Bahrain Medical Bulletin, Volume 17, Number 3, September 1995

Original

Serum Ribonuclease Activities and Body Weight Loss

Essam M Karawya, PhD*

The levels of serum ribonucleases (RNase) were determined in a control group of healthy adults and in groups of patients suffering from body weight loss secondary to Insulin Dependent Diabetes Mellitus, hyperthyroidism and hypothyroidism. The study also included a group of premature infants with body weight much less than normal. The results obtained support the correlation between the rise of serum ribonuclease levels and the extent of weight loss. These results also emphasise the importance of measuring the specific activities of both the alkaline and the acidic RNases and calculating the ratio of these activities for the correct assessment of the extent of weight loss in a certain pathological condition. Bahrain Med Bull 1995;17:

Ribonucleases have been detected, identified and characterised in several organs and animal body fluids[1-15]. The ribonuclease (RNase) activity of the three human body fluids; serum, CSF and urine, is chromatographically heterogenous[16]. However, the extent and physical bases of this heterogeneity are unknown. Serum, for instance, contains at least six species of RNase activities separable by phosphocellulose column chromatography[1,2,17,18]. These six species of serum RNase activities have been categorized in two major classes distinguished by their pH optima for depolymerisation of RNA; acidic (pH 6.5) and alkaline (pH 8.5)[18].

The levels of serum RNase activities have been noticed to increase in several diseases, such as malignant neoplasia[19-22], renal insufficiencies[23], pancreatic disorders and leukemia[3,19,21,22,23,24]. These changes in serum RNase activities have been thought of as potential diagnostic tools. However, the significant discrepancies seen in the available reports led investigators to consider other factors such as nutritional status and renal function in the interpretation and evaluation of RNase levels in the sera of cancer patients. Frequent elevation of serum RNase levels in a variety of malignant conditions has been suggested to be modulated by the nutritional status of the patients as measured by percent body weight loss[21,25-28]. This view is supported by previous reports suggesting

* Department of Clinical Biochemistry
College of Medicine
Abha, P O Box 641
Saudi Arabia

that RNase level may serve as a useful measure of the nutritional status in animals, children and adult humans with non-malignant diseases[29,30,31]. Therefore, clinicians and investigators have become aware of the need for a reliable test to assess the nutritional status of cancer patients where abnormal metabolism is frequently seen and weight loss is associated with an adverse prognosis[32-34].

In this communication we tested this hypothesis of the possible relationship between serum RNase levels and body weight loss in some cases of endocrine imbalance which are known to affect body weight such as "Insulin Dependent Diabetes Mellitus" (IDDM), hypo/hyperthyroidism and also the serum RNase levels in premature full term babies compared with normal babies.

METHODS

Reagents: 0.2 g yeast RNA (from Sigma Chemical Co., St Louis, MO, USA) was dissolved in 100 ml of 50 mM Tris-HCL buffer (pH 8.0) and dialysed against excess of 50 mM Tris-cl, pH 8.0 at 4oC for 72 hours with several changes of the dialysis medium. Another 0.2 g of yeast RNA was dissolved in 100 ml of 50 mM sodium phosphate, pH 6.5 and dialysed as above. The serum RNase activities (alkaline and acidic) were performed in microscale using the modified method published by Akagi et al as follows[1]:

a) Alkaline RNase activity: 95 ul of yeast RNA (in alkaline buffer) were pipetted in an Eppendorf tube, mixed with 5 ul serum and incubated at 37o for 30 minutes. For blank and control 5 ul H20 and 5 ul of10 ug/ml bovine pancreatic RNase (Sigma Chemical Co.) was used in place of serum, respectively. After incubation the reaction was stopped by adding 400 ul ice cold 1 M HCI in 75% ethanol. The tubes kept on ice for one hour and centrifuged for 10 minutes in an Eppendorf centrifuge. 0.2 ml of the clear supernatant was diluted 5 times with 0.8 ml of double distilled water and the absorbance at 260 nm measured.

b) Acidic RNase activity: The same procedure was used to measure acidic RNase activity except using the RNA substrate solution in 50 mM sodium phosphate buffer, pH 6.5. One unit of RNase activity is the amount of enzyme which produce acid soluble oligonucleotides equivalent to A260 of 1.0/min. at a certain pH value at 37oC in 1ml reaction volume. Specific activity was expressed as units/mg serum protein. Serum protein concentration was determined by the Biuret method35 using standard human serum from Boehringer Manneheim Co., Germany.

