Evaluation of The Anti-Newcastle Disease Vaccine Serum (ANDVS) on Canine Distemper

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Original Article

Keywords: Dog, Distemper, Anti-Newcastle disease vaccine serum, RT-PCR, Distemper treatment

Posted Date: February 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-158117/v1

Version of Record: A version of this preprint was published at Veterinaria México OA on November 15th, 2022. See the published version at https://doi.org/10.22201/fmvz.24486760e.2022.1044.

Abstract

Distemper is a contagious, highly lethal, and almost incurable viral disease in dogs and other terrestrial carnivores. This study aimed to treat distemper in dogs with a novel method. Two healthy 10 and 12- month male dogs of mixed breed and weighed 31 and 33 Kg were prepared. A 1000-dose vial of Newcastle disease vaccine (Lasota strain) was then diluted in 6 ml normal saline and 3 ml of it was injected into the cephalic vein of each dog. Eleven hours later, the dogs were anesthetized and 20% of their blood volume was collected. Blood sera were separated and used as anti- Newcastle disease vaccine serum (ANDVS) to treat dogs suspected to have canine distemper. Dogs whose distemper was finally diagnosed by RT-PCR were grouped in the following seven treatment groups: 1- ANDVS; 2- ANDVS+ Cotrimoxazole; 3- ANDVS+ Penicillin-Gentamicin; 4- ANDVS+ Cefazolin-Amikacin; 5- Cotrimoxazole; 6- Penicillin-Gentamycin; 7- Cefazolin-Amikacin. Then the mortality rate of dogs, correlation between distemper and sex, breed, and age, and effects of distemper on hematological factors and vital signs were evaluated. The recovery rate in the ANDVS+ Cotrimoxazole and the ANDVS+ Cefazolin-Amikacin groups were higher than the ANDVS group ($P<0.05$). There was also a significant difference between the mortality rate in distemper positive and negative dogs. It is concluded that although ANDVS alone has no effect on the treatment of distemper, along with some antibiotics such as cotrimoxazole or cefazolin+ amikacin significantly improved the recovery rate compared to antibiotics used alone.

Introduction

Canine distemper (CD) is a common fatal disease caused by canine distemper virus (CDV) (Taylor et al. 1991). It is the second most deadly viral disease after rabies in dogs (Latha et al. 2007). CDV belongs to the Morbillivirus genus from Paramyxoviridae family (Greene 2012) and is antigenically related to human measles virus (HMV) (Hall et al. 1980; Taylor et al. 1991); with the difference that it has more tropism to the nervous system (Greene 2012) and causes encephalomyelitis in half of the dogs (Appel and Gillespie 1972; Mamaev et al. 1996).

Currently, there is not any specific antiviral treatment against CD (Latha et al. 2007) and only supportive therapy including fluid, antibiotic and corticosteroid therapy are available. Although the dog may recover with supportive treatment, the prognosis of nervous signs is poor, and the symptoms are usually irreversible, and euthanasia is often recommended. Even in the absence of neurological symptoms, the owner should be warned about complications that may affect the animal in the future (Greene 2012). As a result, it is necessary to find an effective treatment to reduce the death rate and its complications.

Dr. Sears, a private sector veterinarian in Lancaster, USA, used anti-Newcastle disease vaccine serum to treat distemper. In his method, the vaccine of Newcastle disease was injected into a donor dog, and 10 to 12 hours later the serum of the dog was prepared and subcutaneously injected into dogs with distemper. He found that this treatment was very effective and increased the recovery rate of CD even in dogs with neurological signs. In this method, the precise mechanism of treatment with anti-Newcastle disease vaccine serum is unclear. Certainly, antibodies do not play a role in the treatment because they do not seem to be produced in the donor dog within 10 to 12 hours after the injection of the vaccine instead it takes about two

weeks to appear in the blood. In this method, it is hypothesized that a series of unknown cytokines stimulate the immune system, triggering a rapid immune response and quickly destroying the distemper virus (Sears 2009). Despite various empirical clinical reports, there is no definite scientific report on the treatment of distemper by anti-Newcastle disease vaccine serum. Therefore, in the present study, the effects of anti-Newcastle disease vaccine serum on the treatment of distemper were investigated.

