Original Article

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Biochemistry Section

Study of Biomarkers in Cancer Patients Treated with Radiotherapy: A Case-control Study

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ABSTRACT

Introduction: Blood based biomarkers can be useful as prognostic markers because these biomarkers can reflect variations in the tumour microenvironment and the immune system of the host. This study investigates some biomarkers in cancer patients experienced with radiotherapy to evaluate the effect of radiotherapy on the body.

Aim: To assess radiotherapy induced changes in the biomarkers like serum total protein, albumin, creatinine and urea in cancer patients and compare to the healthy controls.

Materials and Methods: This was a case-control study, conducted in the Biochemistry Department, Baroda Medical College, Gujarat, India, from October 2017 to August 2018. This study enrolled 537 patients, who underwent radiation therapy irrespective of any cancer and 537 healthy controls without any history of cancer or radiation therapy. Serum total protein, albumin, creatinine and urea were measured. Statistical analysis was performed by descriptive statistics, independent t-test, frequency, Pearson's correlation coefficient (r).

Results: In each of the groups, out of 537 subjects 185 subjects were females, 61.30±9.03 years was the mean age for females, and 352 subjects were males with a mean age of 61.02±8.93 years. Albumin had negative statistically significant correlation with all parameters. But total protein which was also composed of other acute phase positive reactants had higher values compared to control group which was statistically significant ($p<0.001$).

Conclusion: It was found that patients with cancer, undergoing radiotherapy, suffered from radiation induced changes such as acute phase response, host defense mechanism etc., causing changes in the body which is reflected by biomarkers.

Keywords: Acute phase response protein, C-reactive protein, Host defense protein, Immunity

INTRODUCTION

Blood based protein biological markers can be implemented as prognostic markers because they have the power to study both contrasts in the tumour context and the host defense system [1]. In the face of its name, the acute phase response is linked to a wide range of illnesses, including trauma, infarction, infection, inflammatory problems, and other systemic autoimmune and neoplastic conditions, in addition to acute inflammatory states. Proteins in the acute phase are those whose serum concentrations increase or drop by at least 25% during inflammatory circumstances [1,2]. Such Acute Phase Reactants Proteins (APRPs) are positive or negative acute phase proteins, positive proteins are C-reactive protein (CRP), serum amyloid A, haptoglobin, α1-acid Glycoprotein (AGP), α2-macroglobulin, fibrinogen, complement (C3, C4) and negative proteins are transferrin, albumin, transthyretin, retinol-binding protein [3,4]. Changes in APRPs levels are thought to be the result of hepatocytes being overfed, owing to the impact of cytokines released by macrophages, monocytes and a number of other cells during the inflammatory process. Furthermore, the key inducer of most APRPs is interleukin (IL)-6, many cancer cells produce IL-6, the pro-inflammatory cytokine which triggers the release of Host Defense Proteins (HDPs) via an APRP. These cytokines also inhibit albumin synthesis, which is referred to as a "negative APRPs" because its levels fall as a result of inflammation. Different inflammatory situations cause different patterns of cytokine production. In cancer, IL-2 and IL-6 may have pleiotropic effects [5,6].

Sometimes biomarkers are inducible in response to the stimuli. The messenger molecules such as cytokines are highly important in the scoring of the inflammatory response to self or not-self danger molecules. Anti-cancer host defense mechanisms are scrutinised to symbolise and immune surveillance. However, evidence is growing that parts of the Acute Phase Protein Response (APPR) provide anti-cancer host protection as well. The HDPs have been shown to be cytotoxic to tumour cells via cancer cell membrane lysis or cancer cell apoptosis [7]. The CRP is a serum protein that is increased in a number of diseases, including cancer. Furthermore, some evidence suggests that CRP levels are associated with a variety of acute inflammatory disorders in cancer patients receiving radiation therapy, and that they are remarkably connected with radiation dose. Pentraxins such as Serum Amyloid P (SAP; also known as PTX2) modulate diverse senses of the innate immune systems [8].

Eminent retaliations are achieved to tumour treatment by Photodynamic Therapy (PDT) in the form of inflammation and immune response. Genetically enhanced regulations of Nitric Oxide (N_2O) pathways favours irradiation induced angiogenesis which is an important step in proving our theory for the augmented level of protein during radiotherapy [9]. Rise in endothelial nitric oxide synthase influence and covalent modifications have played a role by stimulation of the proangiogenic N_2O pathway. Furthermore, the idea that limits these provascular effects of irradiation by inhibiting N_2O production opens up new possibilities for the combined use of antiangiogenic therapies and radiotherapy in clinical practice [10].

