Chapter

Role of Trace Elements in Breast Cancer and Their Characterization Using X-Ray Fluorescence Techniques

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Abstract

Breast cancer is the most common serious disease that occurs in the human body. Trace elements have an important function in biological and metabolism processes including activation or inhibition of enzymatic reaction, reactive oxygen species (ROS), competition between trace elements and metal proteins for binding positions and modifications in the permeability of cellular membranes which influence carcinogenic processes. A significant association between the abnormal concentration of trace elements and breast cancer has been found in many studies using XRF techniques like energy dispersive X-ray fluorescence (EDXRF), particle induced X-ray emission (PIXE), total reflection X-ray fluorescence (TXRF), wavelength dispersive X-ray fluorescence (WDXRF) and synchrotron induced X-ray fluorescence (SRIXE). This chapter considers trace elements like Fe, Cu, Zn, Cr, Cl, Ca, P, S, K, Na, Mg, Se, As and Sr. from the standpoint of their role as either inhibitory or causative agents of breast cancer. XRF techniques and sample preparation methods for analysis of biological samples are also reviewed.

Keywords: breast cancer, trace elements and XRF techniques

1. Introduction

In the human body, sometimes cells begin to grow out of control and divide into a large number of abnormal cells usually known as cancer cells. Cancer is a multifactorial complex disease. These cancer cells can spread or metastasize from one part of the body to another part and damage the patient's quality of life. Cancer is of various types like kidney cancer, colon cancer and lung cancer, etc. Among all these, breast cancer is the most common that occurs in the human body system. Generally, this type of cancer is commonly found in women. It is widely accepted that breast cancer is hormonally influenced, with most of the risk factors associated with the exposure of breast to stimulatory effects of female reproductive hormones, mostly estrogens, leading to increased cellular proliferation due to which normal cells gets converted into breast cancerous cells. Only 5–10% of all breast cancer cases are due to genetic factors i.e., the inheritance of mutations in breast cancer susceptible genes (BRCA1 and BRCA2). Rest of the cases are having hormonal and

non-hormonal non-genetic risk factors. Non-hormonal cases also indirectly tied to modulation of estrogens exposure.

Breast cancer is the most diagnosed cancer among females worldwide, having recent estimates of 2.1 million new incidence and 630,000 deaths in 2018 [1]. Most incidences of breast cancer are reported in the countries with higher Human Development Index (HDI) alleging westernization of lifestyle linked to menstrual characteristic (age at menarche and menopause and type of menopause), Reproduction factors (older ages at first birth, nulliparity, giving fewer births), exogeneous hormone intake (oral contraceptive pills, Menopausal harmonic therapy), medication (fertility drugs, Diethylstilbesterol), nutrition factors (high fat intake during adolescence, alcohol), anthropometric factors (rapid height growth during childhood and adolescence, high body mass index (BMI), body fat distribution), smoking [2]. Engagement in regular physical activities, to avoid consumption of alcoholic beverages, Breastfeeding with longer duration, balanced diet (fruits and vegetable, soy) is some of the important factors to reduce the risk of breast cancer. Although breast cancer incident rates are highest in economically developed than developing countries, the reverse is true for mortality rates, reflecting limited screening and less effective treatments in such areas. In year 2018, highest incidence rate has been recorded in Australia/New Zealand while highest mortality has been estimated in Melanesia [1].

Trace elements and their role in the cancer process have been a matter of great concern and early reports given by various researchers have proved that there is a relation between trace elements and cancer which play a key role in the biological and metabolic processes in the human body. It is reasonable to assume that the abnormal levels of these trace elements lead to the development of cancer in the human body system. Furthermore, the excess and deficiency of trace elements induce the formation of reactive oxygen species (ROS). It is believed that ROS lead to the formation of almost all types of cancer. Generally, these are divided into two groups: (a) Free oxygen radicals (b) non-radicals. The International Agency for Research on Cancer (IARC) has suggested the list of elements that show the carcinogenetic properties. These elements are Be, Cr, Co, Ni, As, Cd, Sb, Pb, Hg, Pt, Mn, Fe, Cu, Zn, Se and Sr. The abnormal levels of ROS disturb the biological processes and metabolic activities which results in the unchecked normal cells growth into cancerous cells [3]. For the detection of these trace elements, analytical techniques like energy dispersive X-ray fluorescence (EDXRF), total reflection X-ray fluorescence (TXRF), synchrotron induced X-ray fluorescence (SIXRF) and proton induced X-ray fluorescence (PIXE) have been widely used [4–9].

The aim of the present chapter is to discuss the role of trace elements in human breast cancer and the techniques used for quantitative elemental analysis of cancer samples.

2. Methods and materials

X-ray Fluorescence (XRF) is a well-established non-destructive analytical technique for quantitative as well as qualitative determination of elemental composition in samples independent of their physical and chemical forms. In XRF, either electrons or photons (X-rays/γ-rays) used as the excitation source, are incident on the sample thereby exciting the atoms of the elements present in the sample [10, 11]. The intensity of characteristic X-rays and scattered photons resulting from the photon-atom interaction processes are detected and measured using energy dispersive X-ray fluorescence (EDXRF) spectrometers. In EDXRF, the characteristic X-rays are not diffracted spatially and are detected by a detector with signal processing

