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The status of pro-inflammatory cytokines TNF-α and IL-6 in the cerebrospinal fluid of dogs with nervous distemper

Marjan Mahmoodabadi[1], Hadi Mohebalian[2*] and Ali Asghar Sarchahi[3]

Abstract

Background Canine distemper virus (CDV) is a multisystemic disease in dogs that causes severe neurological signs due to viral replication in neurons and glial cells; this leads to gray matter lesions and demyelination. The resulting inflammation causes additional harm to the affected tissues. This study aimed to investigate the relationship between pro-inflammatory cytokines Tumor Necrosis Factor (TNF-α) and interleukin (IL-6) levels in Cerebrospinal fluid (CSF) and the neurological form of distemper disease in dogs.

Results The study included 23 CDV-infected dogs with neurological signs, 10 diseased but CDV-negative (non-infected) dogs, and 12 healthy controls. CSF samples were analyzed using Reverse Transcription Polymerase Chain Reaction (RT-PCR) for CDV sequences and the sandwich enzyme-linked immunosorbent assay (ELISA) method for quantifying IL-6 and TNF-α levels. While a significant increase in CSF cytokine levels was observed between the CDV-infected and healthy controls, no significant differences were detected between the CDV-infected and non-infected diseased groups.

Conclusions The elevated levels of IL-6 and TNF-α in the CSF of dogs with nervous distemper suggest their involvement in disease pathogenesis. However, their lack of specificity limits their use as reliable diagnostic biomarkers for distinguishing nervous distemper from other neurological conditions in dogs. Further research is needed to identify more accurate and specific biomarkers for the precise diagnosis and effective management of CDV infection.

Keywords Distemper, Neurological signs, Cerebrospinal fluids, Pro-inflammatory cytokines

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Introduction

Canine distemper is a viral disease caused by the canine distemper virus, a member of the *morbillivirus* genus within the *Paramyxoviridae* family [1]. CDV and other morbilliviruses, such as Measles virus (MeV) and Rinderpest virus (RPV), specifically target the lymphatic system and strongly suppress the immune system. Infection with CDV leads to a prolonged disruption of immune function, resulting in immunosuppression, loss of lymphocytes, reduced white blood cell count, and heightened vulnerability to opportunistic infection [2]. CDV is a virus with a protective outer layer and a negative-sense single-strand RNA; it is composed of six structural proteins; the nucleocapsid (N), the phosphoprotein (P), the large (L), the matrix (M), the hemagglutinin (H), and the fusion (F) protein [3–5].

CDV spreads rapidly in lymphoid tissues by selectively binding to the membrane glycoprotein SLAM (signaling lymphocyte activation molecule) through its F and H proteins [3–5]. The H protein of CDV interacts with one or multiple receptors, facilitating cellular attachment and initiating the activation of the F protein by tissue-specific proteases, ultimately resulting in cellular infection [6, 7]. CDV infects resident T-cells and B-cells via SLAM, resulting in viral replication and primary viremia [8]. Transmission of CDV primarily occurs by aerosols and affects the upper respiratory tract. Within the initial 24 h after infection, the virus multiplies in macrophages and circulating B and T cells. Subsequently, viral particles travel through the lymphatic pathway to the bronchial lymph nodes and tonsils [9, 10]. CDV then spreads across various epithelial tissues and the central nervous system (CNS) via the bloodstream or CSF [10]. During acute CDV infection, the upregulation of SLAM in immune cells within the CNS facilitates viral replication in the brain. The virus can enter the CSF and spread to various brain structures, such as optic fibers and nerves, rostral medullary velum, cerebral peduncles, and spinal cord, either as a free virus or associated with lymphocytes. This results in lesions in these areas, contributing to the disease's pathogenesis [3–5]. The virus can infect several types of cells, including epithelial, lymphoid, and neural cells. As a result, it causes a widespread infection involving the respiratory, gastrointestinal, urinary, lymphatic, cutaneous, skeletal, and central nervous systems [11, 12]. During the early and late convalescent phases of CDV, affected dogs often show a higher frequency of respiratory, gastrointestinal, and neurological signs. The most commonly observed clinical signs associated with canine distemper include fever, coughing, ocular-nasal discharge, diarrhea, lymphopenia, cutaneous hyperplasia, tremors, and paralysis [13]. Neurological signs vary and may include hyperesthesia, ataxia, seizures, myoclonus, cerebellar and vestibular diseases, paraparesis, and tetraparesis [11]. The differences in neurological signs seen in dogs may be linked to the infecting viral strains [14].