Radiation therapy for cancer treatment invariably exposes normal tissues; as a result, symptoms associated with normal tissue damage arise during the course of therapy, weeks after therapy, or months or years later. Many biomarkers play a role in the risk and severity of normal tissue reactions, and these parameters differ by location [11]. The impact of curative radiotherapy is primarily determined by the total dose delivered in the targeted volume, but there are two types of side effects (acute and late) that occur during and after radiotherapy, with radiation induced late complications (LC) being of particular interest due to their irreversibility and potential impact on quality of life [12].

As there is no study done to show the correlation between biomarkers like serum albumin, protein, creatinine and urea in the specific area of study. The aim of the present study was to measure these biomarkers in cancer patients treated with radiotherapy to assess the effects of radiotherapy on the body.

MATERIALS AND METHODS

This case-control study was carried out between October 2017 to August 2018 in the Biochemistry Department, Baroda Medical College, Gujarat, India. Ethical clearance was obtained from the Institutional Ethics Committee (HREC/UGPG/BMC/SESSION 9/12).

Inclusion criteria: This study had enrolled 537 patients of radiation therapy irrespective of any cancer and 537 age and sex matched healthy controls without any history of cancers and radiation were include in this study.

Exclusion criteria: Patients with pre-existing hepatic and renal disorders were excluded from both the case and control groups were exclude in this study.

Sample size calculation: In the absence of the similar previous case-control study, we done a small pilot study and sample size calculated using reference Kelsey's formula for sample size calculation. The sample size calculated was 500 so sample size was taken 537 per group to allow some extreme outliers. Power of this study was 80% [13].

Study Procedure

A total of 1074 participants were included in this study, with 537 in the case group (group 1) and 537 in the control group (group 2).

After taking informed consents 5 mL of venous blood samples were taken with an aseptic sample collection technique following proper disinfection of venipuncture site. The samples were collected in either sitting or supine position, then sent to centrifuge machine and centrifuged at 1500 rpm for 20 minutes at room temperature to obtain serum for the measurements of serum total protein, albumin, creatinine and urea.

Urea was estimated by the colourimetric kinetic urease method. In this method, urea is decomposed into ammonia ion and carbon dioxide by urease, ammonium ion reacts with phenol and produce colour. Creatinine was estimated by the colourimetric kinetic Jaffe reaction method. In an alkaline media, creatinine generates a quantitative orange colour when combined with picric acid. Total protein was estimated by the colourimetric biuret method. In the biuret method copper salts in an alkaline solution generate a purple complex with peptide bonds in the biuret method. Albumin was estimated by colorimetric the Bromocresol Green (BCG). In this method albumin and BCG form a chromophore that can be detected at 620 nm. A fully automated analyzer was used to estimate all of the tests by colorimetric methods [14].

STATISTICAL ANALYSIS

MedCalc and excel 2019 (commercially available statistical software) were used to conduct the analysis. A statistically significant p-value of less than 0.05 was used. All of the aforementioned was demonstrated through the use of descriptive statistics, independent t-tests, frequency, and Pearson's correlation coefficients (r).

RESULTS

A total of 1074 participants were included in this study, with 537 in the case group and 537 in the control group. In each of the groups, out of 537 subjects 185 subjects were females, 61.30±9.03 years was the mean age for females and 352 subjects were males with a mean age of 61.02±8.93 years. The case and control groups were not significantly different in terms of age distributions (p-value >0.05). However, males had a considerably higher prevalence of cancer than females in the case group (p<0.05).

Comparison of variables by student's t-test and correlation of variables: In group statistics, the t-test was used for biological variables such as albumin, total protein, urea and creatinine.

The [Table/Fig-1] shows specifics of the case and the control groups' various characteristics. There are statically significant differences in the means of the albumin, total protein, urea and creatinine between the case group and the control group (p<0.001).