electronics. An entire spectrum can be acquired virtually simultaneously in the EDXRF technique so that the detection of most of the elements across the periodic table can be possible within a few seconds. It is mainly a non-destructive chemical analysis technique and a great variety of non-portable and portable experimental set-ups are available. The triaxial geometry of non-portable spectrometers allows radiations (not monochromatic) of selected energy range, depending upon the secondary target. Si (Li) or Ge detectors, cooled by liquid nitrogen [12, 13], are generally used in these types of set-ups. As far as portable spectrometers are concerned, radioactive sources or X-ray tubes are commonly used. To reduce the noise level, detectors are cooled by the Peltier effect. These types of detectors have limitation of poor sensitivity, so trace elements of biological samples were studied less [14]. The wavelength dispersive X-ray fluorescence (WDXRF) technique is very important for the routine elemental analysis for the quality control of various materials. This technique is based on the Bragg's law, $n\lambda = 2d\sin\theta$, where n is an integer determined by the order of diffraction, θ is the scattering angle, d is the inter-planar distance of crystal lattice and λ is the wavelength of impinging radiation. On being incident on a crystalline sample, they are scattered in a peculiar fashion by the atoms undergoing constructive interference, which occurs only if the electromagnetic radiation or subatomic particle waves have the wavelength comparable to the atomic spacing. In this technique, amplification in the signal is obtained due to constructive interference of detected X-rays which obey the Bragg's condition. The crystal monochromator is one of the key parts in WD spectrometers. WDXRF technique uses optimized analyzer crystals and detectors to separate and count the emitted discrete X-ray wavelengths using diffraction from a crystal with a very high degree of resolution. This technique is more stable for analytical accuracy and precision even in performing the chemical analysis as compared to others. Total reflection X-ray fluorescence (TXRF) [15] and micro X-ray fluorescence (μXRF) [16] are the advanced variants of EDXRF. TXRF utilizes the property of total external reflection. In TXRF a fine, collimated and almost parallel X-ray beam from the X-ray tube falls on a smooth polished surface of the target sample in the form of a thin layer of a few nm thickness, at a grazing angle below the critical angle of the surface and gets totally reflected. Due to this condition, a totally reflected beam reduces scattering and absorption of the incident beam in the photon absorption matrix of a sample. This leads to a largely enhanced peak to background ratio, significantly amended fluorescence yield and consequently much better sensitivities to elements present even in ultra-trace levels. The improved detection limits of TXRF make it a valuable tool for trace and ultra-trace element analysis. The TXRF technique is more sensitive due to the use of glancing angle and destructive to some extent as compared to both the EDXRF and WDXRF techniques. **Figures 1** and **2** show the X-ray emission spectra of normal and abnormal breast tissue/blood samples obtained by non-destructive EDXRF, WDXRF and TXRF techniques. In μXRF, X-rays generated by the X-ray tube are converged at a small region ~10 μm on the sample surface by polycapillary lens (an X-ray focusing system) that exploits the phenomenon of multiple total external reflection in array of small hollow glass tubes [17]. The polycapillary lens increases the intensity and spatial resolution of X-rays irradiating on the sample. Also, irradiating the sample with X-rays micro focused only on the target position enhances the signal-to-background ratio by reducing fluorescence X-rays generated from adjacent areas. The photon microprobe is the best technique of the future for material information because of the very low deposit in the matter and its variety of interactions. One can use this technique in various element mapping applications of X-ray fluorescence.

Synchrotron induced X-ray fluorescence (SIXRF) offers distinct advantages over other XRF techniques as the synchrotron radiation has been used as a

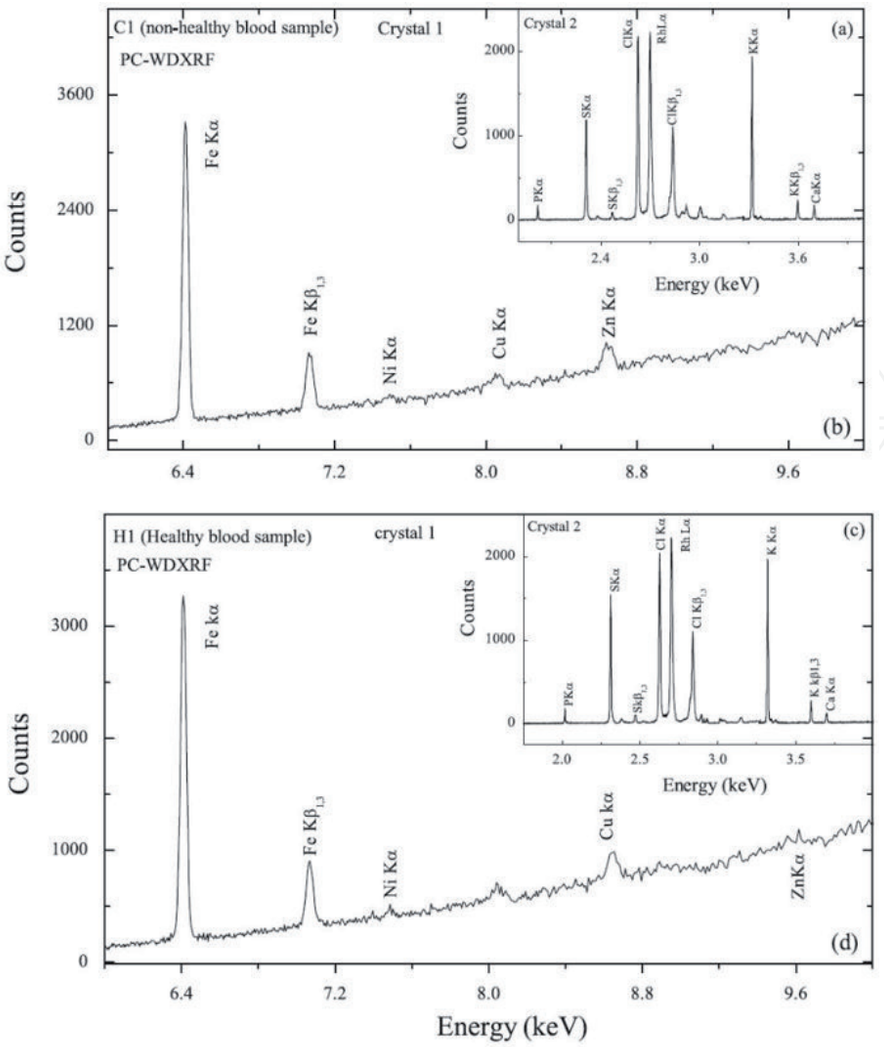

Figure 1.
Insert (a) and (c) show the spectrum of non-healthy (C1) and healthy (H1) blood samples of patients taken by crystal 2. Caption (b) and (d) represent spectrum of non-healthy (C1) and healthy (H1) blood samples of patients taken by crystal 1 of PC-WDXRF spectrometer [32].