Experimental data were expressed as the mean´ standard deviation. Statistic differences were tested using the nonparametric U-test, Mann and Whitney, at 5% level of significance.

Patients: Serum samples were collected from 27 normal control Saudis (College students and employees) ranging in age from 20 to 40 years and body weights from 70-80 kg with no weight loss during the previous 2-3 years. Serum was also collected from 27 patients recently diagnosed as insulin dependent diabetes mellitus (IDDM) before starting any treatment and with observed loss of 15-30% of their regular body weight. The sampling also included 29 patients with hyperthyroidism (serum free T4 higher than 5 ng/dl) before any medical intervention and 28 patients with hypothyroidism (serum free T3 and T4 less than normal and TSH levels higher than 7 uU/ml) 6-12 months after treating earlier hypothyroidism. These patients have been experiencing significant weight loss, 20-30%, in conjunction with the course of their illness. The study also included a small group of premature full term infants (body weight less than 3kg) and 10 normal infants with normal body weight as a control group.

RESULTS

The activities (mean´ SD) of both alkaline and acidic RNase were determined and expressed as units as well as specific activities (units/mg serum protein). Table 1 shows the results of serum RNase levels in a control group (A) and a group of patients with diabetes mellitus type 1 (IDDM).

Table 1
Comparison of RNase activities of a control group and patients with Diabetes Mellitus type 1 (IDDM)

	Alkaline RNase (units)*	Alkaline RNase specific activity u/mg protein	Acidic RNase (units)*	Acidic RNase specific activity u/mg protein	Alkaline SP activity/ acid SP activity
A-Control (n=27)					
Range	2.09-4.44	0.62-1.13	0.53-3.64	0.16-0.88	1.21-3.88
Mean±SD	3.30±0.56	0.93±0.15	2.11±0.70	0.59±0.18	1.75±0.62
B-Diabetes Mellitus (n=27)					
Range	2.98-7.00	0.88-2.47	1.80-6.03	0.53-2.13	1.16-1.90
Mean±SD	4.85±1.52	0.98±0.33	3.35±1.05	1.42±0.30	0.87±0.25
Z	5.50**	5.91**	4.35**	4.85**	0.92

* One unit is the amount of RNase which produces acid soluble oligonucleotides equivalent to A260 of 1.0/min at 370C in a 1 ml reaction volume.
** P<0.05

For healthy individuals (n=27), the alkaline RNase levels were 3.30±0.56 units or 0.93±0.15 u/mg protein, while the acidic RNase levels were 2.11±0.70 units or 0.59±0.18 u/mg protein. The ratios of the alkaline RNase/acidic RNase activity for this control group were 1.75±0.62. In patients with IDDM (Group B, Table 1; n = 27) the alkaline RNase activity units were 4.85±0.87 or 1.41±0.30 u/mg protein which were significantly higher than the control group. The acidic RNase activities were 3.35±1.05 units or 0.98±0.33 u/mg protein which were also significantly higher than the control group. However, the ratio of alkaline RNase/acidic RNase activities was non-significantly lower than the control group.

Table 2
Serum alkaline and acidic RNase activities of two groups of patients. Group A with Hyperthyroidism and Group B with Hypothyroidism

	Alkaline RNase (units)*	Alkaline RNase specific activity u/mg protein	Acidic RNase (units)*	Acidic RNase specific activity u/mg protein	Alkaline SP activity/ acid SP activity
A-Hyperthyroidism (n=29)					
Range	1.71-4.91	0.45-1.97	1.71-4.28	0.46-1.58	0.94-1.68
Mean±SD	3.90±1.38	0.94±0.21	2.88±0.24	1.28±0.61	0.68±0.29
B-Hypothyroidism (n=26)					

Range	2.83-5.06	0.88-1.66	1.26-4.24	0.40-1.31	1.14-2.55
Mean±SD	3.951.59±0.34	0.78±0.23	2.61±0.74	1.18±0.19	0.61±
Z	3.27**	4.53**	2.29**	2.97**	0.60

* One unit is the amount of RNase which produces acid soluble oligonucleotides equivalent to A260 of 1.0/min at 370C in a 1 ml reaction volume.
** P<0.05

Table 2 shows the RNase levels in serum from patients with hyperthyroidism (Group A, n=29) and hypothyroidism (Group B, n=26). The alkaline RNase activities in hyperthyroidism were 3.900.68 ± units or 1.280.29 ± u/mg protein which were significantly higher than the control group. The ratio of the alkaline RNase/acidic RNase activities were 1.380.21 ± which were significantly lower than the control group. The serum alkaline RNase of patients with hypothyroidism (Group B of Table 2, n=26) were 3.950.61 ± units or 1.180.19 ± u/mg protein which were significantly higher than the control group. The acidic RNase levels were 2.610.74 ± units or 0.780.23 ± u/mg protein which were also significantly higher than the control group. The ratios of alkaline RNase/acidic RNase activities, however, were 1.590.34 ± which were non-significantly lower than the control group.

