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ORIGINAL ARTICLE

Lipid Peroxidation and Thymidine Phosphorylase expression in Prostate Carcinoma

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Aim: To understand the association between markers of oxidative stress and angiogenesis in relation to disease progression, clinical stage and cytological grade in patho-physiology of prostate carcinoma.

Patients and Methods: Case control study comprised of 50 prostate carcinoma patients along with 20 age and sex-matched healthy subjects as controls. Levels of malondialdehyde were measured to study the oxidative stress status in the study subjects. Angiogenesis was evaluated by studying the activity of Thymidine Phosphorylase/Platelet derived endothelial cell growth factor.

Results: The levels of markers of oxidative stress along with the activity of thymidine phosphorylase were found to be significantly higher in the study subjects in comparison to healthy controls. The results indicate oxidative stress and angiogenesis activity increase progressively with the increase in staging and progression of disease.

Conclusion: Oxidative stress and expression of angiogenesis activity points clearly that with the progression of oxidative stress there is a simultaneous progression of angiogenesis in relation to disease progression, clinical stage and cytological grade in the pathophysiology of prostate carcinoma.

Key words: Lipid peroxidation, Thymidine Phosphorylase activity, Prostate carcinoma

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Cells undergo oxidative stress when levels of "Reactive Oxygen Species" (ROS) exceed the counter-regulatory antioxidant capacity of the cells, either generated from elevated production and accumulation of ROS or from a diminution in cellular antioxidant defenses. ROS initiate autocatalytic lipid peroxidation, which generates a large variety of potential genotoxic breakdown

products, including alkoxyl radicals ($LO^{\cdot}$), peroxyl radicals ($LOO^{\cdot}$), and aldehydes, such as malondialdehyde (MDA). MDA has been used for many years as a convenient biomarker for lipid peroxidation because of its facile reaction with thiobarbituric acid to form an intensely colored chromogen (Janero,1990). These oxidatively modified products may affect several functions in cancer cells or tumor tissue such as cell proliferation, promotion of mutations and genetic instability. Inherent oxidative stress is a potent factor of angiogenesis and is considered to be involved in patho-physiology of cancers (Kuroki et al 1996).

Angiogenesis is a complex, dynamic process that involves multiple pathways that converge to affect carcinogenesis, proliferation, and tumor growth. During the process of tumor angiogenesis, many of growth factors secreted by the tumor cells, endothelial cells and supporting cells are required for the angiogenesis. There is now enough evidence that the growth of solid tumors is also angiogenesis dependent. The degree of angiogenic activity in a tumour can be determined directly by determining the degree of vascularization within that tumour, the intratumoural microvessel density or indirectly by assaying known angiogenic stimulatory factors. In addition to simulators many angiogenic inhibitors have been identified. Angiogenesis is a dynamic balance of angiogenic stimulators and inhibitors mediating interactions between tumour cells, endothelial cells, and the surrounding extra cellular matrix. Understanding these interactions and the various controls of angiogenesis such as oncogenes and hypoxic regulation, points to anti-angiogenesis becoming an extremely promising future therapy.

Many angiogenic factors have been identified such as VEGF, Fibroblast growth factors, Thymidine Phosphorylase/Platelet derive endothelial cell growth factor, Angiogenin, Heptocyte growth factor, interleukin-8, Placental growth factor, Prostaglandin E-1 and E2, transforming growth factor, tumour necrosis factor-α. Elevated levels of angiogenic stimulators correlate with poor outcome in a number of tumours. Thymidine phosphorylase is an enzyme; it is also angiogenic and induces endothelial cell migration in *vitro.* Elevated levels of the Thymidine Phosphorylase have been demonstrated in many tumours including bladder, breast and ovarian cancers.

The present study was undertaken in order to investigate the role of oxidative stress and expression of thymidine phosphorylase activity of cells in stimulating angiogenesis in prostate carcinoma. In addition, thymidine phosphorylase expression was analysed for possible associations with histological grade and PSA expression.

MATERIALS AND METHODS

This study was conducted in Department of General Medicine, and Department of Urology, University Hospital, Banaras Hindu University in collaboration with Department of Biophysics, Institute of Medical Sciences, Banaras Hindu University.