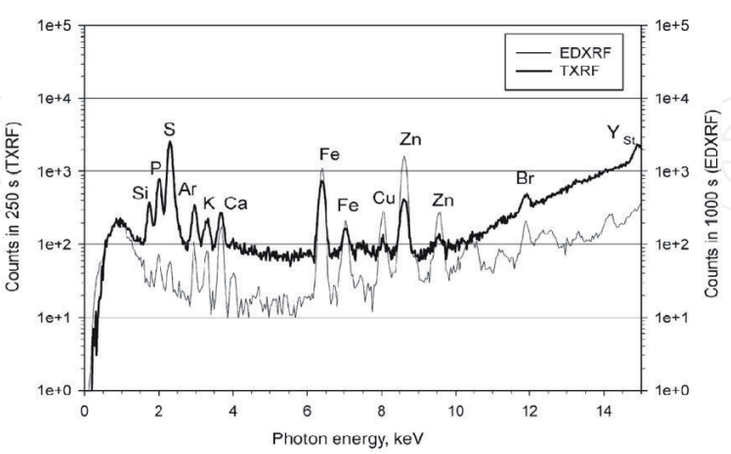

Figure 2.
X-ray emission spectra of normal and abnormal breast tissue/blood samples obtained by non-destructive EDXRF and TXRF techniques [31].

powerful X-ray source [18]. The synchrotron radiation source is characterized by a high degree of polarization and pulse height, high collimation, low emittance, reliability in energy by monochromatized emission. It has become an important tool in various fields of research. The synchrotron source provides the combination

of high flux and low divergence which is crucial for the massive success of experiments in the field of SIXRF. An important property of the synchrotron radiation is linear polarization which allows the SIXRF setup to detect the concentrations of specific elements present at trace levels due to a significant reduction in background level produced from Compton scattering. Almost complete repression of Compton scattering can be achieved by locating the detector at 90° with respect to the synchrotron beam in the plane of polarization. Hence the improved detection limit can be obtained using synchrotron radiation. The directionality and brightness of synchrotron radiation provide a superlative capability for micro-beam analysis. **Figure 3** represents the X-ray emission spectra of normal and cancerous blood serum by non-destructive synchrotron based XRF technique.

Particle induced X-ray emission (PIXE) is well known technique for the elemental analysis and high cross-sections of the elements. In recent years, most of the scientists use this technique for the biological samples. Due to the low level of continuum background, it produced better results than other techniques. PIXE opens up a new era in the field of biological samples where measurements of low Z elements are possible at microscale level. It is also well known that the microbeam PIXE is the technique that offers results in ppm level with high sensitivity and the

