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CORRELATION ANALYSIS OF INORGANIC ELEMENTS IN WHOLE BLOOD OF GRMD DOGS USING INAA

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ABSTRACT

Instrumental neutron activation analysis technique (INAA) has been used to determine Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S concentrations in whole blood of GRMD dogs' samples from control, carrier and affected dogs (treated and untreated). The GRMD dogs are relevant as an experimental model for Duchenne Muscular Dystrophy (DMD), which is the most severe and prevalent type of muscular dystrophy. Muscle weakness, premature death and instability of the membrane that involves the muscle fibers - causing functional/structural abnormalities and cell death - are main characteristics of this genetic disease. To show in more details the alterations that this disease may cause in whole blood, correlations matrixes were generated for control, carried and affected groups permitting a comparison between them. The results emphasize physiologic differences for K and Mg and suggest that Br results are altered, emphasizing a constant monitoring need. Other than that, these results may help the researchers to evaluate the efficiency of new treatments and to compare the advantages of different treatment approaches before performing tests in patients with muscular dystrophy.

1. INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is the most severe and prevalent type of muscular dystrophy. Muscle weakness, premature death and instability of the membrane that involves the muscle fibers - causing functional/structural abnormalities and cell death - are main characteristics of this genetic disease. DMD is a dysfunction that affects approximately 1 in every 3500 - 6000 newborn boys in the world [1,2]. In general, males are affected and only approximately 10% of female carriers can show some disease manifestations. The worldwide incidence of genetic disorders among newborns in United States and in Europe is 3%. In Brazil, according to the latest census conducted by the IBGE (Instituto Brasileiro de Geografia e Estatística), this percentage means more than 5 million people will be affected, and in São Paulo State, the number will be about 1 million [3]. While there is no cure for DMD, substantial strategic research activities along with a search for new therapies are currently being tested in clinical trials and animal models [4].

Now, in this investigation, the purpose was to investigate GRMD (Golden Retriever Muscular Dystrophy) dogs blood submitted to hASCs treatment, using Instrumental Neutron Activation Analysis (INAA) and Energy Dispersive X-Ray Fluorescence (ED-XRF) analytical techniques. These analytic procedures are complementary and appropriate for blood analysis [5,6,7]. We intend to improve the understanding of this treatment regarding to

ions and metals (excess and deficiency) correlations during hASCs transplantations. These analyses were performed over 2 years at regular intervals (every 6 months).

2. EXPERIMENTAL PROCEDURE

To perform this investigation, blood samples from 12 Golden Retriever male dogs (5 control 7 carrier and 2 affected treated and 4 affected untreated,) at the average age of 7.1 ± 1.3 years old and weight of 28 ± 5 kg were collected. A detail explanation on the hASCs therapy, sample preparation, INAA and ED-XRF techniques used in this investigation can be found in the reference [8].

3. RESULTS AND DISCUSSION

After established the biological samples concentrations [8], it is possible to construct the Pearson's correlation matrix [9,10], which provides correlation coefficients for each element in whole blood for control, carrier and affected (treated and untreated) groups. The correlation matrices in Tables 1, shown for GRMD (control male group) and for Tables 2 and 3, are shown for GRMD (affected treated group – C9 and C16, respectively). The main correlation coefficients are highlighted.

Table 1 – Correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S (gkg^{-1}) in whole blood of GRMD control dogs

Control	Br	Ca	Cl	Cr	Fe	K	Mg	Na	P	S
Br	**1**	0.42	0.46	0.01	-0.29	-0.07	**-0.63**	0.66	0.37	-0.20
Ca		**1**	-0.35	**0.34**	0.70	0.70	-0.41	-0.02	-0.60	-0.20
Cl			**1**	**0.38**	-0.50	-0.24	-0.51	**0.94**	0.42	-0.10
Cr				**1**	**0.60**	**0.75**	-0.43	0.47	**-0.68**	-0.02
Fe					**1**	**0.91**	-0.04	-0.33	**-0.99**	0.00
K						**1**	-0.06	-0.08	**-0.92**	0.25
Mg							**1**	-0.76	0.01	0.77
Na								**1**	0.28	-0.30
P									**1**	-0.04
S										**1**

Table 2 – Correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S (gkg^{-1}) in whole blood of GRMD affected treated dogs (C9)

C9	Br	Ca	Cl	Cr	Fe	K	Mg	Na	P	S
Br	**1**	0.00	-0.34	-0.63	0.87	0.02	**-0.05**	0.09	0.21	-0.64
Ca		**1**	-0.61	**0.09**	0.43	0.56	-0.60	0.19	0.08	-0.43
Cl			**1**	**0.70**	-0.48	0.16	0.29	**0.54**	0.13	0.90
Cr				**1**	**-0.46**	**0.52**	-0.01	0.68	**0.01**	0.77
Fe					**1**	**0.43**	-0.47	0.29	**0.44**	-0.63
K						**1**	-0.76	0.85	**0.68**	0.27
Mg							**1**	-0.32	-0.83	0.01
Na								**1**	0.46	0.44
P									**1**	0.26
S										**1**

Table 3 – Correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S (gkg^{-1}) in whole blood of GRMD affected treated dogs (C16)