Selection of cases

Case control study comprised of clinically suspected and histopathologically confirmed 50 cases of carcinoma prostate. Patients of suspected carcinoma prostate were subjected to various investigations such as Serum Prostate Specific Antigen titre, Transrectal ultrasound (TRUS), USG abdomen, TRUS biopsy, Bone Scan, Chest X-ray, CT scan abdomen.

Collection of samples

5 ml of blood was collected by vene-puncture

with proper aseptic technique in a plain sterile vial. Serum was separated by centrifugation at low speed and stored at -70^0 degree until analyzed. Blood samples from the study subjects were taken with the informed consent of each individual purely for research purpose.

Estimation of Lipid Peroxidation level

Assay of oxidative damage in the serum of the patients as well as healthy control samples was assessed by measurement of products of lipid peroxidation in serum by the thiobarbituric acid (TBA) method (Burge and Aust, 1978). MDA, which is a stable end product of fatty acid peroxidation, reacts with TBA at acidic conditions to form a complex that has maximum absorbance at 532 nm.

Estimation of Thymidine phosphorylase (PDECGF) level

Thymidine phosphorylase **(**TP) ELISA is an enzyme linked immunosorbent assay for the quantitative in vitro determination of natural and recombinant human Thymidine phosphorylase (PDECGF) in research samples of tumor, lysates from tumor cell lines, peripheral blood cells, or serum within antibody pre coated micro titers plates (MTPs).

In the first step TP is bound to anti TP coated surface of the micro titer plate by taking 50 µl of standard and serum samples from controls / patients. Following the washing step, the peroxidase (HRP) conjugated second antibody is added to the wells and the plate was incubated at room temperature for one hour with constant shaking. Following the washing step the peroxidase bound in the complex is developed by the substrate ABTS by mixing with 100 µl of sample buffer. The plate was incubated at room temp for one hour with constant shaking for the binding to the coated wells. The color intensity is proportional to the concentration of TP.

Statistical Methods

Study data was presented as percentage and Mean ±SD. Student 't' test was used for the mean difference between two groups for unpaired cases. Chi-Square was used to test significance of difference among two proportions. The level of significance was considered at 5% as cut off point.

RESULTS

Patients in the study group comprising of 50 cases of carcinoma prostate along with twenty age and sex matched healthy subjects as controls were inducted the for study. Carcinoma prostate (Ca Prostate) had three subgroups: Adenocarcinoma, Transitional cell carcinoma and Squamous cell carcinoma. The age and sex wise analysis of the controls, cases and their subgroups did not reveal any statistical significant difference in distribution; thus ruling out the effect of sex and age as confounding factors.

The levels of Malondialdehyde in Carcinoma Prostate group were found to be elevated in comparison to controls indicating the higher level of oxidative damage. The difference between these values was statistically significant as shown in Table 1. A significant increase in the activity of serum thymidine phosphorylase in the carcinoma prostate cases in comparison to controls groups was observed. History of smoking revealed that only 23% patients were smokers, this signifies that smoking may not be risk factor for prostate cancer. Most of the cases presented were of localized disease.

Serum thymidine phosporylase measurement was done in controls and prostate carcinoma

patient group and its relationship with Clinical stage, Gleason score, Prostate volume, Serum prostate specific antigen was investigated (Table 2). Significant difference (P < 0.001) in the activity of thymidine phosporylase in carcinoma prostate with respect to control groups indicates the role of thymidine phosphorylase in carcinoma prostate. Serum thymidine phosphorylase was significantly higher in patients with metastasis prostate cancer in comparison to localized disease and healthy controls. PSA level greater than 20 ng /ml had significantly higher serum thymidine phosphorylase activity then the patients with PSA level < 20 ng / ml suggest that serum thymidine phosphorylase activity is elevated in disseminated disease.