Dogs with CDV infection exhibited expression of both pro- and anti-inflammatory cytokines in the brain, including IL-1β, IL-6, IL-12, and TNF-α. This expression may be directly induced by the virus or triggered via autocrine and paracrine immune signaling [15–17]. Notably, increased IL-6, TNF-α, and IL-1 levels correlate with viral replication and may contribute to pathogenesis through cytokine-mediated mechanisms [18]. TNF-α is mainly produced by astrocytes and appears to have a vital function in the development of early demyelination, as evidenced in the case of multiple sclerosis [19]. While there is no apparent link between cytokine mRNA transcripts and respiratory or gastrointestinal symptoms, central nervous system demyelination is often accompanied by elevated levels of IL-1, IL-12, TNF-α, and transforming growth factor (TGF)-β in the bloodstream of dogs with natural CDV infection [16]. TNF-α and IL-6 mRNA, which are linked to illness exacerbation, as well as IL-10 and TGF-mRNA, which indicate remission, are frequently found together in the CSF of spontaneously infected dogs [20].

Canine Distemper virus is a worldwide enzootic disease that is difficult to diagnose early due to the variety of clinical signs, with very high morbidity and mortality, especially in unvaccinated animals [21]. Although routine laboratory assessments and clinical findings can provide valuable insights for an initial diagnosis of CDV, they are insufficient for confirming the disease [22]. Reverse transcription polymerase chain reaction (RT-PCR) has been used to detect the presence of CDV sequences in whole blood, buffy coat cells, serum, and CSF in dogs with systemic or nervous distemper [23]. Among these, CSF samples demonstrate higher sensitivity for detecting CDV than mucosal swabs or whole blood samples, using both rapid and RT-PCR assays [22]. The conventional belief that the CNS is immune-privileged is currently being challenged. This is because CNS disease often leads to molecular and morphological responses and interactions among different resident CNS cells in response to tissue stress and injury [24, 25]. Within this framework, cytokines, which are extremely powerful immune agents, play a crucial role in mediating the processes of immunology and immunopathology in different CNS disorders [26]. Accurate diagnosis is crucial for effective treatment and to avoid misdiagnosis. This study aimed to assess the pro-inflammatory cytokines TNF-α and IL-6 levels in CSF samples from dogs with confirmed CDV infection. Cytokine levels were measured using sandwich ELISA to evaluate their potential role as distinguishing markers in neurological distemper.

Table 1 Demographic and clinical characteristics of suspected CDV-infected dogs, including age, sex, breed, vaccination status, origin, laboratory findings, clinical signs, outcome, and detection of CDV by rapid test kits and RT-PCR (from CSF samples)