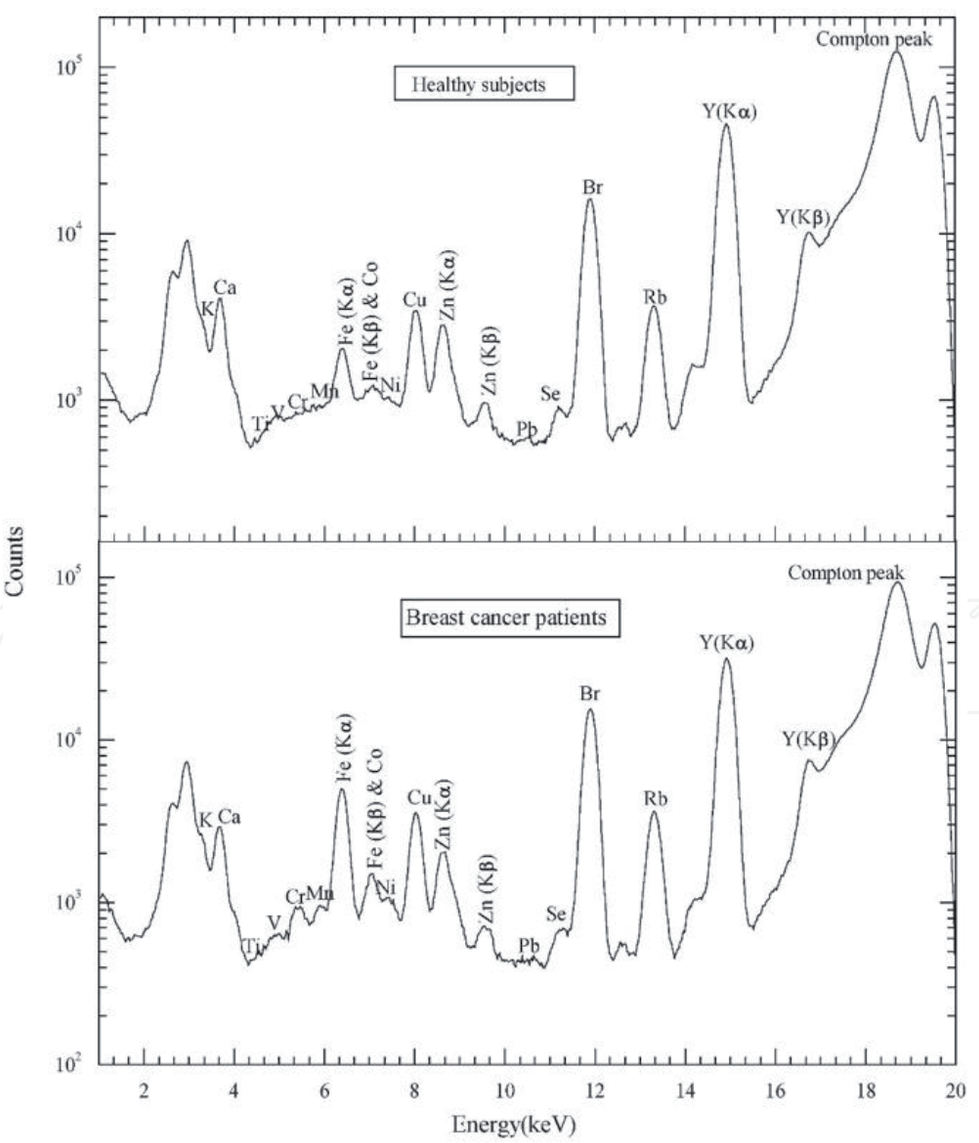

Figure 3.
X-ray emission spectra of normal and cancerous blood serum by non-destructive synchrotron based XRF technique [38].

size of the beam is smaller than the biological sample cell dimensions. In PIXE, the active protons (MeV) excite the target atoms to produce the X-ray spectrum by the inner shell decay process producing X-ray. The emitted X-ray energies are the characteristics of the elements and are proportional to the mass of that element present in the sample for further analysis [19–21]. **Figure 4** illustrates the normal and abnormal breast cancerous tissue obtained from PIXE spectrum. X-Ray absorption spectroscopy is a technique in which a core electron is excited to an empty state of LUMO and continuum, known as X-Ray Absorption Near Edge Spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS), respectively [22]. Mostly synchrotron is used as X-Ray source, but laboratory based commercial system are also available [23]. XANES spectra reveals average oxidation state, local coordination environment, chemical speciation and symmetry of the metal site while the EXAFS delineates the identity, number, distance of neighboring or adjacent atoms from the excited atom [24, 25]. XAS techniques acquire ascendency over X-Ray crystallographic techniques in the sense that local structural information around an element can be unraveled even from disordered samples such as powders and solutions. XANES spectroscopy has been used to examine the oxidation state of Zn, Fe and Cu in the normal and primary invasive breast cancer tissues [26].

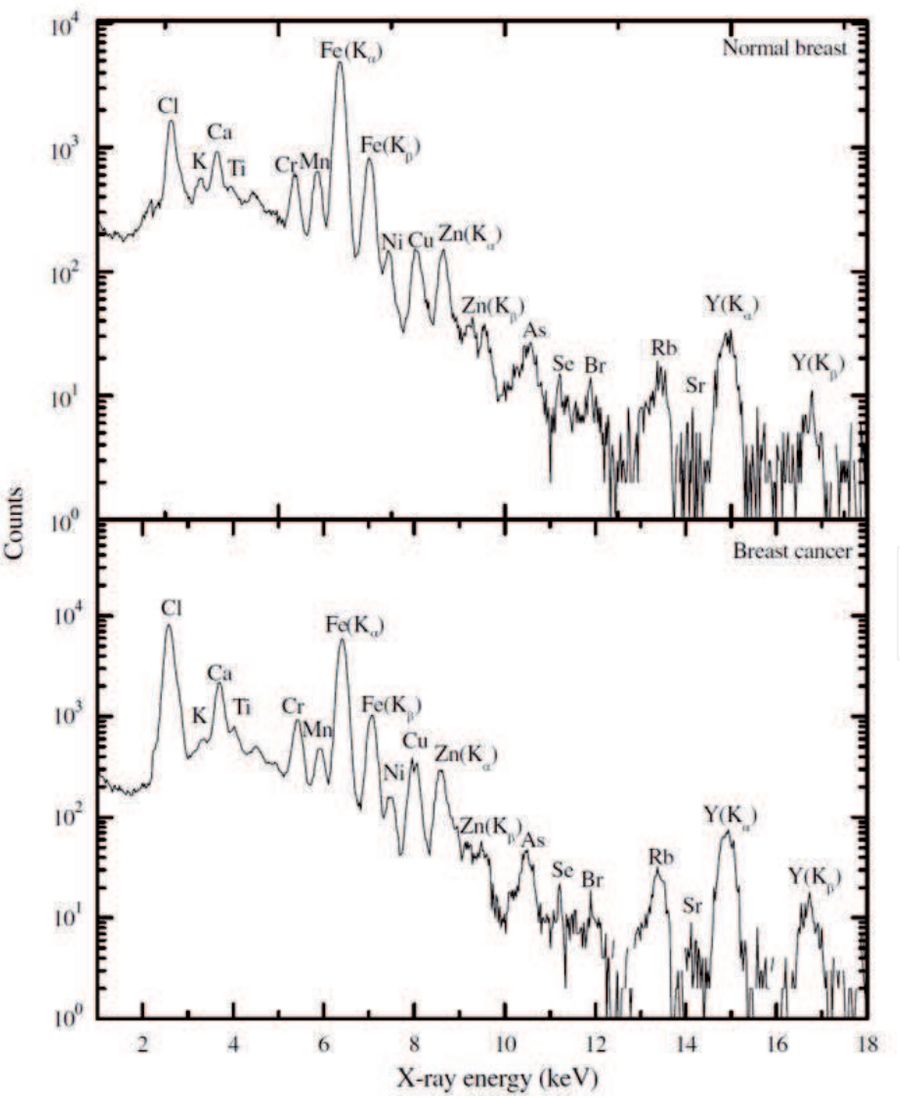

Figure 4.
Normal and abnormal breast cancerous tissue obtained from PIXE spectrum [37].