Materials And Methods

The study protocol was assessed by the Research Committee of the Faculty of Veterinary Medicine and approved by the Research Ethics Committee of Ferdowsi University of Mashhad (Approval ID: IR.UM.REC.1399.121). Two 10 and 12 months old mixed-breed dogs weighing 31 and 33 Kg without a history of vaccination were provided and dewormed with Praziquantel Forte (Alfasan, Holand) (Praziquantel, Pyrantel Pamoate, and Febantel, one tablet per 10kg). The dogs were kept in College Hospital for two weeks and their health status were checked. A 1000-dose vial of Newcastle disease vaccine (Lasota strain, Razi Institute; Karaj; Iran) was dissolved in 6 ml normal saline. Then, an IV catheter was placed in the cephalic vein of the dogs and 3 ml of the dissolved vaccine were injected into each dog. After 11 hours, the animals were anesthetized and 20% of their blood volume was extracted. Blood was poured into 10 ml tubes without anticoagulant and centrifuged after clotting. Sera were separated and frozen in glass tubes in plastic bags. These sera were then tested for the treatment of dogs affected by distemper referred to the Veterinary Teaching Hospital.

The treatment protocol was explained to the animal's owners and was started with their consent. A detailed history of each dog, including previous vaccination and antiparasitic treatments were recorded and treatment was performed only on dogs not receiving the distemper vaccine. After observation and detection of distemper symptoms (gastrointestinal, respiratory, cutaneous, neurological and systemic), 2 ml of blood samples were obtained from each dog for hematological evaluation; and 6 ml of blood was obtained and stored in EDTA-treated tubes at minus 80°C for definitive diagnosis of distemper by RT-PCR. The conjunctival swaps were also obtained for early diagnosis using rapid kit tests (Anigen Rapid CDV Ag Test Kit, Korea). In this study, all statistical evaluations were performed based on the RT-PCR positive results.

For RT-PCR in Briefly, RNA was extracted from whole blood by blood RNA isolation kit (–– –, —, –) according to the manufacturer's instructions. For negative control, we used DEPC- treated water. The RNA quantity and quality were analyzed by spectrophotometer (Thermo Scientific NanoDrop) and electrophoresis in 1% agarose gel using loading buffers 6x (DENAzist, Mashhad, Iran) and stained with DNA green viewer (Pars Tous Biotechnology, Mashhad, Iran).

Immediately after RNA extraction, the cDNA was synthesized using Parstous DNA synthesis kit (–, –) according to the manufacturer's instruction.

PCR was performed using oligonucleotides primer pair I, II, III and housekeeping primer (GAPDH) for amplification of the CDV nucleoprotein (NP) gene sequences (Frisk et al. 1999) synthesized by Humanizing Genomic Macrogen, South Korea. PCR was performed with negative (DEPC treated water) and positive (a sample whose PCR products were sequenced, Bioneer, South Korea) controls. The PCR products were

analyzed by electrophoresis in 1.5% agarose gel in 1x TBE buffer after staining with the green viewer and visualizing under UV light.

