Analysis of trace element levels in the nails in patients with colorectal cancer: Consider to the effects of chemotherapy or radiotherapy

Huynh Truc Phuong (✉ htphuong@hcmus.edu.vn)
University of Science, VNU-HCM, Vietnam

Dinh Thanh Binh
Dong Nai General Hospital, Bien Hoa City

Nguyen Thi Truc Linh
University of Science, VNU-HCM, Vietnam

Truong Thi Hong Loan
University of Science, VNU-HCM, Vietnam

Nguyen Van Hanh
Oncology Hospital, Ho Chi Minh City

Ho Manh Dung
Center for Nuclear Techniques, Ho Chi Minh City

Nguyen Van Dong
University of Science, VNU-HCM, Vietnam

Tran Tuan Anh
Nuclear Research Institute, Da Lat City

Research Article

Keywords: trace elements, nails, chemotherapy, radiotherapy, colorectal cancer risk.

DOI: https://doi.org/10.21203/rs.3.rs-119274/v1

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Abstract

Nails are considered as suitable biological materials for the diagnosis of diseases by measuring trace element levels. This study aimed to evaluate the role of trace elements in detecting the risk of colorectal cancer. Evaluating the effects of cancer treatment with chemotherapy or radiotherapy was also the aim of this study. The levels of trace elements in the nails of 104 patients with colorectal cancer and 112 healthy subjects were analyzed using the k0-standardization method of neutron activation analysis. The results showed that there were significant differences of the following elements, As, Fe, Hg, Sc, Se, and Zn between the control and the patient groups, while it was not the case for the elements Br, Co, and Cr. Furthermore, this study showed that there was no significant difference in the levels of the obtained trace elements in both colon and rectal cancer patient groups between untreated and treated subjects. We conclude that the levels of As, Hg, Fe, Se, and Zn in integrated samples of the fingernail and toenails may be used to evaluate the colorectal cancer risk, and they were not affected by chemotherapy or radiotherapy.

Introduction

Colorectal cancer is one of the most common types of cancer in the world. Colorectal cancer can be caused by the intake of trace elements into the human body from the environment, drinking water, food, pesticides and fertilizers, and its incidence is higher in industrialized countries\(^1,2\). Trace elements play an important role in the human body. A deficiency or elevation in trace element levels in the body may be associated with the risk and development of chronic diseases, including cancer\(^3,4\). To estimate the risk of cancer caused by trace elements, many studies have measured their levels in the tissues of the human body\(^5-11\). Milde et al.\(^7\) investigated trace element levels in blood serum and colon tissue in colorectal cancer patients. They showed that the levels of Se and Zn were significantly different between cancer patients and healthy subjects. Alimonti et al.\(^1\) have shown that the elements such as Co, Cr, and Cu had significantly different levels between polyps and normal tissue in patients with colorectal polyps. Fernandez-Banares et al.\(^12\) showed that serum Se level was associated with the risk of colorectal adenoma in those living in geographical areas with low selenium concentrations. Meanwhile, Wallace et al.\(^13\) indicated that serum selenium concentration was associated with the risk of recurrent colorectal adenoma. Some of the works listed above suggested that tissue or serum could be one of the good biological samples for assessing the exposure to trace elements of the human body. However, these samples can only be assessed over short-term exposure, are difficult to store, and complex in sampling.

Garland et al.\(^14\), Ka He\(^3\), and Przybylowicz et al.\(^8\) have shown that nails were the most suitable source of biological material for the assessment of trace element levels in the human body. Nails often reflect the long-term exposure to trace elements\(^15\); they can be stored for a long time; and are easy in sample processing. Previously published studies on trace elements in the nails showed that there was a relationship between the trace element levels and the risk of cancer\(^16-21\). Johnson et al.\(^20\) evaluated the levels of the following elements: As, Cr, and Ni in toenails, and they showed that there was a relationship
between lung cancer and these element levels. Campos et al.[19] indicated that Zn levels in toenail had a protective effect on gastric cancer. Vance et al.[22] studied the chemical composition of the nail in Alzheimer’s disease patients. The results showed that Br, K, and Zn levels were higher in those patients than in the control subjects, whereas Hg was lower than that in the controls. Garland et al.[23] studied the level of As, Cu, Cr, Fe, and Zn in toenails of women with breast cancer and control subjects, and they showed that none of these trace elements could support the hypothesis that breast cancer changes the composition of these elements in the nails. Rodushkin and Axelsson[24] reported that the mass fraction of Cd in the fingernails of smokers was 10 times higher than that in non-smokers. The levels of chemical elements such as La, Ce, Pr, and Nd also showed some increase in the fingernails from smokers. Meanwhile, Huynh et al.[21] have shown the elements such as Cr, Fe, and Zn were associated with risk of breast cancer by examining trace element levels in the fingernail of women with this disease.

