on mice and found to be an effective hypoglycaemic agent. Further research is needed to elucidate the active constituent, which has an antidiabetic effect.

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Received 30 January 2006; revised accepted 18 March 2008

Biochemical changes in neutrophils of cervical cancer patients treated with ^{60}Co

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Cervical carcinoma is the second most common malignancy of the female genital tract in India. The highest incidence occurs at Chennai. This study was conducted on 30 women with biopsy-proved squamous cell carcinoma of the cervix of stage IIb. The neutrophil count increased significantly in cancer patients compared to control subjects. Total protein, glycogen and total lipid increased in neutrophils of cervical cancer patients. The level of cholesterol, triglycerides and fatty acids increased significantly in neutrophils of such patients compared to control subjects. The activity of alkaline phosphatase increased significantly in cervical cancer patients. Upon treatment with cobalt-60, these changes were brought to near-normal levels. This study highlights the impairment in the neutrophil function in cervical cancer patients, which may lead to reduced immune status.

Keywords: Absolute neutrophil count, cervical cancer, glycogen, lipids, neutrophil alkaline phosphatase.

CERVICAL cancer is one of the most common malignancies among women[1]. In India, cervical cancer ranks first among different forms of cancers[2]. Incidence of cervical cancer is high among the rural population[3]. This could be due to difference in lifestyle, economic status, education, personal hygiene and healthcare. Cervical cancer is generally associated with HPV infection[4]. Radiotherapy is the primary local treatment for most patients with loco regionally advanced cervical carcinoma. Radioactive isotopes of cobalt-60 (^{60}Co) and gold-98 (^{98}Au) are used in the treatment of cervical cancer. Cobalt-60 is preferred because it is easy to handle and less expensive than gold.

Neutrophils (polymorpho nuclear leucocytes; PMNs) constitute the 'first line of defence' against infectious agents or 'non-self' substances that penetrate the body's physical barriers. This is the most abundant leukocyte present in the blood with mean concentration of 4.4×10^6 cells/ml. Neutrophils are derived from the common hematopoietic stem cell. Their development in the bone marrow takes approximately 10–14 days. The bone marrow produces approximately 1×10^9 neutrophils/kg/day[5]. As the neutrophils mature, they develop the capacity to enter the blood through increasing formability and

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Box 1. Proforma of radiation-treated cervical cancer patients.

Name	:	Age	:	Height :	Weight:
C/S No	:	F	:	Blood group: A/B/AB/O + or –	
Address	:				

Monthly income (Rs)
<500
500–1000
1000–2000
>2000

Age at which she
Matured
Married
First childbirth

Education
Uneducated
Pre-Degree
Degree

Menopause attained

Professional

Menstrual cycle
Bleeding cycle
Contraceptive Y/N
Diagnosis TNM
History of cancer – pre-family
Secondary from other disease
Radiation treatment started on
Dosage/15 days once

Diet
Veg/Non-veg
Mixed
Fish/tea/coffee/tobacco/alcohol

through changes in the adhesion protein in their surface membranes. Agents such as G-CSF, CM-CSF, corticosteroids or endotoxins can stimulate the release of neutrophils from the marrow.

The most important role of neutrophils is in the defence of tissues outside the blood. Neutrophils phagocytose the invading infectious agents and neutralize them. Since it has been reported by various authors that cervical cancer could arise due to microbial infection and as neutrophils are the defence cells involved in counteracting the infection, the role of neutrophils in cervical cancer, their biochemical changes and the effect of ^{60}Co radiotherapy was assessed in this study.

The study was conducted on 30 women with biopsy-proven squamous cell carcinoma of the cervix with clinical stage II-b, registered at the Department of Radiation Oncology, Government Royapettah Hospital, Chennai. Details about age, family history, weight, height etc. of the patients were registered as shown in the proforma (Box 1). The age range between 45 and 55 years and the women weighed about 55 ± 5 kg. The control group consisted of 30 women with age, weight and sex-matched healthy volunteers. Patients were given external radiation using ^{60}Co at a dosage of 50 Gy in 25 sittings for a period of 3 months.

