Nitrogen to Creatine ratio (BUN/CREA), Creatine Kinase (CK) were measured. In the 3rd group of healthy cats, complete blood count values and serum biochemical parameters were measured once.

Statistical analysis

Descriptive statistics of the data obtained in the study were performed. After the distribution of the numerical data was checked, it was determined that they did not show normal distribution. Kuruskall-vallis ANOVA test was performed for the data that did not

show normal distribution. In all analyses, *P* values less than 0.05 were considered statistically significant and analyses were performed in SPSS 19.0 (IBM, USA).

RESULTS

It was observed that 2 of the 7 cats in the 1st group were female (28.5%) and 3 of the 7 cats in the 2nd group were female (42.85%). All cats in the 1st and 2nd groups were unvaccinated, 3 of the cats in the 3rd group (42.85%) were vaccinated and all cats in the study were 2-6 months old. It is known that 71.42% of the cats in our study were tabby and stray animals. In addition, 85.71% of all cats in the study were domestic and mixed breeds. As a result of the treatment, respectively 1 cat from group 1 and 2 died. The cat in group 1 died on the 4th day and the cat in group 2 died on the 3rd day. The symptoms of the patients in group 1 and group 2 were anorexia (85.71%), lethargy (64.28%), vomiting (57.14%) and diarrhoea (50%). In the haemogram results of the cats included in the study, it was determined that the WBC count on day 0 of all cats (100%) in the 1st group was below the reference values, the WBC count on day 0 of 7 cats in the 2nd group was normal in 3 (42.85%), low in 3 (42.85%), high in 1 (14.28%) and all cats (100%) in the 3rd group were within the range of reference values. In group 1 patients, WBC, LYM, GRAN values were found to be $P < 0.05$ between days 0 and 7 and a statistically significant difference was observed. In group 2 patients, WBC, LYM, GRAN values were calculated as $P > 0.05$ between days 0, 3 and 7 and no significant difference was observed. In group 3 patients, no statistical measurement was made between days because blood counts were performed once. WBC value was found to be $P < 0.05$ between group 1 and group 3 on day 0 and a statistically significant difference was observed. LYM value was found to be $P < 0.05$ between group 1 and group 3, group 2 and group 3 on day 0 and a statistically significant difference was observed. LYM value was found to be $P < 0.05$ between group 1 and group 3 on the 3rd and 7th days and a statistically significant difference was observed. GRAN value was found to be $P < 0.05$ between group 1 and group 3 on day 0 and a statistically significant difference was observed.

In the comparison of serum biochemical parameters within days, $P > 0.05$ was calculated in all measurements and no statistically significant difference was observed. In the comparison of serum biochemical parameters between groups, BUN value was found to be $P < 0.05$ between the 1st and 2nd group on day 0 and a statistically significant difference was observed. AMY value was found to be $P < 0.05$ between the 1st and 3rd groups on days 0 and 3 and a statistically significant difference was observed. CHOL value was found to be $P < 0.05$ between the 1st and 3rd groups on day 0 and a statistically significant difference was observed. ALT value was found to be $P < 0.05$ between the 1st and 3rd groups on the 3rd day and a statistically significant difference was observed.

Table 1. Statistical analysis of WBC counts on days 0, 3 and 7.

Groups	N	Parameters	Mean & Standard error
Group 1	7	WBC 0.day[a]	1.8743 ± 0.64149
	7	WBC 3. day[b]	6.1514 ± 2.32351
	6	WBC 7. day[a]	27.1817 ± 5.18801
Group 2	6	WBC 0. day[a]	4.6233 ± 1.33584
	6	WBC 3. day[b]	7.5417 ± 1.80608
	5	WBC 7. day[c]	18.054 ± 8.95391
Group 3	7	WBC[d]	11.4757 ± 0.94104
Group 1	6	LYM 0. day[a]	0.9417 ± 0.3048
	7	LYM 3. day[b]	1.9957 ± 0.61128
	6	LYM 7. day[a]	10.47 ± 2.10223
Group 2	6	LYM 0. day[a]	1.5633 ± 0.29016
	6	LYM 3. day[b]	4.5 ± 1.09254
	5	LYM 7. day[c]	6.608 ± 1.93499
Group 3	7	WBC[d]	4.6757 ± 0.4685
Group 1	6	GRAN 0. day[a]	0.845 ± 0.30235
	7	GRAN 3. day[b]	3.1057 ± 1.36879
	6	GRAN 7. day[a]	10.125 ± 2.40687
Group 2	6	GRAN 0. day[a]	2.3783 ± 0.93821
	6	GRAN 3. day[b]	2.0333 ± 0.77292
	5	GRAN 7. day[c]	8.212 ± 5.73109
Group 3	7	WBC[d]	5.1486 ± 0.71265

[a,b,c,d]Values shown with the same letters are statistically significant ($P < 0.05$). Different letters are statistically insignificant ($P > 0.05$).