Table 3
Serum alkaline and acidic RNase activities for a control group of infants (A) with normal body weight and a group of premature infants (B)

	Alkaline RNase (units)*	Alkaline RNase specific activity u/mg protein	Acidic RNase (units)*	Acidic RNase specific activity u/mg protein	Alkaline SP activity/ acid SP activity
A-Control (n=10)					
Range	2.29-3.48	0.59-1.15	1.02-2.33	0.26-0.74	1.50-2.77
Mean±SD	2.992.07±0.38	0.47±0.14	1.50±0.41	0.94±0.17	0.40±
B-Premature Infants (n=4)					
Range	3.97-6.08	2.31-3.29	2.28-4.38	1.23-1.97	1.39-1.88
Mean±SD	4.661.65±0.23	1.67±0.32	2.92±0.99	2.70±0.43	0.96±
Z	2.83**	2.83**	2.69**	2.83**	1.84

* One unit is the amount of RNase which produces acid soluble oligonucleotides equivalent to A260 of 1.0/min at 370C in a 1 ml reaction volume.
** P<0.05

The levels of serum RNase activities of premature infants (n=4) were compared with the values of control healthy infants (n=10) with normal body weights, and summarised in Table 3. The activities of alkaline RNase units were 4.660.96 ± or 2.700.43 ± u/mg protein which were significantly higher than the control values. The levels of acidic RNase activities were 2.920.99 ± units or 1.67 ± 0.32 u/mg protein which were again significantly higher than the corresponding values for the control group. The ratios of alkaline RNase activities/acidic

RNase activities were 1.650.23 ´ which were non-significantly lower than the control values.

DISCUSSION

Serum ribonuclease levels have been suggested to correlate with the nutritional status of some cancer patients as measured by percent ideal body weight or percent weight loss[25]. Therefore, this study was undertaken to assess this correlation in well defined pathological conditions, other than cancer, where body weight loss is profound. The results shown in Table 1 indicate that the levels of circulating RNase in IDDM were significantly higher than the control group, with higher increase in the acidic RNase levels than the increase in alkaline RNase levels as indicated by the significant decrease of the ratio of alkaline RNase activities/acidic RNase activities indicating excess increase of the acidic RNase which originate from liver or spleen[16]. The weight loss is a common feature of IDDM when it develops sub acutely over a period of weeks. The weight loss is initially due to depletion of water, glycogen and triglyceride stores. Chronic weight loss due to reduced muscle mass occurs as amino acids are diverted to form glucose and ketone bodies[36]. This remarkable weight loss in IDDM may be a major contributing factor for elevating serum RNase activity. On the other hand, renal failure due to micro-vascular disease is a leading cause of death in type 1 diabetes[37]. The diabetic nephropathy, with the resulting renal failure, may also explain the high levels of serum acidic RNase activity similar to other previous reports in non-diabetic renal failure patients[23,28]. There are only a few situations which are marked by excessive caloric intake accompanied by loss of weight. Hyperthyroidism and diabetes mellitus are the most common. The weight loss, often in the face of hyperphagia, is a characteristic feature of thyrotoxicosis and patients tend to experience severe weight loss that mimic neoplasms[39]. The results shown in Table 2 indicate that thyrotoxicosis significantly increased the activities of serum RNases with significant decrease in the ratio of alkaline to acidic RNase activity. These results may best be explained as the result of weight loss following the increased basal metabolic rate and occasional fever associated with severe hyperthyroidism[40]. The results of group B (Table 2) are for patients with hypothyroidism, marked by lower serum levels of T4 and higher TSH than normal. As shown in Part B of Table 2, the hypothyroidism significantly increased the serum levels of RNases but with less elevation of the acidic form of RNase than in hyperthyroidism. Keeping in mind the possible correlation between weight loss and the serum levels of RNase, the results shown are not unexpected. Thinking of the pathogenesis of hypothyroidism, the most frequent causes are related to treatment given for primary hyperthyroidism[41]. Graves's disease may sometimes progress to an eventual hypothyroid state, without any medical intervention[41]. It has been reported that patients with hypothyroidism often gain weight after a long, several years, gradual slow withdrawal of thyroid hormones[41]. The patients with hypothyroidism included in this group, were mostly recently recognised before reaching the late stage of gaining weight. Therefore, the raised levels of serum RNases can still be related to tissue destruction accompanying the earlier stage of weight loss.

