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Studies on changes in trace elemental content of serum of uterine cervix cancer patients using PIXE

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Abstract The objective of this study was to evaluate the levels of trace elements in blood sera of uterine cervix cancer patients, analyze their alteration with respect to healthy controls and identify the best predictors amongst these for disease occurrence and progression. Particle induced X-ray emission (PIXE) technique was used in this work to identify and quantify trace elements Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br in the blood sera of uterine cervix cancer subjects and healthy control subjects. The observed alterations are discussed with respect to the possible mechanisms by which these elements might influence the carcinogenic process.

Keywords PIXE · Trace elements · Uterine cervix cancer

Introduction

With changing lifestyle patterns and environmental conditions human beings have become vulnerable to either a deficit or an excess of available trace elements. Trace elements are undoubtedly important for the vital processes of life, but even more important is the fact that their functional forms and their characteristic concentrations must be maintained within narrow limits. Even for essential elements there is always an optimum range of concentration in the diet, below which deficiency symptoms occur, and above which symptoms of toxicity begin. Moreover, discoveries that both essential and nonessential trace elements can markedly influence several other key biological events such as cell cycle regulation, processes involved in replication or transcription, inhibition or activation of several enzymatic reactions, activation of growth factors, and factors involved with apoptosis, have strengthened convictions that variations of trace elemental concentrations in both the extracellular and intracellular environment may markedly influence cancer risk. It is therefore reasonable to postulate that trace minerals would exert action, directly or indirectly, on the carcinogenic process.

Uterine cervix cancer is the cancer that develops in the lining of the cervix, the lower part of the uterus that enters the vagina. It is the second most common cancer in women worldwide [1] and is a leading cause of cancer-related deaths in women in underdeveloped countries. Worldwide, approximately 500,000 cases of cervical cancer are diagnosed each year. Among the several risk factors associated with cervical cancer, infection with human papilloma viruses (HPVs) is the main factor [2, 3]. In recent years, numerous studies were carried out to understand the etiology of uterine cervix cancer [4–7], but the molecular mechanisms involved in initiation and progression of cervical cancer are still not well understood. Therefore, the need of the hour is to simultaneously screen a patient for multiple biomarkers and adopt multidisciplinary treatment strategies.

This study was undertaken with an aim to evaluate the levels of trace elements in blood sera of cervical cancer patients, analyze the alteration with respect to healthy

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controls and establish the role played by them in the initiation, promotion and inhibition of cancer. Here an attempt is also made to correlate the occurrence of trace elements to the clinical stage of tumour and thereby identify the best predictors amongst these for disease occurrence and progression. Particle induced X-ray emission (PIXE) technique was employed in the present work to analyse trace elemental content in the serum samples. PIXE has rapidly gained acceptance as a valuable analytical tool, because of the ever-increasing need for elemental analysis of very small amounts of sample. Respected for its practical accuracy and detection range of parts per million, PIXE has enjoyed a secure place in the analytical arsenal of the nuclear physics laboratory. Due to its high sensitivity and multi-elemental analysis capability, PIXE has found application in the trace elemental analysis of samples from almost every conceivable field of scientific or technical interest.

Materials and methods

Study subjects

Forty-nine newly diagnosed, histologically proven, uterine cervix cancer patients (mean age = 47 ± 10 years) were inducted in this study. These uterine cervix cancer patients were further classified as having limited disease (stage I: 9 patients and stage II: 12 patients) or advanced disease (stage III: 28 patients). Thirty age-matched, apparently healthy female volunteers who had not had any medication served as controls (mean age 45 ± 10 years). All healthy subjects, who served as controls, and the cervix cancer patients were recruited into the study after obtaining their informed consent. In order to avoid the influence of diet and environmental factors on the trace elemental content of serum samples, all the studied subjects were selected from the same region.

Sample collection and preparation

The blood samples of the studied subjects were collected from Lion's Cancer Treatment and Research Centre, Visakhapatnam only after obtaining a written consent from the research centre's ethics committee. Six to eight milliliters (ml) of whole blood was obtained from an antecubital vein of each of the patients and control subjects. These blood samples were collected in separate vacutainer tubes and then centrifuged at 3000 rpm for 10–15 min. For each sample, the supernatant containing the serum was aspirated with an air displacement pipette and stored in a trace element free plastic container at −20 °C until further biochemical determinations.

