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Role of Osteopontin in Diagnosis and Prognostication of Cervical Cancer Patients

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ABSTRACT

Osteopontin, a member of small integrin binding ligand N-linked glycoprotein (SIBLING) family, has been discussed as a plasma surrogate marker of tumor hypoxia. Its mediates wound healing, cell survival and tumor progression. Elevated plasma osteopontin levels have been detected in a variety of human cancers which has been correlated with tumor progression and metastasis. Plasma collected from a total of 38 histopathologically diagnosed cases of cervical carcinoma before and after start of chemotherapy. Enzyme Linked Immunosorbent Assay (ELISA) method is used to estimate the levels of osteopontin in serum. In the end of study we concluded that plasma osteopontin levels were significantly elevated in cervical cancer patients and no significant correlation of plasma osteopontin levels seen with increasing tumour stage & histopathological grade.

Keywords: ELISA, cervical cancer, osteopontin

INTRODUCTION

Osteopontin is mainly expressed by bone, kidney and epithelial tissues. It is found in several biological fluids including human plasma, breast milk & urine. Its functions include role in bone metabolism, immune regulation, wound healing, cell survival and tumor progression. Cancer of cervix continues to be the major cause of death from gynecological cancers worldwide. It is the most common genital cancer encountered in clinical practice in India. In cervical cancer a number of tumor markers have been investigated such as CEA & CA-125 in case of cervical adenocarcinoma while for squamous cell carcinoma, SCC antigen & CEA were recommended. Clinical use of these markers in cancer management has been restricted by their lack of sensitivity and specificity. In this context it is important to search for new prognostic and predictive markers that can reflect amount of tumor burden or identify patients at risk for tumor recurrence.

Material and Methods

It's a prospective cohort study of 38 cases; histopathological proven cases of carcinoma cervix with no concurrent illness, no previous history of malignancy are included with age group 18-70yrs. A serum sample was collected before and after one month of completion of chemotherapy against a control group who are females of 15 yrs age. Blood samples were collected from study & control group using heparin or EDTA as an anticoagulant. Following collection, blood was centrifuged for 20 minute at 3000 rpm. Supernatant plasma was separated and stored at -20oC. Clinical data of patient were taken on a preformed proforma. Enzyme Linked Immunosorbent Assay (ELISA)- quantitative

sandwich enzyme immunoassay technique was used to estimate the osteopontin levels in serum.

Results:

A total of 38 women with different stages of carcinoma cervix were enrolled in the study. The age of patients ranged from 28 years to 90 years. Maximum numbers of subjects were aged between 31-40 and 51-60 years. Only 2 (5.3%) patients were upto 30 years of age while only 4 (10.5%) were above 60 years of age. The mean age of patients was 49.00±12.34 (SD) years. Majority of subjects were para 4 or above (60.6%). There were only 5 (13.1%) patients with para 1 and para 2. Majority of subjects were postmenopausal (65.8%). There were 4 (10.5%) in premenopausal stage and 9 (23.7%) were in perimenopausal stage. Majority of subjects were in FIGO Stage II (n=21; 55.3%). Of these 21, there were 2 patients in FIGO Stage IIa and 19 patients in FIGO Stage IIb. The single patient in Stage I was Stage Ib. There were 16 patients in Stage III, among these 1 patient was Stage IIIa and remaining was Stage IIIb. According to histological type, all except 2 (5.3%) patients were squamous cell carcinoma. Majority of cases were moderately differentiated type (78.9%). There were only 2 (5.3%) poorly differentiated and 6 (15.8%) well differentiated cases Before treatment plasma OPN levels ranged from 107 to 457 ng/ml with a mean value of 283.20±111.29 ng/ml and a median value of 270. On assessment of the distribution for normality using Kolmogorov-Smirnov test the distribution was observed to symmetric and normal (p=0.200)(Figure 1-A). A total of 15 demographically matched controls were also assessed for plasma OPN levels. In control group, the plasma OPN values ranged from 35 to 200.50 ng/ml with a mean value of 98.85±40.43 ng/ml. The median value observed was 94.25. As depicted in the box plot , there were three cases in whom the plasma OPN values have been depicted as extreme/outlying value. On checking the normality of the distribution using Kolmogorov-Smirnov test, the data was found to be symmetric and normal (p=0.200)(Figure-1 B). Intergroup comparison of plasma OPN levels in cases and controls revealed the mean values in cases to be significantly higher as compared to that of control (p<0.001). Plasma OPN levels were maximum in patients with parity P4 and minimum in patient with parity P1. From Para 1 to Para 4, an increase in plasma OPN values was observed followed by a decrease in >P4 status. However, the association was not significant statistically. A significant association between age and plasma OPN levels was observed. The mean value was minimum in women <30 years of age and maximum among those in agegroup 41-50 years (F=4.775; p=0.004). The trend observed was – an increase in mean plasma OPN levels up to age group 41-50 years followed by a stepped regression. Though the mean plasma OPN levels in Stage II subjects were lower as compared to those in Stage III, yet the difference was not significant statistically (p=0.313). Mean plasma OPN levels were maximum in poorly differentiated grade patients and minimum in moderately differentiated grades. However, the difference among grades was not significant statistically (Table -1).