Several studies have been listed to show that the fingernails and toenails were among the biological materials used to predict trace element exposure and cancer risk. However, these studies have often used either fingernail or toenail samples to evaluate the trace element levels. In such cases, samples were often collected in small weight fractions that resulted in insufficient level of many elements for the detection limit of the analysis. Therefore, the analysis of the trace elements chosen in the integration of the fingernail and toenail should be conducted to increase the sensitivity of the analysis. Moreover, by reviewing previous studies on the trace elements and cancer risk, including colorectal cancer, Navarro Silvera and Rohan[25] have suggested that one needs to conduct a deeper research on the epidemiologic evidence in the future. Hornik et al.[26] indicated that in order to understand the role of biologically important trace elements in carcinogenic processes and how these are influenced by the medical treatment (chemotherapy and radiation), further examinations are to be conducted.

In line with previous studies, the goal of this study was 1) to evaluate the differences in the trace elements between the fingernails and toenails of healthy human subjects, 2) to evaluate the differences in the levels of trace elements in the integrated samples of fingernails and toenails of colorectal cancer patients and those of the healthy subjects, and 3) to investigate the significant differences in trace element levels between treated and untreated groups of colorectal cancer patients.

**Results**

**Trace element levels in the fingernails and toenails in healthy human subjects**

Table 1 shows the observed trace element levels in the fingernails and toenails of both healthy women and men were measured. For the elements whose levels were below the detection limit, a half of the detection limit value was calculated. Through the two-tailed *t*-test, we found there was no significant difference in all observed elements between the fingernail and toenail in both genders (*p* > 0.05).

**Table 1.** The trace element levels (mg/g) in the fingernails and toenails of healthy human subjects
Curiously, the results of this study showed that there was no significant difference in the observed trace element levels in the fingernail and toenail between the women and men (p > 0.05) (Table 2).

Table 2. A comparison of trace element levels (mg/g) between the fingernails and toenails of healthy human subjects

| Element | Women (n = 30) | Men (n = 30) | p-value | Women (n = 30) | Men (n = 30) | p-value |
|---------|---------------|--------------|---------|---------------|--------------|---------|
|         | Fingernail (n = 30) | Toenail (n = 30) |         | Fingernail (n = 30) | Toenail (n = 30) |         |
| As      | 0.36 (0.18) | 0.33 (0.19) | 0.58    | 0.34 (0.20) | 0.35 (0.16) | 0.82    |
| Br      | 0.97 (0.58) | 0.91 (0.45) | 0.65    | 0.84 (0.38) | 0.89 (0.34) | 0.58    |
| Co      | 0.11 (0.07) | 0.12 (0.07) | 0.58    | 0.10 (0.05) | 0.13 (0.08) | 0.11    |
| Cr      | 0.52 (0.31) | 0.55 (0.42) | 0.81    | 0.60 (0.28) | 0.65 (0.42) | 0.54    |
| Hg      | 0.31 (0.13) | 0.27 (0.12) | 0.21    | 0.33 (0.16) | 0.30 (0.17) | 0.53    |
| Fe      | 70.2 (33.7) | 72.0 (31.1) | 0.83    | 67.3 (29.8) | 79.7 (38.0) | 0.17    |
| Sc      | 0.012 (0.009)| 0.015 (0.014)| 0.25    | 0.011 (0.009)| 0.018 (0.017)| 0.06    |
| Se      | 0.96 (0.38) | 0.92 (0.51) | 0.82    | 0.97 (0.40) | 1.08 (0.51) | 0.34    |
| Zn      | 106.2 (38.1)| 122.7 (40.5)| 0.11    | 110.4 (40.8)| 111.2 (39.3)| 0.94    |

From the above studies, we found that the analysis of certain trace element levels did not depend on gender and the type of the nail: fingernail or toenail. This proves that the integration of the fingernail and toenail samples can be used to analyze trace element levels for the evaluation of the metal exposure of the human body.