The study was approved by the ethical committee of the hospital and all the women gave their written consent for providing blood samples. 5 ml of blood was collected before and after 3 months of radiation treatment. Neutrophils were isolated by dextran sedimentation followed by ficoll paque density gradient centrifugation as described below.

Total WBC count was done according to the method of Chesbrough and McArthur[6]. Differential count was done using Leishman stain. Absolute neutrophil count (ANC) is determined by multiplying the total WBC count by the percentage of neutrophils.

$$\text{ANC} = \text{WBC} \times \text{per cent neutrophils}.$$

Neutrophils were isolated by the method of Boyum[7] using dextran sedimentation followed by ficoll paque density gradient centrifugation. Neutrophils were freeze-thawed three times and centrifuged. The particle-free supernatant was used for the estimation of total protein[8] and glycogen[9]. Total lipid content was estimated by the method of Folch[10] after extracting lipids with chloroform and methanol mixture.

Lipids were extracted from the suspensions immediately after the final washing using chloroform–methanol mixture (2 : 1). This extract was then analysed for cholesterol[11], phospholipids[12], triglycerides[13] and free fatty acids[14].

Neutrophils were lysed with 0.2% Triton X-100 and then utilized for the assay of alkaline phosphatase (ALP) using the method of King[15].

All quantitative estimations were made on 30 patients in each group. The values are expressed as mean ± SD. Statistical analysis was done using Student's *t* test and the *p* value was arrived at to assess the statistical significance of the changes observed. A *P* value less than 0.02 was considered significant.

Table 1 shows ANC and neutrophil count (NC) in normal subjects, cervical cancer patients and in ^{60}Co-treated subjects. ANC was found to be significantly de-

creased ($P < 0.001$) in cervical cancer patients compared to normal subjects, whereas its level increased significantly ($P < 0.001$) upon radiotherapy. NC increased significantly compared to healthy subjects. A further significant increase ($P < 0.001$) was noticed in ^{60}Co-treated subjects.

Table 2 shows the biochemical constituents like total protein, glycogen and total lipid present in neutrophils of normal subjects, cervical cancer patients and ^{60}Co-treated subjects. It can be observed from Table 2 that the levels of total protein, glycogen and total lipid increased significantly ($P < 0.001$) in neutrophils of cervical cancer patients compared to healthy controls. On treatment with ^{60}Co-radiotherapy, the levels of total protein and total lipids increased further compared to cervical cancer patients. However, the glycogen content decreased drastically ($P < 0.001$) in neutrophils of ^{60}Co-treated subjects.

Table 3 shows the lipid profile in neutrophils of normal subjects, cervical cancer patients and ^{60}Co-treated subjects. The level of cholesterol, triglycerides and free fatty acids increased significantly ($P < 0.001$) in neutrophils of cervical cancer patients compared to healthy controls. Upon ^{60}Co radiotherapy, the level of cholesterol, triglycerides, free fatty acids decreased significantly ($P < 0.001$) compared to cervical cancer patients, showing that the treatment was effective. However, the levels of these remained still higher compared to normal subjects.

The level of phospholipids decreased significantly ($P < 0.001$) in neutrophils of cervical cancer patients compared to normal subjects. It increased significantly ($P < 0.001$) in ^{60}Co-treated subjects.