Among the several sample and target preparation procedures for biological samples, the one best suited for PIXE analysis is the well established procedure of pulverizing the sample and then pressing it into pellets [8]. Aliquots of 2 ml serum standard were taken and a known quantity of 1000 ppm yttrium stock solution was added for quantitative analysis as internal standard. After thorough homogenisation, the resulting solution was rapidly frozen at liquid nitrogen temperature. Subsequently the serum samples were lyophilized for 48 h at a temperature of −52 °C in a freeze drier. The time was optimized for total removal of the water. Prior to analysis, the freeze dried sample was powdered in an agate mortar and the powder obtained was stored under controlled humidity conditions. In order to overcome the problem of insulating nature of the biological samples, 80 mg of high purity graphite powder was added to 120 mg of the powdered serum sample. The mixture was homogenized in the agate mortar and the resulting sample weighing ∼200 mg was pressed into a pellet of 12 mm diameter and about 2 mm thickness, using a 10 ton hydraulic press. Special attention was paid to avoid contamination during the whole procedure, especially during sample preparation. After pelletising, these samples were carefully placed in polystyrene vials until assayed. The pellets were then used as targets for the PIXE experiment.

Sample irradiation

The PIXE measurements were performed using a 3 MV Pelletron Accelerator at the Institute of Physics in Bhubaneswar, India. A proton beam of 2.5 MeV energy and collimated to a diameter of 2 mm irradiated the studied targets at an angle of 45°. A typical acquisition time of 1800 s was used for each target so as to ensure good statistics. The characteristic X-ray spectra were registered with a Si(Li) semiconductor detector (energy resolution 180 eV FWHM at 5.9 keV) positioned perpendicular to the beam axis and coupled to standard electronics and a personal computer based multi channel analyzer. Spectral data was then transferred to a computer where further data analyses were carried out.

Data analysis

The PIXE spectra analyses were performed using GUPIX software package [9, 10]. Using this software package, trace elements titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, and bromine were identified and their concentrations were estimated in every sample of all the studied groups. Comparing the concentration of yttrium obtained in the present work with the known concentration of yttrium

