

Effect of radiotherapy and chemotherapy on serum glycoproteins of gynecological cancers

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Abstract

Glycoproteins are end moieties of the carbohydrate chains are biologically important and essential for functions of glycoconjugates and are reported to be altered in cancer patients. The effect of chemotherapy and radiotherapy on cervical, ovarian and endometrial cancers gynecological cancers were evaluated by the estimation of serum glycoprotein levels including total sialic acid, protein bound fucose and hexose. In patients of gynecological cancers, the levels of total sialic acid, protein bound hexose and fucose were significantly higher as compared to the normal subjects. There is a statistical significance (0.000*) between the levels of serum glycoproteins before and after treatment. The mean and standard deviation of the glycoproteins before and after treatment indicates that there is a statistical significance between two therapies ($p < 0.05$) with respect to the total sialic acid, protein bound hexose and fucose and it is also observed that chemotherapy is more effective than radiotherapy in cervical cancer, where as radiotherapy is more effective for ovarian and endometrial cancer. The results demonstrated that chemotherapy shows better response to cervical cancer where as radiotherapy for ovarian and endometrial cancer.

INTRODUCTION

Glycosylation is one of the most frequently occurring or post-translational modifications made to proteins and lipids in the secretion machinery of the cell, with resultant carbohydrate side chains to have very complex oligosaccharide sequences and concomitant structural diversity^[1]. More than half of known protein sequences can potentially be glycosylated^[2] and are called glycoproteins. Glycoproteins can be simply defined as proteins that have carbohydrate moiety covalently attached to their peptide portion and are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes. These are organic compounds, composed of both a protein and carbohydrate monosaccharides, usually hexose, hexosamine, fucose and sialic acid, joined together covalently linked to polypeptide chain.

Majority of the presently known tumour markers are glycoprotein in nature. Various investigators have reported striking differences in cell surface carbohydrate structure in malignant cells^[3]. Further, molecular changes in carbohydrate antigens have been reported to be associated with cancer^[4]. Altered glycosylation of glycoconjugates is one of the important molecular changes that accompany malignant transformation^[5].

The metastasis of the tumor is most important process for tumor spreading, which involves the multistep series of adhesive events and signaling events^[6] in a variety of inflammatory and thrombotic disorders^[7]. The interactions between tumor cells and endothelial cells, the attachment and invasion of tumor cells through the endothelium via binding of selectins to their ligands, may be an important step in the metastatic process^[8]. The level of different types of serum glycoproteins are maintained within a narrow range in health^[9], but is elevated in many pathological conditions including tuberculosis^[10], autoimmune disease^[11], cardiovascular disease^[12], cancer of cervix, uterus and breasts^[13].

Numerous studies have addressed themselves to the quantification of serum or plasma sialic acid in various disease states^[16]. Elevation of serum sialic acid was reported in patients with cervical cancer. It is therefore concluded that this factor can be used for the prognosis in treatment monitoring^[17,18]. Hence, the present study was aimed at to determine the response of chemotherapy and radiotherapy in three types of gynecological cancers by the estimation of serum glycoproteins Total sialic acid, Protein bound Hexose and Protein bound Fucose.

MATERIALS AND METHODS

SAMPLES COLLECTION

All the serum samples were collected from the Govt. General hospital (GGH), Guntur, Andhra Pradesh, India. The samples were collected from the various types of gynecological cancers (N=152) includes Cervical (N=60), Ovarian (N=52) and Endometrial (N=40) cancers with a mean age of 51.8 years and the cancer was confirmed by the biopsy report given by the department of Pathology, GGH. Simultaneously Serum was also collected from the age matched healthy individuals and served as healthy controls (N=25). The characteristic features of the three different types of cancers with stages are summarized in Table 1.

Consent letter from patients was obtained. Before and after treatment (Radiotherapy, N=76 and Chemotherapy, N=76) 3 ml of blood sample were collected. All the serum samples were stored at -20°C for further analysis. Biochemical analysis was performed on serum samples for the estimation of protein bound Hexose (PBH), protein bound Fucose (PBF) and Total Sialic Acid (TSA).

The present work was approved by the Institutional ethical committee (IEC), S.V Medical College, Tirupati.