The last group included in this study was a small number (n=4) of premature infants with body weight at birth of 2.0 to 2.3 Kg. The serum RNase levels of these premature infants were significantly higher than a control group (n=10) of infants with normal body weight of 3.50 to 4.0 Kg. These results in Table 3 also shows that the ratio of activities of the alkaline to the acidic RNase were significantly less than the control group which means a profound elevation of the acidic RNase activity.

These results, taken altogether, support the suggested correlation between the serum RNase levels and the weight loss. Also, the results emphasise the importance of measuring the specific activities of both the alkaline and the

acidic RNases and calculating the ratio of these activities for the correct assessment of the extent of weight loss in a certain pathological condition.

CONCLUSION

A close correlation between serum ribonuclease levels and the body weight loss, associated with endocrine imbalance was observed. Serum RNase levels can now provide a useful tool for the assessment of the nutritional status in these clinical conditions. This initial report should be followed by detailed investigations involving the different serum RNase species. Analysis, characterisation and monitoring these different serum RNase species will certainly provide valuable information for the correct assessment of the extent of body weight loss.

ACKNOWLEDGEMENTS

The author wishes to thank the CMRC, College of Medicine at Abha, for supporting this study. We are grateful to Dr A Abdel-Hameed, Department of Medicine at Khamis Mushayt Hospital and Dr A Mahfouz for their great help for this study.

REFERENCES

1. Akagi K, Murai K, Hirao N, et al. Purification and properties of alkaline ribonuclease from human serum. Biochim Biophys Acta 1976;442:368-78.

2 Akagi K, Yamanaka M, Murai K, et al. Purification and properties of acid ribonucleases in human serum and leukocytes. Cancer Res 1978;38:2136-67.

3. Bardon A, Sierakowska H, Shugar D. Purification and properties of human acidthermo stable ribonuclease and diagnosis of childhood pancreatic fibrosis. Clin Chim Acta 1976;67:231-43.

4. Delaney R. Chemical, physical and enzymatic properties of several human ribonucleases. Biochemistry 1963;2:438-44.

5. Frank JJ, Levy CC. Properties of a human liver ribonuclease. J Biol Chem 1976; 251:5745-51.

6. Metais P, Mandel P. Activite ribonucleasique du serum humain. Bull Soc Chim Biol 1955;37:999-1007.

7. Naskalski J. A comparison of the isoenzymic patterns of urinary ribonucleases in healthy subjects and patients with chronic granulocytic leukemia. Pol Med J 1972; 11:827-34.

8. Neuwelt EA, Frank JJ and Levy CC. Purification of human spleen ribonuclease by immunoabsorption: similarity of the enzyme with human liver ribonuclease. J Biol Chem 1976;251:5752-58.

9. Neuwelt EA, Schmuckler M, Niziak Ms, et al. The immunological characterisation of several human ribonucleases by using primary binding tests. Biochem J 1977;163:419-26.

10. Neuwelt EA, Boguski MS, Frank JJ, et al. Possible sites of origin of human plasma ribonuclease as evidence by isolation and partial characterisation of ribonucleases from several human tissue sources. Cancer Res 1978;38:88-93.

11. Rabin EZ, Weinberger V, Tattrie B. Ribonuclease activity in human serum, cerebrospinal fluid and urine. Clin Chim Acta 1977;78:235-42.

12. Reddi KK. Nature and possible origin of human serum ribonuclease. Biochem Biophys Res Commun 1975;67:110-18.

13. Reddi KK. Purification and properties of a ribonuclease in human urine that hydrolyses polycytidylic acid. Prep Biochem 1977;7:283-99.

14. Schmuckler M, Jewett PB, Levy CC. The effects of polyamines on a residue-specific human plasma ribonuclease. J Biol Chem 1975;250:2206-12.