Dogs with all of the following conditions were included in the design. 1. Dogs which had at least two symptoms of distemper. 2. Dogs whose RT-PCR tests were positive 3. Dogs that had not received any antibiotics before the visit. 4. Dogs that completed the course of treatment. The dogs were randomly (according to the order of the groups) divided into seven groups. 1) Anti-Newcastle disease vaccine serum (ANDVS), 2) ANDVS + Cotrimoxazole (ANDVS + CoTrim), 3) ANDVS + Penicillin- Gentamicin (ANDVS + PG), 4) ANDVS + Cefazolin-Amikacin (ANDVS + CA), 5) Cotrimoxazole (CoTrim), 6) Penicillin- Gentamicin (PG), 7) Cefazolin-Amikacin (CA). The amount of serum in the ANDVS- receiving groups was 1ml/dog + 2.2ml/10kg BW subcutaneously q12h for three times (Sears 2009). Therefore, for example, a 10 kg dog received 3.2 ml of anti-Newcastle disease vaccine serum each time. The dose of cotrimoxazole (Co-Trimoxazole 24%, Kela Laboratoria N.V., Hoogstraten- Belgium) was 15–30 mg/kg intramuscularly every 12 hours, cefazolin (Cefzolix, Jaber ibn Hayyan, Tehran- Iran) 20–30 mg/kg intravenously or intramuscularly every 8 hours, amikacin (Ipacin, Caspian Tamin, Tehran- Iran) 15–30 mg/kg intravenously or intramuscularly every 24 hours, penicillin (Pen 800 Vial, Jaber ibn Hayyan, Tehran- Iran) 20000–40000 IU/kg intramuscularly every 24 hours, and gentamicin (Gentadic, Caspian Tamin, Tehran- Iran) 6–8 mg/kg intravenously or intramuscularly every 24 hours. The antibiotics were administered for at least one week. If it was necessary, the dogs of all groups were also treated with supportive treatments (fluid therapy, B complex, antiemetics). Finally, with appropriate follow-up, the rate of total recovery (and mortality) of dogs, as well as the effects of distemper on hematological factors and vital signs and correlation of distemper with sex, breed, and age were evaluated. Improvement in symptom was recorded daily until the end of the medication period. Also, one and three months after treatment, the condition of the dogs was monitored for recurrence of the symptoms or becoming a nervous form.

Statistical analysis

The total recovery (and mortality) rate of CD was reported as frequency rate. The efficacy of each drug groups and comparison between treatment groups, correlation of distemper with sex, age and breeds of dogs (in breeds that contain at least 3 dogs) evaluated by Chi-square method and comparison of hematological indices and vital signs between groups evaluated by one way ANOVA. A level of 0.05% was considered significant.

Results

The number of dogs, and recovery and fatalities rate in each group are presented in Table 1. 120 dogs suspected of having distemper were participated in this study. 60 out of 120 dogs recovered and 60 dogs died. RT-PCR was performed on 113 dogs, and was positive in 66 dogs. Out of 66 dogs, 22 (33.3%) recovered and 44 dogs (66.7%) died (Table 1). Chi-square comparison showed that there was a significant difference between mortality (and recovery) rate of distemper positive and negative dogs confirmed by RT-PCR ($p < 0.0001$).

Table 1. The number of dogs recovered and died in treated groups

RT-PCR			Groups							Total
			ANDVS	ANDVS + CoTrim	ANDVS + PG	ANDVS + CA	CoTrim	PG	CA	
Positive	Outcome	Live	0	8	3	5	2	2	2	22
		Dead	8	5	9	5	7	5	5	44
	Total		8	13	12	10	9	7	7	66
Negative	Outcome	Live	0	0	1	3	6	8	15	33
		Dead	3	2	1	1	3	0	4	14
	Total		3	2	2	4	9	8	19	47
Total	Outcome	Live	0	8	4	8	8	10	17	55
		Dead	11	7	10	6	10	5	9	58
	Total		11	15	14	14	18	15	26	113

ANDVS, Anti-Newcastle disease vaccine serum; CoTrim, Cotrimoxazole; PG, Penicillin- Gentamicin; CA, Cefazolin-Amikacin.

The results also showed a significant difference in the mortality rate of distemper positive dogs among treatment groups (P = 0.042). Paired comparison between treatment groups showed that the mortality rate in group 1 (ANDVS) was higher than that of ANDVS+CoTrim (P = 0.007) and ANDVS+CA (P = 0.036) groups. None of the distemper positive dogs in the ANDVS group (Group 1) recovered. On the other hand, 8 out of 13 dogs in group 2 (ANDVS+CoTrim) recovered (61.5%), which is significantly higher than dogs in group 1 that received only ANDVS (P = 0.007). However, although the recovery rate in dogs treated with ANDVS+CoTrim (group 2) was higher than that of dogs treated with CoTrim (group 5), this difference was not significant but approached the borderline of significance (P = 0.063). In the other groups that received a combination of antibiotics and ANDVS, in group 4 (ANDVS+CA), the recovery rate was 50% (5 out of 10), which is higher than that of CA (28.6%) group. In group 3 (ANDVS+PG) the recovery rate was 25%, which is higher than that of group 1 (0%) but does not differ from group 6 (PG) which has improved by 28.6% (Fig 1).