PROTEIN BOUND HEXOSE

Hexose was estimated by the method of Mukesh Nandave et

Table 1. Characteristics of three types of gynecological cancer patients and healthy controls

S.No	Character	Patients (no)	Controls
1	Median Age (Range)	51.8 Yr (30-75 Yrs)	50.2 Yr (30-73 Yrs)
2	Type of Cancers	152	25
	Ca Cervical	60	
	Ca Ovarian	52	
	Ca Endometrial	40	
3	Cancer Stage		
	IIA	12	
	IIB	14	
	IIIA	24	
	IIIB	17	
	IV	8	
4	Histological features		
	SCC	24	
	DSCC	12	
	M.D/WDSCC	6/15	
	Du SCC	5	
	In. SCC	4	
	Un-identified	10	
5	Treatment		
	Chemo therapy (Cisplatin+Mitomycin)	76	
	Radiotherapy	76	

Note: SCC: Squamous Cell Carcinoma; DSCC: Differentiated Squamous Cell Carcinoma; M.D/WD SCC: Moderately and Well Differentiated Squamous Cell Carcinoma; Du SCC: Ductal Squamous Cell Carcinoma; In. SCC: Intermediate Squamous Cell Carcinoma.

al.,^[19]. Hexose moiety present in the glycoprotein was precipitated by ethanol at room temperature and it was determined by orcinol reaction at 540nm.

PROTEIN BOUND FUCOSE

Protein bound Fucose was estimated by Mukesh Nandave et al.,^[19] method which leads to the determination of methyl pentose present in the serum. In this method the serum sample was heated with H₂SO₄ for 5-20 min followed by the addition of Cystein. Methyl pentose value was determined by measuring the optical density at 396 and 430nm. Because fucose has been demonstrated in the serum glycoproteins or related mucoid it appearance reasonable to report methyl pentose value as fucose.

ESTIMATION OF TSA BY THE PERIODIC ACID RESORCINOL MICROASSAY

Total Sialic acid was estimated by Surangkul (2001)^[20], 40µl of pure standard Sialic acid sample with different concentrations (2-10 µg/well) were added to the first row of the 96-well micro titer plate (Polystyrene, Nunc) and the remaining rows will be filled with 40 µL of each patients serum sample, all the samples were maintained as replicates. Then, 50 µL of 1.3mM Periodic Acid (PA) was added to each well and mixed thoroughly for 5min

on a ice box for 60min, and 100 µl of 0.6 g/dL resorcinol reagent was added to each well. The micro titer plate was covered with glass plate and heated 80°C for 60 min in a water bath, cooled down to room temperature. Then, 100µL of 95% tetra-butyl alcohol was added to each well and the contents were mixed, read the absorbance at 620nm by a micro titer plate reader (Bio Rad, Germany).

STATISTICAL ANALYSIS

The entire analysis has been carried out using PASW SPSS-18 statistical software. The aim of the statistical analysis was to compare the influence of each therapy separately in the three glycoproteins. The second one is to compare and to know which kind of therapy will give effective results in reducing the risk of cancer. This was achieved and observed in each parameter of respective cancer types. Paired samples t test and Independent samples t-test were used respectively to achieve the above two objectives.

RESULTS

The serum levels of glycoprotein in three types of cancers were compared with those of healthy individuals. In all cases the median of the glycoprotein levels in cancer patients were higher

Table 2. Serum levels of three glycoproteins before and after treatment (Chemotherapy) in three gynecological cancers

Type of Cancer Parameter		Mean \pm Std. Deviation	t-stat & p-value
Cervix	PBH	Before	180.18 \pm 12.49
		After	148.70 \pm 6.42
	PBF	Before	20.14 \pm 2.15
		After	15.11 \pm 0.77
	TSA	Before	135.67 \pm 4.10
		After	108.20 \pm 1.29
Ovarian	PBH	Before	187.07 \pm 10.06
		After	155.81 \pm 3.98
	PBF	Before	23.65 \pm 2.07
		After	14.79 \pm 0.84
	TSA	Before	154.41 \pm 7.53
		After	115.36 \pm 6.10
Endometrium	PBH	Before	176.09 \pm 7.09
		After	125.44 \pm 5.34
	PBF	Before	21.95 \pm 4.00
		After	16.18 \pm 0.65
	TSA	Before	124.17 \pm 2.79
		After	75.77 \pm 3.70

than the corresponding healthy subjects.