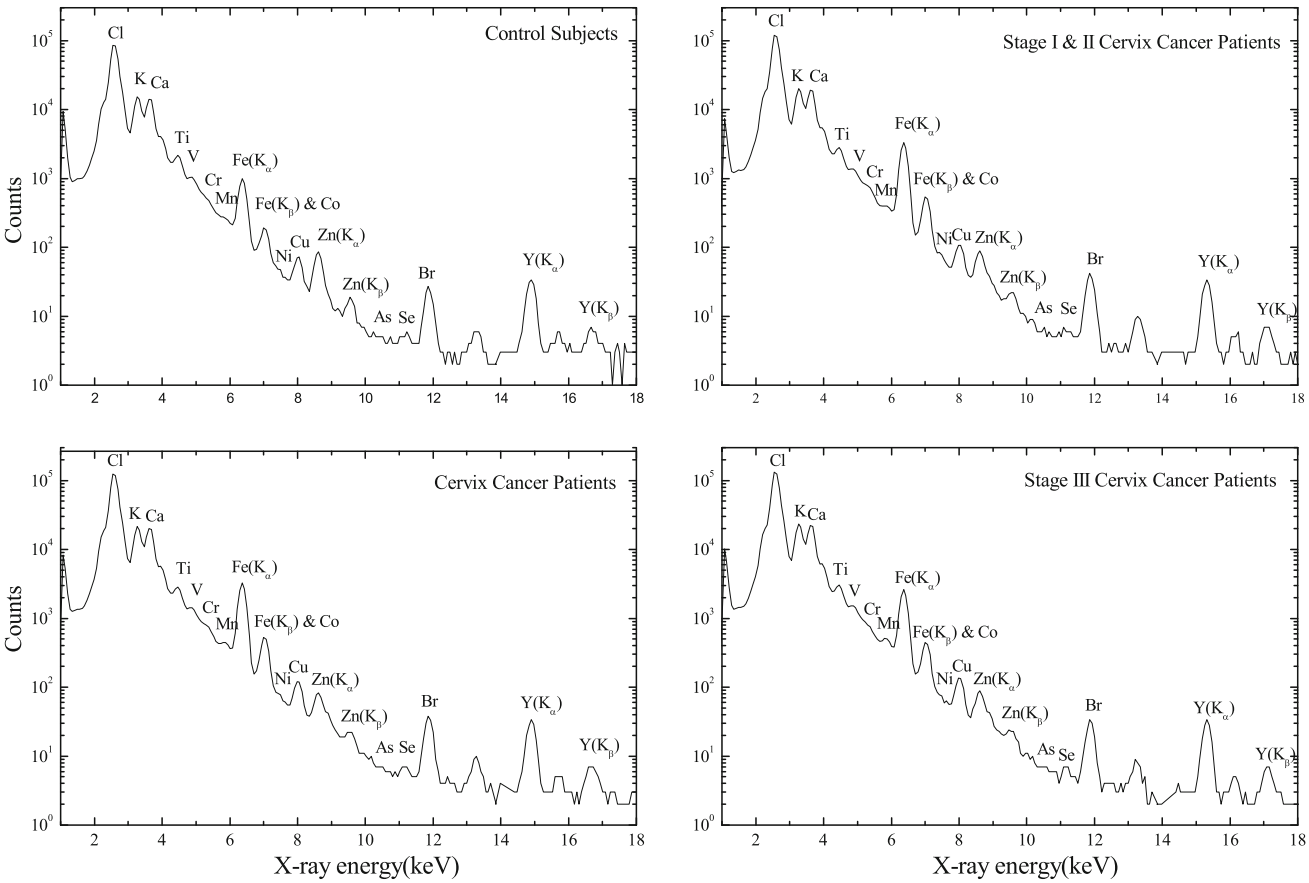

Fig. 1 PIXE spectra for control subjects, cervix cancer patients, Stage I & II patients, and Stage III patients

added to the samples, the reliability of the input parameters was checked. Analysis of International Atomic Energy Agency certified reference material (CRM)-animal blood (Sample No A-13) and National Institute for Standards and Techniques CRM-bovine liver (Sample No. 1577b) was also carried out in the same experimental conditions as that of the serum samples in order to ensure the reliability of this method in reproducing concentrations of low Z elements. The results obtained for these CRM's are discussed in our earlier works [11, 12]. This shows the accuracy and reliability of the present experimental set up and use of GUPIX software.

Statistical analysis

A detailed statistical evaluation of the blood serum of normal and cancer subjects was performed in this work for all the identified elements by using STATISTICA software. The concentrations of trace elements present in different samples were averaged group-wise. The Mann–Whitney test, a nonparametric statistical unpaired-sample test was used to examine the data for significant differences in serum trace elemental content between the various studied groups.

Results and discussion

The typical PIXE spectra recorded for control subjects and cervix cancer patients are displayed in Fig. 1. The uterine cervix cancer patients were further classified as having limited disease (stage I and II) or advanced disease (stage III). The typical PIXE spectra recorded for patients with limited disease and advanced disease are also depicted in Fig. 1. Table 1 presents the average concentrations and associated standard deviations of the identified trace elements for the different studied subjects. The p values, indicating the significant differences between the compared groups for each element are also given in the same table.

From Table 1, it is observed that the elemental profiles in blood serum of cervix cancer patients displayed increased concentrations of Cr, Mn, Fe, Ni, Cu, As and lowered concentrations of V, Co, Zn, Se, and Br in comparison with control subjects. However, this variation was significant only for Cr ($p < 0.05$), Fe ($p < 0.005$), Ni ($p < 0.05$), Cu ($p < 0.0001$), Zn ($p < 0.00001$), and Se ($p < 0.005$). When considered by stage of disease, it can be observed from Table 1, that the levels of serum Ti, Mn, Cu, and As were higher while those of serum Cr, Fe, Co, Ni, Se, and Br were