These results revealed that dogs that received ANDVS with cotrimoxazole showed the greatest improvement (61.5%), followed by dogs received ANDVS with cefazolin-amikacin (50%). These results also showed that the mortality rate of distemper in dogs confirmed by RT-PCR is high (44 out of 66 which is equivalent to 66.7%).

Of the 66 cases whose distemper were confirmed, 39 (59.1%) were under six months old, 20 (30.3%) were between 6 and 12 months, and 7 (10.6%) were between 1 and 5 years old. 27 out of 39 (69.2%) dogs with distemper who were less than 6 months old died. In dogs between 6 and 12 months, 11 dogs out of 20 (55%)

and in dogs between 1 to 5 years, six out of 7 (85.7%) died. There was no significant difference in mortality rates between different ages (Table 2).

Table 2. The effect of distemper on different ages of dogs

RT-PCR			age (Month)				Total
			<6	6-12	12-60	>60	
positive	outcome	live	12	9	1		22
		Dead	27	11	6		44
	Total		39	20	7		66
negative	outcome	live	15	4	13	1	33
		Dead	11	2	1	0	14
	Total		26	6	14	1	47
Total	outcome	live	27	13	14	1	55
		Dead	38	13	7	0	58
	Total		65	26	21	1	113

Of the 66 dogs whose RT-PCR were positive, the sex of one dog was unknown. Of the remaining 65 cases, 36 (55.4%) were female and 29 (44.6%) were male (Table 3). 15 out of 36 female dogs recovered (41.7%), while the recovery rate of male dogs was 24.1% (7 out of 29). Although the recovery rate among female dogs was almost twice that of males, this difference was not statistically significant ($p = 0.138$).

Of the 66 dogs whose distemper was positive, the breed of one dog was not recorded. The remaining 65 dogs were of 11 breeds. Of these, only Afghan (a local breed), German Shepherd, Husky, Mixed, Spitz and Terrier breeds had more than three patients and were statistically analyzed (Table 4). The results showed a significant difference in mortality (and recovery) rate between breeds ($p = 0.030$). Accordingly, the number of distemper-related deaths in the German Shepherd breed was lower than other breeds and the highest mortality rate was in the Afghan and Spitz breeds, in which all six infected dogs died.

Table 3: The distemper positivity in male and female dogs

		Sex (%)		Total
		Female	Male	
Outcome	Live	15 (41.7)	7 (24.1)	22
	Dead	21 (58.3)	22 (75.9)	43
	Total	36 (55.4)	29 (44.6)	65

Table 4: The mortality (and recovery) rate of different breeds in distemper positive dogs

RT-PCR			breeds						
			Afghan	German Shepherd	Husky	Mixed	Spitz	Terrier	Total
positive	outcome	live	0	2	1	14	0	2	19
		dead	6	1	3	21	6	2	39
	Total		6	3	4	35	6	4	58

Mean heart rate, respiratory rate, body temperature, red blood cells and white blood cells are listed in Table 5. Hematocrit (28.83 ± 6.98) and RBC count (4.80 ± 1.05) decreased significantly in distemper positive dogs compared to distemper negative dogs (P = 0.012 and P = 0.015, respectively). Other hematological indices and vital signs were not significantly different between distemper positive and distemper negative dogs (P> 0.05).

Of the 60 cases whose RT-PCR were positive, rapid kit test was positive in 51 (85%) dogs. On the other hand, out of 36 cases in which RT-PCR was negative, rapid kit tests were negative in 29 (81%) cases. Therefore, the sensitivity and specificity of rapid kit test were 85% and 81%, respectively. Thus, there is a strong significant relationship between rapid kit test and RT-PCR (P <0.0001, Phi test = 0.649).