Breast tissue samples are generally collected from mastectomies, lumpectomies and breast reduction surgery [27–29]. Since, among the healthy population, a significant variation of trace elemental levels is found, generally healthy tissues from areas distant to malignant tissues/neoplastic are also collected from the same individual for comparison. For measurements like SIXRF to be performed directly on the wet tissues, collected tissue Samples are washed with milli-Q water to remove any stain of blood and are stored in formalin (10% formaldehyde in water) at room temperature or kept frozen at <−40°C until the analysis [27, 29, 30]. For EDXRF measurements, tissue samples are lyophilised for at −60°C and low pressure, approximately 10^{-1} atm, where the low temperature ensures the retention of volatile elements like Arsenic (As) and Mercury (Hg). These dried tissue samples are milled by freezer mill cooled by liquid nitrogen and pressed into pellets without any further chemical treatment or additive [7]. For techniques TXRF, requiring thin sections, tissue samples are cut into small pieces and excised into cylindrical pieces after cooling with liquid nitrogen. The excised tissue is then cut with a cooled microtome to get thin sections. Thin section is positioned in the centre of the sample carrier which is made non-hydrophobic by means of alcoholic silicon solution and then dried. Thin sections are spiked with internal standard solution and are again dried [31]. Various researchers have also used breast cancerous blood and blood serum for trace elements determination. For determination of trace element in human blood samples 4 ml of blood was mixed with 1350 mg cellulose and freeze dried. A fine powder of this mixture was pressed into pellets and used for WD-XRF measurements [32]. Blood serum samples are also lyophilized, and a small pellet is made which has been used for SIXRF in study [33]. For PIXE analysis of these serum samples, to make the sample conducting, graphite powder is also added and and pelletized [34]. **Table 1** shows the trace elements levels reported in the literature for normal and abnormal human breast cancer using XRF techniques. The values are given in μg/g.

2.1 Quantification of trace elements

2.1.1 WDXRF

The quantitative elemental analysis of different blood samples (normal and abnormal) was performed by using a commercial WDXRF spectrometer equipped with different anodes X-ray tubes (Rh/Ag/W), a gas flow proportional (FP) counter and a scintillation counter (SC) as photon detectors. The mass concentrations of different elements present in samples were determined using advanced software package available with the spectrometer. The intensity of X-ray lines for the specific element is determined with Lachance-Traill method which is defined as

$$I_g = D_g C_g \times \left(1 + \sum_{w \neq g} R_{gw} . m_w \right) \tag{1}$$

where, I_g corresponds to the intensity of the specific element g, C_g refers to the measured concentration of the corresponding element, D_g is the instrumental calibration coefficient for the given element. The term m_w denotes the concentration of the other element w and R_{gw} is the inter-element matrix coefficients. This software enables to evaluate the accurate concentrations of different elements ranging from Be to U present in unknown samples by incorporating corrections due to the matrix effects. The data acquisition time for each target was kept usually as ~20 minutes to collect good statistics under different X-ray peaks arising from different elements present in each sample.

Elements	Na	Mg	P	S	Cl	K	Ca	Cr	Fe	Cu	Zn	As	Se	Sr	Ref.	Analytical Technique
Normal									11.6	0.3	2.7					
Abnormal									18.8	0.9	6.5				[27]	SR-XRF
Normal						438			14.1	0.3	2.9					
Abnormal						1112			21.7	1	6.9				[29]	SR-XRF, EDXRD
Normal						210			7	0.29	1.8					
Abnormal						1032			18.8	0.95	7.7				[29]	SR-XRF, EDXRD
Normal							726		9.0	0.6	3.8					
Abnormal							2400		25.3	1.8	12.9				[30]	SR-XRF
Normal					3999.9	1381.0	157.1	32.0	299.5	42.0	56.2	2.57	0.7	6.7		
Abnormal					6815.4	1550.8	480.2	52.7	376.3	60.7	126.2	4.12	1.3	13.7	[37]	PIXE
Normal				295		17	153		5	2	6					
Abnormal				3240		165	1270		98	14	39	—	—	—	[7]	TXRF
Normal						112	970		32	35	31			6		
Abnormal						360	1530		147	27	64			8	[7]	EDXRF
Normal						1975	1259	3.2	28.1	14.8	19.2	0.06	1.7			
Abnormal						2175	1320	2.9	56.3	19.5	9.8	0.07	0.8		[33]	SR-XRF
Normal								18.6	291	24.6	38.5	0.9	2.5			
Abnormal								10.4	355	32.3	13	0.7	1.5		[34]	PIXE
Normal	1437.2	66.7	354.5	1618.6	4805.5	3164	131.5		1231.9	19.9	30.4					
Abnormal	2494.1	83	590.7	2631.6	7372.9	4309.5	294.8		1885	36.1	57.5				[32]	WDXRF
Normal				1908		3.0	100.6		3.4	1.7	16.9					
Abnormal				2132		4.4	127.4		8.7	3.3	11.4				[39]	PIXE

Table 1.
Trace elements levels reported in the literature for normal and abnormal human breast cancer using XRF techniques. the values are given in μg/g.

2.1.2 For TXRF

The qualitative and quantitative analysis of this technique requires an internal standard (U) solution of known concentration with associating its concentration relative to the net intensities found in the analyte by using the following equation

$$C_G = \frac{\frac{N_P}{S_P}}{\frac{N_R}{S_R}} C_U \tag{2}$$

Where, C_G/ C_U are the concentration of the analyte / known internal standard solution. The term N_P/ C_U refer to the net intensity of the given analyte and S_R/C_U correspond to the relative sensitivity of the analyte/known standard solution.

