

Serum Alkaline Phosphatase and Placental Alkaline Phosphatase Activities in Monitoring Therapy of Stage III Cervical Carcinoma

EVRE III SERVİKS KANSERİNDE TEDAVİNİN TAKİBİNDE
SERUM ALKALEN FOSFATAZ VE PLASENTAL ALKALEN FOSFATAZ AKTİVİTELERİ

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Summary

Serum alkaline phosphatase (ALP) and placental alkaline phosphatase (PLAP) activities were determined in 68 patients with cervical carcinoma. Blood samples from each patient were collected when they first admitted to hospital and at the end of radiotherapy protocol. Patients were subdivided into two groups as responders and non-responders according to their response to treatment. It was concluded that serum ALP activity was not effective in evaluating the disease status, because serum ALP activities were significantly decreased in both groups after radiotherapy. However, it was found that patients that had higher serum ALP activities at first admission to hospital had poorer prognosis.

Serum PLAP activities were found to be a better marker to indicate disease activity. On the other hand, no significant difference between serum PLAP activities of responders and non-responders were found at the time of diagnosis.

These results suggest that simultaneous evaluation of serum ALP and PLAP activities might be helpful in both evaluation of prognosis and monitoring response to treatment.

Key Words: Alkaline phosphatase,
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Özet

Servikal karsinoma tanısı almış 68 hastada serum alkaline fosfataz (ALP) ve plasental alkaline fosfataz (PLAP) aktiviteleri tayin edildi. Hastaların hepsinden hem ilk tanı aldıkları zaman hem de radyoterapi protokolü uygulandıktan sonra kan örnekleri alındı. Tedaviye verdikleri yanıtlara göre hastalar yanıt verenler ve vermeyenler olmak üzere 2 gruba ayrıldı. Serum ALP aktivitesinin hastalığı değerlendirmede etkili bir marker olmadığına karar verildi, çünkü her iki grupta da serum ALP aktiviteleri radyoterapi sonrasında anlamlı oranda düşük bulundu. Buna karşın, ilk başvuru sırasında serum ALP aktivitesi daha yüksek hastaların daha kötü prognoz gösterdikleri saptandı.

Serum PLAP aktivite ölçümünün hastalık aktivitesini göstermede daha değerli bir marker olduğu tesbit edildi. Öte yandan, tedaviye yanıt verenler ve vermeyenlerin serum PLAP aktiviteleri arasında ilk tanı anında herhangi bir anlamlı fark bulunmadı.

Bu sonuçlar, serviks kanserli hastalarda serum ALP ve PLAP aktivitelerinin beraber ölçülmesinin hem hastalığın prognozu hakkında bilgi verebileceğini hem de tedaviye verilen yanıtın değerlendirilmesinde yararlı olabileceğini düşündürmektedir.

Anahtar Kelimeler: Alkalen fosfataz, Plasental alkaline fosfataz, Servikal karsinoma

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The uterine cervix is of major interest and importance to almost every obstetrician and gynecologist as it represents a common focus for the development of malignant tissue (1). Cervical carcinoma is still one of the most prevalent carcinoma in women all around the world and the prognosis of

the disease is strictly related to the early diagnosis, treatment and careful monitoring of patients after the treatment. Currently, the authors notice the importance of close follow-up of patients with monthly examinations including vaginal smear, Schiller test and colposcopy during the first year after the treatment has been completed. Despite the intense clinical trials, a reliable biochemical tumor marker for the evaluation of disease status in cervical carcinoma has not been described yet (1).

ALP [orthophosphoric-monoester phosphohydrolase (alkaline optimum) EC.3.1.3.1] an enzyme which catalyzes the hydrolytic cleavage of phosphate esters at basic pH values has received extensive attention because of its utility and importance in clinical diagnosis of many diseases (2, 3). Numerous researchers have demonstrated the clinical significance of variations of serum alkaline phosphatase activities in various malignancies (2, 4). PLAP, the heat-stable isoenzyme of alkaline phosphatase expressed by placental syncytiotrophoblasts cannot be detected in the sera of healthy individuals except for pregnant women. As pregnancy proceeds, the activity of serum PLAP increases, reaching its highest concentrations by the third trimester of pregnancy (5-7). Interest in PLAP grew, after Fishman et al (6) had discovered Regan isoenzyme in the serum and tumor tissue of a patient with bronchogenic carcinoma, with its properties including heat-stability, inhibition by L-phenylalanine, optimum pH and electrophoretic migration identical to those of PLAP (8, 9). Many reports have confirmed the presence of PLAP in sera and tissues of patients with various malignancies including germ-cell, ovarian, lung, cervix and breast tumors (10, 11).

The aim of the current study was to determine the potential use of serum ALP and PLAP activity measurements in monitoring the effectiveness of radiotherapy in patients with Stage III cervical carcinoma.

Materials and Methods

A total of 68 patients with stage-III cervical carcinoma (mean age \pm S.D.=53.3 \pm 5.28 years) were selected from 374 suspected cases who were admitted to Ankara University Faculty of Medicine Department of Obstetrics and Gynecology from

1994 to 1996. All cases of invasive carcinoma of the cervix were diagnosed as untreated stage-III squamous cell cervical carcinoma according to their clinical examinations and histopathological reports.