Case No.	Age(months)	Sex	Breed	Vaccinated	Origin	Clinical findings & signs onset to referral to the clinic (days)		Major laboratory findings	RT-PCR assay	IC assay		Outcome
						Systemic	Neurologic		CSF samples	CSF samples	Mucosal	
960,278	5	Female	Mixed	No	Stray	Yes (data unavailable)	Yes (data unavailable)	Neutrophilia-monocytosis-lymphocytosis-regenerative anemia	+	+	–	Dead after 2 days
960,336	24	Female	German shepherd	Yes	Private owner	Yes (30)	Yes (5)	Lymphopenia-mild regenerative anemia	+	+	–	Euthanasia after 14 d
960,504	5	Male	Mixed	No	Private owner	No	Yes (30)	Mild regenerative anemia	+	+	–	Owner unresponsive
961,006	4.5	Male	Mixed	No	Shelter	Yes (data unavailable)	Yes (data unavailable)	Data unavailable	+	+	–	Euthanasia
961,012	2.5	Female	Husky	No	Private owner	Yes (17)	Yes (2)	Lymphopenia-moderate degenerative anemia	+	+	+	Owner unresponsive
970,597	12	Female	Mixed	No	Private owner	Yes (360)	Yes (360)	Lymphopenia-mild regenerative anemia	+	+	–	Live
961,155	8	Female	German	No	Private owner	No	Yes (2)	Normal	+	+	–	Live
970,945	5	Male	Mixed	No	Private owner	Yes (45)	Yes (data unavailable)	Data unavailable	+	+	–	Dead after 17 days
970,953	6.5	Male	Sarabi	No	Private owner	Yes (4)	Yes (4)	Lymphopenia-mild regenerative anemia	+	+	+	Dead after 10 days
980,012	–	Male	Mixed	No	Private owner	Yes (60)	Yes (60)	Degenerative anemia, lymphopenia	+	–	–	Owner unresponsive
960,276	36	Female	German shepherd	No	Private owner	No	Yes (30)	Mild regenerative anemia, monocytopenia	+	+	–	Dead after 14 days
960,495	5	Male	Mixed	Data unavailable	Private owner	No	Yes (30)	Degenerative anemia	+	+	–	Live
4	96	Female	German shepherd	No	Shelter	No	Yes (360)	Mild regenerative anemia, lymphocytosis, neutrophilia, monocytosis, thrombocytosis	+	–	–	Dead after 60 days
960,258	4.5	Male	Mixed	No	Private owner	Yes (10)	Yes (4)	Data unavailable	+	+	+	Live
960,599	8	Female	Mixed	No	Shelter	No	Yes (45)	Mild regenerative anemia, lymphopenia	+	+	–	Dead after 97 days
961,153	48	Male	Terrier	No	Private owner	Yes (60)	Yes (60)	Regenerative anemia, lymphopenia, thrombocytosis	+	+	–	Live
1208	2.5	Male	Mixed	Data unavailable	Stray	Yes (data unavailable)	Yes (data unavailable)	Data unavailable	+	+	Not evaluated	Euthanasia
970,519	8	Female	Mixed	Yes	Private owner	No	Yes (7)	Lymphopenia-monocytosis, mild degenerative anemia	+	+	–	Euthanasia
971,087	4.5	Male	Mixed	No	Shelter	No	Yes (30)	Mild regenerative anemia	+	+	–	Euthanasia

Table 1 (continued)

Case No.	Age(months)	Sex	Breed	Vaccinated	Origin	Clinical findings & signs onset to referral to the clinic (days)		Major laboratory findings	RT-PCR assay	IC assay		Outcome
						Systemic	Neurologic		CSF samples	CSF samples	Mucosal	
82.2	4	Female	Spitz	No	Private owner	Yes (14)	Yes (data unavailable)	Data unavailable	+	+	–	Owner unresponsive
980,127	48	Male	Mixed	No	Private owner	Yes (14)	Yes (2)	Lymphopenia, severe regenerative anemia	+	–	–	Owner unresponsive
971,158	12	Male	Mixed	No	Private owner	Yes (14)	Yes (14)	Mild regenerative anemia	+	+	–	Live
1209	7	Female	German Shepherd	Yes	Private owner	Yes (20)	Yes (13)	Lymphopenia, mild regenerative anemia	+	–	–	Dead after 3 days

Materials and methods

Study design and subject

A case-control study was conducted at the veterinary hospital of Ferdowsi University of Mashhad in Iran. The study protocol was assessed by the Faculty of Veterinary Medicine research committee and approved by the Research Ethics Committee of Ferdowsi University of Mashhad (Approval ID: IR.UM.REC.1399.120). The study included 45 dogs: 23 CDV-infected with neurological signs (study group), 10 non-infected diseased dogs (control group 1), and 12 healthy dogs (control group 2) admitted to the Veterinary Teaching Hospital. A comprehensive history was collected from the owners, which included information on recent vaccinations, living conditions, and the onset date of symptoms. Additionally, a complete physical examination was conducted on the dogs, and their data are presented in Table 1. Efforts were made to rule out other diseases that could present similar clinical signs to canine distemper virus (CDV); this was accomplished through detailed histories, assessment of clinical signs, physical examinations, monitoring of disease progression, hematological evaluations, and the use of Rapid IC antigen test kits. Consent was then obtained from the owners, and samples were collected following routine hospital procedures. Dogs were anesthetized with 10% ketamine hydrochloride and 2% Acepromazine intravenously before sample collection. The area between the occipital bone and atlas wings was shaved, cleaned, disinfected, and prepared for CSF collection. Under anesthesia, CSF samples were collected from all dogs following standard procedures. A 1 ml aliquot of CSF was stored at -80 °C for further analysis.

