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Effect of Radiotherapy on Tissue Damage, Innate and Humoral Immunity in Malignant Condition

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ABSTRACT

Radiation oncology is a widely accepted subject in all over the world for the treatment of malignancy. To study the innate as well as humoral immune response before and after radiation therapy in patients and also to estimate the level of tissue damage in them the present study was performed. The extent of tissue damage was determined by measuring the C-reactive protein and immunologic defense was estimated by measuring complement C3 for innate resistance and immunoglobulin IgG for the humoral response. The complement C3, IgG and C-reactive protein levels of cancer patients were significantly higher than the normal level. More increase in serum C-reactive protein level in every patient after radiotherapy was observed. In majority of the cases decrease of C3 and IgG level was estimated after prolonged radiation treatment. This indicated that radiation therapy, despite being a successful treatment for cancer, causes massive tissue damage; and cellular as well as humoral immunity remains at a low level after radiotherapy. Age is a crucial factor to tolerate such prolonged therapy treatment. Prolonged application of radiotherapy may also cause side effects on normal immune system as they are already in immune deficient state due to the life threatening neoplastic disease.

Key words: Cancer, C-reactive protein, complement C3, immunoglobulin IgG

INTRODUCTION

Radiation therapy or radiation oncology is the medical use of ionizing radiation as a part of cancer treatment to control malignancy. It may be used for curative or adjuvant cancer treatment, or as palliative or therapeutic treatment (Rath and Mohanti, 2000). Since radiation therapy is not specific, it kills cancerous cell along with few normal healthy cells and thus at times it leads to massive tissue damage depending on the dose and course of radiation therapy which leads to many complications. The extent of tissue damage can be determined by measuring the C-reactive Protein (CRP) level, an acute phase protein produced by liver or adipocytes, in the patient's serum (Pepys and Hirschfield, 2003). CRP seems to assist in complement binding to foreign and damaged cells, it enhances phagocytosis by macrophages and play an important role in innate immunity as an early defense system against infections (Erlinger *et al*., 2004).

As a major defense mechanism against cancer, host immunological surveillance is composed of a cellular immunity as well as humoral immunity including antibody and a complement system (Matsutani *et al*., 1984). Complement system is an important mediator in the immunologic defense of the body. It is reasonable, therefore, to investigate the possible implications of the complement

system in diseases in which immunologic phenomena are important (Hu *et al*., 1988). Complement serves as an important mediator in non-specific (innate) resistance by amplifying the humoral response and converting it into an effective defense mechanism to destroy invading microorganisms by enhancing phagocytosis (Bjorge *et al*., 2005). Complement proteins are produced in inactive forms and then become active following enzymatic cleavage by alternative or classical pathway or by lectin protein. After complement activation membrane attack complex is formed which mediates cell lyses. Thus to increase the immune response as defense mechanism the complement C3 has central role in formation of membrane attack complex as well as in opsonization. Humoral immune response is mediated by secreted immunoglobulins produced by B lymphocyte lineage. IgG being most abundant, constitute about 80% of the total serum immunoglobulin (Matsumotoa *et al*., 2006) and thus in the present study it is used as an important parameter to represent humoral immunity.

The present investigation is aimed to study the immune response, innate as well as humoral, before and after radiation therapy in patients and also to estimate the level of tissue damage in them.

MATERIALS AND METHODS

A total of 15 cases were enrolled for each investigation from patients attending to Barasat Cancer Research and Welfare Centre, a prime health centre covering many districts of West Bengal. The malignancy was diagnosed by various investigations like radio-imaging, cytology and histo-pathological examinations by physicians. Controls (n = 15) were randomly selected patients admitted to the same hospitals as the cases during the same time period. They were frequency matched to cases by age, sex and selected from hospital admission lists. Written informed consent was obtained from all participants in accordance with the guidelines from hospital center review board. Blood samples were collected from cases and controls both before and after the commencement of radiation treatment. Then blood samples (1 mL) were centrifuged at 3000 rpm for 15 min and clear serum was collected.

Determination of CRP level: To estimate tissue damage by radiation therapy CRP level in the serum samples was assayed using turbidimetric immunoassay, based on the principle of agglutination reaction, as per manufacturer instruction (Tulip Diagnostics, India). Activation buffer (500 μL) and latex reagent (50 μL) were mixed properly, incubated at 37°C for 10 min and used as working solution. The serum sample (3 μL) was added to the working solution and the CRP concentration was estimated by spectrophotometric reading at 546 nm.

Determination of complement C3 level: To study the effect of prolonged application of radiotherapy in cancer patients on their innate immunity, concentration of complement C3 was compared in serum of cancer patient before radiotherapy and after 2-6 dozes of treatment. Radial Immuno Diffusion (RID) plate (DIFFU-plate) containing uniform mono-specific antiserum directed against complement C3 protein in agarose gel layer is used (Biocientifica S.A., Argentina). The serum samples (5 μL each) were filled on the wells of agarose gel. Wet cotton was placed at the centre of the RID plate to avoid agarose dehydration. The plate is tightly closed and incubated at room temperature for 48 h. Radial diffusion of protein out of the well into the surrounding gel leads to the formation of a visible precipitation ring by reaction between protein C3 and antiserum. The diameter of the precipitation ring is proportionate to the protein concentration and the concentration was determined by the corresponding reference table.