15. Ukita T, Takahashi T, Waku K, et al. Research on pancreatic ribonuclease. J Biochem (Tokyo) 1964;55:293-302.

16. Blank A, Dekker CA. Ribonucleases of human serum, urine, cerebrospinal fluid and leukocytes. Activity staining following electrophoresis in sodium dodecyl sulfate polyacrylamide gel. Biochemistry 1981;20:2261-7.

17. Sznajd J, Naskalski JW. Ribonuclease from human granulocytes. Biochim Biophys Acta 1973;302:282-92.

18. Ressler N, Olivero E, Thompson GR, et al. Investigation of ribonuclease isozymes by an electrophoretic ultraviolet method. Nature 1966;210:695-8.

19. Akagi K, Yamanaka M, Murai K, et al. Serum acid ribonuclease in myelogenous leukemia. Cancer Res 1978;38:2168-73.

20. Fink K, Adams WS and Skoog WA. Serum ribonuclease in multiple myeloma. Am J Med 1971;50:450-7.

21. Reddi KK, Holland JF. Elevated serum ribonuclease in patients with pancreatic cancer. Proc Natl Acad Sci USA 1976;73:2308-10.

22. Kottel RH, Hock SO, Parsons RH, et al. Serum ribonuclease activity in cancer patients, Br J Cancer 1978;38:280-6.

23. Humphrey RL, Karpetsky TP, Neuwelt EA, et al. Levels of serum ribonuclease as an indicator of renal insufficiency in patients with leukemia. Cancer Res 1977;37:2015-22.

24. Levy AL, Rottino A. Effect of disease states on ribonuclease concentration of body fluids. Clin Chem 1960;6:43-51.

25. Chlebowski RT, A Bramson SB, Bateman JR, et al. Influence of nutritional status on circulatory ribonuclease c levels in patients with cancer. Cancer 1985;55:427-31.

26. Peterson LM. Serum RNase in the diagnosis of pancreatic carcinoma. Proc Natl Acad Sci USA 1979;76:2630-4.

27. Marabella PC, Tritsch GL, Moore RH, et al. Serum ribonuclease in patients with lung carcinoma. J Surg Oncol 1976;8:501-5.

28. Maor D, Klein ME, Kenady DE, et al. Carcinoma of the lung and cigarette smoking: effect on serum ribonuclease activity. JAMA 1978;239:2766-8.

29. Zigman S, Allison JB. Ribonuclease activity of protein- depleted and tumour-bearing rats. Cancer Res 1959;10:1105-8.

30. Siguleum DM, Brasel JA, Velasco EG, et al. Plasma and urine RNase as a measure of nutritional status in children. Am J Clin Nutr 1973;26:793-7.

31. Reddy V, Mohanram M, Prabhavathi P. Alkaline ribonuclease activity in plasma and leukocytes of malnourished women Nutr Metab 1978;22:357-61.

32. Dewys WD, Begg C, Lavin PT. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Am J Med 1980;69:491-8.

33. Chlebowski RT, Heber D. Hypogonadism in male patients with metastatic cancer prior to chemotherapy. Cancer Res 1982;42:2495-8.

34. Chebowski RT, Heber D and Block JB. Serial assessment of glucose metabolism in patients with cancer cachexia. Clin Res 1982;30:69.

35. Flack CP, Woollen JW. Prevention of interference by destran with biuret-type assay of serum proteins. Clin Chem 1984;30(4):559-61.

36. Karam JH, Salber PR and Forsham PH. Pancreatic hormones and diabetes mellitus. In: Greenspan FS, ed. Basic and clinical endocrinolgy. 3rd ed. Norwald, Connecticut: San Mateo, California, 1991:6111.

37. Najarian JS, Sutherland DE, Simmons RL, et al. Ten year experience with renal transplantation in juvenile onset diabetes. Ann Surg 1979;190:487-500.

38. Akagi K, Tsuji H, Kajiwara E, et al. Activities of serum acid ribonucleases in patients with malignant neoplasms or with renal failure. Clin Chim Acta 1983;135:83-87.

39. Thomas FB, Mazzaferri EL, Skillman TG. A pathetic thyrotoxicosis: A distinct clinical and laboratory entity. Ann Intern Med 1970;72:679-86.

40. Greenspan FS, Rapoport B. Thyroid gland. In: Greenspan FS, ed. Basic and clinical endocrinology 3rd ed. Norwald, Connecticut: San Mateo, California, 1991:49-59.

41. Daggett PR. The Thyroid. In: clinical endocrinology. London: Edward Arnold Publishers Ltd, 1981:49-59.