Table 1 Concentrations (in μg/g) of trace elements in the blood sera of control subjects, cervix cancer patients, Stage I & II patients, and Stage III patients

Trace elements	Control subjects $C_{CS} \pm SD$ (ppm)	Cervix cancer patients		Stage I & II cervix cancer patients		Stage III cervix cancer patients	
		$C_{CP} \pm SD$ (ppm)	p [a] $_{CP,CS}$	$C_{I\&II} \pm SD$ (ppm)	p [b] $_{I\&II,CS}$	$C_{III} \pm SD$ (ppm)	p $_{III,}$ [c] $_{CS}$
Ti	415 ± 17	420 ± 15	>0.05	395 ± 15	<0.05	439 ± 16	>0.05
V	32.0 ± 12.5	28.9 ± 11.0	>0.05	28.2 ± 10.1	>0.05	29.5 ± 11.6	>0.05
Cr	18.6 ± 7.8	26.6 ± 6.7	<0.05	33 ± 6.4	<0.05	21.9 ± 7	>0.05
Mn	29.8 ± 5.7	39.0 ± 5.1	>0.05	26.5 ± 4.8	<0.05	48.4 ± 5.3	<0.0005
Fe	291 ± 7	555 ± 7	<0.005	633 ± 7.8	<0.01	497 ± 7	<0.05
Co	2.9 ± 1.1	1.7 ± 0.5	>0.05	3.7 ± 0.5	>0.05	0.2 ± 0.5	>0.05
Ni	7.2 ± 3.2	9.6 ± 2.9	<0.05	11.5 ± 3.1	<0.05	8.2 ± 2.9	>0.05
Cu	24.6 ± 3.4	34.8 ± 3.2	<0.0001	29.6 ± 3.1	<0.05	38.7 ± 3.2	<0.00005
Zn	38.5 ± 3.9	22.8 ± 3.2	<0.00001	23.5 ± 3.2	<0.0005	22.2 ± 3.2	<0.0005
As	0.9 ± 0.5	1.1 ± 0.9	>0.05	0.4 ± 0.6	>0.05	1.7 ± 1.2	>0.05
Se	2.5 ± 2.5	1.9 ± 1.7	<0.005	2.1 ± 1.9	<0.05	1.7 ± 1.5	<0.005
Br	46.1 ± 7.1	40.5 ± 5.8	>0.05	48.6 ± 6.3	>0.05	34.4 ± 5.4	<0.05

C_{CS}, C_{CP}, $C_{I\&II}$, C_{III} Concentration of trace elements in control subjects, newly diagnosed cervix cancer patients, Stage-I & II cervix cancer patients, and Stage-III cervix cancer patients

SD Standard Deviation

[a] $p_{CP,CS}$: stands for the p value between newly diagnosed cervix cancer patients and Control subjects

[b] $p_{I\&II,CS}$: stands for the p value between Stage-I & II cervix cancer patients and Control subjects

[c] $p_{III,CS}$: stands for the p value between Stage-III cervix cancer patients and Control subjects

lower in Stage III patients than in Stage I and Stage II patients. There was a trend to elevation in serum Cu levels and depression in serum Se levels as the disease progresses. The serum Zn levels were found to be significantly low in both the patients groups with respect to control subjects. However, no significant alteration in the serum Zn content was found among the cervix cancer patients with limited disease (Stage I and II) and advanced disease (Stage III).

The present findings of elevated Fe and Cu levels in the serum of cervix cancer patients suggest that excess Fe and Cu might have led to the initiation and promotion of cancer in these patients by causing oxidative DNA damage [13–15] and consequently enhancing the susceptibility to cancer. The role of Fe and Cu in tumour progression through angiogenesis [16–19] also correlates with the high levels of these elements observed in the serum of cancer patients. Elevated levels of Cr and Ni observed in the sera of cervix cancer patients could have resulted in the inception and development of cancer in these patients because both Cr and Ni are well-known carcinogenic agents [20–23]. They exhibit their carcinogenicity by causing several DNA lesions [24–26] and activating transcription factors [27, 28].