Determination of IgG level: By detecting the change in the level of serum IgG, before and after the therapy, the effect of radiation therapy on humoral immunity was studied. RID plate (DIFFU-plate) containing mono-specific IgG antiserum were used (Biocientifica S.A., Argentina) and the same protocol stated above was used for the investigation.

RESULTS

Present investigation highlighted that malignancy may directly affect immunoglobulin production through immuno-suppression. The concentration of CRP was greater in cancer patients as compared with healthy one (0.6 mg dL^{-1}). The study also revealed even more increase in serum CRP level in every patient after radiotherapy (Table 1).

In case of complement, the serum C3 level was higher than normal level (80-160 mg dL^{-1}) in all the patients, except one case, showing malignancy before radiation therapy. After 2-3 radiotherapy dozes, out of 15 patients 6 patients (40%) showed increased C3 level. Among remaining 9 patients (60%) the C3 level decreased drastically after 5-6 prolonged therapy treatments, of which in 4 cases C3 level after radiation treatment was below normal level (Table 1).

Result revealed that IgG level was higher in all the patients before radiation treatment than the normal range (600-1650 mg dL^{-1}). This level was again increased in few patients after radiotherapy, but in significant number of patients IgG level decreased. Out of 15 patients the concentration of IgG in serum decreased after radiation therapy in 10 patients (66.7%) as compared with before radiation therapy. Moreover, among these 10 patients, the serum IgG level dramatically decreases beyond the normal level in 4 patients and thus considered to be effective. These patients were exposed to prolonged radiation treatment and they

Table 1: Level of CRP, complement C3 and IgG before and after radiation therapy

	Before radiation therapy					After radiation therapy						
	Concentration of Complement C3		Level of IgG			Concentration of Complement C3		Level of IgG		Normal		
Level of CRP (mg dL^{-1})	Diameter (mm)	Level (mg dL^{-1})	Diameter (mm)	Level (mg dL^{-1})	Level of CRP (mg dL^{-1})	Diameter (mm)	Level (mg dL^{-1})	Diameter (mm)	Level (mg dL^{-1})	CRP (mg dL^{-1})	C3 (mg dL^{-1})	IgG (mg dL^{-1})
1.2	5.7	111.4	6.3	1129.3	6.9	6.5	162.9	7.0	1498.7	0.6	80-160	600-1650
0.8	5.2	82.7	5.8	889.2	7.4	5.7	111.4	6.8	1389.2			
1.6	4.8	61.5	6.2	1079.7	6.5	5.3	88.2	6.9	1443.6			
0.9	6.0	129.9	7.0	1498.7	4.2	6.1	136.3	8.0	1389.2			
0.7	5.5	99.6	5.5	754.7	3.8	6.3	149.4	6.1	1030.8			
1.2	5.4	93.8	6.0	982.8	6.5	5.9	123.7	6.5	1230.9			
2.3	5.8	117.5	6.5	1230.9	5.4	5.5	99.6	5.9	935.6			
2.5	5.3	88.2	8.0	2094.0	5.9	4.2	33.0	6.7	1335.6			
1.1	5.7	111.4	6.8	1389.2	2.6	4.9	66.7	6.2	1079.7			
1.5	6.1	136.3	5.9	935.6	4.8	5.5	99.6	5.6	798.7			
1.4	6.0	129.9	7.2	1611.4	2.3	5.5	99.6	6.5	1230.9			
1.7	5.5	99.6	6.0	982.8	2.8	4.1	28.7	4.5	357.8			
2.4	5.8	117.5	5.4	711.4	5.0	4.4	42.1	4.3	288.8			
1.9	5.9	123.7	5.7	843.5	3.6	4.3	37.5	4.4	322.5			
2.2	6.1	136.3	5.5	754.4	4.9	5.2	82.7	4.5	357.8			

belong to age group 55-70 years. In case of remaining 5 patients (33.3%) the IgG concentration has increased after radiation therapy (Table 1). These patients belong to the age group 25-45 years.

