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Research Article

Determination of the concentration of Fe, Se, and Zn elements in nails of Vietnamese women with breast cancer using k_0-INAA method

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ABSTRACT

Introduction: Breast cancer is the most common type of cancer resulting in death. Trace elements of Fe, Se, and Zn can play a key role in the onset and prevention of breast cancer. Trace elements in the fingernails may be used as bioindicators for breast cancer diagnosis. The purpose of this work is to determine the concentrations of Fe, Se, and Zn in the fingernails of women with breast cancer and healthy women, which used to find the difference and correlation of these elements in the fingernail. **Methods:** This research was approved by the Ethics Committee of Dong Nai General Hospital. The fingernail collected from 29 women with breast cancer and 30 healthy women, who are the same age and living in Dong Nai province, Vietnam. The concentrations of Fe, Se, and Zn in the fingernails were determined using the k_0-INAA method. The analytical data were evaluated using some statistical analysis for the correlation of trace elements in the fingernails of both groups. **Results:** As a result, the mean concentrations of Fe, Se, and Zn in fingernails of women with breast cancer were 102.87 μg/g, 0.75 μg/g and 65.49 μg/g, respectively, while those of healthy women were 69.74 μg/g, 0.78 μg/g and 107.75 μg/g. The assessment of these elements in fingernails for both two sample groups, including t-test and correlation coefficients, was also carried out in this study. As a result, the significant difference (P<0.05) was found for the Fe and Zn in fingernails of women with breast cancer, while those of Se was not found. The correlation between Se and Zn was found in both groups. The correlation between Fe and Zn was found in fingernails of healthy women, but it disappears in the women with breast cancer. **Conclusions:** From our findings, it can be concluded that Fe and Zn significantly associated with the risk of breast cancer, while Se is not associated.

Key words: Essential elements in fingernails, breast cancer, the k0-INAA method

INTRODUCTION

Vietnam is one of the countries with the cancer rate at high levels. Prof. Nguyen Chan Hung suggested that, up to 2020, cancer is a reasonable cause of death for 115,000 people per year in Vietnam, approximately 315 people per day. In those, breast cancer is one of the common cancer types cause leading death for Vietnamese women. The reason for cancer-causing maybe start from the intake of the toxic elements into the human body, and/or it also can be due to reducing the concentration of essential elements in tissues.

Trace elements entered the body by eating, activities, and living environment. The trace elements are accumulated in human body tissues like hair and nails that have distinct advantages of the application as biomarkers. Therefore, they are recognized as biological tools for disease diagnosis. A large amount of evidence suggested that overload or deficiency of certain heavy metals linked with the risk of chronic diseases, including cancer and other ones [1,2].

Many studies claimed that there is a relationship between trace elements and cancer risk [3–5]. Breast cancer is the third most common cancer worldwide, and the most common cancer among women [6]. Its incidence increases with age, with greater frequency at menopause. It was reported that the lower level of Zn may be associated with an increased risk of breast cancer [3], while the lower levels of Zn and Se, and the high level of Fe may be associated with an increased risk of prostate cancer [7]. The most accurate way to determine the level of trace elements in the body is the analysis of hair and nails [8]. Different analysis methods can be used to identify and measure trace elements, such as Total reflection X-Ray Fluorescence spectroscopy (TXRF) [9], Atomic Absorption Spectroscopy (AAS) [10–12], Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) [13–15], Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) [16,17], and Instrumental Neutron

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Activation Analysis (INAA) [18–20]. Mostly these methods are required for standard reference materials in comparison with analyzing samples. Meanwhile, the k_0-INAA method is one of the methods which give high sensitivity and accuracy and not required for standard reference materials [21,22].

In this study, the k_0-INAA standardization method was chosen for the determination of the concentration of Fe, Se, and Zn in the nails of Vietnamese women with breast cancer and healthy. Besides, the statistical analysis was also considered in this study, such as the significant differences and correlation coefficients between the elements in the fingernails of both sample groups.

MATERIALS - METHODS

This research was approved by the Ethics Committee of Dong Nai General Hospital.

Sample collection

The present study was conducted in Dong Nai province in the southeast of Vietnam, where locates many large industrial zones of the country. The study had involved a group of breast cancer females and a group of healthy persons as reference. The former group included twenty-nine female patients whose ages between 45 and 60, treated breast cancer at the Oncology Department of the General Hospital Dong Nai. The latter group was healthy women whose the same ages as the first group and living also in Dong Nai province. All the patients in the study were in first stage breast cancer and had not treated by chemical or radiation. The mean age was 47.28 for both studied groups. The fingernail samples were collected from August 2017 to February 2018 on the persons who had been aware of the investigation.