The observed low levels of Zn and Se in the sera of cervix cancer patients included in this study lend support to the hypothesis that low Zn and Se status enhances the risk of developing cancer through the increase in oxidative stress and DNA damage [29–31]. Zinc and Se provide considerable protection against cancer via their antioxidant effects [29, 32]. Their deficiency causes a decline in immunological competence [33–36] and leads to alterations in cell cycle arrest and apoptosis [37–40]. Selenium is also found to act as an inhibitor of angiogenesis [41], initiate antagonistic action against a number of carcinogenic and toxic metals [42], and inhibit the activation of certain transcription factors [39]. This apparent diversity in the effects of Zn and Se on cancer cells explains how their deficiency might lead to the initiation and promotion of cancer. Zinc levels in the serum of cervix cancer patients did not seem to vary with the stage of disease. These results suggest that lowered Zn status in cancer patients might have lead to the initiation and promotion of cancer in them, rather than being a consequence of cancer.

The serum Cu to Zn ratio was significantly higher for cervix cancer patients than for the control group (1.53 ± 0.26 vs. 0.64 ± 0.11). Patients with advanced stages of the disease had more elevated Cu to Zn ratio than patients with limited disease (1.74 ± 0.29 vs. 1.26 ± 0.22). These results indicate that the serum Cu to Zn ratio might be used as a valuable predictor for the occurrence and progression of cervical cancer. The present observation of high Cu to Zn ratio in cervix cancer patients with respect to the values in control

subjects is in agreement with the observations of others [43, 44].

Conclusions

Particle induced X-ray emission (PIXE), a well established method for elemental analysis, was used in this work to identify and quantify trace elements in the blood sera of uterine cervix cancer patients and healthy control subjects. Moreover, the variation of trace elemental content in the sera of cervix cancer patients with the clinical stage of disease was also studied. The results obtained in this study have helped in elucidating the effects of altered homeostasis of trace elements Cr, Fe, Ni, Cu, Zn and Se in the etiology of cervix cancer. The positive association between serum Cu and Se levels and the stage of uterine cervix cancer observed in this study imply that serum Cu and Se levels may provide an effective means of evaluating the extent of uterine cervical cancer. Although these results provide a scientific basis for the supplementation of Zn and Se in nutrition of cervix cancer patients, it is suggested that incorporation of Se and Zn supplementation into an overall therapeutic strategy is not warranted until all the aspects of trace element metabolism in cervix cancer patients are clearly sorted out.

The considerably higher serum Cu to Zn ratios observed in the cervix cancer groups with respect to that of the control subjects indicate that the serum Cu to Zn ratio might be used as a valuable predictor of the presence of cancer. A good correlation was established between the Cu to Zn ratio and the stage of the disease in cervix cancer patients suggesting that estimation of serum Cu to Zn ratio may provide an effective means of evaluating the extent of uterine cervical cancer. It is hoped that concerted efforts in this direction would definitely help in early detection and management of this widely prevalent disease and in monitoring the efficacy of treatment.

