Praca oryginalna *Original paper*

Adenosine deaminase in the diagnosis of white muscle diseases in lambs*)

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Diagnostic parameters as an addition to routine enzymes used in the diagnosis of white muscle diseases in lambs: adenosine deaminase

Summary

The aim of the study was to investigate the importance of ADA serum and its isoenzyme activity in diagnosing white muscle disease in lambs. The animal material used in the study was forty seven Akkaraman lambs - twenty seven of which had clinical signs of white muscle disease (white muscle disease group) and twenty healthy lambs (control group). Blood samples were taken and Se levels, CK, LDH, AST, ADA and ADA izoenzyme activity were determined. Following this, the animals in the white muscle disease group received 1 mg sodium selenit + 60 mg vitamin E intramuscularly as a single dose. Fourteen days following sodyum selenit + vitamin E application blood samples were again taken and the same analysis repeated. Apart from ADA2 activity, other enzyme activities were significantly high ($p<0.001$), and serum Se concentrations were significantly low ($p<0.001$) in animals of the white muscle disease group compared to the control group. When the parameters obtained from the white muscle disease group were compared before and after treatment it was indicated that all enzyme parameters decreased significantly ($p<0.001$) after treatment. On the other hand, serum Se concentration increased ($p<0.01$) after treatment in the diseased group. The results confirm that determining ADA serum and its isoenzymes, together with CK, LDH and AST values seems to be useful in diagnosing white muscle disease.

Keywords: Adenosine deaminase, white muscle disease

White muscle disease (WMD) is an enzootic nutritional disorder, which is characterized by degenarations in muscles, heart and liver, and mostly seen at 3-8 weeks after birth (2, 3, 5, 15). In its etiology, selenium (Se) deficiency and/or vitamin E reported to play role, and it is seen in all over the world (2, 5, 9, 13, 15). The disease develope in animals eating forage grown on Se-deficient soils or it may be causing by antagonistic effects of various metals (silver, copper, cobalt, cadmium, mercury) (5). Furthermore, if sheep, fed with rations deficient in Se and vitamin E during long winter season and giving birth to lambs, WMD may also occur in these lambs or sheep (2, 3, 5, 9). Additionally, placental transport deficiency or Se metabolism disorders may also cause the diseases development even if rations are sufficient with regard to Se (4).

The diagnosis of WMD is based on clinical, pathological and laboratory findings. Clinical findings of the disease are; stiffly moving, recumbency, short and vertical step, fall down to back extremities during walking, unable to stand on back limbs, dog like sitting and unable to stand at all. There are some diseases which show similar clinical findings. Therefore, it is hard to diagnose WMD only on the clinical bases (2, 5, 15). In studies concerning WMD have been shown correlation between clinical signs, pathological findings and serum enzyme activities (4, 6). However, in clinical diagnosis and pathological examination in all animals are impossible. Therefore, beneficial effect of histopathological examination is limited (5, 6). On the other hand, in the laboratory diagnosis of the disease, reduction in the Se and glutathione peroxydase level (6, 14), reduction (6) or unchange (13) in the vitamin E level, increase of the creatine kinase (CK), aspartate amino transpherase (AST), lactade dehydrogenase (LDH) and alanine aminotranspherase (ALT) have been reported (4, 6, 13, 14, 18). Furthermore, in the diagnosis and prognosis of subclinical and clinical WMD, determination of muscle originated enzymes (CK, AST, LDH) reported to be more practical and reliable (6, 13, 15, 18). In addition to above muscle originated enzymes, adenosine deaminase (ADA, EC 3.5.4.4) has also been reported to be present in the muscle (17, 21, 22, 24, 25). But, it is not

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clear whether its activities are affected due to degenerations occurred in skeletal muscles such as WMD, and it is not known whether it can or can't be used as an extra criteria in the diagnosis of this disease.

ADA is an enzyme in the purine nucleoside salvage pathway which catalyses hydrolytic deamination of either adenosine or 2-deoxyadenosine to produce respectively inosine and deoxyinosine (22, 25). In mammals, ADA is present in all organs including myocardium, muscles and liver (21, 24, 25). It has also been detected in cytoplasm, mitochondria and nucleus of the cells (25). ADA activity in the myocardium reported to be twice higher than in the *m. soleus* and 3.5 times higher than in *m. quadriceps* in rats (17).