The nail donors were asked to clean their hands with fresh water, then with distilled water. The fingernail from 10 fingers was taken by a stainless-steel snail clipper, kept in a pre-cleaned plastic bag, and stored in ambient laboratory conditions. The information on the nail donors, including name, ages, place of living, health conditions, *etc.* was recorded.

Sample preparation

For neutron activation analysis, the fingernail samples were treated as described elsewhere [6,19,23,24]. Briefly, the treatment procedure for fingernail based on 5 steps as follows: i) The fingernail samples were first kept soaked in distilled water for 10 minutes followed by another 5 minutes in rubbing alcohol with light shaking. This step was to reduce the risks of microbiological activities from fungi and bacteria. ii) The fingernail samples were triplicated soaked in acetone with ultrasonic agitation (Model B2510-DTH, Branson, USA) for one minute. For each replicate, the acetone was discarded, and new acetone was added to the nail sample. iii) The fingernail samples were treated in the same manner with step 2, using 2% Triton X100 (CAS 9002-93-1, Merck KGaA, Germany) instead of acetone. iv) The fingernail samples were triplicated cleaned by soaking in distilled water and ultrasonic agitation for one minute. v) The cleaned fingernail samples pre-dried by placing on the filter paper for 12 hours at ambient temperature.

For neutron activation analysis, approximately 30-70 mg of each sample was placed in a cleaned polyethylene bag and sealed before irradiation. The certified reference materials, namely, NIST 1566b (Oyster Tissue) and NIST 1577a (Bovine Liver) were also used as quality control samples. For dry based calculation, the humidity of fingernail samples and certified reference materials - NIST 1566b, and NIST 1577a were measured (Model MB45, Ohaus, USA). For this determination, approximately 80 mg of NIST 1566b and 130 mg of NIST 1577a were dried in an oven (Model UFB 500, Memmert, Australia) at 80^{o}C for 12 hours. The humidity was 4.1% and 11% for NIST 1566b and NIST 1577a, respectively. For the fingernail samples, the humidity was between 8.2% and 10.7%.

Irradiation, measurements, and calculation

The samples were divided into two groups. The first group was included with 29 fingernail samples from patients, NIST 1566b, cleaning blank (~ 123 mg) and Al-0.1%Au (wire form, ~ 3.6 mg) as a neutron flux monitor. The second group was included with 30 fingernail samples of healthy women and NIST 1577a. Samples from each sample group were placed together, wrapped with aluminum foil and placed in an aluminum irradiation device called "rabbit". The neutron irradiation was performed in the Dalat Nuclear Reactor, Vietnam for 10 hours under a thermal neutron flux of 3.2×10^{12} $n.cm^{-2}.s^{-1}$. The deviation of the epithermal neutron spectrum and the ratio of thermal/epithermal neutron fluxes were a = 0.071 $\pm$ 0.001, and f = 39.5 $\pm$ 0.4, respectively.

The irradiated, the sample was measured using a gamma-ray spectrometer with HPGe detector (Canberra, USA) which its resolution (FWHM) of 1.9 keV at 1332.5 keV peak of ^{60}Co. Each sample was counted in a time of 10 hours, after 12 days of decay. At the full energy peak of radioisotopes, the net area was obtained using software GENIE 2000. For the fingernail samples, the full energy peaks of ^{59}Fe (192.3 keV and

1099.3 keV), ^{75}Se (279.5 keV) and ^{65}Zn (1115.5 keV) were measured. The element as Fe, Se, and Zn were also detected in the blank, but them at low levels (level of $\mu g.kg^{-1}$) and ignorable. The k_0-INAA standardization method was used to calculate the concentration of elements [21,22].

Data and statistical analysis

The element concentrations were expressed as the arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, and median which calculated using Microsoft Office Excel. The statistical significance of mean values between cancer and the healthy group was determined by applying Student's t-test. When a probability value (P-value) is smaller than 0.05, the difference was considered to be significant. Besides, the compositional relationships among elements in fingernails are evaluated by using the correlation matrix.

