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Decreased serum adenosine deaminase activities in van cats with feline retroviruses infections

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Summary

This aim of the study was to determine the activity of ADA serum and isoenzyme in feline retroviral infections. The study involved 6 FeLV seropositive, 4 FIV seropositive and 10 healthy seronegative cats aged between 1-8 years. Haematological, serum enzyme acivity (AST; ALT; ALP; GGT) as well as ADA serum and isoenzyme activity were determined in all the cats. Haematological parameters were within the normal range except for the platelet count in FIV seropositive cats ($p<0.05$). Serum enzyme activity was unchanged except for the AST concentration in FeLV and FIV seropositive cats compared to the healthy subjects. ADA serum and ADA1 concentrations were lower in the seropositive group than in the seronegative group. However, the decrease in ADA serum and ADA1 concentrations in FIV seropositive cats ($p<0.01$) was more significant than that of FeLV seropositive cats ($p<0.05$). In conclusion, decreased ADA and ADA1 activity in feline retrovirus infections may be significant.

Keywords: Adenosine deaminase, feline

Adenosine deaminase (ADA, EC 3.5.4.4) is an enzyme in the purine nucleoside salvage pathway which catalyses the converts adenosine to inosine (1, 4, 22, 32). In mammals, ADA is present in all organs, although the highest activity has been found in lymphoid tissues (1, 4, 31). It has also been shown that there is a relation between cellular immune response, lymphoreticular cell activity and ADA activity (4, 32). Two ADA isoenzymes are known as ADA 1 and ADA 2 in domestic animals (31). It is suggested that increased ADA 1 is derived mainly from injured tissues and cells or lymphocyte and neutrophil, while increased ADA 2 may be an indicator of monocyte-macrophage activation or turnover (8, 17, 35). On the contrary, deficiency of ADA is associated with inhibition of lymphocyte proliferation and differentation (3, 16, 18). Moreover, it is suggested that decreased ADA activity could be reflects in the dysfunction of cell-mediated immunity (3, 4, 16, 30, 32). Furthermore, it is reported that a deficit of this enzyme's activity could constitute a direct cause of immune dysfunction or could reflect a deviation of cellular function or genetic change that would reach simultaneously the immune capacity and enzymatic activity (2). The genetic deficiency of ADA results in severe combined immunodeficiency disease (SCID), and levels of ADA isoenzymes in the SCID are found to be clinically correlated with the severity of diseases in patients (21, 34). Inhibition of ADA also results in significant immunosuppression (21, 23).

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are retroviruses that establish long-term persistent infections in cats (33, 37). Retroviruses of cats are found worldwide under natural conditions (11, 37). Both viruses are associated with a wide range of diseases in domestic cats, including tumors and immunodeficiency (33). Symptomatic stages of both FeLV and FIV infections are frequently associated with hematologic abnormalities, particularly leukopenia and anemia (27). But, cats infected with feline retroviruses may remain asymptomatic for long periods of time (26). Therefore, cats in the asymptomatic stage of infections rarely have hematologic abnormalities (27).

Retroviruses have been reported to alter ADA activity in tumoral cell. Especially, immunosuppressions occurs commonly in associations with tumors and haematologic abnormalities induced by retroviruses (6, 11, 33, 34). The similarity of FeLV- and, in particular, FIV-induced acquired immunodeficiencies to human immunodeficiency virus-1 (HIV-1)-induced acquired immune deficiency syndrome (AIDS) has established both feline viruses as important animal models (33). It has been reported that serum ADA activity is either increase (5, 34) or decrease (22, 32) in HIV infections. But, change of ADA levels in serum correlated with the clinical conditions of the patients with these diseases (5, 34). Patients with the HIV infections reportedly have investigated ADA activity in serum (5, 22, 32, 34), however, levels of serum ADA in cats infected with the FeLV and FIV infections is not known. For this reason, in the present study, serum ADA activity was investigated in feline retrovirus infections.

Material and methods

Animal materials. The study was performed on 10 Van cats that with retroviruses infections from Yüzüncü Yıl University

Van Cat Research Center and home-grown animals. Seronegative healthy cats (n = 10) were used controls.