Therefore, in the present study, in addition to practical, reliable and well known muscled originated enzymes (CK, AST, LDH) which were used in the diagnosis and prognosis of WMD, determination of serum ADA and ADA isoenzymes has any impact on the diagnosis of the disease were aimed to investigate.

Material and methods

Animal materials. This study was performed in a group of 27 lambs (white muscle group) showing clinical signs of white muscle diseases. These lambs were chosen from herds (a total 250 lambs) which did not receive vitamin E and/or Se for prevention. For control; 20 lambs from the same location were also used, but these control animals received vitamin E and/or Se as preventive medicine according to their owners long before they used in this study. All animals used in this study were aged between 1-3 mounths and were in Akkaraman breed.

Study design and treatment. Firstly; from all lambs, blood samples were taken through *v. jugularis* to the tubes which contained no anticoagulant. Then, white muscle diseased group received 1 mg sodium selenite plus 60 mg vitamin E (Eselen®-Vetaş™/Turkey) only once intramuscularly. Blood samples were again taken from white muscle diseased group 14 days after drug application, but not from control group. All obtained blood samples were centrifuged at 3000 rpm/minute for 10 minutes to obtained serum samples. The serum samples were stored at –20°C until they used for analysis.

Analysis of biochemical parameters. The serum samples were analysed for Se concentrations using atomic absorption spectrophotometer (Solar AA Spectrometers®-Thermo Elk. Co. /UK) according to the method reported by Galgon and Frank (16). Serum CK (Randox CK-110), LDH (Randox LD-401) and AST (Randox AS-521) levels was determined spectrophotometrically (Boehringer-Mannhein Photometer 5010, Mainhein-Germany) using commercial kits. The serum total ADA activity was also determined spectrophotometrically and described by Giusti and Galanti (8). To distinguish between 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 only of the ADA1 isoenzymes and a concentration of 200 µmol/L was used in the reaction solution (23). In EHNA's presence, only ADA2 iso-enzyme is active. The ADA1 activity is then calculated by subtracting the ADA2 isoenzyme activity from the total ADA activity as reported by Ungerer et all. (23).

Statistical analysis. For statistical analysis, paired t test was used to determine the difference between the values obtained before and after treatment. For this purpose, SPSS packet program was used (20).

Results and discussion

White muscle disease is a nutritional disease seen in all over the world and cause reduction in the output, therefore, important economic waste occur (2, 5, 15). Vitamin E and/or Se deficiency in the animal ration known to play role in development. Although preventive precautions can be taken to overcome this problem, other problems such as placental transport deficiencies or abnormalities in the Se metabolism in the placenta reported to play role in the development of the disorder (4). Therefore, requirement of a safe and easy diagnosis is becoming much more important for this disorder.

In the present study, Se concentration and some enzyme activity values for white muscle disease and control group are given in tab. 1. In diseased group, serum Se concentration obtained before treatment were significantly lower ($p < 0.001$) compared to the values obtained from control group. Furthermore, Se concentration in diseased group were also significantly increased ($p < 0.001$) after treatment compared to the values obtained before treatment. Hovewer, when the values obtained after treatment which got from the

Tab. 1. Serum Se concentration and enzyme activity levels in lambs with white muscle disease and control groups

Parameters	Control Group $\bar{x} \pm SX$ (n = 20) (XMin-XMax)	White Muscle Diseased Group $\bar{x} \pm SX$ (n = 27) (XMin-XMax)	
		Before Treatment	After Treatment
Se (ng/ml)	106.0 ± 2.33 (80.8-122.2)	53.9 ± 2.47[a] (32.8-72.1)	96.2 ± 1.09*[c] (86.3-104.4)
CK (IU/L)	27.25 ± 2.85 (13-60)	3351.53 ± 552.97[a] (850-14970)	51.46 ± 2.09*[b] (32-72)
LDH (IU/L)	341.40 ± 18.61 (213-475)	3525.06 ± 245.50[a] (1350-5310)	429.23 ± 6.68*[b] (388-527)
AST (IU/L)	43.40 ± 3.14 (20-63)	273.56 ± 12.87[a] (105-442)	34.50 ± 1.84* (18-65)
ADA (IU/L)	7.83 ± 0.59 (2.85-14.13)	16.36 ± 0.23[a] (13.52-18.75)	14.40 ± 0.16*[b] (12.62-15.83)
ADA1 (IU/L)	7.31 ± 0.47 (3.22-13.75)	15.63 ± 0.21[a] (13.28-18.25)	13.93 ± 0.15*[b] (12.35-15.64)
ADA2 (IU/L)	0.52 ± 0.09 (0-1.67)	0.73 ± 0.05 (0-1.15)	0.47 ± 0.03* (0-0.68)