Table 2 and 3 shows that there is a statistical significance (0.000*) between the levels of serum glycoprotein (PBH, PBF and TSA) before and after treatment (radiotherapy and chemotherapy) in all the three types of gynecological cancers. The present investigations also focused in reporting the actual improvement in the patients status; this can be met by considering the comparison strategy between the chemotherapy and radiotherapy in each cancer type for all the three parameters.

COMPARISON BETWEEN SERUM GLYCOPROTEINS IN RESPONSE TO TREATMENT (RADIOTHERAPY AND CHEMOTHERAPY) IN 3 TYPES OF GYNECOLOGICAL CANCER

In Cervical cancer (figure 1), it is observed that there is a statistical significance between two therapies ($p < 0.05$) with respect to the parameters TSA, PBH and PBF and it is also observed that chemotherapy is more effective than radiotherapy, this was stated by observing the mean and standard deviation values.

In Ovarian cancer (figure 2), it is observed that there is a statistical significance between two treatments ($p < 0.05$) with

respect to parameters TSA, PBF and there was no significance with respect to parameter PBH and the response was observed to be same with the both therapies. Again, considering the significant parameters TSA and PBF, radiotherapy is more effect than chemotherapy.

In Endometrial cancer (figure 3), it is observed that there is a statistical significance between two therapies ($p < 0.05$) with respect to the parameters TSA, PBH and PBF and it is also observed that radiotherapy is more effective than chemotherapy, this was stated by observing the mean and standard deviation values.

DISCUSSION

Glycosylation has been demonstrated to play a critical role during malignant transformation^[21, 22]. It is well established that the gynecological cancer patients had significantly higher levels of different forms of glycoproteins including TSA, PBH and PBF as compared to the controls^[23, 24]. In the present study the results in serum levels of glycoproteins were in accordance with the reports from the studies of Paszakowaska^[24].

A well established data were focused only on the levels of glycoproteins expressed in healthy controls and gynecological

Table 3. Serum levels of three glycoproteins before and after treatment (Radiotherapy) in three gynecological cancers

Type of cancer	parameter		Mean± Std. Error Mean	t value & p_value
Cervix	PBH	Before	185.28±1.48	38.64
		After	131.67±0.47	0.000*
	PBF	Before	19.95±0.41	26.20
		After	10.92±0.24	0.000*
	TSA	Before	134.57±0.70	39.18
		After	96.31±0.66	0.000*
Ovarian	PBH	Before	190.82±1.29	16.17
		After	164.82±0.80	0.000*
	PBF	Before	24.21±0.33	16.86
		After	17.18±0.22	0.000*
	TSA	Before	155.56±1.52	17.54
		After	127.02±0.86	0.000*
Endometrium	PBH	Before	172.74±1.13	29.17
		After	142.75±0.95	0.000*
	PBF	Before	20.83±0.88	1.69
		After	19.33±0.17	0.107*
	TSA	Before	123.84±0.55	16.50
		After	102.47±1.28	0.000*

cancers rather than the expression after the treatment. It is important to note that the prognostic importance of the glycoproteins, which can be estimated after the treatment of either radiotherapy or chemotherapy. In the present study response of the treatment has been estimated on levels of glycoproteins in three different gynecological cancers. Chemotherapy shows good response to the cervical cancer patients because it shows significant (0.000*) decreased levels of TSA, PBH and PBF mean values with 27.46±4.06, 31.47±13.24, 5.02±2.12 respectively when compared to radiotherapy respectively. Hence the data indicates that the chemotherapy shows good response to cervical cancer patients than the radiotherapy related to glycoproteins. In ovarian cancer, radiotherapy shows good response when compared to the chemotherapy, since it shows significant (0.000*) decreased levels of TSA, PBH and PBF mean values with 28.53±8.29, 25.99±8.19 and 7.02±2.12. Hence radiotherapy shows good response to ovarian cancer patients. Similar results were observed in endometrial cancers, in which the radiotherapy shows good response when compared to the chemotherapy, since it shows significant (0.000*) decreased levels of TSA, PBH and PBF mean values.