C16	Br	Ca	Cl	Cr	Fe	K	Mg	Na	P	S
Br	**1**	0.60	0.68	0.25	-0.22	0.55	**-0.62**	0.65	0.17	0.75
Ca		**1**	0.52	**-0.30**	0.49	-0.16	-0.61	0.67	-0.17	0.69
Cl			**1**	**0.63**	0.35	0.26	-0.53	**0.98**	0.40	0.13
Cr				**1**	**-0.03**	**0.61**	-0.26	0.45	**0.80**	-0.36
Fe					**1**	**-0.48**	-0.31	0.48	**0.12**	-0.21
K						**1**	-0.58	0.10	**0.75**	0.34
Mg							**1**	-0.55	-0.63	-0.61
Na								**1**	0.27	0.20
P									**1**	-0.11
S										**1**

The correlation matrices in Tables 4, 5, 6 and 7, are shown the results for GRMD affected untreated group C3, C8, C10 and C11, respectively. The main correlation coefficients are highlighted.

Table 4 – Correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S (gkg^{-1}) in whole blood of GRMD affected nontreated dogs (C3)

C3	Br	Ca	Cl	Cr	Fe	K	Mg	Na	P	S
Br	**1**	0.49	0.93	0.74	-0.51	0.17	0.47	0.83	0.70	0.11
Ca		**1**	0.34	0.52	0.17	0.27	0.20	0.46	0.86	-0.51
Cl			**1**	0.44	-0.60	-0.20	0.46	0.67	0.45	0.27
Cr				**1**	-0.12	0.79	0.23	0.84	0.86	-0.26
Fe					**1**	0.25	-0.87	-0.01	0.12	-0.90
K						**1**	-0.05	0.45	0.60	-0.42
Mg							**1**	-0.06	0.13	0.71
Na								**1**	0.80	-0.36
P									**1**	-0.53
S										**1**

Table 5 – Correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S (gkg^{-1}) in whole blood of GRMD affected nontreated dogs (C8)

C8	Br	Ca	Cl	Cr	Fe	K	Mg	Na	P	S
Br	**1**	0.23	0.69	0.95	0.11	-0.04	0.51	0.61	0.11	-0.08
Ca		**1**	-0.54	0.27	-0.85	-0.09	0.53	0.25	0.03	-0.11
Cl			**1**	0.60	0.75	-0.04	0.05	0.29	0.04	-0.03
Cr				**1**	-0.02	0.08	0.69	0.70	-0.03	0.22
Fe					**1**	-0.35	-0.29	-0.36	-0.22	-0.17
K						**1**	-0.24	0.74	0.63	0.48
Mg							**1**	0.24	-0.61	0.46
Na								**1**	0.53	0.34
P									**1**	-0.38
S										**1**

Table 6 – Correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S (gkg^{-1}) in whole blood of GRMD affected nontreated dogs (C10)

C10	Br	Ca	Cl	Cr	Fe	K	Mg	Na	P	S
Br	**1**	-0.31	0.11	-0.33	-0.40	-0.50	0.54	0.10	-0.67	-0.32
Ca		**1**	-0.20	-0.49	0.89	0.41	-0.58	0.16	0.68	0.90
Cl			**1**	0.57	-0.61	0.02	0.52	0.92	-0.69	-0.39
Cr				**1**	-0.59	-0.34	0.59	0.26	-0.40	-0.35
Fe					**1**	0.36	-0.73	-0.29	0.91	0.91
K						**1**	-0.83	0.23	0.45	0.06
Mg							**1**	0.27	-0.83	-0.40
Na								**1**	-0.48	-0.11
P									**1**	0.73
S										**1**

Table 7 – Correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S (gkg^{-1}) in whole blood of GRMD affected nontreated dogs (C11)

C11	Br	Ca	Cl	Cr	Fe	K	Mg	Na	P	S
Br	**1**	-0.02	0.86	0.30	-0.50	0.77	-0.18	0.69	-0.03	-0.37
Ca		**1**	0.20	0.02	-0.27	-0.16	-0.68	0.70	-0.08	0.74
Cl			**1**	0.48	-0.81	0.46	-0.08	0.76	-0.46	-0.31
Cr				**1**	-0.11	0.50	-0.32	0.21	-0.03	0.15
Fe					**1**	0.12	-0.27	-0.59	0.85	0.40
K						**1**	-0.47	0.40	0.53	-0.04
Mg							**1**	-0.58	-0.59	-0.84
Na								**1**	-0.13	0.24
P									**1**	0.45
S										**1**

Predominant correlations for control group (Table 1) are expressed by Mg:Br (-0.63) and K:Fe (0.91) which are not kept in the affected treated group, however the correlations between Mg:Br (-0.05) and K:Fe (+0.43) for C9 (Table 2) and Mg:Br (-0.62) and K:Fe (-0.48) for C16 (Table 3) show a weak and inverted coefficients.

Related to the GRMD untreated group (Tables 4,5,6, 7) the correlations were substantially altered, for that reason a study case by case need to be done.

4. CONCLUSIONS

In this study the correlation coefficients of Br, Ca, Cl, Cr, Fe, K, Mg, Na, P and S, in whole blood for GRMD control, carrier, treated and untreated dogs were obtained. These results may help the researchers to evaluate the efficiency of new treatments and to compare the advantages of different treatment approaches before performing tests in patients with muscular dystrophy. Besides, the alteration in some correlation coefficients data among the elements in the health status and in the diseased status indicates a connection between Br, K, and Mg elements in whole blood.

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