POST-TREATMENT ASSESSMENT- A total of 20 subjects who were given chemo-radiotherapy, could be followed & assessed clinically as well as by estimating plasma OPN levels, for response to therapy. Out of 20, 16 patients showed complete response and 4 patients showed partial response clinically. Following treatment a mean decrease of 58.07±26.68 ng/ml in plasma OPN levels was observed. Statistically, this change was significant (p<0.001) (Table-2). A significant difference in % decrease in plasma OPN levels was observed between 2 response categories (p=0.043) (Table -3) (Figure-2). In both Stage II and Stage III subjects a significant decrease in plasma OPN levels was observed (p<0.001) following treatment (Table-4) (Figure-3).

Discussion:

Majority of studies concluded the pivotal role of osteopontin (OPN) (glycoprotein) in tumor progression and metastasis [1-4,8,9]. Luigi et al proposed that there may be other forms of OPN that elicit distinct effect in different tissue and tumor hence favour pleiotropic activity of OPN [1]. Few studies are concordance with the study done by Luigi et al OPN has been studied as a blood tumour marker since the mid-1990. Various studies suggests that OPN blood levels may have a potential as a prognostic or diagnostic marker in eosopahgus ,head and neck cancer [5-7,10,12]. However mechanisms by which OPN may enhance malignancy are still unclear [13]. In cervical cancer majority studies have