DISCUSSION

The present investigation revealed that complement C3, IgG and CRP levels of cancer patients were significantly higher than those of the healthy subjects which is at par as reported in early studies (Bjorge *et al.*, 2005; Erlinger *et al.*, 2004; Rhaegen *et al.*, 1976). The high IgG and complement levels in neoplastic disease as compared with normal may be caused by the continued presence of a tumor mass which serves as an antigenic stimulus for continued antibody production. The antigen-antibody complexes require immunoglobulin and complement which causes an increased production to maintain normal levels (Hu *et al.*, 1988). CRP is a member of the class of acute phase reactants as its level rise dramatically during inflammatory processes occurring in the body (Erlinger *et al.*, 2004). This increment is due to a rise in the plasma concentration of IL-6 which is produced predominantly by macrophages as well as adipocytes (Pepys and Hirschfield, 2003). The present investigation revealed that radiation therapy, despite being a successful treatment for cancer, causes massive tissue damage which is evident from the elevated level of CRP in the serum of the patients after radiation. This may lead to great suffering of patients from many short term and long term side effects. This observation puts a question mark on the efficiency of radiotherapy treatment when the health of the patient is in stake.

The term complement defines it as the activity of blood serum that completes the action of antibodies. The increase of complement activity was dependent on the stage of the disease and on the therapy and C3 were the most representative values for this stage-linked increase (Bjorge *et al.*, 2005; Rhaegen *et al.*, 1976). The elevated complement level in cancer patients may be explained by the concept that complement activity rises to compensate for depressed cell-mediated immunity, in order to preserve the activity of the biophylaxis mechanism against cancer (Matsutani *et al.*, 1984). Fall in C3 level decreases the opsonin and anaphylatoxic activity of C3 as well as affect membrane attack complex formation. The majority of patients with C3 deficiency after radiation therapy showed higher incidences of infection, recurrent bacterial infection and may have immune-complex disease. This result supports the earlier observation that cellular immunity remains at a low level after radiotherapy (Hu *et al.*, 1988). But the level of IgG has increased in few patients' indicating no immune suppression in them.

The decreased levels of IgG, complement C3 and increased levels of CRP indicate that due to prolonged radiation therapy such patients may be prone to other bacterial infection, immune complex diseases or it may also induce a state of secondary immuno-deficiency leading to an unwanted consequence. Though therapeutic approaches are actually aimed at increasing immune response to the malignant or cancer cells, but prolonged application of radiotherapy may cause side effects on normal immune system, as they are already in immune deficiency state due to the life threatening neoplastic disease (Rhaegen *et al.*, 1976). This observation was in line with the fact that somatic mutation may results from the irradiation cause an impairment of immune function (Stone *et al.*, 1994). Irradiation can also cause a decrease in immune competency which in tern can accelerate aging (Stone *et al.*, 1994). The number of dozes given in radiotherapy depends on the stage and type of cancer, age, present health condition of the patients. Age is a crucial factor to tolerate such prolonged therapy treatment. Thus close monitoring of patients undergoing radiotherapy is very important and prolonged exposure should be avoided.

CONCLUSION

The present investigation revealed the fact that though radiation therapy is an important means for controlling cancer disease, but it can cause tissue damage, harm cellular and humoral immunity and also short term and long term side effects. It may also induce a state of secondary immuno-deficiency by decreasing immune-competency. Thus the success of radiotherapy depends on the close monitoring of patients with efficient treatment by selection of doses. The present study is the unique approach to contribution in this direction towards the understanding.

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REFERENCES

Bjorge, L., J. Hakulinen, O.K. Vintermyr, H. Jarva, T.S. Jensen, O.E. Iversen and S. Meri, 2005. Ascitic complement system in ovarian cancer. BJC, 92: 895-905.

Erlinger, T.P., E.A. Platz, N. Rifai and K.J. Helzlsouer, 2004. C-reactive protein and the risk of incident colorectal cancer. JAMA, 291: 585-590.

Hu, D.E., X.S. Ling, J. Hu, B.L. Li, X.F. Wang, Y.G. Shen and J. Ye, 1988. The effects of radiotherapy on the immune system of patients with nasopharyngeal carcinoma. Br. J. Radiol., 61: 305-308.

Matsumotoa, K., T. Yasugia, A. Okib, T. Fujiic and C. Nagatad *et al.*, 2006. IgG antibodies to HPV16, 52, 58 and 6 L1-capsids and spontaneous regression of cervical intraepithelial neoplasia. Cancer Lett., 231: 309-313.

Matsutani, M., T. Suzuki, T. Hori, H. Terao, K. Takakura and K. Nishioka, 1984. Cellular immunity and complement levels in hosts with brain tumours. Neurosurg. Rev., 7: 29-35.

Pepys, M.B. and G.M. Hirschfield, 2003. C-reactive protein: A critical update. J. Clin. Invest., 111: 1805-1812.

Rath, G.K. and B.K. Mohanti, 2000. Textbook of Radiation Oncology: Principles and Practice. BI Churchil Livingstone, New Delhi, India.

Rhaegen, H., W.E. Ock, J. Decree and F. Vekbruccen, 1976. Increase of serum complement levels in cancer patients with progressing tumors. Cancer, 38: 1608-1613.

Stone, W.H., D.G. Saphire, S.M. Hackleman, A.M. Braun and P. Pennington *et al.*, 1994. Effect of radiation and age on immunoglobulin levels in rhesus monkeys (*Macaca mulatta*). Radiat. Res., 138: 401-408.