Explanations: a – before treatment, statistical importance between control and white muscle diseased group $p < 0.001$; b – after treatment, statistical importance between control and white muscle diseased group $p < 0.001$; c – after treatment, statistical importance between control and white muscle diseased group $p < 0.01$; *statistical importance of white muscle diseased group before and after treatment $p < 0.001$

diseased group compared to the values obtained from control group; Se level were still lower ($p < 0.01$). Blood Se concentration below 50 ng/ml is reported to be the sign of Se deficiency, 50-100 ng/ml is critical values, and over 100 ng/ml reported to be normal values for Se (15). In the present study, control group values were in normal borders as reported above literature (15). On the other hand, values obtained from white muscle diseased group before treatment were in the critical level (15). But, after treatment Se concentrations in the white muscle diseased group approached to the reference values (15) for healthy animals.

In the present study, CK, LDH and AST enzyme activities obtained from diseased group before treatment were significantly ($p < 0.001$) higher than the same values obtained from control group. After Se plus vitamin E treatment of the diseased animals, above enzyme activities reduced and approached to control group values (tab. 1). When the values obtained from diseased group after treatment compared to the values obtained from control group; CK and LDH levels were still higher ($p < 0.01$). But, AST levels of the diseased group obtained after treatment were not statistically different compared to the control values (tab. 1). These findings were in agreement with the several researchers findings with regard to CK, LDH and AST (13-15). Similarly, Sekin et al. (18) reported CK, LDH and AST values in clinical white muscle diseased lambs as 2797.00 ± 953.89 IU/L, 2260.90 ± 435.32 IU/L and 908.10 ± 301.30 IU/L, respectively before treatment and 7 days after treatment as 45.10 ± 5.56 IU/L, 587.90 ± 18.60 IU/L and 36.80 ± 3.29 IU/L, respectively.

Due to muscle and liver degeneration in this disorder, CK, AST and LDH activities have been reported to increase in white muscle disease by several workers (6, 13, 14, 18). Among these enzymes, especially CK well known to be specific for heart and skeletal muscles, and due to the severity of damage in the muscle cell membranes its concentrations in the blood reported to increase. But its half-life is between 2-4 hours. Therefore, its plasma levels characteristically drop quickly unless there is continued myodegeneration (6, 15). The other muscle damage indicator is AST. But, it also represent not only muscle damage but also liver damage. Therefore, it isn't as reliable as CK for the diagnosis of white muscle disease (6, 15). Similarly, LDH reported to be very specific for nutritional myopathie. But when it compared to other enzymes; its sensibility low and its activity reported to increase liver diseases as well (6). On the other hand, Fry et al. (6) reported that CK, ALT, LDH and AST combinations can safely be used in the diagnosis of nutritional myopathie. Furthermore, increase in the CK, AST and LDH reported to be related directly with the muscle degeneration degrees (4, 6).

ADA reported to be present in the myocardium, muscles and liver. Its presence in the muscles is related to muscle fibres (17, 21, 24). Martinez et al. (12) reported two tipe of ADA having 31.000 and 180.000 kDa molecular weight in cattle skeletal muscles. The ADA having low molecular weight were found more often than the other one and although it is present in a very low concentrations, it is reported to be very active enzyme (12). In contrast, Ungerer et al. (22) reported that ADA having 35.000 kDa molecular weight not 31.000 kDa is the ADA 1 isoenzyme.

In the present study, total serum ADA enzyme activity were 16.36 ± 0.23 IU/L, ADA1 were 15.63 ± 0.21 IU/L and ADA2 were 0.73 ± 0.05 IU/L before treatment in the white muscle diseased group. On the other hand, the same values after treatment respectively were 14.40 ± 0.16 IU/L, 13.93 ± 0.15 IU/L and 0.47 ± 0.03 IU/L. When these values were analysed statistically, values obtained before treatment except ADA2 were significantly higher ($p < 0.001$) compared to the same values obtained from control animals. Furthermore, when values obtained before treatment and after treatment compared; all values including ADA2 observed to significantly decrease ($p < 0.001$) after treatment compared to the same values obtained before treatment. Increase ADA and ADA1 activity before treatment and decrease the same parameters after treatment were synchronous with the CK, LDH and AST enzyme activities before and after treatment (tab. 1).

