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ORIGINAL ARTICLE

A NOVEL ALKALINE PHOSPHATASE ENZYME IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS' SERUM

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ABSTRACT: Alkaline phosphatase (ALP) is a non-specific member of a group of phosphomonoesterase enzymes generating an organic radical and inorganic phosphate due to catalyzing the hydrolysis of phosphate esters in an alkaline environment. The aim of the present study was to investigate the clinical usefulness of characterization of alkaline phosphatase isoenzymes, as metastases markers. The alkaline phosphatase activity of the Human Serum has been partially characterized in patients with lymphoblast leukemia's acute diseases and the '*in vitro*' effect of heavy metals on enzymatic activity has been analyzed. The optimal pH was 10.2 for alkaline phosphatase activity. The effect of heavy metals is dependent on the serum of patients analyzed. Zn^{+2} showed the highest inhibition for normal serum enzyme activity but Mn^{+2} showed the highest inhibition for lymphoblast leukemia's acute cancer serum enzyme and the parameter *K*m For normal serum and cancer serum was 18 and 20 respectively. This is a different and unique isoenzyme which may use as a diagnostic marker in lymphoblast leukemia's acute patients due to increase in its activity, different k_m value, different pH optima, different effect of Zn^{+2} and Mg^{+2} comparing to another members of phosphomonoesterase group.

KEYWORDS: Alkaline Phosphatases, pH, Metals, Human, Serum, lymphoblastic leukaemias acute.

INTRODUCTION

Alkaline phosphatases (EC 3.1.3.1) constitute a group of phosphomonoesterase enzyme generating an organic radical and inorganic phosphate due to catalyzing the hydrolysis phosphate esters at an alkaline pH (Reichling and Kaplan, 1982). The tissue unspecific alkaline phosphatase (ALP) is expressed mainly in bones, liver, and in lesser amounts from intestines, placenta and kidneys of healthy adults (Friedman *et al.*, 1996). Markedly elevated serum ALP, hyper alkaline phosphatasemia, is seen predominantly with more specific disorders, including, malignant biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, hepatic lymphoma, extra hepatic bile obstruction, infiltrative liver disease, hepatitis, sarcoidosis and cancers (Neuschhwande-Terti, 1995). There are anecdotal reports and small studies suggesting that, a new alkaline phosphatase isoenzyme is useful for the diagnosis and clinical evaluation of patients with lymphoblastic leukaemias acute cancer (Whitby and Moss, 1975).

The aim of the present study was to investigate the clinical usefulness of characterization of alkaline phosphatase isoenzymes, as metastases markers.

PATIENTS AND MTHODS

Total of 255 patients diagnosed lymphoblastic leukaemias acute cancer of Shefa Hospital, Ahvaz, Iran from January 2005 to December 2010, were enrolled in this study. 5 mL venous blood was obtained and alkaline phosphatase was determined according to the procedure described by Walter and Schutt (Walter and Schutt, 1974). To determine the effect of metals (Mn^{+2} and Mg^{+2}), the concentration, in the final volume, ranged between 5 mM. The buffers employed in the analysis of metal effects were 0.1 M phosphate at the optimum pH for serum patients. The enzyme activity and *K*m parameters for normal and cancer serum calculate at optimum pH and room temperature,

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with substrate concentrations at 5 mM and an incubation time of 60 min. At the end of incubation period, the reaction was stopped immediately by placing the tubes on ice and adding 2 ml 0.1 N NaOH. The absorbance of *p*-nitro phenol released was recorded at 405 nm. The serum from 255 healthy volunteers was also used as the control.

