LEVELS OF SERUM CORTISOL IN CANCER PATIENTS

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ABSTRACT*: Serum cortisol level estimations were carried out in thirty five cancer patients of different categories and in a control group of nine normal subjects. The different categories of cancer patients includeded acute lymphoblastic leukemia, breast cancer, non-hodgkin's lymphoma, stomach cancer and lung cancer. The levels of cortisol have been investigated with the help of Enzyme Linked Immunosorbent Assay (ELISA). The levels of cortisol of cancer patients were compared with the control subjects. It was found that mean serum cortisol levels, both in the morning and evening were significantly elevated in cancer patients than in control subjects. These results are discussed in the light of previous reports in different types of cancer patients.*

Key Words: Cancer, ELISA, Cortisol.

INTRODUCTION

Cancer is an abnormal condition that accounts for some 13% of all human deaths in the world. The World Health Organization went on to predict that number of people who would die from cancer would grow to 11.4 million in 2030 [1]. Cancer is a progressive disease, and most cancer morbidity and mortality occurs upon spread of the tumor from an original localized site [2].

There have been several reports indicating that plasma cortisol levels in patients with cancer are higher than those in control groups. A previous report demonstrated high cortisol levels in prostate cancer patients [3]. It has been found that plasma cortisol levels, both in morning and evening, were increased in lung cancer patients than in control subjects [4]. In a study patients of breast cancer showed elevated mean cortisol levels than control group [5]. In another study it has been reported that plasma cortisol levels were elevated in polycystic ovary syndrome female patients than in healthy controls [6]. Another study showed that patients suffering from adrenal cancer have significantly elevated cortisol level than healthy controls [7]. In another study elevated plasma cortisol was observed in children and adolescents with Cushing's disease [8]. Substantially increased levels of cortisol were observed in patients of colorectal, gastric and pancreatic cancer than control group [9]. Some previous studies also showed that the cortisol level was high in case of the tumors of the pancreas [10]. Patients with cancer demonstrated elevated prevalence rates for depression and plasma concentrations of cortisol in these patients showed higher level [11]. The present study was conducted to assess the relationship of the plasma cortisol levels and disease of cancer.

MATERIALS AND METHODS

The present study is based upon thirty five cancer patients and nine normal subjects belonging to different age groups. All the cancer patients were selected from Institute of Nuclear Medicine and Oncology (INMOL) who were already diagnosed by oncologists. The cancer patients were categorized into different groups on the basis of the type of cancer. Control subjects of same age groups were selected from the population of Lahore Pakistan. A structured questionnaire was used to collect detailed information. Blood samples of the cancer patients and normal healthy persons were drawn in morning and evening by the venipuncture method. 3ml of venous blood was taken in a vacuum container. The serum was separated and stored into the labeled tubes at -20^{o}C.

Serum cortisol was determined by commercially available Monobind ELISA Kit (Monobind Inc. USA) employing the method of Burtis and Ashweed [12]. Cortisol enzyme reagent, cortisol biotin reagent, wash concentrate solution and stop solution provided in above mentioned Kit were used for estimation of serum cortisol level. Before proceeding with the assay, all reagents, serum references and controls were brought to room temperature (20 – 27 ^{o}C). The microplate wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate. 25µl of the appropriate serum reference, control and patient specimen was pipetted into the assigned well. 50µl of the working cortisol enzyme reagent was added to all wells. The microplate was swirled gently for 20 – 30 seconds to mix. 50µl of cortisol biotin reagent was added to all wells. The microplate was swirled gently for 20 – 30 seconds to mix. The microplate was covered and incubated for 60 minutes at room temperature. The contents of the microplate were discarded by decantation. After decantation, the plate was blotted dry with absorbent paper. 300µl of wash buffer was added, and was aspirated. The washing was repeated for two additional times. 100µl of working substrate solution was added to all wells. Reagents were added in the same order to minimize reaction time differences between wells. The microplate was incubated at room temperature for 15 minutes. 50µl of stop solution was added to each well and mixed it gently for 15 – 20 seconds. The absorbance was read in each well at 450 nm in a microplate reader.