Table 2. P values of complete blood count results between groups on days 0. 3 and 7.

Parameter	Days	Group 1 – Group 2	Group1 - Group 3	Group 2 - Group 3
WBC	0. day	0.715	0.001	0.058
	3.day	0.088	0.088	0.088
	7. day	0.051	0.051	0.051
LYM	0. day	1.000	0.002	0.027
	3. day	0.208	0.030	1.000
	7. day	1.000	0.021	0.366
GRAN	0. day	1.000	0.006	0.102
	3. day	0.057	0.057	0.057
	7. day	0.118	0.118	0.118

DISCUSSION

Panleukopenia is a viral disease that has been widespread since the beginning of the 20th century and causes severe leukopenia, gastroenteritis and nervous symptoms in cats [1]. Human migration has further increased the spread of this disease by dispersing pathogens, creating larger and more interconnected populations [21]. Despite the widespread use of effective vaccines against FPV, which is associated with high morbidity and mortality, studies have shown that cat populations are not well protected in many countries. In a study of 350 cats, the prevalence of antibodies against FPV was found to be 71% [19]. It was reported that 184 (60.3%) of 244 cats with FPV had never been vaccinated, while 73 (39.7%) had been vaccinated at least once [9,18,20]. In this study, similar to other studies, it was determined that all cats in the 1st and 2nd groups were unvaccinated and 3 cats (42.85%) in the 3rd group were vaccinated. There was no significant relationship between the severity of the disease and the cat being unvaccinated [5]. The incidence of FPV decreases as the age of the animal increases. In cats aged 3-5 months, the amount of antibody from the mother decreases [22]. In this study, it was determined that the age of all FPV Ag positive cats included in group 1 and group 2 was 2-6 months. It has been reported that 40% of patients with FPV are male and 60% are female [8]. In a study, it was reported that 59.5% of the patients were male and 40.5% were female [9]. It was determined that 2 of 7 cats in group 1 were female (28.5%) and 3 of 7 cats in group 2 were female (42.85%). This study had a similar gender distribution with other studies. When the breeds in group 1 and group 2 consisting of cats with FPV included in the study were examined, it was seen that 14.28% were exotic breeds and the remaining patients were domestic and mixed breeds. In a study it was reported that approximately 15% of the patients were exotic breeds and the majority were mixed and domestic breeds [8]. In another study it was reported that more than 90% of the sick cats were domestic and mixed breeds [9]. It was reported that in a study involving 9 cats of domestic breeds, 6 of them had contact with the external environment, while 3 of them were shelter animals [2]. It was observed that 71.42% of the cats in our study were tabby cats and animals living on the street. Our study was found to be compatible with other studies. According to Kruse *et al*. [9] and Citravoa *et al*. [2], such results occurred due to the environmental conditions depending on the locations of the study, the presence of stray animals living on the street, the low rate of exotic species being stray and living on the street, and the high vaccination rates. It is thought that the higher proportion of cats living on the streets in Turkey and the low vaccination rate of these cats cause a difference in the spread of the disease and the breeds in which it is seen. Subacute, peracute, acute and perinatal clinical forms can be seen in cats with FPV. The most common symptoms in the acute form are fever, lethargy, anorexia, vomiting and watery, fibrinous and/or haemorrhagic diarrhoea, severe

leucopenia and dehydration [15,20,22]. It has been reported that leucopenia in 55% of cats with FPV, lethargy in 77.5%, anorexia and intermittent vomiting in 88.5%, and fever in all cases [2]. In another study it was reported that 93.61% of the cats included in the patient groups had leukopenia, 82.97% had neutropenia, 93% had anorexia and lethargy, 72.72% had vomiting and 45.55% had diarrhoea [8]. In this study, when the symptoms of the cats with FPV in group 1 and group 2 were examined at the time they were brought to the clinic, anorexia was observed in 85.71%, lethargy in 64.28%, vomiting in 57.14% and diarrhoea in 50%. Clinical findings were found to be compatible with the literature.