Trace element levels in nails of untreated patients

In this study, the fingernails and toenails of each participant were integrated into a sample (nails for short). Table 3 shows the levels of observed trace elements from the nails of the healthy human subjects, untreated colon cancer patients, and untreated rectal cancer patients. In there, the values such as minimum, maximum, mean, and standard deviation (SD) are shown. For the elements whose levels were below the detection limit, half of the detection limit value was calculated.
Table 3. Trace element levels (mg/g) in the nails of untreated patients and healthy human subjects

|       | As  | Br  | Co  | Cr  | Hg  | Fe  | Sc  | Se  | Zn  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Healthy (n = 30) |     |     |     |     |     |     |     |     |     |
| Min   | 0.05 | 0.36 | 0.01 | 0.08 | 0.14 | 8.1  | 0.001 | 0.16 | 16.5 |
| Max   | 0.95 | 2.49 | 0.19 | 1.79 | 0.73 | 195.5 | 0.049 | 1.95 | 383.3 |
| Mean  | 0.30 | 1.07 | 0.08 | 0.43 | 0.37 | 62.9  | 0.013 | 0.78 | 109.7 |
| SD    | 0.26 | 0.47 | 0.05 | 0.32 | 0.14 | 46.4  | 0.010 | 0.41 | 63.9 |
| Colon (n = 24) |     |     |     |     |     |     |     |     |     |
| Min   | 0.07 | 0.62 | 0.02 | 0.08 | 0.15 | 26.5  | 0.008 | 0.45 | 74.1 |
| Max   | 1.15 | 2.21 | 0.24 | 1.23 | 1.98 | 219.0 | 0.122 | 2.88 | 256.2 |
| Mean  | 0.53 | 1.23 | 0.07 | 0.42 | 0.56 | 92.3  | 0.026 | 1.34 | 164.2 |
| SD    | 0.33 | 0.33 | 0.06 | 0.28 | 0.39 | 48.4  | 0.022 | 0.79 | 39.2 |
| p-value | 0.009 | 0.163 | 0.768 | 0.857 | 0.028 | 0.029 | 0.015 | 0.003 | <0.001 |
| Rectal (n = 24) |     |     |     |     |     |     |     |     |     |
| Min   | 0.14 | 0.36 | 0.02 | 0.22 | 0.19 | 26.4  | 0.002 | 0.11 | 88.1 |
| Max   | 1.03 | 2.95 | 0.18 | 1.46 | 2.12 | 207.2 | 0.058 | 1.91 | 349.3 |
| Mean  | 0.47 | 1.41 | 0.08 | 0.52 | 0.66 | 99.7  | 0.029 | 1.18 | 164.2 |
| SD    | 0.27 | 0.81 | 0.04 | 0.28 | 0.47 | 44.4  | 0.017 | 0.56 | 69.5 |
| p-value | 0.031 | 0.100 | 0.685 | 0.313 | 0.014 | 0.003 | <0.001 | 0.009 | 0.008 |

This analysis showed that in both patient groups, the minimum and maximum values of the observed element levels were higher than those in healthy subjects. The mean values of the elements including As, Cr, Hg, Fe, Sc, Se, and Zn in the patient group were higher than those in the healthy group.

Using the two-tailed $t$-test, this study showed that, with the exception of Br, Co, and Cr, the remaining elements showed significant differences between healthy subjects and the patients with colorectal cancer who untreated by chemotherapy or radiotherapy (Table 3) (p < 0.05).

**The significant differences in trace element levels between treated and untreated patient groups**

In this experiment, the trace element levels in the nails of both colon and rectal cancer patients were analyzed and assessed with regard to significant differences between treated and the untreated patients. Table 4 shows the mean values of the element levels analyzed and the $p$-value of the two-tailed $t$-test. In the both colon and rectal cancer patients, this study showed that there was no significant difference in observed trace element levels between treated and untreated groups (p < 0.05). This presumably suggests that the treatment with chemotherapy or radiotherapy did not affect for trace element levels in the nails.