Table 5: Mean ± standard deviation of vital signs and hematological factors in distemper positive and negative dogs tested with RT-PCR.

Factors	RT-PCR	N	Mean	Std. Deviation	P value
Heart rate	positive	53	111.23	28.89	.057
	negative	39	126.59	42.81	
Respiratory rate	positive	46	42.50	20.33	.968
	negative	30	42.30	21.17	
Temperature	positive	58	38.86	1.51	.561
	negative	44	38.71	1.06	
Hematocrit	positive	60	28.83	6.98	.012
	negative	44	32.72	8.53	
RBC	positive	60	4.80	1.05	.015
	negative	44	5.37	1.29	
WBC	positive	60	12581.67	9045.69	.413
	negative	44	14134.09	10114.53	
neutrophil	positive	59	10515.36	7767.19	.607
	negative	44	11389.25	9382.73	
lymphocyte	positive	56	1210.11	1499.69	.302
	negative	43	1499.21	1186.68	
monocyte	positive	56	973.93	1068.85	.538
	negative	43	853.93	785.41	
Band cell	positive	26	416.38	379.99	.888
	negative	17	399.06	407.33	

Discussion

Distemper is a fatal disease that is very difficult to treat. Many efforts have been made to treat distemper, but little success has been achieved. Dal Pozzo et al. (2010) investigated the antiviral effect of EICAR (a ribavirin analogue) against the canine distemper virus in vitro. They have claimed that the use of EICAR for treatment is inconsistent, but this study provides new insights into the use of EICAR against CDV as an antivirus (Dal Pozzo et al. 2010).

Bogdanchikova *et al.* (2016) examined the silver nanoparticles (which block viral attachment to the host cell) in the treatment of distemper in dogs and showed that silver nanoparticles are highly effective in the treatment of the non-neural form of distemper and provides up to 90% recovery without complications; however, it has very little effect in treating the neurological form of the disease (Bogdanchikova et al. 2016).

Teixeira et al. (2009) using the essence of SAINT GERMAIN as a supplement to supportive treatment studied the recovery rate of canine distemper in Brazil and showed that this formulation was very effective in treating distemper. One week after treatment, the nervous symptoms of 10 dogs improved so that they could walk without assistance (Teixeira et al. 2009).

Liu et al. (2016) used the porcine anti-canine distemper virus IgG and F(ab') 2 antibody fragments as passive immunotherapy to treat infected puppies exhibiting respiratory signs without neurological signs of distemper and showed that 76% of pups (19 out of 25) survived. However, 8 of 25 (32%) pups showed neurological symptoms during treatment (Liu et al. 2016).

Dr. Sears, a private sector veterinarian in Lancaster, USA, based on his personal experience has stated that anti-Newcastle disease vaccine serum (ANDVS) produced in dogs can improve dogs with and without nervous signs of distemper (Sears 2009; edbondny 2016); However, to our knowledge, there is still no scientific report on the effect of ANDVS on CD in dogs.

The results of the present study showed that ANDVS (group 1) alone has no effect in the treatment of dogs with distemper, because all puppies treated with this serum died. However, ANDVS in combination with cotrimoxazole caused a 61.5% improvement in distemper-positive dogs, whereas only 22.2% of dogs treated with cotrimoxazole alone (group 5) recovered. These results suggest that although ANDVS alone may not be effective in treating distemper, in combination with some antibiotics it may increase their therapeutic effects. This synergistic increase was also seen in the combination of ANDVS with cefazolin-amikacin (group 4). Because the cefazolin-amikacin (group 7) caused a 28.6% improvement, while the combination of ANDVS with cefazolin-amikacin resulted in a 50% improvement in dogs. Moreover, in dogs receiving penicillin-gentamicin, the ANDVS did not increase the recovery rate of these dogs. Since these differences are not significant, it is impossible to judge the healing effect of the ANDVS. One of the weak points of this study is that although a relatively large number of dogs with distemper have been tested and treated, the number of dogs in each group is low due to the large number of groups. Hence, few cases in each group affected the final result. However, this result could give researchers insight that in the future they will work on more dogs to find a better approach.