Many secondary infections such as mouth ulcers, iritis, icterus, otitis are the most common findings associated with the decrease in the immunity of cats with panleukopenia and the decrease in leukocyte count [22]. Jaundice was observed in the mucous membranes of 1 cat in the 2nd group on the 3rd day of treatment. Mortality reaches up to 100% in the peracute form and 25-90% in the acute form [13]. As a result of the treatment, 1 cat each from group 1 and 2 died. The cat in group 1 died on the 4th day of treatment and the cat in group 2 died on the 3rd day. Mortality rate was 14.28% in both groups. There was no difference in mortality between the group treated with filgrastim and the group treated only with supportive treatment. On the other hand, it was reported in a study that the addition of Filgrastim to treatment significantly affected mortality [17]. Similarly to our results, it was reported that no significant effect on mortality was observed when Filgrastim was added to symptomatic treatment [8]. This significant result in the study conducted by Rice [17] is thought to be influenced by parameters such as the severity of clinical symptoms, the age of the animals and how long after the onset of the disease the treatment was started. Symptoms are usually observed 2-7 days after transmission [3]. Therefore, infection may develop later in animals newly introduced to public living areas or homes. Total leucocyte count is between 4,000-8,000/μL in subclinical cases. Leukocyte count can be 200 WBC/μL 4-6 days after infection. Neutrophil count loss may decrease up to 4,000 cells per day [22]. All cats (100%) in group 1 had WBC counts below the reference values in the complete blood count on day 0. The WBC count on day 0 of 7 cats in group 2 was found to be normal in 3 (42.85%), low in 3 (42.85%) and high in 1 (14.28%) of them compared to the reference value. All of the cats in the 3rd group (100%) were found to have WBC counts within the range of reference values. Leucocytes (4,000-6,000) can be reintroduced into the circulation on a daily basis. In this way, it can be up to 35,000 WBC/μL within 3-4 days [22]. Since it is not known on which day of the infection the patients were brought to our clinic, it is thought that WBC values may have been normal or high in some animals. It has been reported that there may be a rapid increase in neutrophil counts in cats with FPV treated with filgrastim [16]. Other studies have shown that there was no significant difference in clinical findings or neutrophil counts between treatment and control groups [11]. It has been reported that G-CSF injections may also increase blood neutrophil counts by accelerating the maturation of progenitor cells, but this effect requires at least 2 to 3 days to be measurable. The normal physiological duration of granulopoiesis in the bone marrow can be reduced from 3 to 5 days to 1 day [7]. It has been reported that the most beneficial effects of G-CSF can be achieved in Parvovirus-exposed animals if they can be treated before leukaemia develops. In animals dying with severe leukaopenia, very few granulopoietic progenitor cells with Human Granulocyte Colony Stimulating Factor (rhG-CSF) receptors were reported to survive and the bone marrow failed to respond to endogenous or synthetic exogenous G-CSF [11]. In this study, it was observed that the use of Filgrastim (Group 1) in cats with FPV produced statistically significant results on WBC ($P < 0.009$), lymphocytes ($P < 0.009$) and granulocytes ($P < 0.003$) between days 0 and 7.

This shows that the use of Filgrastim in addition to supportive therapy increases the number of lymphocytes and granulocytes [17]. Serum biochemistry parameters are not specific for FPV. Hypoalbuminaemia is the most common abnormality, probably due to decreased protein intake and leakage from mucosal lesions into the gastrointestinal tract [9]. rhG-CSF treatments in humans can cause slight changes in measured biochemical parameters [4]. However in a study it was reported that no biochemical changes were observed [11]. In the statistical study between the groups, AMY value was found to be $P < 0.05$ between the 1st and 3rd groups on the 0th and 3rd day and a statistically significant difference

was observed. ALT value was found to be $P < 0.05$ between the 1st and 3rd groups on the 3rd day and a statistically significant difference was observed. BUN value was found to be $P < 0.05$ between the 1st and 2nd groups on day 0 and a statistically significant difference was observed. CHOL value was found to be $P < 0.05$ between the 1st and 3rd group on day 0 and a statistically significant difference was observed. In addition, $P > 0.05$ was calculated in serum biochemical parameters on days 0, 3 and 7 and no significant difference was observed. Similar to the literature, no significant change was observed in biochemical parameters.

CONCLUSION

It was concluded that the use of Filgrastim in addition to supportive treatment had no effect on mortality. It was observed to increase WBC, lymphocyte and granulocyte counts and did not affect serum biochemistry values and is considered to be safely used without side effects. It was concluded that the stage of the disease and the state of the immune system should be taken into consideration before using an immunomodulator such as G-CSF.

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***Ethical approval*.** This study was approved by Aksaray University Animal Experiments Local Ethics Committee with the decision dated 12.09.2023 and the decision No. 40.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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