ADA1 activity compared to ADA2 reported to be much higher in mammals (12, 22). As a matter of fact, in this study ADA2 isoenzyme activity in white muscle diseased group in this study were not different compared to the values obtained from control animals before treatment. Furthermore, ADA2 isoenzyme activity were very low compared to ADA1 values (tab. 1). Therefore, increase in total ADA activity most probably was due to increase in ADA1 isoenzyme activity. According to these results, in the diagnosis of white muscle disease, total ADA and ADA1 activities can be sum up as diagnostic indicator. On the other hand, determination of ADA2 isoenzyme activity can be evaluated as not important in the diagnosis of the disease.

In the diagnosis of heart failure and liver diseases, total ADA enzyme activity together with the other cell damage parameters have been used successfully (25). In acute liver diseases, increase in ADA activity reported to be due to damaged cell and tissues as a result of liver cell necrosis. Furthermore, increase in ADA activity reported to be the sign of the severity of cell necrosis and this increase reported to occur especially in the ADA1 isoenzyme activity (10, 11). Similarly, acute and chronic experimental carbon tetrachloride toxication in dogs, AST, ALT, ALP and ADA enzyme activities reported to increase, and increase in this enzyme activity found to be in parallel with the liver cell damage histopatologically (1). In the present study, CK, LDH and AST values found to be high in white muscle diseased group which are well known to occur due to muscles damage. Furthermore, in this study total ADA

and ADA1 activities also increased in the same animals parallel to the CK, LDH and AST (tab. 1). Therefore, increase in ADA and ADA1 activities were most probably causing by muscle cell damage. In contrast to our findings and other workers (4, 6, 13, 14, 18) results, Rodriquez and Gonzalez (16) have reported decrease in LDH and CK activities in lambs with enzootic muscular dystrophy. But, the same workers reported increase in ADA activity in the same animals.

Sinci et al. (19) used solutions with sodium selenite and without sodium selenite in the treatment of guinea pigs with experimentally made normothermic is chemia. They observed healing ratio of mechanical cardiac functions and compared malondialdehit (MDA) and ADA values as ischemic tissue degeneration indicator. Animals received solutions containing sodium selenite had lower ADA and MDA level, but heart rate, contractile force and other mean heart values found to be higher compared to the other group. According to above study, addition of selenium to reperfusion solutions helped to cardiac functions in a good manner which also showed decrease in post-ischemic myocardial injury in these guinea pigs. In the present study, 1 mg sodium selenite plus 60 mg vitamin E were used to treat diseased animals. Clinical recovery and decrease in the ADA activity after treatment were also seen in the present study as reported by Sinci et all. (19).

In the present study, ADA and its isoenzyme activities except ADA2, serum CK, LDH and AST enzyme activities in white muscle diseased lambs increased synchronously before treatment and decreased after treatment. It was also found that, increase in total ADA activity were due to increase in ADA1 activity. As a result, observed increase in ADA level may be due to this enzyme release from damaged tissues, and in the diagnosis of white muscle disease, serum total ADA and its isoenzyme activities believed to used extra parameters to the well known laboratory tests such as CK, LDH and AST.

