



# Values of alkaline phosphatase and their isoenzyme profiles in patients with cancer in respect to bone and liver metastasis

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## SUMMARY

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**Background:** Alkaline phosphatase is a glycoprotein that catalyzes two kinds of chemical reactions: hydrolysis of phosphorus ester breaking P-O bonds and phospho-transfer reactions in which phosphoric group is transferred to an acceptor molecule. In the human body, ALP exists in multiple molecular forms whose heterogeneity is partly due to genetic factors and partly to posttranslational modifications. The aim was to evaluate a total ALP activity and its isoforms in cancer patients with bone and liver metastasis in comparison to healthy controls.

**Methods:** Human serum was collected from 20 healthy individuals, and 20 cancer patients with bone and liver metastases, with metastases confirmed by ultrasound, computerized tomography and a radiology scan. Determination of ALP was done by the endpoint spectrophotometric method. Isoenzymes were determined by heat inactivation method.

**Results:** In cancer patients, the total ALP activity was significantly higher ( $p < 0.05$ ) compared to healthy controls. In the sera of cancer patients with liver metastases, the remaining ALP activity was two-fold higher in comparison to bone metastases.

**Conclusion:** Determination of ALP isoenzymes is important but a correct clinical interpretation in the context of other analyses is vital for a proper diagnosis of a disease.

**Key words:** Alkaline Phosphatase; Isoenzymes; Neoplasms; Neoplasm Metastasis; Liver Neoplasms; Bone Neoplasms

## INTRODUCTION

Alkaline phosphatase (orthophosphoric-monoester phospho hydrolase EC 3.1.3.1, further in the text ALP) is a glycoprotein that catalyzes two kinds of chemical reactions: hydrolysis of phosphorus ester breaking P-O bonds (hydrolytic activity) and phospho-transfer reactions in which phosphoric group is transferred to an acceptor molecule (1-3). Alkaline phosphatase is present in cell membranes: in proximal kidney tubules, intestinal mucosa, placenta, osteoblasts and bile duct. It was shown that ALP can be found in some body fluids (serum, alveolar surfactant). ALP is bound to the plasma membrane by a glycosylated phosphatidylinositol (GPA) anchor. The hydrophobic diacylglycerol part is immersed in the external layer of the two-layered membrane, and the inositol OH group is bound by a glycosidic bond to glucosamine, which is a part of the glycanic component. Glycerine is bound to ethanolamine, while ethanolamine is bound to C-terminal amino acid of an extracellularly oriented protein. As a part of a membrane, ALP is a tetrameric protein, while in body fluids it is either dimeric or a mixture of tetrameric and dimeric proteins.

In a human body, the ALP "constitutes a system of multiple molecular forms of enzymes in which heterogeneity is partly due to genetic factors and partly to posttranslational modifications" (4). There are four isoenzymes encoded by four different genes. These are tissue non-specific, germ cell, placental and intestinal ALP. The tissue non-specific isoenzymes are subject to posttranslational modification (glycosidation) producing tissue specific isoforms of liver, kidneys and bones. Tissue specific isoforms are heat unstable, while a liver isoform is more stable than a bone isoform (5-7). They are inhibited by urea and levamisole and are resistant to L-phenylalanine.

A regan isoenzyme is also heat stable and it is a result of placental gene depression. It is found in serum of 5 to 15% of patients with different malignant tumours.

The aim of the work was to determine the values of total ALP enzyme and isoenzyme activity in serum of cancer patients with regard to presence of liver and bone metastases.

## PATIENTS AND METHODS

### Subjects

The enzyme values in samples of twenty healthy adults (controls, 10 men and 10 women, aged between 18 and 45 years) and twenty patients with cancer – 10 cancer patients with confirmed liver metastases and 10 bone metastases, were studied. The metastases were confirmed by ultrasound, computerised tomography and a radiology scan, for appropriated localisation. Tumor diagnosis was confirmed using biopsy or surgery section for appropriated tumors. Local ethic committee approved the research in the Clinical Centre of Serbia, Belgrade. Serum from cancer patients was collected by standard procedure and kept frozen at -20°C until use.

### Methods for determination of total alkaline phosphatase activity

ALP values were determined by the end-point spectrophotometric method (8-11). Hydrolysis of 4-nitrophenyl phosphate in the alkaline substrate produces 4-nitrophenyl of yellow colour whose intensity is determined on a spectrophotometer at a wavelength of 405 nm. The activity of alkaline

phosphatase was calculated with molar extinction coefficient and the results of the enzyme activity were given in the International Units.

### Determination of isoenzymes of alkaline phosphatase after heat inactivation

The serum samples were incubated simultaneously at the room temperature, at 56°C and 65°C. After 10 minutes of incubation at 56°C, i.e. 30 minutes at 65°C, the heated samples were cooled at 0°C and the ALP activity was determined in each aliquot (3, 5, 8).

The percentage of the remaining activity is calculated for each aliquot with the following formula

$$\text{The remaining activity} = \frac{\text{activity in the heated aliquot}}{\text{activity in the unheated aliquot}} \times 100$$

Results were presented as the mean and median values.

### Statistical analysis

The data were calculated using Mann-Whitney U-test for determination of a difference between the groups. Statistical diggerence  $p < 0.05$  was used as significant.

## RESULTS

### ALP enzyme activity in serum of healthy controls

Total ALP enzyme activity in sera of healthy women was in the range from 24.55 to 43.47 U/L, (median 33.54U/L) while in sera of men, it varied from 33.14 to 52.6 U/L (median 43.01U/L). No statistically significant difference between the two groups was found ( $p > 0.05$ ). The mean value of the remaining ALP activity after the heat inactivation at 56°C was similar, 27% and 26%, in women and men, respectively (Table 1).