RT-PCR

CDV infection was confirmed using reverse RT-PCR on the CSF samples. An RNA extraction kit (GeneAll Biotechnology Co, Ltd, Seoul, Korea) was used according to the manufacturer's instructions. RNA was converted into cDNA with AccuPower® CycleScript RT PreMix (Bioneer, Daejeon, South Korea), using 20 µL of CSF RNA according to the manufacturer's instructions. Oligonucleotide primer pairs (PP-I, PP-II, and PP-III) were used to amplify specific CDV nucleoprotein (NP) gene regions, producing amplicons of 286, 259, and 899 bp in length, respectively. A separate primer pair was used to amplify the housekeeping (GAPDH) gene sequence [27]. Although the primers and PCR protocol were based on the method described in the reference, minor modifications were made to the cycling conditions to improve amplification efficiency: initial denaturation at 94 °C for 1 min, 37 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 2 min, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 5 min. PCR products were analyzed by electrophoresis on a

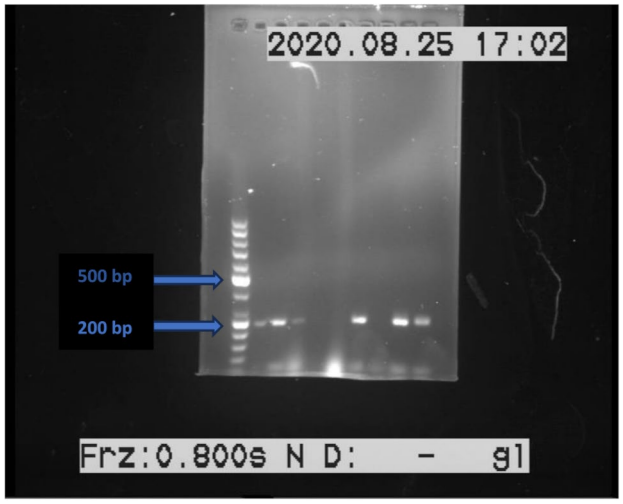

Fig. 1 RT-PCR analysis of CSF samples from CDV-infected dogs. Amplification of the CDV nucleoprotein (NP) gene is shown, with visible bands between 200–220 base pairs, lanes from left to right: 960,336, 980,258, 971,158, 980,276, 961,155, 960,278. GAPDH was used as a housekeeping gene for normalization. PCR products were separated on a 1.5% agarose gel and visualized under UV light

1.5% agarose gel in 1× TBE buffer (pH 8.4), stained with DNA green viewer (Pars Tous Biotechnology, Mashhad, Iran), and visualized under UV light after running at 70 volts for 90 min.

Measurement of IL-6 and TNF-α in CSF samples

A commercial sandwich ELISA kit (Bioassay Technology Company) was used to measure IL-6 and TNF-α levels in the CSF samples. The assay was performed according to the manufacturer's instructions, with standard and blank samples run in triplicate. The standard curve was generated using the concentration standards and their corresponding optical density (OD) values. An ELISA reader was employed to obtain absorbance values, which were then used to determine concentrations based on the standard curve generated from the ELISA assay.

Statistical analysis

Cytokine levels were compared among groups using a one-way analysis of variance (ANOVA). Post-hoc comparisons were conducted to identify differences between specific groups.

Results

In the current study, cerebrospinal fluid (CSF) samples from 23 dogs with confirmed neurological signs of CDV were analyzed using RT-PCR. CDV RNA was detected in all infected dogs, confirming the presence of the virus in the study group. In contrast, no amplification of CDV RNA was observed in either the 10 non-infected (diseased) or 12 healthy control groups. Figure 1 shows the RT-PCR analysis of CSF samples, with visible amplicon bands corresponding to the CDV nucleoprotein gene in the infected samples.

The current study investigated the levels of pro-inflammatory cytokines TNF-α and IL-6 in the CSF of CDV-infected, non-infected, and healthy dogs. A total of 45 dogs were included: 23 CDV-infected dogs with neurological signs, 10 non-infected but diseased dogs (control group 1), and 12 healthy dogs (control group 2).