Study design and laboratory analysis. Blood samples for analysis was taken from the cephalic antebrachial vein. The serum was obtained by centrifugation for 10 minutes at 3000 turns. Serum samples were kept at –20°C until the analysis. Firstly, seropositivity for retrovirus infection was determined by the commercial kits of FeLV p27 antigen (Virachek®/FeLV Synbiotics) and the presence of FIV by FIV p24 antibodies (Virachek®/FIV Synbiotics). The seropositivity of FIV was demonstrated in 4 cats and FeLV antibodies in 6 cats. Two of the FIV positive cats were males and 2 were females and their age varied between 4 and 8 years. Among these cats, only one old female cat had symptoms for FIV (8 years old). Four of the 6 FeLV seropositive cats were males and 2 of them were females. Two of the cats were 5 to 6-year-old and 4 of them were 1 to 2 years of age. No clinical symptoms were observed in seropositive cats for FeLV.

Secondly, seropositive animals was determined hematological parameters and serum enzyme activities. Haematological parameters were determined on EDTA blood using QBCvetautoreader® cell counter (Idexx). The serum total ADA activity was determined by the spectrophotometric method described by Giusti and Galanti (9). To distinguish between the ADA1 and ADA2 forms, the ADA activity was measured using same technique with and without erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). EHNA is a potent inhibitor of only ADA1 isoenzymes and a concentration of 200 μmol/L was used in the reaction solution (35). In its presence, only the ADA2 isoenzyme is active. The ADA1 activity is then calculated by subtracting the ADA2 isoenzyme activity from total ADA activity.

The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) levels were determined spectrophotometrically using commercial kits by spectrophotometer (Photometer 5010, Boehringer-Mannheim).

Statistical analysis. For statistical analysis, Student t test was used to determine the difference between seropositive and seronegative cats. For this purpose, SPSS programme was used (29).

Results and discussion

Genetic deficiency of ADA impairs the development of the immune system. Reports that cell lines transformed by retrovirus have low ADA levels together with clinical similarities between immunosuppression induced by ADA deficiency and that associated with retrovirus infections suggest a potential role for purine metabolism in retrovirus-induced immunosuppression. In addition, enzyme activity levels have been postulated to be of prognostic and therapeutic value in acquired immune deficiency syndrome and adult T cell leukemia (6, 10).

Feline system has been a useful and well-documented animal model for retrovirus associated immunosuppression was used here. Serum adenosine deaminase activities in Van Cats with feline retroviruses infections are summarized in tab. 1. The asymptomatic retroviruses infections in the present study was determined decrease in serum total ADA and ADA1 activities. When the FeLV- and FIV-positive groups were compared with control group, both group cats had lower total ADA and ADA1 activity (tab. 1), but higher AST levels. Especially, FIV-positive cats had significantly lower ($p < 0.01$) ADA and ADA1 activity than the controls and FeLV-positive groups ($p < 0.05$). It has been

Tab. 1. Serum adenosine deaminase activities in van cats with feline retroviruses infections ($\bar{x} \pm Sx$; min.-max.)

Parameters	Control Group (n = 10)	FeLV + Group (n = 6)	FIV + Group (n = 4)
Total ADA (IU/L)	16.17 ± 2.13 (10.02-32.71)	11.22 ± 1.11* (8.07-14.89)	6.15 ± 0.96** (4.17-8.77)
ADA1 (IU/L)	15.02 ± 2.14 (8.21-31.38)	10.36 ± 1.16* (6.74-14.20)	5.08 ± 1.05** (3.76-8.22)
ADA2 (IU/L)	1.15 ± 0.12 (0.55-1.81)	0.86 ± 0.11 (0.55-1.33)	1.07 ± 0.35 (0.41-1.80)