Mean ± SEM of serum cortisol levels of each category of cancer patients and controls were calculated and statistical analysis of the data was carried out by employing Student 't' test.

RESULTS

In the present study the serum cortisol levels of controls and of different categories of cancer patients mentioned below were estimated in morning and evening (Table 1).

Acute Lymphoblastic Leukemia

In the morning, the serum cortisol level in the acute lymphoblastic leukemia patients was elevated as compared to

control subjects. In control subjects these values were 22.16 ± 0.65 µg/dl while in acute lymphoblastic leukemia patients these values were 31.76 ± 2.64 µg/dl. The observed difference was significantly higher (P<0.05) in acute lymphoblastic leukemia patients. In the evening, the serum cortisol level was elevated in acute lymphoblastic leukemia patients as compared to the control subjects. In control subjects these values were 12.36 ± 1.11 µg/dl and in acute lymphoblastic leukemia patients these values were 23.35 ± 1.62 µg/dl. The observed difference was highly significant (P<0.01) in acute lymphoblastic leukemia patients.

Breast Cancer

The serum cortisol level of breast cancer patients was elevated in the morning as compared to serum cortisol level of the controls at the same time. The morning values were 22.16 ± 0.65 µg/dl of the controls and of the breast cancer patients were 44.11 ± 6.24 µg/dl. These values showed a statistically highly significant (P<0.01) elevation in breast cancer patients. The serum cortisol level of breast cancer patients was elevated in the evening as compared to the evening serum cortisol level of the controls. The evening values were 12.36 ± 1.11 µg/dl of the controls and that of the breast cancer patients were 30.10 ± 4.88 µg/dl. These values showed a highly significant (P<0.01) elevation in breast cancer patients.

Non-Hodgkin's Lymphoma

Morning serum cortisol level of the non-Hodgkin's lymphoma patients was elevated than the morning serum cortisol level of normal subjects. The morning values of non-Hodgkin's lymphoma patients were 33.87 ± 5.4 µg/dl and of normal subjects were 22.16 ± 0.65 µg/dl. The morning value of non-Hodgkin's lymphoma patients showed a significant (P<0.01) elevated level of cortisol. Evening serum cortisol level of non-Hodgkin's lymphoma patients was elevated than the evening serum cortisol level of normal subjects. The evening values of non-Hodgkin's lymphoma patients were 27.69 ± 3.99 µg/dl than normal subjects which were 12.36 ± 1.11 µg/dl. The evening value of non-Hodgkin's lymphoma patients showed statistically highly significant (P<0.01) elevated level of cortisol.

Stomach Cancer

The serum cortisol level of stomach cancer patient was raised in the morning as compared to the morning serum cortisol level of the healthy subjects. The cortisol values of the morning were 35.72 ± 5.59 µg/dl in stomach cancer patients. In healthy subjects the morning cortisol values were 22.16 ± 0.65 µg/dl. The observed difference was highly significant (P<0.01) in stomach cancer patients. The serum cortisol level of stomach cancer patients was raised in the evening as compared to the evening serum cortisol level of the healthy subjects. The evening cortisol values of healthy subjects were 12.36 ± 1.11 µg/dl and the evening cortisol values of stomach cancer patients were 30.81 ± 5.97 µg/dl. The observed difference was highly significant (P<0.01) in stomach cancer patients.

Table 1: Mean serum cortisol levels in control subjects and in different categories of cancer patients.

	No.	Serum Cortisol (µg/dl)			
		Morning		Evening	
		Mean ± SEM	SD	Mean ± SEM	SD
Control Subjects	9	22.16 ± 0.65	1.97	12.36 ± 1.11	3.34
Acute Lymphoblastic Leukemia Patients	15	31.76 ± 2.64*	10.22	23.35 ± 1.62**	6.29
Breast Cancer Patients	6	44.11 ± 6.24**	15.24	30.10 ± 4.88**	11.92
Non-Hodgkin's Lymphoma Patients	4	33.87 ± 5.4**	10.8	27.69 ± 3.99 **	7.99
Stomach Cancer Patients	4	35.72 ± 5.59 **	11.19	30.81 ± 5.97 **	11.95
Lung Cancer Patients	6	33.28 ± 5.06 *	12.35	22.96 ± 1.23 **	3.02