The results suggested that the mortality rate of distemper in this study is high (66.7%). There are many reports on the prevalence of the distemper from different countries (Ek-Kommonen et al. 1997; Avizeh et al. 2007; Dezengrini et al. 2007; Mosallanejad et al. 2010; Namroodi et al. 2013; Martinez-Gutierrez and Ruiz-Saenz 2016). In most of these reports, the prevalence of the disease has been examined by serological methods, but there are few reports that show the exact mortality rate of distemper in dogs. A study on necropsy cases reported that 11.7% of necropsy patients were due to distemper (Headley and Graça 2000). In a study, the mortality rate of distemper in vaccinated dogs was estimated at 30 percent. In another study, distemper losses were reported to be up to 50% (Beineke et al. 2009; Greene 2012). In the present study, a relatively high mortality rate was observed in treated dogs (66.7). This suggests that the death of untreated dogs may be much higher. In the present study, in group 1, which was treated with anti-Newcastle disease vaccine serum, despite supportive therapies, the mortality rate due to not using appropriate antibiotics was up to 100%. This also highlights the importance of secondary bacterial infections in increasing the mortality

rate of disease. The distemper virus may cause immune suppression, creating the opportunity for the growth of bacteria and the deaths caused by them (Ek-Kommonen et al. 1997; Blancou 2004).

In the present study, 59% of patients with the definitive diagnosis of distemper were under six months old. However, there was no significant difference in mortality rate at different ages. This indicates that there is a high risk of death for dogs of all ages. The cause of most infections at young ages is due to the lack of development of the immune system in these dogs (Appel and Summers 1995; Martella et al. 2008).

In this study, due to the distribution of dogs in 11 breeds, we were not able to properly investigate the effects of breed on distemper deaths because there was only one infected dog in some breeds so that these breeds were excluded from statistical comparison and only breeds with more than 3 patients were participated in statistical analysis. Moreover, in the case of these breeds, the total number of dogs was few. Other studies have reported that brachiocephalic dogs are generally more resistant and less likely to develop distemper (Latha et al. 2007). In our study opposite was true as German Shepherd breed was more resistant, which is a dolichocephalic dog. It should be noted that the Afghan breed, which is a strong, large and a local dog, is more sensitive to distemper.

In the present study, although most hematological factors were decreased in distemper positive dogs, only hematocrit and total red blood cell count were significantly reduced compared to distemper negative dogs. The effect of distemper on different hematological factors has been reported in various studies. Most studies have suggested that distemper decreases lymphocytes (Appel and Summers 1995; Larson and Schultz 2006; Greene 2012), but in the present study, the decrease in lymphocytes was not statistically significant. One reason may be the wide range of lymphocytes in dogs with distemper, so that in some distemper positive dogs the total number of leukocytes increases and consequently the number of lymphocytes also increases; In contrast, in some dogs with distemper, the total leukocyte count decreases and subsequently lymphocyte count decreases. When the values of these dogs are averaged, they neutralize each other's effects to some extent.

In the present study, a high correlation was found between RT-PCR and rapid conjunctival kits ($P< 0.0001$) and this correlation was relatively strong (Phi test = 0.649). The sensitivity and specificity of conjunctival kits compared to RT-PCR were 85% and 81%, respectively. These results showed that CD can be diagnosed in many patients (85% of cases) referred to the clinic by performing a conjunctival kit, which is 81% correct if the test is negative. An *et al.* (2008) also reported that the sensitivity and specificity of rapid kits are 100% (An et al. 2008). Since the rapid diagnosis of canine distemper can lead to better treatment and prevention of the disease, the use of these kits for rapid diagnosis is very important.