RESULTS

Although the ALP activities of normal and abnormal sera are low, we could measure the Human serum and of hydrolysis of *p*-nitro phenol without difficulty (see Table 1). The activity of alkaline phosphatase of all diseases was higher than that of alkaline phosphatase in all tissue (Table1). The lowest Km is found in the Human liver, and the highest in the Scrobicularia plana Gill. For ALP of lymphoblastic leukemia's acute cancer serum, *K*m was 18 µM. The percentage of inhibition with 5 mM of metals (Zn^{+2}, Mg^{+2}) on normal serum lymphoblastic leukemia's acute alkaline phosphatase was 12.63 and 19.80%, respectively. The percentage of inhibition with 5 mM of metals (Zn^{+2}, Mg^{+2}) on lymphoblastic leukemia's acute alkaline phosphatase was 13.76% and 12.84% respectively (Table 2). Table 3 shows pH vs. enzymatic activity of serum of the lymphoblastic leukemia's acute normal, patients and some sources of alkaline phosphatase. We can find so many peaks in all tissues and diseases, the first at pH 8.5 for Scrobicularia plana Gill, Digestive Gland, Mantle of Clam, Siphon of Clam, spider venom, the second at pH 9.4 for Human Serum, the third at pH 10.0 for smooth muscle mouse, gastric and Vas Deferens, fourth at pH 10.1 for kidney cortex and, kidney Medulla, fifth at pH 10.4 for bone, plasma, urine, Kidney of Frog, sixth at pH 10.5 for Human thyroid and seventh pH 10.7 for Human Placental (Table 3). The apparent *K*m and pH of so many of alkaline phosphatases, normal serum and lymphoblastic leukaemias acute cancer serum are reported in Table 3.

Table 1: Alkaline phosphatase activity for Human Normal Serum and lymphoblastic leukaemias acute diseases

Different Alkaline phosphatase	Enzyme activity (Unit/mL)
Human Normal Serum	251.5
Human cancer Serum	456.3

Table 2: Influence of several compounds in the alkaline activities

Source of Alkaline Phosphatase		Metal Ions		Reference
		Zn++	Mg++	
	Human thyroid	-	20%	Klipatrick and Crofton, 1981
	Human Serum	-	100%	Vinet *et al.*, 1978
	Human gastric	-	50%	Kwan and Ito, 1987
	Vas Deferens	-	65%	Kwan and Ito, 1987
	Chelon labrosus Gill	39%	-	Mazorraa *et al.*, 2002
	Frog Kidney	-	87%	Whinnie *et al.*, 1971
	Escherichia coli	6%	6%	Trotman and Greenwood, 1971
	Smooth muscle mouse	-	74%	Whitmore and Goldberg1972
	Spider venom	84%	166%	Rodrigues *et al.*, 2006
	kidney Medulla	-	100%	Koyama *et al.*, 1988
	spider venom	18%	212%	Rodrigues *et al.*, 2006
Present Study	Normal serum with 5mM of Metals	2.63%	9.80%	
	Cancer serum with 5mM of Metals	3.76%	2.84%	

Table 3: Optimum pH and Km value parameters of alkaline phosphatase activity

Sours of Alkaline Phosphatase	Km (µM)	pH	Reference
Human Placental		10.7	Metaye *et al.*, 1988
Human liver	0.1		Vinet *et al.*, 1978
kidney cortex	0.67	10.1	Koyama *et al.*, 1988
kidney Medulla	0.56	10.1	Koyama *et al.*, 1988
human thyroid	0.9	10.5	Klipatrick and Crofton, 1981
Kidney tissues of R. philippinarum	-	10.5	Blasco *et al.*, 1993
Human Serum	-	9.4	Vinet *et al.*, 1978
Human Intestinal	0.15	-	Vinet *et al.*, 1978
Lumen	2.51	-	Miura *et al.*, 1990
Brush	2.41	-	Miura *et al.*, 1990
Cytosol	2.48	-	Miura *et al.*, 1990
Chelon labrosus gill	-	10.75	Belloc and Gallis, 1980
Scrobicularia plana Gill	4.52	8.5	Mazorraa *et al.*, 2002
Digestive Gland	2.48	8.5	Mazorraa *et al.*, 2002
Mantle of Clam	2.88	8.5	Mazorraa *et al.*, 2002
Siphon of Clam	2.48	8.5	Mazorraa *et al.*, 2002
spider venom	-	8.5	Rodrigues *et al.*, 2006

	Frog bone	-	10.4	Whinnie *et al.*, 1971
	Frog plasma	-	10.4	Whinnie *et al.*, 1971
	Frog urine	-	10.4	Whinnie *et al.*, 1971
	Frog Kidney	-	10.4	Whinnie *et al.*, 1971
	Smooth muscle mouse	-	10	Whitmore and Goldberg1972
	Lymph	2.50	-	Miura *et al.*, 1990
	gastric	-	10	Kwan and Ito, 1987
	Vas Deferens	-	10	Kwan and Ito, 1987
Present Study	Human Normal Serum	2	10.5	
	Human cancer Serum	1.8	10.8	