TNF-α levels in the CSF were significantly higher in both CDV-infected dogs (mean: 17.6 pg/mL) and non-infected diseased dogs (mean: 16.9 pg/mL) compared to healthy controls ($P < 0.001$ for both comparisons), as shown in Table 2. However, no statistically significant

Table 2 Compares TNF-α and IL-6 in the studied groups. Values are expressed as mean±sem

Multiple comparison							
Dependent variable	**(I)group**	**(J) group**	**Mean Difference (I-J)**	**Std. Error**	**Sig**	**95% Confidence interval**	
						Lower Bound	**Upper Bound**
TNF-α	CDV-infected group	Control group (healthy)	14.48116	2.27728	0.000	8.8024	20.1599
		Non-infected group	0.68783	2.42231	1.000	-5.3526	6.7283
	Control group (healthy)	CDV-infected group	-14.48116	2.27728	0.000	-20.1599	-8.8024
		Non-infected group	-13.79333	2.73816	0.000	-20.6214	-6.9653
	Non-infected group	CDV-infected group	−0.68783	3.11695	1.000	-6.7283	5.3526
		Control group (healthy)	13.79333	2.79753	0.000	6.9653	20.6214
IL-6	CDV-infected group	Control group (healthy)	1.8225	0.67331	0.029	0.1435	3.5015
		Non-infected group	-1.0809	0.71619	0.416	-2.8668	0.7051
	Control group (healthy)	CDV-infected group	-1.8225	0.67331	0.029	-3.5015	−0.1435
		Non-infected group	-2.9033	0.80957	0.003	-4.9221	−0.8845
	Non-infected group	CDV-infected group	1.0809	0.71619	0.416	−0.7051	2.8668
		Control group (healthy)	2.9033	0.80957	0.003	0.8845	4.9221

Based on observed means

The error term is Mean Square (Error) = 3.57

The mean difference is significant at the 0.05 level

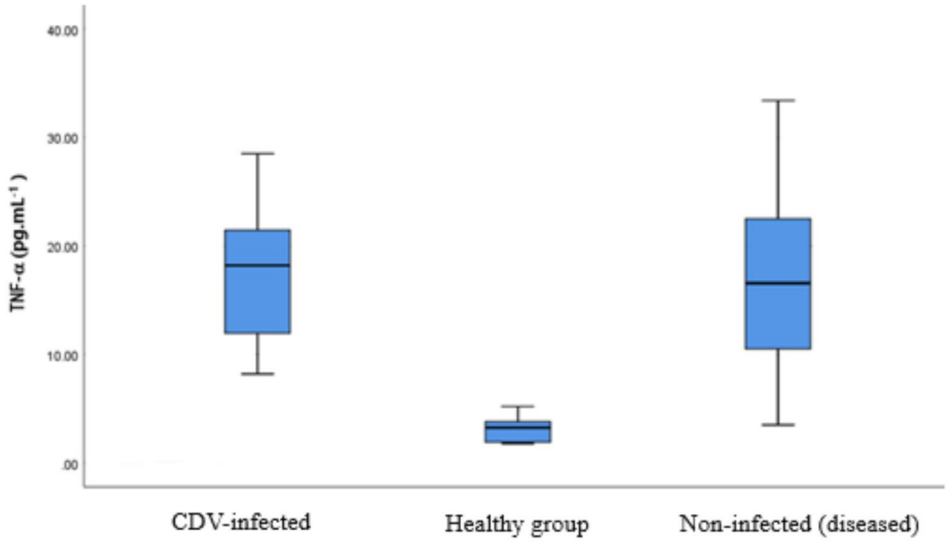

Fig. 2 The box plot shows TNF-α concentrations in three different groups ($p < 0.05$)