The mechanism of increased serum glycoproteins concentration in malignancy conditions is unclear, but several

explanations have been proposed. These include, spontaneous release of aberrant Sialic acid-containing cell surface glycoconjugates, increased concentrations and/or glycosylation of normal serum glycoproteins, and secondary inflammatory reactions leading to elevated glycoproteins of gynecological cancers^[25]. A limitation in using sialic acid as a tumor marker is that it has an acute phase reactant and most of the proteins that increase in acute phase are sialylated. Therefore, most investigators indicated that sialic acid and other proteins could be used in monitoring of cancer patients and follow up after therapy. Moreover, the elevated expression of these glycoproteins has decreased after the treatment, this is because of the removal of proliferated tumor cells in response to either chemotherapy or radiotherapy. In the present investigation, chemotherapy shows good response to cervical cancer where as radiotherapy for ovarian and endometrial cancer.

CONCLUSION

An admirable discrimination was found between before and after treatment of the three types of glycoprotein levels in patients with gynecological cancers. Since, the present investigation was concluded that chemotherapy shows effective response to cervical cancer where as radiotherapy for ovarian and

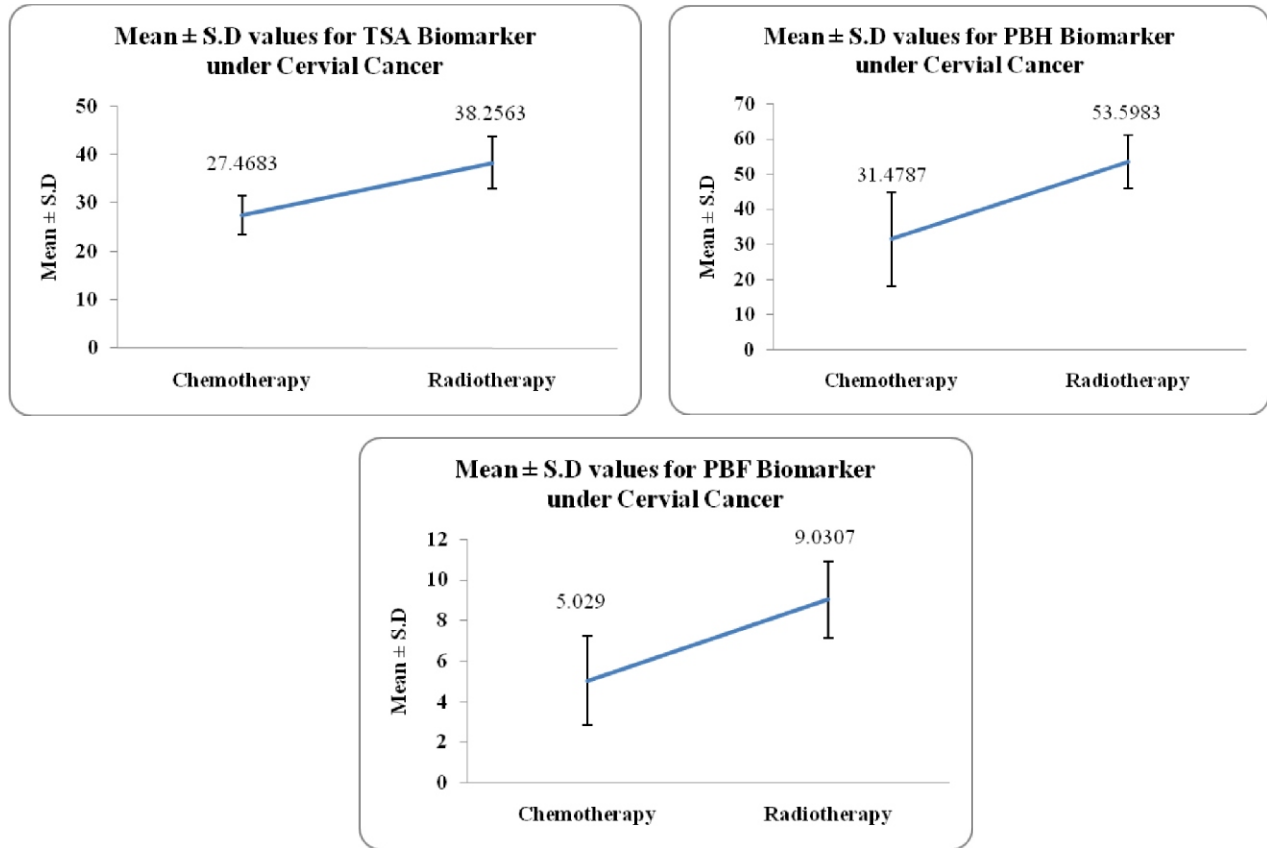


Fig. 1. Effect of Chemo and Radiotherapy on serum TSA, PBH and PBF respectively in cervical cancer represented by Whisker line graphs.

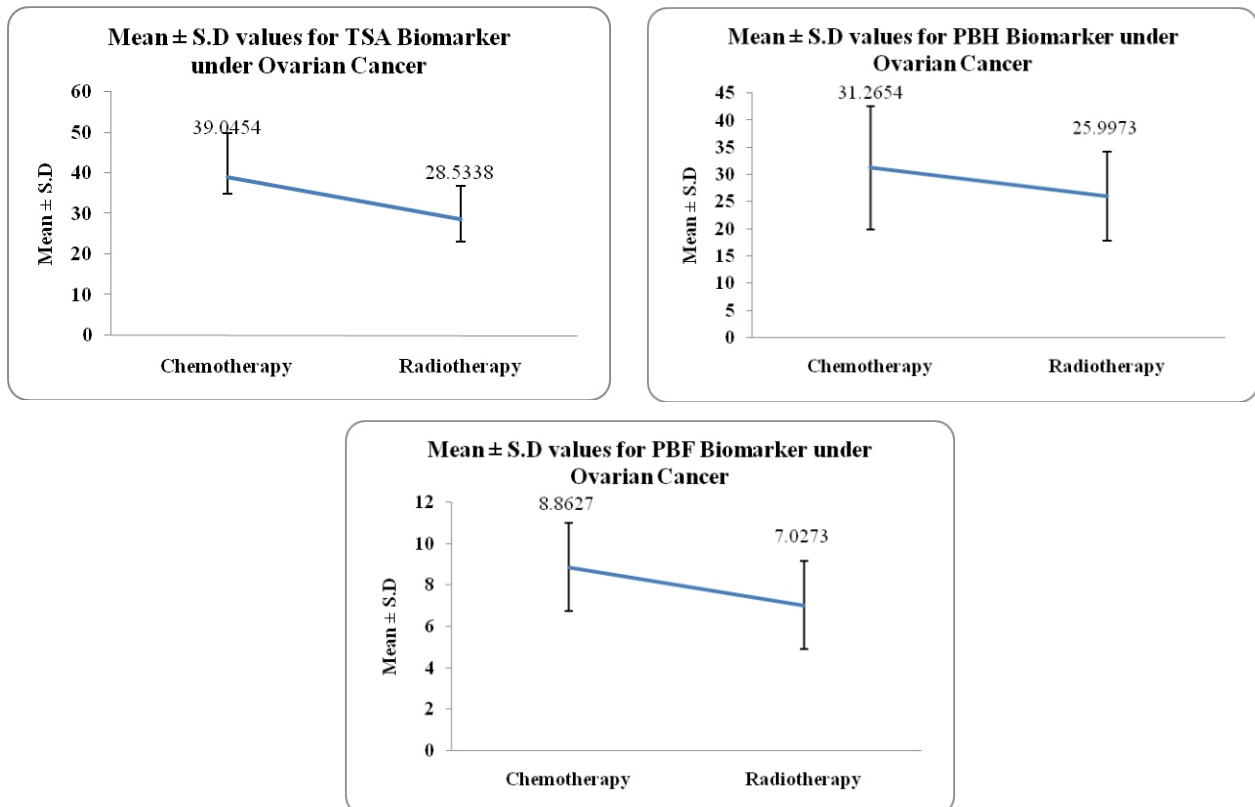


Fig. 2. Effect of Chemo and Radiotherapy on serum TSA, PBH and PBF respectively in Ovarian cancer represented by Whisker line graphs.

