Comparative Evaluation of Metal Imbalances in the Blood of Thyroid Cancer Patients in Comparison with Controls

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Comparative Evaluation of Metal Imbalances in the Blood of Thyroid Cancer Patients in Comparison with Controls

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IN THE NAME OF ALLAH,

THE MOST MERCIFUL,

THE MOST KIND

DEDICATED

TO

MY LOVING PARENTS & SISTERS

DECLARATION

This is to certify that this dissertation entitled ***"Comparative Evaluation of Metal Imbalances in the Blood of Thyroid Cancer Patients in Comparison with Control"*** by ***Miss Kalsoom Bibi*** is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the dissertation requirements for the degree of ***Master of Philosophy*** in ***Analytical/ Inorganic Chemistry***.

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ABSTRACT

Cancer incidence and mortality rates have been increasing rapidly worldwide. A growing body of evidence revealed that exposure to trace metal is the most important aetiology for the development of cancer. Therefore, present study was designed to evaluate the imbalances of selected essential and toxic metals (Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Sr and Zn) in the blood of newly diagnosed thyroid cancer patients in comparison with their counterpart healthy subjects/controls. Concentration of the metals was quantified by flame atomic absorption spectrometry by employing nitric acid and perchloric acid based wet digestion method. Average levels of Na (1404 μg/g), K (1322 μg/g), Fe (309.6 μg/g), Ca (35.65 μg/g), Mg (25.94 μg/g) and Cr (14.68 μg/g) were found to be higher in the blood of patients than controls. Correlation study showed significantly strong relationships ($r > 0.500$) between Zn-Cd, Mn-Cd and Mg-Fe in the blood of thyroid cancer patients while Zn-Mg, Sr-Cd, Mn-Cr and Na-K show strong correlations in the blood of healthy donors. Significant variations in the trace metal levels were observed with the gender, habitat, food habits and smoking habits of both donor groups. Metal levels exhibited considerable differences with the stages of thyroid cancer and the age-groups of the subjects. Principal component analysis (PCA) and cluster analysis (CA) of metal data manifested significantly divergent apportionment of the metals in the blood of both the donor groups.

Chapter 1

INTRODUCTION

1.1 Thyroid Cancer

Thyroid cancer refers to uncontrolled or abnormal growth of cells in thyroid gland which is located in front part of neck and it has two lobes (NCI, 2015). It has two types of cells; Follicular cells and C-cells. Former cells control the metabolism by using iodine from blood, while latter cells make a hormone called Calcitonin that controls the calcium usage. Prime function of thyroid gland is to secrete hormones that are used to regulate and control the daily energy requirements (ACS, 2016). There are four major types of thyroid cancer; (i) Papillary thyroid cancer (ii) Follicular thyroid cancer (iii) Medullary thyroid cancer (iv) Anaplastic thyroid cancer (NCI, 2016). Papillary thyroid cancer develops in one lobe of the thyroid gland and it is usually not fatal and can be easily treated. It spread very slowly but it can spread to lymph node in the neck (ACS, 2016). Second most common type is follicular thyroid cancer which is well differentiated tumour. It originates from follicular cells. About 95% of the cancer cases are of papillary and follicular thyroid cancer. The incidence of follicular thyroid cancer is more in females than males. Mostly it cannot spread to distant area (Norman, 2016). Medullary thyroid cancer develops from C-cells of thyroid gland. About 4% of the thyroid cancer is medullary thyroid cancer. One of the reasons of medullary thyroid cancer is the mutation in a gene that is passed from parents to child. The fourth type of thyroid cancer is anaplastic thyroid cancer, which is named as undifferentiated carcinoma because the cells does not resemble to normal thyroid cells. It accounts for 2% of all thyroid cancer. Mostly it is considered that this type of thyroid cancer develop from already existing papillary or follicular carcinoma and is very difficult to treat (ACS, 2016).

There are different systems for staging of thyroid cancer; one of the systems is TNM system which is developed by American Joint Committee on Cancer (AJCC). According to AJCC there are three categories; T, N and M. T stands for tumour and use to tell about the size of primary tumour, while N stands for node which describes that whether tumour spread to regional lymph node or not. M stands for metastases, and it tells whether cancer spread to distant area of the body or not. There is another system of staging; Stage-I, Stage-II, Stage-III and Stage-IV. In stage-I tumour is localized with size

of less than 2 cm and does not spread to other body parts or lymph node. Stage-II has to meet either of the two conditions; first condition is that the tumour's diameter range from 2 to 4 cm and should limited to the thyroid region only. It is not spread to distant region and not even to regional lymph node. The second condition is that the tumour's size is greater than 4 cm and started to grown outside the thyroid gland. Stage-III has to fulfil either of following criteria; the tumour has not spread to nearby lymph node or other body parts but has grown outside the thyroid and its diameter is larger than 4 cm or tumour can spread to lymph node in the neck but can't spread any further, and tumour can be of any size. Stage-IV is further subdivided on the basis that where it is spread. In stage-IV (A) cancer is not spread to distant body part but it is grown beyond the thyroid gland and spread to the region such as neck, upper chest etc. In stage-IV (B) the tumour has spread to large blood vessels and spine but still not to the distant body parts. In stage-IV (C) tumour cells are metastasized and they spread to distant body parts (AJCC, 2016).