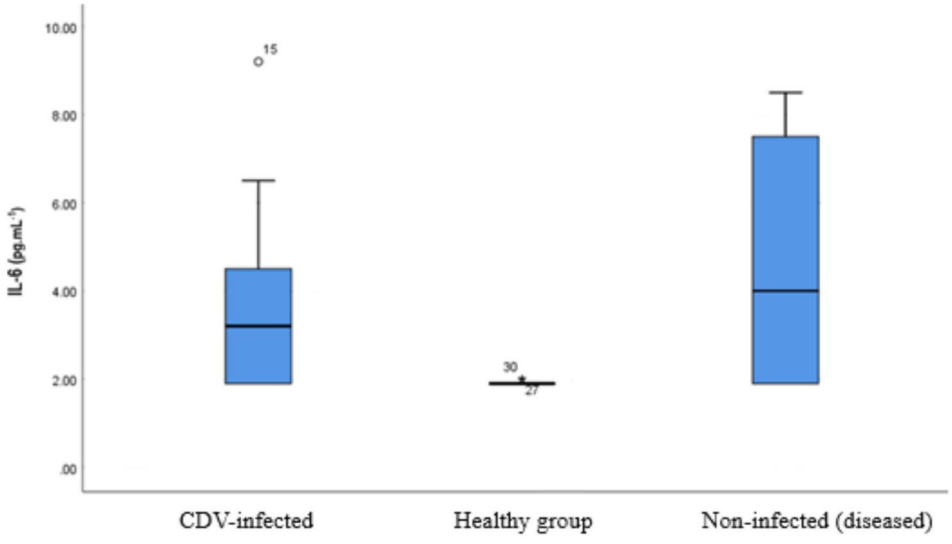

Fig. 3 The box diagram illustrates the levels of IL-6 in the three studied groups

difference was found between the CDV-infected and non-infected groups ($P = 1.000$) despite the slightly higher mean in the infected group. These findings are further illustrated in Fig. 2.

The present study revealed a significant difference in IL-6 levels between CDV-infected and healthy dogs ($P = 0.029$) Table 2. Furthermore, our results indicated substantial differences in the IL-6 levels between non-infected dogs and healthy dogs ($P = 0.003$), suggesting that the assumption of equal means between these groups is invalid. However, the levels of IL-6 in the CSF of the CDV-infected group compared to the non-infected group were not significant ($P = 0.416$) Table 2. These findings suggest that the role of IL-6 may be shared across various neurological disorders and may lack specificity to be considered as a specific diagnostic marker for nervous distemper (Fig. 3).

Discussion

There are ongoing uncertainties regarding how viral persistence affects the onset of neurological signs associated with canine distemper virus (CDV) infection [6]. Cytokines play a crucial role in causing inflammation and degeneration in the affected central nervous system of dogs with distemper disease. The interaction between viruses and cytokines is complex. Viruses can trigger cytokine expression but also suppress cytokine activity through multiple pathways [28, 29]. It seems CDV infection starts a series of events leading to the activation of pro-inflammatory cytokines such as TNF-α and IL-6