Table 4 shows the activity of ALP in neutrophils of normal subjects, cervical cancer patients and ^{60}Co-treated subjects. The activity of ALP had elevated significantly ($P < 0.001$) in cervical cancer patients compared to healthy controls. The activity decreased significantly upon radio-

Table 1. Absolute neutrophil count (ANC) and neutrophil count (NC) in normal subjects, cervical cancer patients and ^{60}Co-treated subjects

Parameter	Normal subjects	Cervical cancer patients	^{60}Co-treated subjects
ANC	3.85 ± 0.10	(a)* 3.06 ± 0.20	(b)* (c)* 3.42 ± 0.18
NC in percentage	45 ± 0.1	(a)* 53 ± 0.3	(b)* (c)* 59 ± 0.5

Values are expressed as mean ± SD. *$P < 0.001$.
(a) Comparison between normal subjects and cervical cancer patients.
(b) Comparison between cervical cancer patients and ^{60}Co-treated subjects.
(c) Comparison between normal and ^{60}Co-treated subjects.

Table 2. Biochemical constituents present in neutrophils of normal subjects, cervical cancer patients and ^{60}Co-treated subjects

Parameter	Normal subjects	Cervical cancer patients	^{60}Co-treated subjects
Total protein (mg/10^9 cells)	4.1 ± 0.3	(a)* 4.4 ± 0.1	(b)** (c)* 4.6 ± 0.4
Glycogen (nmol/mg protein)	180 ± 10	(a)* 240 ± 18	(b)* (c)* 205 ± 15
Total lipids (mg/10^9 cells)	1.71 ± 0.09	(a)* 2.5 ± 0.10	(b)* (c)* 2.8 ± 0.18

Values are expressed as mean ± SD. *$P < 0.001$, **$P < 0.01$.
(a) Comparison between normal subjects and cervical cancer patients.
(b) Comparison between cervical cancer patients and ^{60}Co-treated subjects.
(c) Comparison between normal and ^{60}Co-treated subjects.

Table 3. Lipid profile in neutrophils of normal subjects, cervical cancer patients and ^{60}Co-treated subjects

Parameter	Normal subjects	Cervical cancer patients	^{60}Co-treated subjects
Total cholesterol (nmol/10^9 cells)	270 ± 15	(a)* 320 ± 21	(b)* (c)* 300 ± 18
Phospholipids (nmol/10^9 cells)	958 ± 40	(a)* 870 ± 50	(b)** (c)^{NS} 920 ± 80
Triglycerides (nmol/10^9 cells)	70 ± 3	(a)* 98 ± 5	(b)$^{\#}$ (c)* 92 ± 8
Free fatty acids (nmol/10^9 cells)	96 ± 5	(a)* 105 ± 7	(b)* (c)* 120 ± 9
C/P ratio	0.28 ± 0.01	(a)* 0.368 ± 0.02	(b)* (c)* 0.326 ± 0.02

Values are expressed as mean ± SD. *$P < 0.001$; **$P < 0.01$; $^{\#}P < 0.02$; NS, Non-significant.
(a) Comparison between normal subjects and cervical cancer patients.
(b) Comparison between cervical cancer patients and ^{60}Co-treated subjects.
(c) Comparison between normal and ^{60}Co-treated subjects.

Table 4. Activity of alkaline phosphatase in neutrophils of normal subjects, cervical cancer patients and ^{60}Co-treated subjects

Parameter	Normal subjects	Cervical cancer patients	^{60}Co-treated subjects
Alkaline phosphatase (U/10^9 cells)	1.42 ± 0.08	(a)* 5.2 ± 0.2	(b)* (c)* 4.3 ± 0.3

Values are expressed as mean ± SD. *$P < 0.001$.
(a) Comparison between normal subjects and cervical cancer patients.
(b) Comparison between cervical cancer patients and ^{60}Co-treated subjects.
(c) Comparison between normal and ^{60}Co-treated subjects.

therapy, but remained higher compared to normal subjects.

Neutrophils constitute the first line of defence against infectious agents. These are small cells about 9–10 μm in diameter and the most abundant leukocytes in the blood. They also participate in many types of immunologic and inflammatory responses. The absolute neutrophils present in the blood can be calculated by multiplying the total WBC by the percentage of PMNs.