Table 4. Statistically significant differences in trace element levels (mg/g) between the treated and untreated patient groups
| Element | Mean (SD) | p-value | Mean (SD) | p-value |
|---------|-----------|---------|-----------|---------|
| As      | Untreated | Treated | Untreated | Treated |
|         | (n = 24)  | (n = 36) | (n = 20)  | (n = 24) |
| As      | 0.53 (0.33) | 0.62 (0.34) | 0.31       | 0.47 (0.26) | 0.65 (0.41) | 0.08 |
| Br      | 1.23 (0.33) | 1.38 (0.88) | 0.35       | 1.41 (0.81) | 1.33 (0.78) | 0.73 |
| Co      | 0.07 (0.06) | 0.06 (0.04) | 0.61       | 0.08 (0.04) | 0.08 (0.04) | 0.89 |
| Cr      | 0.42 (0.28) | 0.43 (0.19) | 0.92       | 0.52 (0.28) | 0.46 (0.28) | 0.49 |
| Hg      | 0.55 (0.40) | 0.60 (0.40) | 0.59       | 0.66 (0.47) | 0.58 (0.42) | 0.56 |
| Fe      | 92.3 (48.4) | 86.4 (49.6) | 0.65       | 99.7 (44.4) | 86.6 (46.3) | 0.34 |
| Sc      | 0.026 (0.022) | 0.032 (0.014) | 0.24       | 0.029 (0.017) | 0.030 (0.017) | 0.93 |
| Se      | 1.36 (0.79) | 1.02 (0.50) | 0.07       | 1.18 (0.56) | 0.98 (0.46) | 0.21 |
| Zn      | 164.2 (39.2) | 146.4 (41.4) | 0.10       | 164.2 (69.5) | 169.8 (87.2) | 0.81 |

**Discussion**

The levels of the trace elements in the integrated samples of fingernail and toenail in healthy human subjects observed in this study were comparable to those shown in previous studies\(^{27-30}\). In this study, the mean values of trace element levels in the fingernails and toenails were similar to those shown in the international survey by Takagi et al.\(^{28}\). In the fingernails of both men and women, except for As and Fe, the mean values for the rest of the elements were the same as those measured in the fingernails by Vance et al.\(^{27}\). Meanwhile, As and Fe levels were in agreement with those obtained by Biswas et al.\(^{31}\). The level of Zn in toenail was also in agreement with that obtained by Campos et al.\(^{19}\). The levels of As and Cr in toenail were comparable with those obtained by Johnson et al.\(^{20}\). The levels of the elements including Cr, Co, Fe, Se, and Zn in toenails were consistent with those obtained in both men and women by Sureda et al.\(^{29}\). From the results of observed trace element levels in this study (Table 1 and Table 2) and the previous studies mentioned above, it can be assumed that the selected trace elements did not depend on the source, fingernails and toenails, nor did they depend on the gender.

Currently, in our literature search, we have not found any claims that have investigated the trace element levels in the nails of colorectal cancer patients. Therefore, it was difficult to compare the results obtained in our study with those in other studies. The Se level in the nails of the untreated colon and rectal cancer patients was twice as high as that measured in the toenails by van den Brandt et al.\(^{16}\), although it was the same as that measured by Garland et al.\(^{32}\). There were significant differences in the levels of the elements, including As, Fe, Hg, Sc, Se, and Zn, found in this study, while for the elements such as Br, Co, and Cr there were no significant differences (Table 3). The levels of Co, Cr, Fe, and Se in the nails of patients were found to be of the same degree order of their values in colorectal tumors \(^{6}\). Meanwhile, the levels of elements such as Co, Cr, Fe, Hg, Se, and Zn had the same degree order compared to those in...
colorectal polyps\(^1\). Compared with colon tissue as studied by Middle et al.\(^7\), the Se level in the nails of colon cancer patients had the same mean value, whereas the Zn level was approximately twice as high. Juloski et al.\(^33\) reported that Zn and Se levels significantly differed between the malignant tissue of colorectal cancer and adjacent healthy bowel tissue. This was consistent with our results in the case of Zn and Se levels in the nails. Nail Se and Fe levels have also been linked to the development of stomach cancer\(^34\).