Explanations: * – $p < 0.05$, ** – $p < 0.01$

reported that serum ADA activity is either increase (5, 34) or decrease (22, 32) in HIV infections. But, change of ADA levels in serum correlated with the clinical conditions of the patients with these diseases (5, 34). Increased ADA enzyme activities in HIV was attributed to cytopenia (especially lymphopenia) (13, 32) while decreased activities was associated with defective peripheral T lymphocyte function (4, 21, 28). Immunsupression was reported in both situation (21, 32). The present study was carried out in FeLV and FIV seropositive asymptomatic cars. In both positive group cats, no significant differences were found in haematological parameters (haematocrit, haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte, total and differential leukocyte counts), although there were decreased platelet counts (279.0×10^9/L, $p < 0.05$) in FIV-positive group compared to control group (414.7×10^9/L). Although platelet count decreased the figure found in this study fell within the reference range reported for cats (15). Only abnormalities in FIV seropositive cats are thrombocytopenia, thought to be secondary to the peripheral destruction of platelets (26). Furthermore, no significant differences were found in the total leukocyte and absolute differential counts. Haematological findings obtained in this study corraletes with previous study (25-27) where cytopenia was not a laboratory finding in retrovirus infected asymptomatic cats.

This study did not evaluate lymphocyte subsets. However, this parameter was previously determined in immunopathogenesis of feline retrovirus infection and changes in lymphocyte subsets were determined (11, 33). These studies disclosed a lymphopenia resulting form significant decrease in lymphocyte subset (T, CD4+, CD8+ and B cell) in acute infection and especially decrease in CD4+ may have caused in immunsuppression (11, 12, 33). But, alternatively, in cats infected with less acutely immunosuppressive infection, leukocyte and lymphocyte numbers return to normal or near normal values throughout a latency period of months to years before CD4+ and CD8+ T cells gradually but progressivelly decrease (12). Futhermore, cats that develop immune or latent infection may have transient lymphopenia, but usually are not neutropenic or thrombocytopenic (24). For all that latently infected cats are immunosuppressed, compared with nonexposed cats (14, 24). Similarly, cytopenia and differentiation of lymphocyte subsets were not found in HIV carrier (36). For this reason, although asymptomatic feline retrovirus infections in this study were not detected lymphopenia, decreased serum ADA and ADA1 activity may be explained by immunosuppressive effect of the protein p15E during chronic

retrovirus infection (11), functional defects of peripheral T-lymphocytes (32), depressed activities of ADA in lymphocytes (21), decreased mitogen proliferative responses (14) and produced lower IL-2 than do noninfected cats (20). But, it is also unlikely that the low ADA values are related to immune impairment, because high-risk HIV seronegative and healthy HIV-seropositive groups do not exhibit demonstrably impaired immunity (17). These findings are compatible with the observation that low ADA activities are sufficient to restore immunity in partly deficient ADA patients (7, 19).

In our study, FIV-positive cats had significantly lower ($p < 0.01$) ADA and ADA1 activity than the controls and FeLV-positive groups ($p < 0.05$) (tab. 1). This situation may be explained by to the different immunosuppressive effect of FeLV and FIV on the lymphocytes and FIV infection was seen most often higher old aged cats compared to FeLV infected cats.

In the present study, one cat with clinical findings and FIV-seropositive was observed leukocytosis (20.5×10^9/L) as a result of increased lymphocyte/monocyte counts (10.2×10^9/L). Furthermore, it's obtained ADA and isoenzymes activity had lowest in the FIV-positive group. In this cat had symptoms including anorexia, conjunctivitis, gingivitis and disintegration of teeth. These findings are in agreement with those of Pedersen et al. (20) where fourth stage of FIV was similar to AIDS related complex in HIV infection. Renouf et al. (21) reported that patients with AIDS-related complex group were general depression of ADA in lymphocytes. Increased lymphocyte/monocyte count despite decreased ADA and isoenzyme activites in FIV seropositive cat may be explained by the study of Renouf et al. (21).

In this study, FIV-positive cats showed higher AST levels (56.00 ± 4.41 IU/L, $p < 0.05$) than the controls (35.20 ± 4.85 IU/L), FeLV-positive group cats had more higher AST levels (71.66 ± 7.39 IU/l, $p < 0.01$) compared to FIV-positive cats. Elevated AST levels in this study may be explained by microhemolytic processes not detected by the naked eye possibly caused by adsorbsion of virus to the surface of erythrocytes (11). Other serum enzyme levels were within normal limits in all groups.

In this study, decreased serum ADA and ADA1 enzyme activities were determined in FeLV and FIV seropositive asymptomatic cats. As a result, decreased ADA activities may be of diagnostic value in feline retrovirus infection and further studies should evaluate serum and lymphocyte ADA activities in feline retrovirus infection.

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