2.1.3 For PIXE

The multi-element analysis of unknown samples can be obtained by using available commercial peak fitting software GUPIXWIN (University of Guelph, Ontario, Canada) which is given as

$$P_z = P_t(Z)\frac{4\pi mzZ_x e}{\sigma_x^z d\varphi eff_p Q N_A} \tag{3}$$

The term P_t *(Z)* is the counts of the X-ray fluorescent spectra for any atomic number (Z), $d\varphi$, eff_p, Q, Z_x and N_A are the solid angle of the X-ray detector, efficiency, integrated projectile charge, projectile charge, and Avogadro's number, respectively.

2.1.4 For EDXRF

In this type of technique, the elemental concentration of unknown samples is evaluated by

$$m_i = \frac{N_{jk}}{I_o G \in_{jk} \beta_{jk} \sigma_{jk}} \tag{4}$$

Where, m_k denotes the concentration of k^{th} element present in the sample, N_{jk} is the net rate for the j^{th} group of X-rays of k^{th} element, $\in_{jk}$ is the detector efficiency for the j^{th} X-ray energy of the k^{th} element, I_oG is the intensity of the exciting radiation incident on the sample visible to the detector, σ_{jk} is the X-ray fluorescence cross section of the j^{th} X-rays of the k^{th} element at the incident photon energy, and β_{jk} is the self-absorption correction factor which accounts for the absorption of the incident and the emitted characteristic X-rays lying under the ith peak of k^{th} element within the target.

3. Role of trace elements

In the past, various researchers have reported a high level of iron in human breast cancer tissues as compared to the normal one [27–30, 35–38]. Elevated iron levels are also reported in in blood [32, 34] and scalp hair [39] of breast cancer patients using PIXE and WDXRF. Iron (Fe) plays a vital role in the human body

and is an essential element. Normally, the human body contains 4–5 g of iron out of which 1 g is stored in the liver and spleen. The main component forms of iron, hemoglobin and myoglobin help in the growth of cells. The low and high dosage of iron cause various diseases like heart diseases, diabetes, anemia, cancer, listlessness, stomatitis, etc. and promote cancer which damage the tissues and convert hydrogen peroxide to free radical ions via Fenton and Haber-Weiss type reactions. These free radical ions cause DNA strand breaks, sister-chromatid and initiate lipid peroxidation which promotes the growth of cancer [40, 41].

An Element like copper (Cu) is involved in multiple biological processes which promote tumor growth. As far as the role of Cu in breast cancer is concerned, the picture comes out to be rather wavy. Morton K. Schwartz in his research also reported the role of trace elements in cancer [42]. The author mentioned that the Cu level in breast cancer tissue was greater than the normal one. Studies [27, 29, 30, 37, 38] using various techniques are in well agreement with past results. Many studies showed that the level of Cu and in blood serum [32, 34] and in hairs [39] of breast cancer patients are higher as compared to the normal one. The daily requirement of Cu intake is about 2 mg/day and heavy dose ingestion causes various diseases. The role of Cu and its concentration is well explained by many workers [43–45]. The toxicity and abnormal level of Cu element and metabolism processes present in the human blood cause the formation of blood vessels which further results in various types of cancer like breast, brain, gladder, etc. The extra formation of blood vessels in the human body is called Angiogenesis. It plays a vital role in the evolution of cancer cells inside the body. Since blood flows in the whole body, these cells also require blood for their growth, so it gives chemical signals to stimulate Angiogenesis. The matrix metalloproteinase (MMP) family of enzymes degrades the basement membrane and extracellular matrix of tissue inhibitors of metalloproteinase (TIMP). Under critical condition both MMP and TIMP imbalance the tissue and activate angiogenesis which caused breast cancer [46].

For zinc (Zn) element, the concentration of Zn in breast cancer case is slightly large as compared to the normal one. Similar trends were also reported by many researchers by using different techniques and methods [29, 30, 37, 38, 47, 48]. Depressed Zn levels are found in blood sera of breast cancer patients using PIXE [34] but higher levels in blood are found in [32] using WDXRF. This might be understood in terms of biochemical and histological differences between cancerous and normal blood. Like other elements, Zn also plays an important role in the biological, physiological and metabolic processes of the human blood. It is also obligatory for the formation and common function of the cell membrane. Toward the role in cancerous blood or tissues, the statement about the Zn is contradictory. Earlier reports suggested that the abnormal level of Zn leads to carcinogenesis [49]. These inconsistent annotations suggest that the role of Zn may vary from one to another organ depending on various factors like age group, lifestyle, environment changing and diet etc. However, in breast cancer case, level of Zn increases in cancer case rather than the normal case which is explained earlier. Lee et al. [50] also gave evidence on the behavior of Zn in human normal and cancer tissues. In their research, they suggested that the altered Zn homeostatic in breast cancer tissue is responsible for the increased level of Zn in the cancerous case which possibly leads to the growth of breast tumor. Zn is an important trace metal being a cofactor for more than 300 enzymes, and contributes to cellular signaling, proliferation, homeostasis, apoptosis [51, 52]. It is also a structural component of more than ~3000 proteins including metallothionein's, zinc transporters, p53 tumor suppressor and matrix metalloproteases which are involved in carcinogenesis and cancer progression [53]. In particular, p53 activation is important for apoptosis and cell cycle arrest in breast cancer case and protects women from it. Transcription factors,

e.g. nuclear factor-kappa (NF-κB) is activated in the breast cancer and leads to a more aggressive phenotype. Association of Zinc to breast cancer cell inhibits NF-κB [54]. Generally, the level of Zn in breast cancer cases is more. The reason behind them is that Zn is necessary for the cell production in a region adjacent to the tumor due to the presence of MMPs or tissue inhibitors of metalloproteinase. Similarly, the observed correlation between Cu and Zn shows that the ratio of Cu/Zn is more in cancerous blood as compared to the normal blood [34, 48].