DISCUSSION

Many authors have shown that the determination of alkaline phosphatase isoenzyme activity is useful for monitoring and diagnosis and clinical evaluation of patients with different types of carcinomas (Van Hoof *et al.*, 1992). The optimum pHs for ALP of normal serum and lymphoblastic leukemia's acute cancer serum was 10.5 and 10.8 respectively. the highest and lowest value were found for ALP in the lymphoblastic leukaemias acute cancer serum and (Scrobicularia plana Gill, Digestive Gland, Mantle of Clam, Siphon of Clam and spider venom) respectively.

We find the low levels found for ALP activity in normal serum but in patients serum is elevated, the high level ALP activity is a marker for cancer. The alkaline phosphatase activities, pH optimum, km values and also the '*in vitro*' effect of metals of enzymatic activities of the sours of enzyme is affected by the type alkaline phosphatase. The optimal pH ranged between 8.5 and 10.7 for alkaline phosphatase activity. The pH optimum of different sours *Chelon labrosus* gill (Belloc and Gallis, 1982); in the Kidney tissues of *R. philippinarum* (Blasco *et al.*, 1993) and to the kidney and intestine of carp, eels, mice (Yora and Sakagishi, 1986) and whole body homogenates of *Venus gallina* (Koyama *et al.*, 1988) but we find the pH optimum of enzyme 10.2. The pH optimums and the effect of metals profile is recognized as one of the factors used for characterization of isoenzymes. *K*m values varied between organs for alkaline phosphatase activity, it is shown by the clam *R. philippinarum* (Blasco *et al.*, 1993), trout intestinal alkaline phosphatase (Whitemore and Goldberg, 1972), the crab, *C. spidus* (Lovett *et al.*, 1994), Gill, Digestive Gland, Foot, Mantle, Siphon (Mazorraa *et al.*, 2002), Escherichia coli (Trotman and Greenwood, 1971), bone, plasma, urine and kidney of Frog (Whinnie *et al.*, 1971), Kidney and Liver of bird [11] Smooth muscle mouse (Whitemore and Goldberg, 1972), human placental (Metaye *et al.*, 1988), Human liver (Koyama *et al.*, 1988), Human kidney Medulla (Koyama *et al.*, 1988), human thyroid (Kilpatrick and Crofton, 1981), Human Intestinal (Vinet *et al.*, 1978), Human Serum (Maekawa *et al.*, 1985), we find km values for normal serum and cancer serum 20 µM and 18 µM respectively.

The effect of metals was dependent on the source the enzymatic activity analyzed. This finding may be a consequence of the difference in structure. The zinc and magnesium inhibition were quite different between tissues for the alkaline phosphatase. This may be the result of the different structure of the enzyme molecules and their possible interaction with the metal. Zn^{+2} and Mg^{+2} showed the highest inhibition in the normal Human serum and cancer serum respectively. Therefore kinetic analysis of Zn^{+2}, Mg^{+2} inhibitions in lymphoblastic leukemia's acute showed a type of inhibition, indicating the kind of isoenzymes. From this finding, we can diagnosis lymphoblastic leukemia's acute cancer. All highly purified alkaline phosphatases have proved to be Zn (II) metalloenzymes (Coleman and Gettings, 1983). The role of this metal as activator is related to the saturation of Zn (II) binding sites. However, gill, mantle and siphon showed a slight inhibition. This may be a consequence of excessive Zn replacing Mg at binding sites in the ALP (Sastry and Sharma, 1979). For fish, in laboratory conditions, liver alkaline phosphatase activity changes in response to waterborne metal (Lan *et al.*, 1995) making it useful as indicator of metal exposure. Monitoring the characterization of ALP at serum patient's follow-up visit may be economically used as an indicator of cancer. ALP is a simple, low cost, relatively sensitive screening tool for detecting cancer.

CONCLUSION

This is a different and unique isoenzyme which may use as a diagnostic marker in lymphoblastic leukemia's acute patients due to increase in its activity, different k_m value, different pH optima, different effect of Zn^{+2} and Mg^{+2} comparing to another members of phosphomonoesterase group.

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