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References

1. Armstrong EP (2010) J Manag Care Pharm 16:217–230
2. Zur Hausen H (2002) Nat Rev Cancer 2:342–350
3. Kumar V, Abbas AK, Fausto N, Mitchell RN (2007) Robbins Basic Pathology ((8th ed.) ed.) Saunders Elsevier. pp. 718–721. ISBN 978-1-4160-2973-1
4. Kobayashi, Weinberg V, Darragh T, Smith-McCune K (2008) Nature 1:412–420
5. Looi ML, Mohd Dali AZ, Md Ali SA, Wan Ngah WZ, Mohd Yusof YA (2008) Eur J Cancer Prev 17:555–560
6. Di Domenico F, Foppoli C, Coccia R, Perluigi M (2012) Biochim Biophys Acta 1822:737–747
7. Subramanyam D, Subbaiah KV, Rajendra W, Lokanatha V (2013) Exp Oncol 35:97–100
8. Maenhaut W, De Reu L, Vandenhaute J (1984) Nucl Instrum Methods Phys Res B 3:135–140
9. Maxwell JA, Campbell JL, Teesdale WJ (1989) Nucl Instrum Methods Phys Res B 43:218–230
10. Maxwell JA, Campbell JL, Teesdale WJ (1995) Nucl Instrum Methods Phys Res B 95:407–421
11. Naga Raju GJ, John Charles M, Bhuloka Reddy S, Sarita P, Seetharmi Reddy B, Rama Lakshmi PVB, Vijayan V (2005) Nucl Instrum Methods Phys Res B 229:457–464
12. Sarita P, Naga Raju GJ, Pradeep AS, Tapash RR, Bhuloka Reddy S, Vijayan V (2012) J Radioanal Nucl Chem 294:355–361
13. Abalea V, Cillard J, Dubos MP, Anger JP, Cillard P, Morel I (1998) Carcinogenesis 19:1053–1059
14. Theophanides T, Anastassopoulou J (2002) Crit Rev Oncol Hematol 42:57–64
15. Armendariz AD, Vulpe CD (2003) J Nutr 133:203E–282E
16. Norrby K, Mattsby-Baltzer I, Innocenti M, Tuneberg S (2001) Int J Cancer 91:236–240
17. Ziche M, Jones J, Gullino PM (1982) J Natl Cancer Inst 69:475–482
18. Patstone G, Maher P (1996) J Biol Chem 271:3343–3346
19. Brewer GJ, Dick RD, Grover DK, Le Claire V, Tseng M, Wicha M, Pienta K, Redman BG, Jahan T, Sondak VK, Strawderman M, Le Carpentier G, Merajver SD (2000) Clin Cancer Res 6:1–10
20. IARC Monographs on the evaluation of carcinogenic risks of chemicals to humans (1990) Vol. 49 Chromium, Nickel and Welding, International Agency for Research on Cancer, Lyon, France
21. Rojas E, Herrera LA, Poirier LA, Ostrosky-Wegman P (1999) Mutat Res 443:157–181
22. Denkhaus E, Salnikow K (2002) Crit Rev Oncol Hematol 42:35–56
23. Grimsrud TK, Peto J (2006) Occup Environ Med 63:365–366
24. De Flora S, Bagnasco M, Serra D, Zanacchi P (1990) Mutat Res 238:99–172
25. Kasprzak KS (1995) Cancer Invest 13:411–430
26. Krueger I, Mullenders LH, Hartwig A (1999) Carcinogenesis 20:1177–1184
27. Huang X, Klein CB, Costa M (1994) Carcinog (Lond) 15:545–548
28. Wang S, Leonard SS, Ye J, Ding M, Shi X (2000) Am J Physiol Cell Physiol 279:C868–C875
29. Powell SR (2000) J Nutr 130:1447S–1454S
30. Ho E, Courtemanche C, Ames BN (2003) J Nutr 133:2543–2548
31. Patrick L (2004) Altern Med Rev 9:239–258
32. Ganther HE (1999) Carcinogenesis 20:657–666
33. Shankar AH, Prasad AS (1998) Am J Clin Nutr 68:447S–463S
34. Rink L, Gabriel P (2001) Biometals 14:367–383
35. Bhaskaram P (2002) Nutr Rev 60:S40–S45
36. Baum MK, Miguez-Burbano MJ, Campa A, Shor-Posner G (2000) J Infect Dis 182:S69–S73
37. Ho E, Ames BN (2002) Proc Natl Acad Sci U S A 99:16770–16775
38. Ho E (2004) J Nutr Biochem 15:572–578
39. Gasparian AV, Yao YJ, Lu J, Yemelyanov AY, Lyakh LA, Slaga TJ, Budunova IV (2002) Mol Cancer Ther 1:1079–1087
40. Dong Y, Zhang H, Hawthorn L, Ganther HE, Ip C (2003) Cancer Res 63:52–59

41. Combs GF, Clark LC, Turnbull BW (2001) BioFactors 14:153–159
42. Milde D, Novak O, Stuzka V, Vyslouzil K, Machacek J (2001) Biol Trace Elem Res 79:107–114
43. Rosas R, Poo JL, Montemayor A, Isoard F, Majluf A, Labardini J (1995) Rev Invest Clin 47:447–452
44. Cunzhi H, Jiexian J, Xianwen Z, Jingang G, Shumin Z, Lili D (2003) Biol Trace Elem Res 94:113–122