Table 1. Malondialdehyde status and Serum Thymidine Phosporylase (PD ECGF) Activity in study groups

Group	MDA Mean± SD (mmol/L)	p- value	Serum Thymidine Phosporylase (PD -ECGF) Activity Mean± SD (ng/ml)	p-value
Control (20)	1.12±0.38	0.001	253.29± 31.93	0.001
Carcinoma Prostate (50)	3.138± 1.109		366.82±80.35	

Table2. Serum Thymidine Phosporylase (PD ECGF) Activity in carcinoma Prostate patients with respect to tumour staging , PSA and Gleason score.

Group	**PDECGF Activity (ng/ml)**	**p- value**
Tumour Histology		
Adenocarcinoma (47)	361.26± 26.57	Non Significant
Squamous cell carcinoma (02)	351.37±24.15	
Transitional cell carcinoma (01)	342.22±21.74	
Metastasis		
Negative (32)	315.22± 52.44	< 0.001
Positive (18)	346.57± 76.25	
PSA (ng/ml)		
> 20 (14)	351.48±30.88	< 0.001
< 20 (36)	329.27±28.75	
Gleason score		
2-4 (26)	342.58± 24.67	0.218
5-7 (12)	346.37± 27.14	
8-10 (12)	349.26± 29.58	

DISCUSSION

Prostate cancer is the consequence of chromosomal aberration and pathological proliferation of cells of the prostate tissue (Moul et al 2003). Although the specific etiological factors of prostate cancer are not yet known, considerable evidence indicate that both genetic and environment may play a role in the evolution of

prostate cancer. Angiogenesis, essential for tumor formation, can be measured by microvessel density and by measurement of circulating levels of related growth factors, such as Thymidine phosphorylase (PD- ECGF). Several studies suggest that the measurement of circulating levels of PD- ECGF is a useful tool for analyzing prognosis and clinical outcome in many tumors.

Thymidine phosphorylase catalyses the reversible phosphorylation of thymidine to thymine and 2-deoxy-D-ribose-1-phosphate (Fox et al 1995; Griffiths and Statford, 1997). The mechanism of the angiogenic activity is through generation of free radicals by the metabolic product 2-deoxy-D-ribose which was shown to stimulate endothelial cell migration and new blood vessel formation (Moghaddam et al 1997; Brown and Bicknell 1998; Brown et al 2000). The free radicals activate expression of VEGF, interleukin- 8 (IL8) and matrix metalloproteinase-1 (MMP1) (Brown et al 2000).

In the present study age distribution of carcinoma prostate patients (68.32±9.32 years) was well in accordance with accepted peak age of presentation; most of the patients were of adenocarcinoma and 2 of transitional cell and 1 of squmous cell carcinoma. This finding is consistent with the study (Randolph et al 1997) that > 95% of cancer prostate were of adenocarcinoma, in rest 5% Transitional cell carcinoma was more common then squmous cell carcinoma and other types like neuroendocrine type of carcinoma prostate.

Serum thymidine phosporylase activity and oxidative stress levels were higher in patients with carcinoma prostate patients than controls. Additionally, we observed significant association between serum thymidine phosporylase and oxidative stress with certain clinico-pathologic factors including PSA, Stage, Metastasis and Gleason score. Our study demonstrates a significant correlation exist between Serum thymidine phosporylase and oxidative stress levels in prostate carcinoma patients. Significant difference in the activity of thymidine phosphorylase between the carcinoma prostate and control groups indicates thymidine phosphorylase has a role in the disease process. Serum Thymidine phosphorylase was significantly higher in patients with metastasis prostate cancer in comparison to patients of localized disease and healthy controls. The above findings are similar to the results reported in other studies (Sugamoto et al, 1999, Weidner et al 1993).

In conclusion**,** Thymidine phosphorylase/ platelet-derived endothelial cell growth factor (TP/PD-ECGF) is a potent angiogenic molecule stimulating endothelial cell migration and new blood vessel formation (Moghadam et al, 1995). The enzyme's angiogenic activity has been consistently shown in a variety of human malignancies, despite variations of its principal source of production. High thymidine phosphorylase expression was associated with high angiogenesis (Koukourakis et al, 1998). Sugamoto et al (1999) also indicated that thymidine phosphorylase levels are higher in prostate carcinomas than in normal prostatic tissues. Thymidine phosphorylase may be a target for cytotoxic and antiangiogenic therapeutic strategies in prostate tumours.

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