The element chromium (Cr) is also responsible for the formation of breast cancer in human body. Earlier reports suggested that level of trace elements of Cr in breast cancer tissues were significantly higher than the normal ones [37]. But in the research article given by Sarita et al. [34] using PIXE technique, the level of Cr in blood sera was found to be lower in breast cancer case as compared to the normal one. As we know that carcinogenic property of the trace elements depends mainly upon the factors like oxidation states and their chemical structure. In case of Cr element, the hexavalent chromium compounds Cr (VI) are more toxic than trivalent form Cr (III). Cr (VI) is easily absorbed by the body cells and then reduced to the trivalent form i.e. Cr (III). This reduction generates free active oxygen radical as well as glutathionyl radicals which further produces genotoxic effects which are responsible for the formation of breast cancer [55].

Chlorine (Cl) is an invasive chemical used in daily life products. It plays an important role in balancing the body cells and helps to digest the food. In the earlier study, the trends of the Cl element in human cancer tissue and blood were higher than the normal ones [32, 37]. Generally, Cl is present in extracellular fluid. Combination of Cl with organic compounds present in water or soil forms organo-chlorine. These are widely used as standard pesticides e.g. DDT, DDD, Isobenzan, Dicofol, Dieldrin, Eldrin, Lindane, BHC, etc. Carcinogenic and the weak estrogenic and anti-estrogenic hormonal effects of many organochlorines and their hydroxylated metabolites have led many researchers to hypothesize that they increase the risk of breast cancer in humans [56]. These are responsible for impairment or suppression of cell mediated immunity [57], mimicking androgenous hormones and modulating their as well as estrogen hormones metabolism prompting breast cancer development [58].

Since Ca is the major constituent of breast tissue calcification in the form of calcium hydroxyapatite, it has of vital importance in breast cancer. For the blood clotting mechanism, Ca plays an important role as factor IV. The Ca^{2+} cation is involved in various electrochemical mechanisms in the body like neutralization of charge, emulsion stabilization, free energy supply to the body cell. Magalhães et al. [38] mentioned the increased behavior of Ca in their research with TXRF technique. The same trend was also given by various workers with different methods and techniques [30, 37, 59, 60]. Higher Ca levels are found in the scalp hair and blood of breast cancer patients using PIXE and WDXRF in [32, 39]. Cationic calcium homeostasis in the plasma is tightly maintained through absorption, excretion and secretion and storage in bone. High level of calcium in the blood serum, known as hypercalcaemia, is associated with different types of cancer like breast cancer, lung cancer and myeloma, etc. These types of cancer result Ca to leak out from bones to the blood making it heavier than the normal one. This generally happens to the people suffering from cancer in the advance stage. Various analyses like meta-analysis and dose response analysis have been done to establish fact that there is a significant relation between Ca intake and breast cancer risk [61]. In these studies, the researchers suggested the dietary, lifestyle and intake dose of Ca which affect the human body. Having a complex nature, Ca intake is known to be inversely associated to breast cancer risk significantly in pre and postmenopausal women [62]. With rising cases of breast cancer being reported in the literature, the

trace elements like phosphorus (P), sulfur (S), potassium (K) and sodium (Na) and their role should be of greater concern. From the literature, it has been seen that proportion of these elements in breast cancer human blood is slightly higher with respect to the normal human blood. Earlier reports found that the abnormal level of phosphorus may influence breast cancer [32]. Some studies reported a statistically elevated P content in breast tissue compared to normal one using TXRF [31, 38, 48]. The number of in-vitro studies suggested that for cell growth in human body, the inorganic phosphate (Pi) and phosphate are two responsible terms. They both act like a mitogen. This elevated value of Pi promotes cell prolific microenvironment which causes breast tumor. Another study by Wulaningsih and his co-workers showed that when the content of phosphorus along with calcium enters in the human body, it increases the estrogens level which promotes the growth of breast cancer [63, 64].

In a view of sulfur element role, it has been seen from the past studies that the concentration of the sulfur increased in cancerous blood as well as in cancerous tissue than normal ones [31, 32, 38]. Sulfur plays an important role in cell renewal and enables transferring of oxygen from cell membrane. It is widely used in biological processes which act as both fuel and respiratory materials for the human body. Sulfur has an important role in chemotherapy to reduce the size of the breast tumor which uses sulfur containing drugs like Docetaxel, Paclitaxel, Taxanes, Eribulin, etc. [65]. From the past studies, it has been seen that the sulfur is commonly used in some form like organic sulfur to treat cancer which is useful in anti-cancer therapy. The research also claims that the sulfur containing compounds work like an anti-cancer reagent which kills the cancer cells without affecting normal and healthy cells present in the whole body system. Furthermore, organic sulfur compounds like amino acid, methyl-sulfonyl methane and diallyl sulphide, etc. have powerful anti-cancer effect against breast cancer [66].

Elements like potassium (K) and sodium (Na), they both also play a key role in the biological processes present in the human body system. The literature clearly shows that the value of both potassium and sodium in human breast cancer cell and blood increases with respect to normal one [6, 29, 32, 38]. Earlier views on potassium element clearly show that the concentration in human affected from breast cancer is not significantly different from the normal one [37]. This twin behavior of potassium element might depend upon many factors like eating/drinking lifestyle, environment behavior, sample preparation etc. Also, it has been concluded that most of the research has been done on the cancer tissues which are generally not homogeneous. So, for multi-elements detection system and for obtaining better results, samples must be homogeneous. In order to understand the role of potassium element in the cancerous blood, we know that it acts as an electrolyte and present mainly in the form of k^+ ion (cation) inside the human blood. Acid–base and water balance in the tissues and blood is maintained with the help of K^+ in the human body. The role of K^+ in regulating tumor cell proliferation and as anti-apoptotic and pro-apoptotic agent is well established [67]. On its combination with ascorbic acid, the inhibitory effect on the survival of breast cancer cell lines has been observed. Further details are given in Ref. [68]. On the other hand, in the case of sodium element, we clearly have seen from the past studies that the concentration of sodium element increases in case of breast cancer blood of human body [32]. The role of sodium (compound form) in cancerous blood is also a big concern. Since sodium is also present in the human blood in the cation form, it also plays an important role in the metabolism processes in the human body. Researchers reported the activity of Na^+/K^+ adenosine tri-phosphate (ATP) which clearly explain the difference between the concentration of Na + and k + cation in cancerous cells and normal one [69]. Higher Na^+/K^+ ratios have been reported in cancerous blood using WDXRF [32].