Parameters	Case (Mean±SD)	Control (Mean±SD)	p-value*
Albumin (gm/dL)	3.8±0.4	4.3±0.5	<0.001
Total protein (gm/dL)	9.0±0.7	6.5±0.6	<0.001
Urea (mg/dL)	80±10.5	15±5.18	<0.001
Creatinine (mg/dL)	3.0±0.23	1.5±0.15	<0.001

[Table/Fig-1]: Comparison of various parameters in the case and control groups. The independent t-test is used; *p<0.05= Statistically significant

This study also found that there is a negative statistically significant correlation of albumin with creatinine (p<0.0001, r=-0.8284) and with urea (p<0.0001, r=-0.9070) and there is a positive statistically significant correlation of total protein with creatinine (p<0.0001, r= 0.7824) and with urea (p<0.0001, r=0.8587) in the case group as shown in [Table/Fig-2].

Groups	Correlation between	Correlation coefficient r	p-value
Case group	Albumin and creatinine	-0.8284 Negative relationship	<0.0001
	Albumin and urea	-0.9070 Negative relationship	<0.0001
	Protein and creatinine	0.7824 Positive relationship	<0.0001
	Protein and urea	0.8587 Positive relationship	<0.0001

[Table/Fig-2]: Correlation between the biomarkers in the case group. Pearson correlation test; r= Pearson correlation coefficient; p<0.05= Statistically significant

DISCUSSION

In the present study, we had collected biochemical data of radiotherapy patients. The total protein, urea and creatinine were elevated in the case group. In response to radiation, some biochemical changes occurred according to negative and positive acute phase reactants. We also detected low albumin, a negative acute phase reactant in the case group. Nyarota K and Zhou DT, in their study reported that among all cancer patients 28.6% had hypoalbuminaemia and 4.7% had hyperproteinaemia [14]. There was also contrast when they proved that serum albumin and serum total protein levels in the patients with various kinds of cancer did not change significantly. Tolia GM et al., in their study established that CRP, ferritin and albumin were found to be correlated with the acute complication of lung parenchyma radiation induced toxicity, CRP and ferritin were found to be elevated in the immediate postradiotherapy interval (after two months) compared to pre radiotherapy values (p<0.001), and albumin levels were found to be lower (p<0.001). The present study also supported these lower albumin levels after radiotherapy [15]. Kemik O et al., in his study on cachectic patients with colon cancer, demonstrated the link between acute-phase response proteins, cytokines, and hormones and showed that significantly lower levels of the serum albumin were found in patients compared to control subjects (p<0.001) [16]. Results of these studies were comparable with the results of present study.

Pang W et al., apprised that APRP fingerprinting may be considered as supportive cancer biomarkers [17]. Work on the divergent design of immunochemistry and biochemical assay in different types of cancer with distinct stages may be the best intervention in cancer treatment [18,19]. Brøndum L et al., in their study identified an activated immune response in cancer patients [20]. Wang C-Y et

al., found that the elevated CRP values and hypoalbuminaemia in patients with oesophagus cancer submitted to radiotherapy were poor prognostic factors. The finding of hypoalbuminaemia in patients with radiotherapy are supported by the present study [21].

Radiotherapy, mainly gamma radiation modifies the protein structure of albumin thereby decreasing its level in blood and this was shown in a study by Gaber MH, in which reported a decrease in bovine serum albumin after exposure to gamma radiation and the present study also shows decrease in serum albumin levels [22].

Radiation induced renal injury, chemotherapy toxicity are the main responsible factors for urea and creatinine upregulation. Predisposing renal problems can be perceived by renal function tests. Skyscraping ubiquity of hypoalbuminaemia is an agonising scene in respect of prognosis augmenting factors in cancer patients [23]. So, host defense mechanisms, different immunomodulation in response to radiotherapy in cancer patients are adding justifying the changes in the biomarkers in present study.

Limitation(s)

The present study was limited by certain factors. Monitoring Lipopolysaccharide-binding protein, expression of different cytokines, ILs, and various growth factors in favour of acute phase injury prompted by radiation therapy for different organs origin was not considered in the present study. Also, regarding radiation dose, duration, type of cancer, etc., was not evaluated in the present study.

CONCLUSION(S)

The present study suggests that there is negative correlation of albumin with other parameters and statistically significant rise in levels of creatinine and urea in the case group determine theory of nephrotoxicity. Patients with cancer, undergoing radiotherapy, suffered from radiation induced changes such as acute phase response, host defense mechanism etc., causing changes in the body which is reflected by biomarkers. Due to the study's limitations, the specific mechanism is unknown and more research is needed to find out. Therefore, the effects of radiotherapy on the biomarkers should be considered when the treatment for a cancer patient is being planned and initiated.

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