RESULTS

Analytical quality control

After the correction of the blank, the concentration of elements in Certified Reference Materials was calculated. Here, the uncertainty of concentrations obtained was calculated using the propagation of error. **Table 1** and **Table 2** shows the results obtained in the analysis of NIST 1566b Oyster Tissue and NIST 1577a Bovine Liver, respectively. The relative deviation between measured and certified values for NIST 1566b Oyster Tissue and NIST 1577a Bovine Liver were lower than 7% and 9%, respectively. To evaluate the accuracy of the results obtained in the analysis of certified reference materials, the Z-score index was used in this work [25,26]. A result is considered acceptable when the value of Z-score is between -3 and 3. The Z-score values obtained were below 2 indicate that the results obtained are within the range of certified values at a level of significance of 5% [26]. In this work, the Z-score values obtained are also shown in **Table 1** and **Table 1** for NIST 1566b Oyster Tissue and NIST 1577a Bovine Liver, respectively. As a result, all of the elements obtained in the analysis of the certified reference materials were satisfactory. It means that the analysis method in this work was trustworthy.

Elemental concentrations in fingernails

Table 3 and **Table 4** show the results obtained in the analysis of fingernail samples of women with breast cancer and healthy women, respectively. As a result, in the fingernails of both women with cancer and healthy, elements including Fe, Se, and Zn were recognized in this work. The concentration of Se element was obtained at levels of the order of $\mu g.g^{-1}$, while those of elements, including Fe and Zn in order of much higher of $\mu g.g^{-1}$. The results showed that the concentration of Fe in the fingernails of women with breast cancer was higher than of those healthy women, while Zn level was at the lower level. The concentration of Se was the same for both groups. The schemes for comparison on concentrations between the two groups shows in **Figure 1**.

Statistical analysis

Table 5 showed a significant difference in element concentrations in fingernails between breast cancer and healthy women. In this statistical analysis, the t-test, T critical at two tails and P values were carried out with a significant level of 0.05. As a result, there is a significant difference in the concentration of Fe and Zn between two groups ($P < 0.05$), while Se element was not different.

Correlation analysis

In order to examine the interrelationships between elements, the correlation coefficients between determined elements in the fingernails of women with breast cancer and healthy women are represented in **Table 6** and **Table 7**, respectively. In the fingernails of women with breast cancer, strong positive correlations are observed between Zn and Se (**Table 6**). For healthy women, strong positive correlations are found to the elements Zn and Fe, Zn and Se (**Table 7**).

The significant correlation between Fe and Se was not found in both groups. In addition, Zn and Fe have correlated for healthy women, but they were disappeared for women with breast cancer.

DISCUSSION

The metals have diversified biological functions from essential elements to toxic elements, and it is the reason possibly causes cancer or other diseases. The essential metal elements such as Fe, Se, Zn, *etc*., are essential metal elements at common levels. The essential metal elements are very important in the process of metabolism, respiration, and in the process of growing up and death of the cells [27–29]. The change in the concentration of trace elements can be lead to illness or toxicity [27,30,31].

Table 6 and **Table 7** shown that these results are completely suitable for the correlation between Se and Zn. Because, the element zinc (Zn) is exciting for gene

Table 1: **Concentrations of elements in NIST 1566b Oyster Tissue**

Element	Measured value (μg.g^{-1})	Relative deviation (%)	$\|\mathbb{Z}-score\|$	Certified value (μg.g^{-1})
Ag	0.712 ± 0.021	+6.5	2.01	0.666 ± 0.009
As	7.29 ± 0.47	-4.9	0.45	7.65 ± 0.65
Co	0.347 ± 0.040	-6.9	0.59	0.371 ± 0.009
Fe	219.5 ± 17.8	+6.2	0.72	205.8 ± 6.8
K	6229 ± 498	-4.7	0.58	6520 ± 90
Na	3237 ± 162	-1.9	0.35	3297 ± 53
Rb	3.23 ± 1.06	-0.9	0.03	3.26 ± 0.14
Se	1.99 ± 0.21	-3.5	0.27	2.06 ± 0.15
Zn	1376 ± 59	-3.5	0.64	1424 ± 46