* P < 0.05;
** P < 0.01

Lung Cancer

In the morning the serum cortisol level in lung cancer patients was elevated as compared to normal subjects. In normal subjects these values were 22.16 ± 0.65 µg/dl while in lung cancer patients these values were 33.28 ± 5.06 µg/dl. These values showed a highly significant (P<0.05) elevated level of serum cortisol in lung cancer patients. In the evening the serum cortisol level in lung cancer patients was elevated as compared to normal subjects. In normal subjects these values were 12.36 ± 1.11 µg/dl while in lung cancer patients these values were 22.96 ± 1.23 µg/dl. These values showed highly significant (P<0.01) elevated level of serum cortisol in lung cancer patients.

DISCUSSION

In thepresent study the serum cortisol levels in different categories of cancer patients (acute lymphoblastic leukemia, breast cancer, non-Hodgkin's lymphoma, stomach cancer and lung cancer) were studied in morning and evening and compared with normal subjects. The present study demonstrated that the cortisol levels were significantly higher in morning and evening in patients of all categories of cancer (Table 1-6). The results of the present study comparable with the previous reports in literature [4,9,11,13,14]. In these reports, the serum cortisol levels were elevated in morning and evening and these values showed a significant increase in metastatic cancer patients.

In some previous reports patients with metastatic cancer demonstrated elevated prevalence rates for depression. A wide variety of nonspecific stresses have profound effects, many of which are mediated by ACTH and cortisol including surgical stress, trauma and depression [15]. It has been demonstrated that depression is associated with increased

plasma IL-6 concentrations in patients with metastatic cancer [11]. Cortisol production may depend on a direct adrenal stimulation by IL-6. Cancer related hypercortisolemia would depend on alterations of the feedback mechanisms between endocrine and cytokine secretions, occurring in neoplastic disease [16]. Glucocorticoids suppress inflammation and immune reactions. These reactions induce the synthesis of multiple cytokines, and suppressive effects of glucocorticoids have logically been assumed to reflect their ability to inhibit the expression of multiple genes involved in proinflammatory cytokine synthesis. Glucocorticoids upregulate the expression of various cytokine receptors [17]. Patients suffering from breast cancer showed flattened diurnal cortisol rhythm and it is associated with the stress and the disrupted nocturnal sleep. When significant stress is experienced, other control mechanisms are overridden, diurnal variation is disrupted, ACTH immediately increases and peaks within minutes and cortisol peaks shortly after. A wide variety of nonspecific stresses have profound effects, many of which are mediated by ACTH and cortisol including surgical stress, trauma and depression [18]. Psychological distress and depression, which often accompany cancer diagnosis and treatment, can also suppress or dysregulate endocrine and immune function [19]. A report suggests that women with breast cancer show higher stress and by the greater activation of stress hormone altered their functional brain activation profiles which also resulted in verbal memory impairments [13]. Elevated evening cortisol levels may suggest dysregulation of the stress response [20]. Further research is required to establish the relationship of cancer and cortisol production and its effect on body physiology.