[30]. Evaluating cytokines in biological samples, like CSF, is crucial for understanding the immune-inflammatory response and assessing central nervous system (CNS) involvement [30, 31]. A previous study demonstrated that CSF samples exhibit higher sensitivity for detecting CDV than mucosal swabs or whole blood samples, utilizing both rapid antigen and RT-PCR assays [22]. The Khuth study employed the PCR method to analyze the expression of pro-inflammatory cytokines in the brain tissue of infected mice in comparison to sham-inoculated mice with CDV. They found there was a robust correlation between the production of inflammatory cytokines and viral replication in the CNS, observing an increase in the expression of TNF- α and IL-6 mRNAs in all infected brain structures except the brain stem and cerebellum [32]. The role of TNF-α and IL-6 in the CSF of CDV-infected dogs is not fully understood, and their potential use as additional diagnostic biomarkers requires further investigation, particularly when compared to levels in non-infected but diseased dogs. Our results revealed that the concentrations of TNF-α and IL-6 in the CSF of dogs infected with CDV were notably elevated compared to those in healthy dogs. These findings align with previous research. Dinarello and Kiecolt-Glaser's [33, 34] investigations have shown that during the initial stage of morbillivirus infection in dogs, pro-inflammatory cytokines such as IL-6 and TNF-α are prevalent. Similarly, Seible's study found these cytokines were significant during the first 24 h of the CDV Onderstepoort (CDV-OND) strain in vitro infection. The investigation of pro-inflammatory substances in Seible's research was conducted using (RT-PCR) and by analyzing the obtained blood samples [35]. Frisk et al. evaluated cytokine production in the CSF of dogs with natural CDV using RT-PCR. IL-6 transcripts were the most frequently expressed, followed by TNF-α. No cytokine transcripts were found in control dogs. Their study collected CSF samples from dogs within 0 to 72 h post-mortem, which differs from our approach, where CSF samples were obtained from living dogs. Additionally, the RT-PCR method was employed to evaluate the mRNA of cytokines, in contrast to our study, in which ELISA was used to assess the pro-inflammatory concentrations [20]. We found significantly higher concentrations of TNF-α and IL-6 in the CSF of CDV-infected and diseased non-infected dogs compared to healthy controls. These findings align with the results of Merbl et al., who investigated the concentrations of IL-6 and TNF-α in dogs experiencing seizures. The significant positive relation between CSF and serum IL-6 and TNF-α suggests that both cytokines increase systemically and within the CNS during an inflammatory seizure-induced process, which can occur as a neurological sign in CDV infection [36]. In their study, the concentration of cytokines in serum and CSF was measured using ELISA, similar to the approach employed in our study. However, their study focused on dogs with seizures attributable to three distinct etiologies (epileptic seizures of neoplastic, inflammatory, or idiopathic etiology). The research conducted by Song et al. showed that the mRNA expression of IL-6 and TNFα was regulated in peripheral blood lymphocytes (PBL) induced by CDV. In their study, the inflammatory mRNA expression of cytokines was evaluated using RT-PCR. Notably, there was no control group, unlike the current research, which compared CDV-infected groups with both healthy and diseased groups [37]. In the same investigation as ours, Grone et al. [16] examined canine blood samples in three control groups: a healthy and diseased group and a CDV-infected; they detected cytokine mRNA IL-6 transcripts in septic canines and dogs with neurodegeneration. Their results showed that mRNA transcripts of IL-6 and TNF-α were detected in whole blood from dogs with acute distemper. No control animals demonstrated the presence of necrosis factor alpha (TNF-α) mRNA. The mRNA expression of IL-6 in their study in CDV-infected and diseased groups was in line with our results, which evaluated the concentration of these two cytokines in CSF samples, but contrary to our study, they did not detect the transcript of TNF genes in the non-infected (diseased) group. According to the result of Grone's study, TNF-α is frequently seen along with glial fibrillary acidic protein (GFAP) in astrocytes, especially in acute lesions. These findings indicate that astrocytes expressing TNF play a crucial role in the early stage of DL (distemper leuko-encephalomyelitis) and may potentially contribute to the process of demyelination [19] therefore, the group of non-infected (diseased) in their study may account for the variations in results.

In a prior investigation by Markus et al., the development of demyelinating cerebellar lesions in early distemper CNS lesions was examined. Their study revealed that dogs exhibited heightened levels of pro-inflammatory cytokines during the initial phases of canine distemper. The expression levels of different cytokines, such as TNF-α and IL-6, were assessed using RT-PCR. Our study aimed to build upon their finding by investigating cytokine production levels in the CSF of dogs affected by canine distemper, comparing these levels with those of healthy control dogs and non-infected dogs. Our results were consistent with Markus et al., who reported that 94% of dogs with canine distemper exhibited IL-6 expression, while only 17% of the control group showed similar expression [30]. In addition, the results of Beinkie's study, which examined the expression of pro-inflammatory cytokines in nervous canine distemper, were consistent with our findings. They detected Transcripts of precursor cytokines IL-6 and TNF- α in 100% and 80% of CDV-infected dogs, respectively, In comparison to the control

group. Their study utilized Optimal Cutting Temperature (OCT)-embedded frozen tissue blocks from the cerebral cortices of all dogs with distemper leukoencephalitis, evaluated by RT-PCR [38].