**Table 1. Activity of ALP in the serum of healthy men and women**

	Total activity * U/L	Resting activity ** U/L
<b>Men</b>		
Mean (SD)	43.01 (5.84)	11.43 (26%)
Median (range)	43.69 (33.14-52.60)	9.72
N	10	10
<b>Women</b>		
Mean (SD)	33.82 (6.85)	9.43(27%)
Median (range)	33.54 (24.55-43.97)	9.99
N	10	10

\* Incubation at 37°C

\*\*Incubation at 56 °C

### ALP enzyme activity in serum of patients with cancer

The total ALP activity in all patients with malignant diseases (median 126.55 U/L) was significantly increased ( $p < 0.01$ ) compared to healthy people (median 38.61) of the same age. No difference was found between the total ALP in cancer patients with liver and bone metastases ( $p > 0.05$ ). In the sera of cancer patient with a different type of tumour, but with bone metastases, the remaining ALP activity after inactivation at 56°C was 24.28

U/L (Table 2). In the sera of cancer patients with liver metastases the remaining ALP activity was two-fold higher i.e. 55.16 U/L ( $p > 0.05$ , Mann-Whitney U-test). Regan isoenzyme was not found in cancer patients' sera (Table 3).

**Table 2. Activity of ALP in the serum of patients with cancer bone metastasis**

	Total activity * U/L	Resting activity ** U/L (%)
Mean (SD)	127.63 (25.14)	24.23 (19%)
Median (range)	116.57 (106.06-184.06)	24.28
N	10	10

\* Incubation at 37°C

\*\* Incubation at 56 °C

**Table 3. Activity of ALP in the serum of patients with cancer liver metastasis**

	Total activity * U/L	Resting activity ** U/L
Mean (SD)	137.05 (23.63)	53.95 (40%)
Median (range)	134.46 (106.29-192)	55.16
N	10	10

\* Incubation at 37°C

\*\* Incubation at 56 °C

## DISCUSSION

Alkaline phosphatase is a widely used marker for skeletal and hepatobiliary disorders, but its activity is also increased in atherosclerosis and peripheral vascular disease (6-10). In this paper, the values of ALP enzymes and their isoenzymes in healthy adults and cancer patients were studied.

The aim was to standardize the values in relation to controls under our laboratory conditions. The total serum ALP enzyme activity of healthy adults aged from 18 to 45 was used as a control value. ALP isoenzymes were identified by heat inactivation, which is, besides electrophoresis (10, 11), the most frequently applied method. The advantage of this method lies in the fact that it is simple and does not require expensive equipment while the results obtained are usable and clinically useful. However, the method requires a strict control of incubation conditions because of a high temperature coefficient of enzyme heat activation. Previous literature data indicate (5, 11) that application of the heat inactivation method yields similar values to the ones obtained by polyacrylamide gel electrophoresis (10). After ten-minute-incubation at 56°C, the remaining activity in men and women was similar, representing approximately a quarter of median total value. In the literature (1), the remaining activity is around 26-34%, which indicates the existence of a mixed isoenzyme profile (ALP liver and bone isoform). In this paper, heat stable isoenzymes (placental and Regan) were not found in serum of healthy adults of both genders.

Our and previous reports showed that in cancer patients the total ALP enzyme activity was around three-fold higher than the activity in healthy people of the same age (9, 12).

Bone is the most frequent site for metastases in patients with advanced cancer including prostate, breast, lung, thyroid and renal cancers. Approximately 70% of patients with advanced cancer will develop bone metastases (13-17). The assessment of bone metastases by <sup>99m</sup>Tc-based bone scintigraphy is routinely used for the detection of bone metastases (14). Bone scintigraphy offers high sensitivity, but lacks specificity in the detection of

skeletal metastases and the value of bone scanning for detecting disease progression has been questioned on the basis of cost-effectiveness (14). The uncertain scientific and clinical utility of biochemical markers of bone metabolism mean that such approaches have not yet been established as surrogate measurements for clinical efficacy. However, serum biochemical markers can be determined frequently and easily, with negligible disturbance to the patient (14-16, 18-20).

Our data showed that cancer patients with bones metastases mainly had elevated values of bone isoform ALP. Bone metastases cause osteoclastogenesis and bone resorption, disrupting the balance between the osteoblast and osteoclast activity. Bone formation markers are direct or indirect products of osteoblast activity, whereas bone resorption markers are derived from the degradation of skeletal collagen (18, 19). Elevated ALP was due to bone destruction and compensation of osteoblastic activity mediated by an increased production of inflammatory mediators like TNF-alpha (12).

A significant increase of remaining ALP isoform, in patients with liver metastases, which was predominantly increased as results of liver tissue destruction was obtained (9). The Regan isoenzyme was not found in any of the cancer patients' sera.

The ALP was also elevated in patients with osteosarcoma or bone metastases and in patients with osteomalacia and rachitis (19). However, in patients with endocrine system disorders, like primary and secondary hyperparathyroidism, the enzyme values were moderately elevated. The ALP values were also elevated in patients with chronic kidney insufficiency and in patients on haemodialysis, but in these cases, it was due to a decreased elimination from the body (9).

The ALP activity can be decreased in hepatocellular degeneration, where copper ions are assumed, to be competing with zinc ions for the position in the active ALP centre, and where they induce enzyme inactivation. In some metabolic, inherited diseases, like hypophosphatasia (9), synthesis of the ALP encoded by a tissue non-specific gene is probably impossible.

## CONCLUSION

Based on the obtained results, it can be concluded that determination of ALP isoenzymes is important but a correct clinical interpretation of the results in the context of other analyses including CT, scintigraphy and tumor markers is vital for a proper diagnosis of cancer metastases.

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## Conflict of interest

We declare no conflicts of interest

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