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REFERENCES

1. Delfino, M. and Day, M. E., "*Cancer, we live and die by radiation*." MoBeta Publishing, Los Altos, pp. 1-2 (2006).
2. Wells, A., "*Cell Motility in Cancer Invasion and Metastasis*." Springer, The Netherlands, pp. 1-2 (2006).
3. Fabre, B., Grosman, H., Gonzalez, D. Machulsky NF, Repetto EM, Mesh V., Lopez MA., Mazza O., and Berg G., "Prostate Cancer, High Cortisol Levels and Complex Hormonal Interaction." *Asian Pac. J. Cancer Prev.*, **17:** 3167-3171 (2016).
4. Mazzoccoli, G., Carughi, S., De Cata, A., La Viola, M. and Vendemiale, G., "Melatonin and cortisol serum levels in lung cancer patients at different stages of disease." *Med. Sci. Monit*., **11**: 284-288 (2005).
5. Lang, E. V., Berbaum, K. S. and Lutgendorf, S. K., "Large-core breast biopsy: abnormal salivary cortisol profiles associated with uncertainty of diagnosis." *Radiology*, **250**: 631-637 (2009).
6. Gambineri, A., Forlani, G., Munarini, A., Tomassoni, F., Cognigni, G. E., Ciampaglia, W., Pagotto, U., Walker, B. R. and Pasquali, R., "Increased clearance of cortisol by 5 beta-reductase in a subgroup of women with adrenal hyperandrogenism in polycystic ovary syndrome." *J. Endocrinol. Invest*., **32**: 210-308 (2009).
7. Yener, S., Comlekci, A., Akinci, B., Secil, M., Demir, T., Ertilav, S. and Yesil, S., "Non-functioning adrenal incidentalomas are associated with elevated D-dimer lavels." *J. Endocrinol. Invest*., **32**: 338-343 (2009).
8. Hauffa, B. P., Kaplan, S. L. and Grumbach, M. M., "Dissociation between plasma adrenal androgens and cortisol in Cushing's disease and ectopic ACTH-producing tumour: relation to adrenarche." *Lancet*, **1**: 1373-1376 (1984).
9. Zubelewicz-Szkodzinska, B., Muc-Wierzgon, M., Wierzgon, J. and Brodziak, A., "Dynamics of circadian fluctuations in serum concentration of cortisol and TNF-alpha soluble receptors in gastrointestinal cancer patients." *Oncol. Rep*., **8**: 207-212 (2001).
10. Kobakov, G., Dragnev, E., Iordanov, E., Kirov, K. and Kostov, D., "Rare endocrine tumors of the pancreas with secretion of cortisol." *Khirurgiia (Sofiia*), **1-2**: 65-67 (2007).
11. Jehn, C. F., Kuehnhardt, D., Bartholomae, A., Pfeiffer, S., Krebs, M., Regierer, A. C., Schmid, P., Possinger, K. and Flath, B.C., "Biomarkers of depression in cancer patients." *Cancer*, **107**: 2723-2729 (2006).
12. Burtis, C. A. and Ashweed, E. R., "*Textbook of Clinical Chemistry*." W. b. Saunders Company, Philadelphia, pp. 1825-1827 (1994).
13. Kesler, S. R., Bennett, F. C., Mahaffey, M. L. and Sa, D., "Regional brain activation during verbal declarative memory in metastatic breast cancer." *Clin. Cancer Res*., **15**: 6665-6673 (2009).
14. Hamid, Z. A., Ahmad, L. B., Alam, B. K., Shah, A., Ahmad, M., Aejaz, A. S., Iftikhar, B. M., Iqbal, W. A. and Hayat, B. M., "Adrenal insufficiency due to primary bilateral adrenal non-Hodgkin's lymphoma." *Exp. Clin. Endocrinol. Diabetes*, **112**: 462-464 (2004).
15. Luger, A., Deuster, P. and Kyle, S., "Acute hypothalamic pituitary adrenal responses to the stress of treadmill exercise." *New Eng. J. Med*., **316**: 1309-1320 (1987).
16. Lissoni, P., Brivio, F., Fumagalli, L., Messina, G., Secreto, G., Romelli, B., Fumagalli, G., Rovelli, F., Colciago, M. and Brera, G., "Immune and endocrine mechanisms of advanced cancer-related hypercortisolemia." *In Vivo*., **21**: 647-650 (2007).
17. Griffin, J. E. and Ojeda, S,R., "*Textbook of Endocine Physiology*." Oxford University Press, pp. 334-346 (2000).

18. Gosling, J. P. and Basso, L. V., "*Immunoassay: Laboratory Analysis and Clinical Application.*" Butterworth-Heinemann, USA, pp.116-127 (1994).
19. Sephton, S. E., Dhabhar, F. S., Keuroghlian, A. S., Giese-Davis, J., Mcewen, B. S., Ionan, A. C. and Spiegel, D., "Depression, cortisol, and suppressed cell-mediated immunity in metastatic breast cancer." *Brain Behav. Immun*., **23**: 1148-1155 (2009).
20. Costanzo, T.C. and Campbell, T.C., "Stress biomarkers in advanced cancer patients experiencing the pain, fatigue, sleep disturbance symptom cluster." *J. Clin. Oncol.,* **33:** 5 (2015).