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investigated significance of osteopontin by assessing immunohistochemical expression in tumour tissue [14-19]. Only a few investigators have assessed significance of plasma OPN levels[20-21]. This study was carried out to assess plasma OPN levels in cervical cancer patients & evaluate their role. The present study included 38 cases of cervical cancer of different stages & grades and 15 healthy controls. Out of 38 patients, 20 patients who received chemoradiotherapy, could be followed, as few of the remaining patients died, some did not receive chemoradiotherapy and some were lost to follow up (more than 5%). In this study plasma osteopontin levels were found to be significantly higher in cases of cancer cervix (mean 283+111.29 ng/ml) than in healthy controls (98.85+40.43 ng/ml) ($p < .001$). These findings were similar to those of study conducted by Wong et al. who observed increased plasma OPN levels in women with primary cervical carcinoma (median 431 ng/ml, n=198) as compared to control group (median 150 ng/ml, n=100) ($p < .001$) [20]. Similarly study conducted by Hanbyoul cho et al. showed that plasma OPN level in women with cervical cancer (n=81, mean 355.8 ng/ml) were significantly higher than those of healthy controls (n=283, mean 100 ng/ml) ($p < .001$)[21]. Thus OPN may have a role as a potential biomarker in cancer cervix. Regarding correlation of plasma OPN level with parity our study showed an increase in plasma OPN level from parity 1 to parity 4 followed by a decrease in >4 parity. However association was not significant statistically (p=0.182). Hanbyoul Cho et al. also observed no significant correlation of plasma OPN levels with parity [21]. Regarding correlation of plasma osteopontin with age, our study showed a significant association between age & plasma OPN levels with maximum in age group 41-50 year and minimum in women < 30 years of age (p=0.004). However a study conducted by Hanbyoul Cho et al. observed no significant correlation between plasma OPN level in cervical cancer patients and age [21]. No valid explanation for these results was found in the literature. Further we did a comparison of plasma OPN levels among different clinic pathological stages and it was found that although mean plasma OPN levels in stage II were lower as compared to those in stage III, yet the difference was not statistically significant (p=0.313). These findings were similar to those of Y.F. Wong et al. whose study also showed no significant difference in mean plasma OPN levels among primary cervical cancer patients (n=76) in different clinical stages ($p > 0.05$) [19]. However study conducted by Hanbyuol Cho et al. showed a significant correlation with increasing tumour stage (n=97) ($p < 0.001$) [21]. As described in literature, OPN has a role in tumour progression and invasion and hence may have a role as prognostic marker. Cell migration and degradation of the extracellular matrix (ECM) are crucial steps in tumour progression. However more studies including larger sample size are needed to establish its role in prognosis. Similiarly when comparing mean plasma OPN among different histopathological grades (well differentiated, moderately differentiated & poorly differentiated) it was found that mean plasma OPN levels were maximum in poorly differentiated grade and minimum in moderately differentiated grade. However, difference among groups was not significant statistically (p= 0.374). In literature no study has observed a significant correlation between plasma OPN level & increasing grades of cancer cervix. However, study conducted by ZHU Yao-Kui et al. detected significantly higher positive immunostaining rate of OPN in higher grade of cervical cancer than in lower grade [22]. Also study conducted by Tian Xianwen et al. showed a significant difference in positive immunoexpression of OPN in well (38.89%), moderate (53.46%), & poor (93.37%) grade [19]. Further studies evaluating role of plasma OPN in cervix cancer are needed to be explored. In our study majority (78.9%,30/38) of cases were of moderately differentiated grade which lead to an asymmetric distribution. Small sample size or technical error may also be a reason for inconsistent results. On comparing plasma OPN levels before & after chemoradiotherapy in total of 20 cases, a mean decrease of 58.07+26.68 ng/ml was observed and this change was found to be significant ($p < .001$). Further on comparing among 2 response categories, it was found that out of 16 cases who showed complete clinical response, 7 cases (43.75%) developed < 20% decrease in plasma OPN levels and 9 cases (56.25%) developed > 20% decrease. However, in all 4 cases who showed partial / no clinical response, plasma OPN decreased by < 20% only. Difference of % decrease in plasma OPN levels among 2 response categories was statistically significant (p=0.043). These findings concluded that

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plasma OPN may be helpful in predicting response to radiotherapy as well as in monitoring response. Difference in decrease in plasma osteopontin level among cases of stage II (n=12) & stage III (n= 8) was not statistically significant. These results showed discrepancy to those of Miao-sheng et al. who observed that levels of OPN were not significantly different between two groups of before & after radiotherapy (n=71, p>0.05) [16]. This discrepancy may be explained by lost to follow up more than 5% in a time bound study and no defined duration of follow up plasma samples in previous study. further investigation is required to understand the underlying mechanism. More clinical trials are also required to examine the applicability and efficacy of OPN inhibitors in cancer therapy.

Conclusion

Plasma osteopontin levels were significantly elevated in cervical cancer patients as compared to controls. No significant correlation of plasma osteopontin levels with increasing tumour stage & histopathological grade was observed. Significant decrease in plasma osteopontin levels after chemo-radiotherapy was observed. Significant difference in % decrease in plasma osteopontin level between two response categories (complete & partial) was observed. Plasma osteopontin may be useful as a biomarker for detection of cervical cancer as well as in predicting & monitoring response to chemo-radiotherapy. However being a time bound study & having small sample size, its significance and association with prognosis needs to be explored by more studies having higher sample size & good follow up with a specific time period.