The recent research says that the sodium compound form i.e. sodium bicarbonate can be treated as an anti-cancer agent. The research proved that the use of sodium bicarbonate has been beneficial for breast and prostate cancer etc. Most of the work was done on the mice.

Magnesium (Mg) is the second most abundant cation in the body which acts as an activator in about 300 enzymatic reactions. These reactions include conversion of ATP to ADP for cell energy metabolism, DNA replication and repair, protein synthesis, inflammation, proliferation, cell cycle control and apoptosis [70, 71]. Most of these factors are related to carcinogenesis. Progression of breast cancer is also related to Mg where in the proliferative phase of the disease, the neoplastic cells causes an influx of it and hence there is an increase in the intracellular concentration of Mg disturbing its homeostasis [72]. Several studies have investigated the direct association of high magnesium intake and breast cancer risk and an inverse relation has been found. Indirectly, higher Mg intake lowers the C Reactive Protein (CRP) level and decreases the breast cancer risk [73]. Some earlier reports suggested that a significantly low Mg level in serum found with respect to normal tissue [74] while high Mg levels are found in cancerous blood using WDXRF in [32]. It is also observed that the concentrations of magnesium in all blood samples are opposite to the concentration of calcium. Both calcium and magnesium compete for the transporter transient receptor potential melastatin 7 (TRPM7) for their absorption in the tumor membrane. A negative feedback mechanism regulates the level of both. It has been seen that reduced level of magnesium decreases the Mg-ATP levels inside the cell which leads to increase the Ca-ATP levels of the cell and this intracellular Ca increase leads to increased cell proliferation. Ca/Mg ratio is elevated in breast cancer case since Ca concentrations are increased while concentration of Mg is decreased [75].

Selenium (Se) is the important trace mineral for the human body. It is believed that all the enzymes present in the human body system are selenium dependent. The abnormal level of selenium causes many diseases. As far as a disease like breast cancer is concerned, the picture of selenium is not clear. Different studies give different views on the role and presence of selenium in the human body. Using TXRF technique, Magalhães et al. [38] mentioned that the concentration of selenium element increased in breast cancer tissue as compared to the normal one. The observed high level of selenium in breast cancer tissue was also reported in the earlier studies also [37]. Low Se levels are found in the blood sera samples in [34]. In accordance with the hypothesis, finding suggested that the selenium worked as an immune enhancing and antioxidant to reduce the breast cancer [76]. Lifestyle, eating/drinking habits, environment, etc. are deciding factors because most of the selenium found in the human body is from eating/drinking habits etc. An inverse relationship exists between Selenium intake and risk of breast cancer [77]. Also, it might be guessed that it acts like an anti-cancerous agent. We know that selenium is generally absorbed in the body in the form of L-selenomethionine. Earlier studies confirmed that in MCF-7 breast cancer, the therapeutic effect of methyl selenocysteine combined with tamoxifen and imidoselenocarbamate considered as an antitumour agent reduces the growth of breast cancer [78, 79]. Combs et al. [80] in their research article suggested that selenium works as anti-oxidant effects via Glutathione peroxidise (GSH-Px) which further protects the body from damaging effect of free radicals.

Arsenic (As) is one of the most toxic elements found in nature. The main source of arsenic coming in our body is from eating/drinking habits, soil and from plants etc. However, its mechanism and role are not well explained but the epidemiological evidence in the past literature showed the toxicity of arsenic from drinking water causes different types of cancer. From the literature, it has been seen that the level

of arsenic is higher in breast cancer tissue as compared to others by using XRF techniques [37]. Lower serum As concentrations are found in [34]. The reason behind this is that the arsenite generates the effect of estradiol and induces ROS growth, DNA damage and increases c-Myc and heme oxygenase (HO-1) protein levels which lead to tumor cell proliferation and increase in the estrogens level in the body and causes breast cancer in MCF-7 cells. The c-Myc is one of the most commonly activated genes present in advance stages of breast cancer. The study shows that arsenite present in breast cancer MCF-7 cells increases the c-Myc and HO-1 level which results in DNA damage. The high increases in c-Myc and HO-1 level further deactivate the p53 gene and affect the metabolism and biological process if the body results in breast cancer [81]. On the other hand, arsenic trioxide has an antiproliferative effect on human breast cancer MCF-7 cells due to reduction of HERG channels and activation of caspase-3. Generally, HERG belongs to multi-genetic family of voltage gate k + channels and present mostly in the tumor cells, not in normal cells of the human body system [82].

For Strontium, the Department of Health and Human Services determined that stable isotopes of strontium do not play any role in cancer. Its radioactive isotopes ^{89}Sr and ^{90}Sr are important for breast cancer. Earlier studies mentioned the higher level of strontium in breast cancer tissue as compared to the normal tissue [37]. However, the IARC clearly suggested the carcinogenic behavior of radioactive strontium (^{90}Sr) which may cause cancer. The reason is that when ^{90}Sr enters in the body it gets mostly attached on the surface of the bone and soft tissue itself. Due to high dose and radioactive decay property, it combines with the blood or tissue and damages the DNA structure. In the case of ^{89}Sr, the previous study showed that it is more beneficial in breast cancer patients with metastatic bone pain and have similar metabolism function as that of calcium [83].

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