Grone et al. studied tissue samples from CDV-infected dogs. These included primary brain cells (mainly microglia, with fewer astrocytes and neurons), skin fibroblasts, small pieces of the cerebellum, and subcutis. They found that CDV infection of primary brain cells resulted in an increase in mRNA transcripts of IL-6, and TNF protein was identified with a significant increase in CDV-infected cells compared to non-infected cells. However, in dermal fibroblast evaluation, only an increase in TNF transcripts was observed. The expression of TNF protein was limited to a small number of cells, and it increased on days 6 and 9 following infection. In contrast, the increase of TNF in the CSF samples of dogs with CVD in our study, Groen's results indicated the least effect on TNF transcription in Madin Darby canine kidney cells (MDCK) with distemper diagnosis. The only higher mRNA amplification in infected cells was IL-6 on days 6 and 9. In addition, IL-6 protein was present in uninfected MDCK cultures and CDV-infected cultures shortly after infection, which was inconsistent with the absence of increased IL-6 concentration in healthy samples in our study. It appears that the pro-inflammatory cytokines TNF and IL-6 were the most frequently induced by CDV infection in a variety of cell lines. Nevertheless, there are some variations in cell lineage that may be significant in the development of natural diseases. This is particularly evident in the context of MDCK cells and their capacity to resist CDV infection to a certain extent [18]. Interestingly, a contrasting pattern was observed in another study by Dik et al. [21], where serum levels of TNF- α and IL-6 were lower in CDV-positive canines than in healthy dogs. This discrepancy is not entirely evident; however, it may be due to the difference in sample types analyzed (CSF vs. serum) and the immunosuppression of CDV in lymphoid tissues. Additionally, cytokine transcripts were found more frequently in dogs with a low antigen index. In contrast, dogs with a high antigen index showed an absence of cytokine transcripts. This indicates that a high viral load is linked to low expression of cytokines in the peripheral blood. The lack of cytokine transcripts in dogs with a high antigen index may result from the negative impact of canine distemper virus (CDV) on cytokine gene transcription in blood leukocytes [16].

The elevated mortality rates linked to CDV infections necessitate accelerating the diagnosis process to ensure prompt quarantine and treatment for affected dogs [39]. In a study conducted by Kocatürk et al. [40], the authors investigated the differences in cytokine and chemokine profiles, along with inflammatory and antioxidant markers, in serum and CSF samples from CDV-infected dogs exhibiting neurological signs. Their findings demonstrated a significant correlation between specific biomarkers in serum and CSF, particularly IL-6 and monocyte chemoattractant protein-1 (MCP-1), suggesting that serum levels may reflect central nervous system (CNS) changes. Among the cytokines analyzed, only IL-7 (cytokine), IL-8 (chemokine/CXCL8), and MCP-1 (chemokine/CCL2) showed significant differences in serum samples, indicating their potential pathophysiological roles and diagnostic utility in the neurological form of CDV infection. In contrast to our study findings, no statistically significant differences in serum levels of TNF-α and IL-6 between CDV-infected dogs and healthy controls were reported. While our results reinforce the importance of IL-6 and TNF-α as key components of the inflammatory response, we also suggest that a broader cytokine profile may enhance clinical diagnostic capabilities.

Conclusion

This study evaluated the concentrations of two pro-inflammatory cytokines, IL-6 and TNF-α, in the cerebrospinal fluid of dogs with canine distemper virus (CDV) infection. Results showed significantly higher levels of both cytokines in CDV-infected dogs compared to healthy controls, suggesting their involvement in the development and progression of CDV-associated neurological complications. These findings highlight the potential role of IL-6 and TNF-α in guiding treatment decisions and prognosis. However, no significant differences in cytokine levels were found between CDV-infected dogs and non-infected dogs with other neurological conditions (e.g., intervertebral disc disease, epilepsy, generalized paralysis). This lack of specificity limits their value as precise diagnostic biomarkers for CDV.

Given the overlapping inflammatory responses across neurological disorders, future research should investigate additional biomarkers, such as other cytokines or genetic markers with higher specificity and sensitivity. Identifying more reliable indicators will improve diagnostic accuracy, guide clinical management, and ultimately enhance outcomes for dogs affected by CDV.

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Author contributions

Marjan Mahmoodabadi (a), Hadi Mohebalian(b), Ali Asghar Sarchahi(c)a. The First Author has drafted the work, data collection, and data analysis.b. design of the work, interpretation of data, supervisor, revising manuscript.c. Methodology, developed hypothesis, advisor, revising manuscript.

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Data availability
Data is provided within the manuscript.

Declarations

Declarations
This study was discussed and approved by the Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All animal procedures were approved by the institutional Animal care and use Committee at Ferdowsi University of Mashhad and followed international animal care and welfare guidelines.

Ethics approval and consent to participate
All animal procedures were conducted in accordance with the guidelines established by the Animal Care and Use Committee at Ferdowsi University of Mashhad, adhering to international standards for animal care and welfare (Approval ID: IR.UM.REC.1399.120). Prior to commencing any work, informed written consent was obtained from the owners of the dogs involved in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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