The patient group were treated with radiotherapy alone. The dose and duration of radiotherapy were planned and administered individually for each patient by the Department of Radiation Oncology of the same hospital. In the 8th week following the completion of radiotherapy patients were clinically examined and depending on the clinical status of the patient and tumor volume, they were categorized as responders (n=38) and non-responders (n=30). Serum activities of PLAP are known to be affected by smoking habits therefore smokers with a history of more than one package of cigarette per week were excluded (11,12).

Blood samples of the patients were collected when they were admitted to the hospital and following the completion of the radiotherapy protocol (8 weeks after the last therapy). Sera were separated without delay and stored at -20°C until assayed for ALP and PLAP activities. Each sample was analyzed in duplicate. All chemicals were purchased from Sigma Co. (St Louis, MO., USA).

The ALP activities in sera were measured using p-nitrophenylphosphate as substrate, by the method of Bessey et al (13). ALP activity was expressed as international units per milliliter (IU/mL). By definition, 1 Unit (U) of enzyme releases Immoles of p-nitrophenol per minute under the given conditions. Absorbances were read using a Unicam, Helios a UV-Vis spectrophotometer.

For the determination of PLAP activity, sera were incubated at 65°C for 30 minutes and then immediately cooled in ice water. The remaining activity due to PLAP was measured by the method of Bessey et al. (13, 14).

The results were statistically analyzed and the numerical data were expressed as mean \pm SD. Paired samples-t test was performed for the comparison of enzyme activities of each patient in responders and non-responders before and after radiotherapy protocol and a P value more than 0.05 was considered to be statistically non-significant. Mann Whitney-U test was performed to compare the difference of

Table 1. Serum ALP and PLAP activities (Mean \pm SD) in responders and non-responders before and after radiotherapy and statistical significance (paired samples-t test[^]

Groups	ALP (IU/mL)		PLAP(IU/mL)	
	Before treatment	After treatment	Before treatment	After treatment
Responders (n=38)	18.44 \pm 7.59	14.83 \pm 5.07	7.04 \pm 4.68	2.84 \pm 2.36
	P<0.05		P<0.001	
Non-responders (n=30)	27.69 \pm 23.35	15.06 \pm 9.75	5.34 \pm 4.14	7.25 \pm 3.95
	P<0.001		P<0.001	

ALP and PLAP activities before treatment between responders and non-responders.

Results

Serum ALP and PLAP activities before and after radiotherapy protocol are given in Table 1. Both serum ALP and PLAP activities were found to be decreased in responders. However, in non-responders although the ALP activities were decreased PLAP activities continued to increase following the radiotherapy.

The differences and their statistical significance in ALP and PLAP activities of responders and non-responders before radiotherapy were represented in Table 2. The patients with higher ALP at the time of diagnosis showed poorer prognosis.

Discussion

Since present clinical methods are not sufficient for monitoring the healing progress in patients with cervical carcinoma, additional markers are needed to substantiate them. A patient receiving radiotherapy should be closely monitored for response as regression of tumor may be expected to continue for up to 3 months after radiotherapy. Currently, patients are followed by monthly physical examinations and vaginal or cervical smears after radiotherapy has been completed (1). Previous studies on cervical cancer have shown that performing cervical or vaginal cytology alone is not enough for the monitoring of disease. Pateson et al (15) found that at the time of diagnosis, 29% of women with cervical cancer had at least one negative smear within 10 years and 18% of them had a negative smear within 3 years. Therefore in recent years substantial attention has been paid to the fact

Table 2. The comparison of serum ALP and PLAP (mean \pm SD) activities in responders and non-responders before radiotherapy (at time of diagnosis) and statistical significance (Mann Whitney-U test)

	ALP (IU/mL)	PLAP (IU/mL)
Responders (n=30)	18.44 \pm 7.59	5.34 \pm 4.14
Non-Responders (n=38)	27.69 \pm 23.35	7.04 \pm 4.68
	P<0.001	ns

ns: nonsignificant

that Papanicolau smears remain a specific but not a highly sensitive test for cervical carcinoma. The false negative rate is 20% or more in the best of hands (16).

ALP and its isoenzymes are widely used in the diagnosis and management of various diseases. Prohudas et al found that ALP activities could be used in discriminating healthy individuals from patients with cervical carcinoma. The same workers also proved that ALP might be a helpful marker in predicting response to treatment and recurrence of the disease at a preclinical stage (17).

Although Malkin et al and Kellen et al reported increased serum PLAP activities in patients with cervical neoplasms, they did not assess the usefulness of this biochemical marker in monitoring therapy. (18,19). These data are in accordance with our results since we examined both ALP and PLAP activities for the evaluation of response to radiotherapy.

Our data suggests that there are significant differences in both ALP and PLAP activities before and after radiotherapy. However, ALP activity does

not correlate with the response to treatment since its activity continued to decrease in both responders and non-responders after the radiotherapy protocol has been completed. Therefore, serum ALP is not a valuable marker in monitoring the treatment in cervical carcinoma patients. PLAP may be a better marker than ALP in evaluating the response to treatment because serum PLAP activities were found to be significantly increased ($p < 0.001$), in non-responders. However, the ones who had a better response to radiotherapy had significantly decreased PLAP activities ($p < 0.001$).

As might be seen from table-II, ALP was found to be a better prognostic marker than PLAP since the ones with poorer prognosis had higher ALP activities at the time of diagnosis. Therefore, we may say that measurement of serum ALP activity before treatment may be helpful in predicting the prognosis of the disease.

These data may be promising in that, these two tests could be used together to determine the prognosis and response to treatment of patients with cervical carcinoma. Therefore, further prospective long-term studies with larger series will be required to confirm these encouraging results.

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