Table 2: **Concentrations of elements in NIST 1577a Bovine Liver**

Element	Measured value (μg.g^{-1})	Relative deviation (%)	$\|\mathbb{Z}-score\|$	Certified value (μg.g^{-1})
Co	0.23 ± 0.02	+8.7	0.37	0.21 ± 0.05
Fe	204 ± 22	+4.9	0.34	194 ± 20
Na	2442 ± 140	+0.5	0.06	2430 ± 130
Rb	13.1 ± 0.8	+4.6	0.74	12.5 ± 0.1
Se	0.69 ± 0.12	-2.9	0.14	0.71 ± 0.07
Zn	121 ± 4	-1.7	0.22	123 ± 8

Table 3: **Concentration of elements (μg.g^{-1}) in the fingernail samples of women with breast cancer**

Element	No. sample	Arithmetic mean	Standard deviation	Median	Min.	Max.
Fe	20	102.87	37.53	91.220	49.447	188.746
Se	21	0.75	0.30	0.672	0.328	1.550
Zn	26	65.49	23.39	67.452	26.623	103.812

Table 4: **Concentration of elements (μg.g^{-1}) in the fingernail samples of healthy females**

Element	No. sample	Arithmetic mean	Standard deviation	Median	Min.	Max.
Fe	25	69.74	34.21	62.261	26.125	195.477
Se	29	0.78	0.42	0.742	0.161	1.947
Zn	28	107.75	38.95	103.561	50.406	204.000

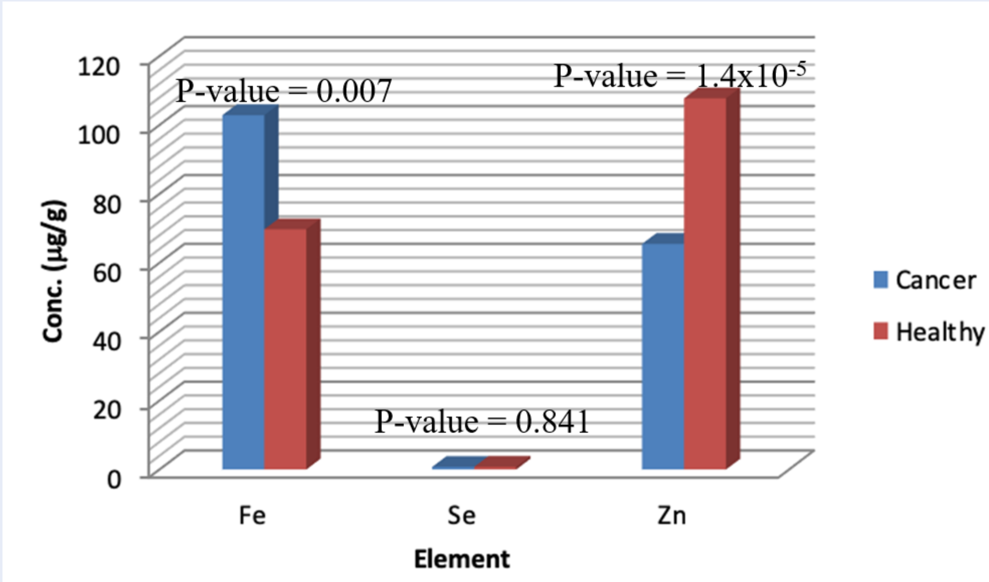

Figure 1: **Scheme for comparison on the element concentrations between cancer and healthy women.**

Table 5: **The statistical parameters of element concentrations in fingernails**

Element	t-test	T critical	P-Value	Sign. different
Fe	3.060	2.021	0.007	Yes
Se	0.295	2.012	0.841	No
Zn	4.872	2.014	1.4x10-5	Yes

Table 6: **Correlation coefficients between elements in fingernails of women with breast cancer**

	Fe	Se	Zn
Fe	1		
Se	0.267	1	
Zn	0.067	0.613	1

Table 7: **Correlation coefficients between elements in fingernails of healthy women**

	Fe	Se	Zn
Fe	1		
Se	0.078	1	
Zn	0.458	0.716	1

transcription and cell proliferation, and increasing of Zn concentration in cells contributed to the multiple - cell processes, even if cells of tumors [32]. While the selenium (Se) has an effect to prevent for development of cancer cells according to a certain mechanism. Selenium (Se) helps for protection and against chromosome injury which may be caused to cancer [33]. For this reason, Zn and Se are always to have a correlation. This result is also found in noncancerous and cancerous breast tissues [34]. In the fingernails of women with breast cancer, the Zn concentration was lower than that of healthy women, while the Se concentration was still not different. Hence, the correlation between Zn and Se was slightly decreased for women with breast cancer. However, these changes were not broken for correlation with them.