References

1. *Altuğ N., Ağaoğlu Z. T.*: Serum adenosine deaminase activity in dogs: It's importance in experimental liver intoxication. Isr. J. Vet. Med. 2000, 55, 129-134.
2. *Aytuğ C. N., Alaçam E., Özkoç Ü., Yalçın B. C., Gökçen H., Türker H.*: Koyun-keçi hastalıkları ve yetiştiriciliği, Teknoğrafik Matbaası, İstanbul 1990.
3. *Beytut E., Karatas F., Beytut E.*: Lambs with white muscle disease and selenium content of soil and meadow hay in the region of Kars, Turkey. The Veterinary Journal 2002, 163, 214-217.
4. *Bostedt H., Schramel P.*: The importance of selenium in the prenatal and postnatal development of calves and lambs. Biol. Trace Elem. Res. 1990, 24, 163-171.
5. *Fraser C. M., Bergeron J. A., Mays A., Aiello S. E.*: The Merck Veterinary Manuel, Merck and Co. Inc., USA 1991.
6. *Fry J. M., Allen J. G., Speijers E. J., Roberts W. D.*: Muscle enzymes in the diagnosis of ovine weaner nutritional myopathy. Aust. Vet. J. 1994, 71, 146-150.
7. *Galgon V., Frank A.*: Automated system for determination of selenium in biological materials, [in:] Bratter P., Schramel P. (ed.): Trace element analytical chemistry in medicine and biology. Walter de Gruyter, Berlin and New York 1988, 84-89.
8. *Giusti G., Galanti B.*: Colorimetric Method, [in:] Bergmeyer H. U. (ed.): Methods of enzymatic analysis, 3rd edn. Verlag Chemie, Weinheim 1984, 315-323.
9. *Keleş İ., Dede S., Keleş H., Değer Y., Altuğ N.*: Studies on some antioxidant vitamin concentrations in lambs with stiff-lamb disease. YYÜ Vet. Fak. Derg. 2000, 11, 79-82.
10. *Kobayashi F., Ikeda T., Marumo F., Sato C.*: Adenosine deaminase isoenzymes in liver disease. Am. J. Gastroenterol. 1993, 88, 266-271.
11. *Kurata N.*: Adenosine deaminase. Nippon Rinsho. 1995, 53, 1178-1183.
12. *Martinez C., Zumalacarrequi J. M., Diez V., Burgos J.*: Bovine skeletal muscle adenosine deaminase purification and some properties. Int. J. Biochem. 1984, 16, 1279-1282.
13. *Nizamlıoğlu M., Tiftik A. M., Turgut K., Tras B.*: The investigation of vitamin E, lactate dehydrogenase, creatine kinase and glutamic oxalasetic transaminase activities in white muscle disease in lambs. Turk J. Vet. Anim. Sci. 1991, 15, 59-64.
14. *Or M. E., Dodurka H. T., Kayar A.*: A diagnostic importance of erythrocyte glutathione peroxidase (GSH-Px) activity and some serum parameters in lambs with white muscle disease. Turk J. Vet. Anim. Sci. 2003, 27, 1-6.
15. *Rodastits O. M., Blood D. C., Gay C.*: Veterinary medicine, Bailliere Tindall, London 1995.
16. *Rodriquez J. Z., Gonzalez J. G.*: Activities of enzymes involved in energy metabolism of churra breed lambs, and changes caused by enzootic muscular dystrophy. Annales de la Faculted de Veterinaria de Leon. 1975, 21, 409-419.
17. *Rutkiewicz J., Gorski J.*: On the role of insulin in regulation of adenosine deaminase activity in rat tissues. Febs Letters. 1990, 271, 79-80.
18. *Sekin S., Voyvoda H., Bildik A., Yur F.*: The importance of creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities in the diagnosis and prognosis of subclinic and clinic white muscle disease in lambs. Turk J. Vet. Anim. Sci. 1996, 20, 225-230.
19. *Sinci V., Gunaydin S., Kalayciođlu S., Soncul H., Gokgoz L., Oz E.*: Effects of selenium enriched reperfusion solutions on isolated guinea pig hearts. Keio J. Med. 1998, 47, 219-222.
20. SPSS: Statistical Package for Social Sciences for Windows. SPSS Inc. Co., USA 2002.
21. *Tanabe T.*: Adenosine deaminase activities in the sera and tissues of animals and their clinical significance. Jap. J. Vet. Res. 1993, 41, 1.
22. *Ungerer J. P. J., Oosthuizen H. M., Bissbort S. H., Vermaak W. J. H.*: Serum adenosine deaminase: isoenzymes and diagnostic application. Clin. Chem. 1992, 37, 1322-1326.
23. *Ungerer J. P. J., Oosthuizen H. M., Retief J. H., Bissbort S. H.*: Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. Chest 1994, 106, 33-37.
24. *Yasuda J., Tanabe T., Hashimoto A., Too K.*: Adenosine deaminase (ADA) activity in tissues and sera from normal and leukaemic cattle. Br. Vet. J. 1996, 152, 485-488.
25. *Zuck V. U., Rotzsch W.*: Die Adenosin Deaminase (ADA)-biologische und medizinisch-diagnostische Bedeutung. Z. Med. Lab. Diagn. 1990, 31, 231-238.

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