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Legends-

Figure-1

A- Kolmogorov-Smirnov test in study group, the distribution was observed to symmetric and normal

B- Kolmogorov-Smirnov test in control group, the data was found to be symmetric and normal

Figure-2 Comparison of % decrease in plasma OPN among 2 response categories

Figure -3 Changes in Plasma OPN levels according to FIGO stage

Table -1 Mean plasma OPN levels among different histological grades of carcinoma cervix

Table 2- Change in Plasma OPN levels following treatment (n=20)

Table 3- Comparison of % decrease in plasma OPN among 2 response categories

Table 4- Change in Plasma OPN levels according to FIGO stage

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Figure-1

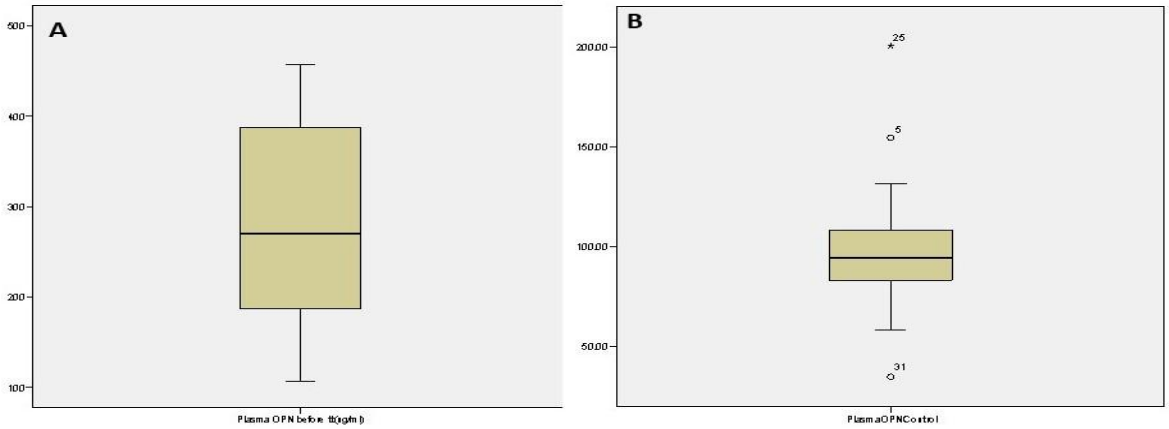

Figure-2

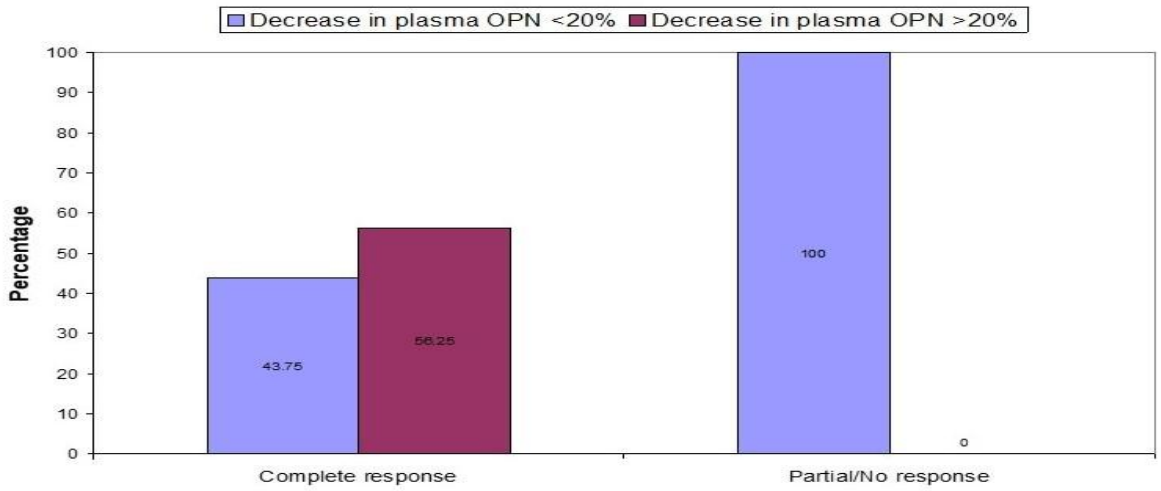

Figure-3

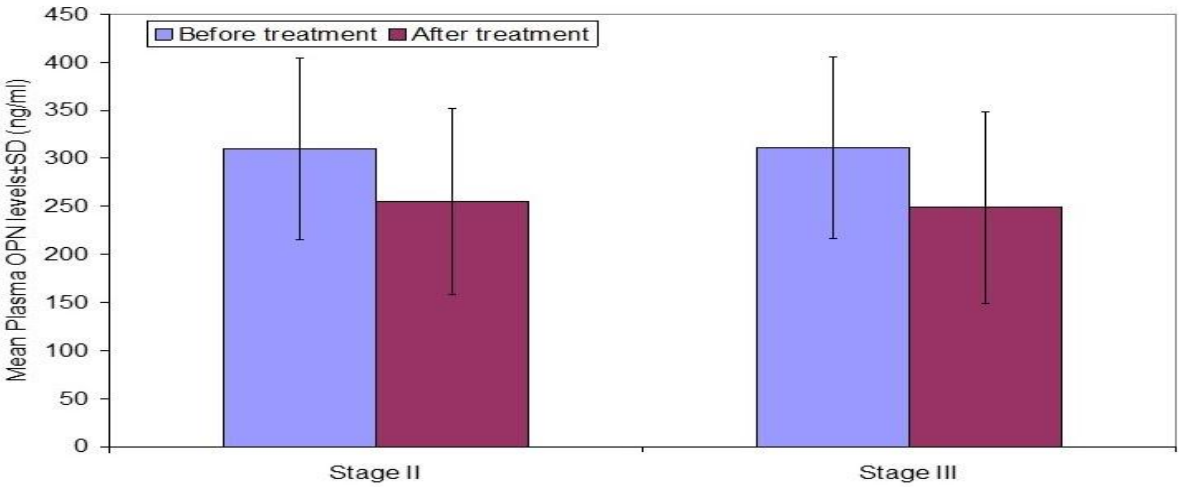

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Table-1

S.No.	Grade	n	Mean	SD	Min	Max
1.	Poorly differentiated	2	391.63	40.835	363	421
2.	Moderately differentiated	30	276.13	108.471	107	457
3.	Well differentiated	6	282.42	134.438	114	450

Table-2

S.No.	Time	Mean	SD
1.	Pre-treatment	310.46	91.98
2.	Post-treatment	252.39	95.33
Change in plasma OPN levels (Mean±SD)		58.07±26.68	
"t"		9.736	
"p"		<0.001	

Table-3

Response categories	Number of subjects	Decrease in plasma OPN ≤20% (n=11)		Decrease in plasma OPN >20% (n=9)		Statistical significance
		No.	%	No.	%	
Complete	16	7	43.75	9	56.25	χ^2 =4.091 p=0.043
Partial/No response	4	4	100.0	0	0	
Total	20	11	100.0	9	100.0	

Table-4

S.No.	FIGO stage	Before treatment		After treatment		Change in OPN levels	
		Mean	SD	Mean	SD	Mean	SD
1.	II (n=12)	310.13	94.69	254.88	97.00	55.25	27.53
t=6.952; p<0.001							
2.	III (n=8)	310.97	94.22	248.66	99.27	62.31	26.58
t=6.631; p<0.001							

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