In this study, the result of the correlation between Zn and Fe was fairly interesting. In researching the correlation between Zn and Fe in the breast tissues of healthy and cancer women, Ammar Mubarak Ebrahim [35] shown that, no correlation between Zn and Fe in the breast tissues of cancer women, but they are correlated in breast tissues of healthy women. This result was agreeing with our study for fingernails. Thus, the evaluation of the correlation between Zn and Fe in fingernails was not different from that in breast tissues. However, there are significant differences in the average concentration of Zn and Fe between fingernails and breast tissues. For the breast tissue of women with breast cancer, the concentration of the element Zn was higher than that of healthy women. It is explained that the development of the tumor was brought about the increasing quantity of cells so that they are needed for the element Zn of transformation and metabolism. In the cancerous cells, increasing element Zn concentration was also represented at other cancers, such as prostate cancer [36], gastric cancer [32,37]. In addition, cancer cells need more blood than that compared with healthy cells, so that the concentration of element Fe has slightly increased, but there is not enough for significant statistical. In breast tissue, the concentration of element Zn was highly increased, while the concentration element Fe was not changed. This is shown that the correlation between Zn and Fe in breast tissues was reversed in comparison with fingernail tissues. In this study, the correlation between Zn and Fe in fingernails was disappeared for women with breast cancer (**Table 6**). In comparison, with healthy women, it was shown that the concentration of element Zn decreased, while the concentration of element Fe increased (**Table 4**). One can understand these cases as follows, because there no blood vessels in fingernail tissues so that the concentration of element Fe in fingernails of women with breast cancer can not similar to cancerous breast tumors. In researching metal exposure in the nails of the population at Punjab, India, Blaurock has shown that the concentration of element Fe in nails of breast cancer patients was much higher than to healthy people and intake Fe into the body due to environmental exposure [38].

Recently, it was not found any research for the evaluation of the concentration of element Zn in nails of women with breast cancer and healthy women. However, a few of research has indicated that, for the patients who contract a chronic As exposure, the concentration of elemental Zn in hair was strongly decreased in comparison with healthy humans [39]. Further, as the above discussions, the concentration of element Zn has increased in tissues of stomach cancer patients [32,37]. However, in the research of Campos *et al.* [40,41] shown that there is a reverse correlation between the concentration of Zn in nails and stomach cancer. In our study, the concentration of Zn in the fingernails of women with breast cancer was lower than that in comparison with healthy women. It was proved that increasing the concentration Zn in cancerous cells leads to deficient at the other organs in the body.

Selenium is considered an essential trace element because it is the primary component of selenoproteins, which have roles in counteracting oxidative stress and regulating the redox status of other molecules [42]. In case–control studies such as those of Van't Veer *et al.* [43] and Ghadirian *et al.* [44], both of which examined the level of Se in the toenail, which is considered more representative of long term Se exposure, found no association with breast cancer risk. This is a good agreement with the obtained results of Se in our study.

CONCLUSION

The present study k_0-INAA method was used to determine the concentrations of Fe, Se, and Zn in fingernail samples of women with breast cancer and healthy women. In the elements determined, the concentration of Fe was found a much higher level in women with breast cancer than those of healthy women, while the concentration of the element Zn was found at a lower level. The element Se was the same in concentration for both sample groups.

The significant difference ($P<0.05$) between cancer and healthy women was found for the elements as Fe and Zn. The results of correlation analysis show that a strong positive correlation is found between Zn and Se in fingernails for both cancer and healthy women, while the correlation between Zn and Fe is found in fingernails for healthy women, but this correlation is disappeared for cancer women.

These results may be concluded that Fe and Zn are significantly associated with breast cancer of the women, while Se is not associated with breast cancer risk. However, because of the small sample size, the results in this paper are insufficient to indicate that the concentration of Fe, Se, and Zn in fingernails can be used as an indicator of breast cancer. Therefore, more evidence is needed to confirm that the elements in this study are associated with breast cancer.

LIST OF ABBREVIATIONS

INAA: Instrumental Neutron Activation Analysis
NIST: National Institute of Standards and Technology
HPGe: High Pure Germanium
FWHM: Full Width at Half Maximun

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, Tran Pham Ngoc Trinh, and Nguyen Thi Truc Linh. Huynh Truc Phuong wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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