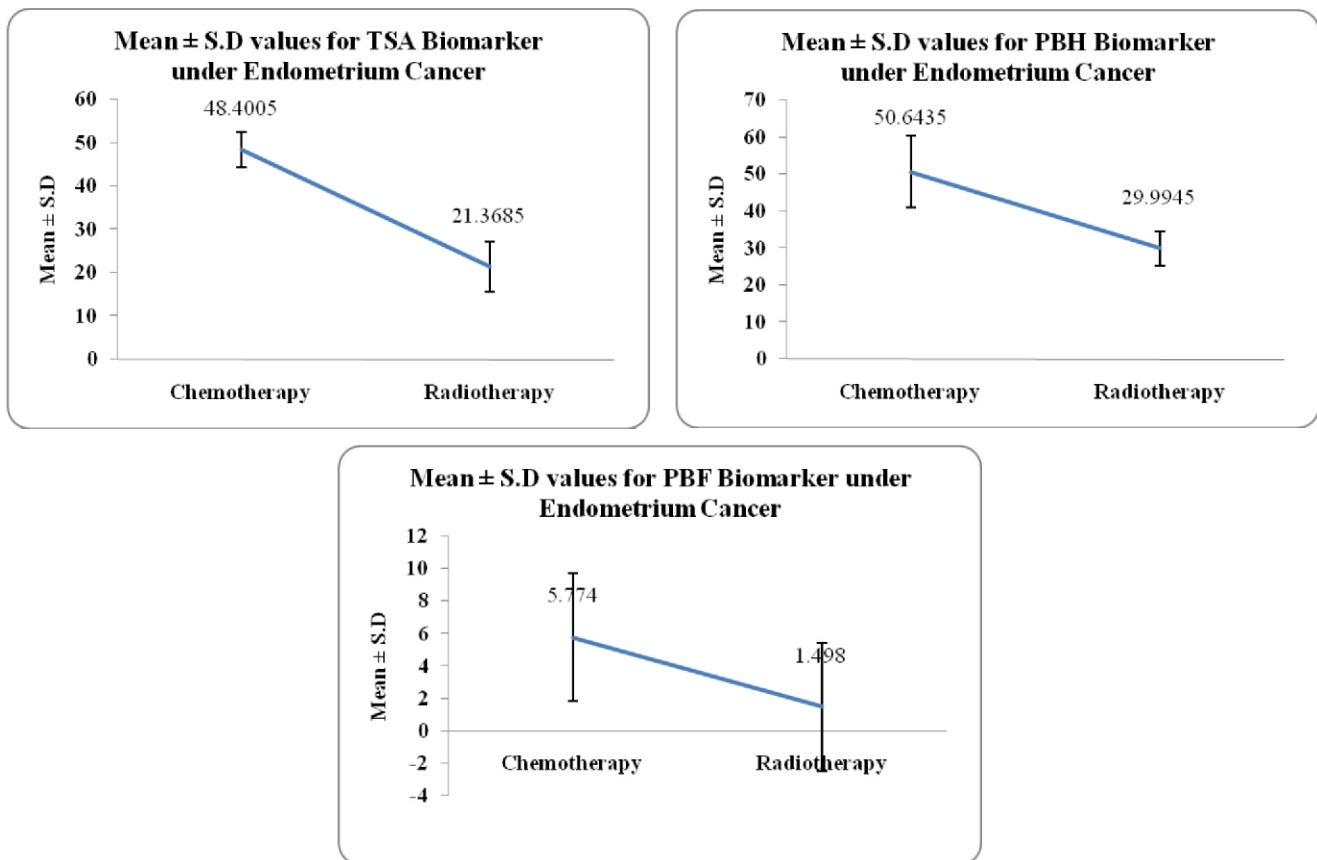


Fig. 3. Effect of Chemo and Radiotherapy on serum TSA, PBH and PBF respectively in Endometrial Cancer represented by Whisker line graphs.

endometrial cancer and should be considered as a supportive evidence of glycoproteins could be used in monitoring of cancer patients and follow up after therapy.

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