Chemotherapy or radiotherapy of colorectal cancer did not affect the levels of the observed elements in this study. All observed elements did not show significant differences between the untreated and treated subjects. In investigating the chemotherapy effect on trace elements in blood serum, Hasan\(^35\) showed that chemotherapy did not augment the rates of trace elements such as Zn, Fe, and Se. However, Ahmadi et al.\(^36\) indicated that the serum Zn and Fe levels in women after chemotherapy were significantly decreased (\(p < 0.001\)), while the serum level of copper increased but was not significant (\(p = 0.676\)). We have not found any claims that have studied the effects of chemotherapy or radiotherapy on trace element levels in the nails.

Toxic elements, such as As and Hg, incorporate into the human body through the air, drinking water, and food\(^37,38\), and important food sources are fish and seafood\(^39\). In this study, As and Hg levels in the nails were found to be significantly different between the healthy group and the patient group, and there was no significant difference between the treated and untreated patients in both colon and rectal cancers. The levels of As and Hg in the patient's nails were higher than those previously found in the unexposed populations\(^27,30,40,41\).

Essential elements such as Fe, Se, and Zn play an important role in metabolism in the human body. A deficiency or excess of these elements can lead to chronic disease, sometimes even leading to cancer. The mean concentrations of these elements were higher in the patient group than in the healthy group. Very few reports on the concentration of trace elements in colon cancer patients are available\(^42,43\). Relative to Fe, previous studies suggested that high levels of this element induced colorectal cancer in animals and raised the risk of colorectal cancer in humans through the formation of OH radicals and suppression of cellular immune functions\(^44,45\). Lee et al.\(^46\) suggested that the intake of dietary heme iron was associated with an increased risk of proximal colon cancer. In this study, the Fe level in the nails in the untreated patient group (both colon and rectal cancers) was approximately 1.4 times higher than that in the healthy group. In the case of the Se level in the nail, many previous studies reported on the Se levels associated with colorectal cancer risk. Ghadirian et al.\(^17\) have observed a statistically significant inverse association between toenail Se level and the risk of colon cancer (\(p = 0.009\)). Meanwhile, van den Brandt et al.\(^16\) and Garland et al.\(^14\) reported that the Se level in the toenail was not associated with the risk of colon or rectal cancer. Our study revealed that there were significant differences in the Se levels in the nail for both colon and rectal cancer. The Se levels in the nail among the untreated patient groups were approximately 1.5 times higher than those in the healthy group. Although Zn is an essential element, a deficiency or excess leads to the risk of chronic diseases, including cancer. Diet is the primary source of
Zn exposure. Average Zn consumption from food ranges from 5.2 to 16.2 mg/day and Zn levels in animal products vary depending on the soil and water concentrations where the animals were raised\textsuperscript{25}. To our knowledge, no studies have examined the Zn level in the nails of colorectal cancer patients. Therefore, the results of this study could not be compared with those of previous studies. Milde et al.\textsuperscript{7} have reported that there was a statistically significant change in Zn level in the serum of colorectal cancer patients in comparison to the control group. Arriola et al.\textsuperscript{6} showed that Zn levels in colorectal tumor tissue were higher than those in the normal tissue. In our study, Zn levels in the nails of patients with colon and rectal cancer were approximately 1.5 times higher than those of the healthy subjects.

In assessing the effect of chemotherapy or radiotherapy on trace elements, we observed that there was no significant difference in Fe, Se, and Zn levels between treated and untreated by chemotherapy or radiotherapy. This was agreed with the studied results by Hasan\textsuperscript{35}.

For other essential elements such as Br, Co, and Cr, no significant differences were found in this study. Since no previous studies have examined the levels of these elements in the nails of colorectal cancer patients, a comparison with previous studies was impossible. In the study of trace element levels in the nails of patients with breast cancer, Huynh et al.\textsuperscript{21} showed that the Br and Co levels did not differ significantly between the control group and the case group, while Cr level did.

In conclusion, this study showed that an integration of fingernail and toenail samples may be used to analyze trace element levels in human disease evaluation. Significant differences in the levels of trace elements including As, Hg, Fe, Sc, Se, and Zn were found between healthy human and colorectal cancer patients. This study showed that there was no significant difference in certain trace element levels between the treated and the untreated patients. The essential elements (including Fe, Se, and Zn) and toxic elements (including As and Hg) were not effected by chemotherapy or radiotherapy. However, due to limited sample size, the results in this paper might be insufficient to provide a reliable statement on the use of the level of trace elements in nails as an indicator of colorectal cancer. Further investigation using a larger scale of sampling are required for the reliable conclusion on the association of trace elements in the nails with colorectal cancer.