In the present study, ANC decreased significantly in cervical cancer patients compared to healthy controls. This may be due to the association between HPV infection and cervical cancer[2]. Various researchers have reported that neutrophils with normal total WBC count exist during cervical cancer[16–18]. Although there was an increase in NC, because of a decrease in WBC count. ANC decreased in cervical cancer patients. Phagocytic activity decreased in cervical cancer patients due to alteration in their composition[16].

Stimulation of neutrophils with lipopolysaccharide causes a twofold increase in the membrane protein[19]. The neutrophils were activated in cervical carcinoma to compensate the immune status[16], the protein content increased significantly in cervical cancer patients compared to controls.

Glycogen metabolism and its regulation are important in neutrophils, as these cells rely primarily on glycolytic mechanism for energy production[20]. Neutrophils have more glycogen content when cultured as monolayers[21]. It has also been reported that neutrophils present at the site of inflammation showed accumulation of glycogen and lipids compared to non-inflammatory neutrophils[22]. Stimulated neutrophils showed an increase in their glycogen levels[23] and also the synthesis of glycogen was induced in and inflammatory neutrophil[22]. As the neutrophils are stimulated in cervical carcinoma, obviously their glycogen content increased and this fact is in agreement with our findings.

Normal PMNs contain lipids about twice as much as lymphocytes. Neutrophils contain 35% phospholipids and 10% cholesterol in contrast to the much higher proportion of each of these lipids in erythrocytes[24]. Various authors[25,26] have reported that the surface membrane of malignant cells differs from its normal mature counterpart in both morphological and functional properties like permeability, deformability, electrical surface charge. This entire membrane defect could be due to alterations in lipid components of the membranes[27]. Normal lipid composition of membranes also plays an important role in successful phagocytic process[28] and any alterations in lipid pattern leads to functional inability of the neutrophils. Hence the lipid pattern of neutrophils during cervical cancer was assessed in this study.

Watkins *et al.*[29] reported a direct relationship between lipid peroxidation and alteration in lipid metabolism. It can be observed from Table 3 that the phospholipid content of neutrophils in cervical cancer patients decreased significantly compared to the healthy controls. This decrease is reflected by an increases in free fatty acid content, as degradation of phospholipids leads to the formation of free fatty acids. Increased lipid peroxides were observed in cervical carcinoma by various workers[30–33], and this also accounts for decrease in phospholipid content.

Any increase in C/P ratio is associated with decreased membrane fluidity. As the C/P ratio increases in neutrophils of cervical cancer patients, the neutrophil membrane fluidity may decrease, which may affect the functioning of the neutrophils.

Human ALP is a phosphatidylinositol-linked glycoprotein[34] that exists in at least four isoforms and is present in many different cell types and tissues, including neutrophils[35]. In neutrophils, ALP is localized mainly in the secretory vesicles[36] and it can be translocated to the plasma membrane during cell stimulation[37].

Neutrophil ALP (NAP) is detectable in differentiated neutrophils and monocytes[38] and it is the product of liver/bone/kidney-type ALP gene[39]. ALP has exhibited remarkable increase in activity in uterine cervical carcinoma[30]. In this present study, we observed an increase in the activity of NAP in cervical cancer patients. NAP is released into the blood stream, perhaps through leakage from damaged or dead neutrophils[40]. It has also been reported that neutrophils may be an important source of increased serum ALP activity or bone-type ALP isoenzyme. The activity of NAP increases in neutrophils isolated from patients suffering from infectious diseases[41].

The increased activity of ALP observed in this study may be due to HPV/HIV infection resulting in cervical carcinoma.

Neutrophils provide defence against infectious agents that penetrate the body's physical barriers. It has been observed from the present study that there is significant increase in NC of cervical cancer patients. These patients showed variations in cholesterol, phospholipids, triglycerides and free fatty acid content of neutrophils. The C/P was also altered leading to functional impairment of neutrophils. The study highlights that cervical cancer patients have reduced immune status because of dearrangement in neutrophil functions.

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Received 10 September 2007; accepted 18 March 2008