It is concluded that the mortality rate of distemper in RT-PCR confirmed dogs is high (66.7%), and that although ANDVS alone has no effect on the treatment of distemper, along with some antibiotics such as cotrimoxazole or cefazolin+ amikacin significantly improved the recovery rate compared to antibiotics used alone.

Declarations

Funding The research council of Ferdowsi University of Mashhad provided financial support in the form of Research Project No. 47896

Conflict of Interest No conflicts of interest have been declared

Ethics approval The study protocol was assessed by the Research Committee of the Faculty of Veterinary Medicine and approved by the Research Ethics Committee of Ferdowsi University of Mashhad (Approval ID: IR.UM.REC.1399.121).

Consent to participate The treatment protocol was explained to the animal's owners and was started with their consent

Consent to Publish Not applicable

Authors' contributions

Conceptualization: Ali Asghar Sarchahi.

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Supervision: Ali Asghar Sarchahi

Availability of data and materials All data generated or analyzed during this study are included in this published article.

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Figures

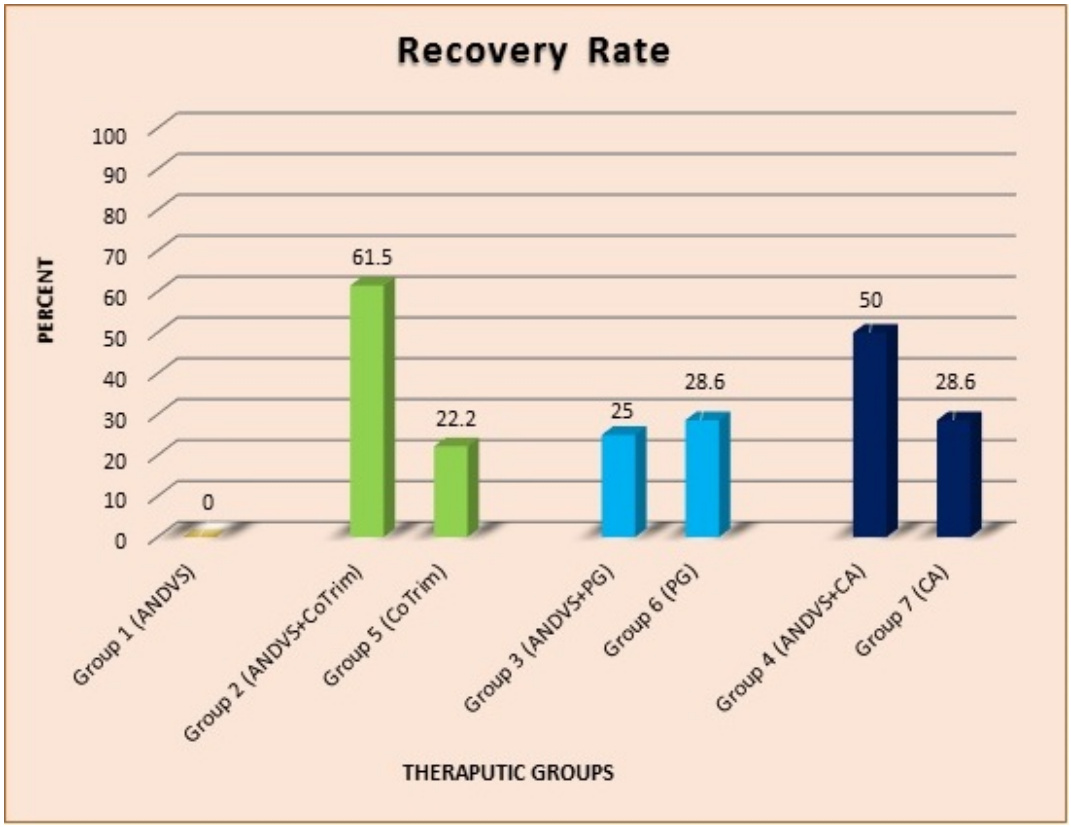

Figure 1

The recovery rate of different treatment groups. The recovery rate was significant between groups 1 and 2 (P= 0.007), and 1 and 4 (P= 0.036)