The strength of this study is that this is the first report on the evaluation of trace elements in the integration of fingernails and toenails samples in colorectal cancer patients. Furthermore, an investigation of the effect of chemotherapy or radiotherapy on trace elements in the nails of patients with colorectal cancer was also carried out in this study. However, there are also difficulties in comparing our results with those obtained from previous studies. A limitation of this study was the small sample size in which trace elements are analyzed.

**Materials And Methods**

This study was approved by the ethics committee of Dong Nai General Hospital, and an informed consent was signed by each study participant. The study has been conducted on the population living in
Dong Nai province, southeast of Vietnam, where many large industrial zones are located.

We confirm that all methods were performed in accordance with the relevant guidelines and regulations.

**Sample preparation**

In this study, nails (an integration of fingernail and toenail) from 104 patients with colorectal cancer (case group), and 60 healthy subjects (control group) were collected. The case group ages were ranging between 32 and 76, and the control group between 36 and 73 years. Classifications such as men, women, colon cancer, rectal cancer, treated (with chemotherapy or radiotherapy), and untreated, were also carried out in this work. In which, untreated patients are those diagnosed with early-stage cancer. Patients, who were treated, were those with stage II and III cancers and had undergone chemotherapy or radiotherapy. Table 5 presents in detail the population parameters obtained for the case group and the control group.

Table 5. Characteristics of the study population

| Variables                        | Case | Control |
|----------------------------------|------|---------|
|                                  | Number (n) | Percent (%) | Number (n) | Percent (%) |
| **Gender**                       |      |         |      |         |
| Men                              | 50   | 48.1    | 30   | 50.0     |
| Women                            | 54   | 51.9    | 30   | 50.0     |
| **Type of cancer**               |      |         |      |         |
| Colon                            | 60   | 57.7    | -    | -        |
| Rectal                           | 44   | 42.3    | -    | -        |
| **Chemotherapy or radiotherapy** |      |         |      |         |
| Treated                          | 60   | 57.7    |      |          |
| Untreated                        | 44   | 42.3    |      |          |
| **Age (mean ± SD)**              | 52.7 ± 14.3 |          | 54.2 ± 8.5 |          |
| p-value                          | 0.76 |         |      |          |

To assess the differences in selected trace element levels between the fingernails and the toenails, 60 healthy individuals were included in this work. Of these, 30 fingernail samples and 30 toenail samples were collected for men. The same amount of samples was collected for women.

Before the sampling, the hands and feet of all participants were washed with acetone and cleaned again with distilled water. Each individual's 10 fingernails and 10 toenails were cut with stainless steel clippers. Each sample was placed in a separate clean plastic bag and sent to the laboratory.

In the laboratory, the samples were treated as described elsewhere\(^ {47-51}\) and as in our previous study\(^ {21}\). It can be briefly described as follows: step 1, the samples were first kept soaked in distilled water for 10 minutes, followed by another 5 minutes in mixing with alcohol with slight shaking; step 2, the samples were soaked to triplicates in acetone with ultrasonic agitation (B2510-DTH, Branson, USA) for one minute; step 3, the samples were treated in the same manner as in the step 2, using 2% Triton X100 (Merck, Germany) instead of the acetone; step 4, the samples were cleaned in triplicates by soaking in distilled water and ultrasonic agitation for one minute; final step, the cleaned samples were pre-dried by placing
them on a filter paper for 12 hours at an ambient temperature. The dry weight of the samples ranging between 40 and 70 mg for the fingernail and toenail, respectively, and between 100 and 120 mg for the integrated samples of fingernail and toenail. The sample was placed in a polyethylene bag before neutron activation.

**Irradiation, measurements, and calculation**

Similar to our previous study,\(^2\) the neutron irradiations were performed for 10 hours on Rotary Rack at a thermal neutron flux of approximately \(3.59 \times 10^{12} \text{ n.cm}^{-2}.\text{s}^{-1}\) of Dalat Nuclear Research Reactor, Vietnam. At this position, the deviation of the epithermal neutron spectrum and the ratio of thermal/epithermal neutron fluxes were \(a = 0.073 \pm 0.001\), and \(f = 37.3 \pm 0.4\), respectively.⁵² The nuclear reactions of interest in this study were \(^{75}\text{As}(n, g)^{76}\text{As}\) \((T = 26.32\ h, E_g = 559.1\ \text{keV})\), \(^{81}\text{Br}(n, g)^{82}\text{Br}\) \((T = 35.30\ h, E_g = 776.5\ \text{keV})\), \(^{59}\text{Co}(n, g)^{60}\text{Co}\) \((T = 5.27\ y, E_g = 1332.5\ \text{keV})\), \(^{50}\text{Cr}(n, g)^{51}\text{Cr}\) \((T = 27.70\ d, E_g = 320.1\ \text{keV})\), \(^{202}\text{Hg}(n, g)^{203}\text{Hg}\) \((T = 46.61\ d, E_g = 279.2\ \text{keV})\), \(^{58}\text{Fe}(n, g)^{59}\text{Fe}\) \((T = 44.50\ d, E_g = 1099.2\ \text{keV})\), \(^{45}\text{Sc}(n, g)^{46}\text{Sc}\) \((T = 83.81\ d, E_g = 889.3\ \text{keV})\), \(^{74}\text{Se}(n, g)^{75}\text{Se}\) \((T = 119.8\ d, E_g = 264.7\ \text{keV})\), and \(^{64}\text{Zn}(n, g)^{65}\text{Zn}\) \((T = 243.9\ d, E_g = 1115.6\ \text{keV})\).

After 12 days of decay, the sample was measured for two hours on HPGe gamma spectroscopy (Canberra, USA) with an energy resolution of 2.0 keV at the 1332.2 keV peak of \(^{60}\text{Co}\). The full-energy peak areas of gamma rays emitted from radioisotopes of interest were obtained using the Genie 2k software.

The concentration of trace elements in each sample were calculated using the \(k_0\)-standardization method of neutron activation analysis.⁵³,⁵⁴ This method can be briefly described as follows, a sample with weight \(w\) is irradiated by a neutron source at thermal neutron position, after irradiation the activities of sample are counted with gamma-ray spectroscopy, then the concentration \(r_a\) of an analytic “a” is obtained from its measured isotope/gamma ray as

\[
\rho_a (\mu g / g) = \frac{N_p / w t^s SDC}{(N_p / W t^s SDC)_{Au}} \times \frac{1}{k_{0,Au}(\alpha)} \times \frac{f + Q_{0,Au}(\alpha)}{f + Q_{0,a}(\alpha)} \times \frac{e_{p,Au}}{e_{p,a}} \times 10^6
\]

where, “Au” refers to the co-irradiated gold monitor \([^{197}\text{Au}(n, g)^{198}\text{Au}, E_g = 411.8\ \text{keV}]\) and \(N_p\) is the net number of counts in the full-energy peak (corrected for pulse losses), \(W\) is the weight of the gold monitor, \(t_m\) is the measuring time, \(S = 1 - \exp(-lt_i)\), \(t_i\) is the irradiation time, \(D = \exp(-lt_d)\), \(t_d\) is the decay time, \(C = [1 - \exp(-lt_m)] / lt_m\), \(l\) is the decay constant, \(Q_0 = l_0 / s_0\) (resonance integral to 2200 ms\(^{-1}\) cross-section ratio), and \(e_{p}\) is the full-energy peak detection efficiency, and \(k_0\) is a combination of nuclear constants and determined experimentally.

**Statistical analysis**
We used a descriptive statistical approach with a two-tailed $t$-test to evaluate the analytical results. $T$-test for two-sample assuming unequal variances were used in this study. A $p < 0.05$ was considered to be significantly different. Mean value, standard deviation (SD), minimum, and maximum were calculated using Excel software version 10.

**Declarations**

**Compliance with Ethical Standards**

- Disclosure and conflict of interest: We have no conflict of interest.
- Informed consent: This study was approved by the ethics committee of Dong Nai General Hospital and participants.

**Author contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Huynh Truc Phuong, Tran Tuan Anh, and Nguyen Thi Truc Linh. The first draft of the manuscript was written by Huynh Truc Phuong. All authors have given approval to the final version of the manuscript.

**Acknowledgements**

This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 103.04-2017.311.

The authors thank the staff of INAA Lab, Dalat Nuclear Research Institute, supported during neutron irradiation. Thank to doctor - nurses in the Department of Oncology, Dong Nai General Hospital, supported for sample collection.

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