1.2 Role of Trace Metals in Cancer

Trace metals are very important for normal functioning but most of them are required in minute amount. Some of the metals are deleterious for human health and they may cause various diseases such as Cancer. Some of the trace metals including As, Cd, Cr and Ni are among the carcinogenic metals, as mentioned by International Agency of Cancer Research (IARC). However some essential metals such as Fe can also be carcinogenic in excess but these can cause cancer only in those who are already suffering from some genetic disease (Durham and Snow, 2006). Some other metals including Cu, Mn, Zn, Co, Cr, Mo and Se are required as enzyme cofactors or prosthetic groups. Any deficiency or excess of these metals may results in some disorders. Other metals, such as Pb, Al and As mostly exhibit toxic effects and are carcinogenic (Florea *et al*., 2012). Essential trace metals are required for the many important functions in the human body. Any deficiency in their desired amount leads to hazardous effects that can be prevented by taking adequate supplementation (Fraga *et al*., 2005). Some metals act as anticancer; e.g., complexes of Pt, Au, Ti and Ga are used for the treatment of cancer (Muhammad and Guo, 2014; Hindo *et al*., 2009).

Carcinogenic metals that are present in occupational and general environments are involved in the increased incidence of cancers (Yang, 2011; Lee *et al*., 2012). Groundwater contamination, metal working, leather tanning, and mining are the main

sources of toxic metal exposure (Yang, 2011; Hopenhayn-Rich *et al*., 1998; Katic *et al*., 2010; Wild *et al*., 2009). In addition, different forms of the metals are added into the medicines (Masur, 2011; Ralph, 2011). The toxic metals can induce cancers especially in the kidney, liver, lung, prostate, and skin (Wang and Shi, 2001; Leonard *et al*., 2004). In the higher oxidation state the oxyanions of V, Cr, Mo, As and Se are stable, so they can cross cell membrane using normal phosphate and/or sulphate transport systems of the cell. These oxyanions can inhibit the enzymes that are involved in catalysing phosphoryl or sulfuryl transfer reactions by acting as phosphate or sulphate analogue (Jennette, 1981). Similarly divalent ions of Be, Mn, Co, Ni, Cd, Hg and Pb are stable but they can mimic essential divalent ions such as Mg, Ca, Fe, Cu, or Zn. Thus normal activity of these species is altered and free radicals may be produced which can damage DNA and other critical cellular molecules. These free radicals formed are considered as main reason of the cancer (Dixon *et al*., 1974).

1.3 Health Effects of Selected Metals

1.3.1 Cadmium

Cadmium is mostly contributed by cigarette smoke, paint additives and batteries. It may be introduced in environment through welding, electroplating, pesticide, fertilizer and nuclear fossil plants. It has long biological life (almost 15 to 25 years), thus there are greater chances of its accumulation than other metals. Exposure to Cd can cause osteoporosis, anaemia, emphysema and renal damage. It can also cause kidney damage, bronchitis, gastrointestinal disorder, bone marrow cancer and increased blood pressure (Singh *et al*, 2011). If level of exposure to Cadmium is very high it can cause lung damage which finally leads to lung cancer (Durham and Snow, 2006). There are increased rate of mortality from lung cancer in Cd exposed workers. International Agency of Cancer on Research (IARC) classified it as human carcinogen in 1993, lower level of Cd cause toxicity in kidney but if exposure is prolonged it leads to kidney failure. In Japan certain areas are rich in Cd concentration; people living in that area suffer from a disease known as itai-itai disease, which is characterized by the epidemic of bone fracture (MCcally, 2002; Järup *et al*., 1998). This disease is mixed up of osteoporosis, osteomalacia and kidney damage. Cadmium exposure also becomes a cause of colorectal cancer (Lamprecht and Lipkin, 2003). Elevated levels of Cd can cause prolonged urinary Ca loss, due to which bones become weak and there are more risks of fractures. Cd is also a

nephrotoxicant (Satarug and Moore, 2004). It is also a root cause of cardiovascular diseases (CVD), stroke and heart failure (Peters *et al*., 2010; Navas-Acien *et al*., 2004; Everett and Frithsen, 2008; Navas-Acien *et al*., 2005). Elevated concentrations can also affects cell cycle progression, proliferation, differentiation, DNA replication and repair (Bertin *et al*., 2006). Its exposure can lead to the cancers of prostate, kidney, liver, hematopoietic system and stomach (Waalkes, 2000; Waalkes and Misra, 1996).

1.3.2 Calcium

Major sources of Ca include milk, cheese, meat, grain, vegetables, fruits, egg, legumes, nuts, seeds, fish and poultry. Milk and milk products contribute about 50% of total dietary Ca (Fleming *et al*., 1994). Women with lower Ca intake in their diet may suffer from osteoporosis or bone loss in any stage of life. Calcium and vitamin-D can also reduce the risk of developing diabetes (Pittas *et al*., 2007; De Boer *et al*., 2008). During pregnancy the increase in Ca intake can reduce the chances of high blood pressure and perinatal morbidity or mortality with no long-term adverse effects (Kumar *et al*., 2009). Calcium and vitamin D can adversely affect the cardiovascular events. High level of Ca intake can lead to increased chances of strokes, which can ultimately leads to death. Calcium supplementation in patients with renal impairment accelerate the vascular calcification and there is increase mortality in both dialysis and pre-dialysis populations (Goodman *et al*., 2000; Block *et al*., 2007; Russo *et al*., 2007; Bolland *et al*., 2011). Decrease in Ca concentration can lead to both colon cancer and kidney stones (Newmark *et al*., 1984). Imbalance in Ca nutrition can adversely affect the tooth structure (Krall *et al*., 2001). Acute deficiency of Ca also termed as hypocalcaemia can lead to serious complications such as anxiety, depression, fatigue, impaired memory, intellectual capacity, personality disturbances, neuromuscular irritability, muscle cramps and paraesthesia (Weaver and Heaney, 2007).

1.3.3 Chromium

Major sources of Cr are mine and minerals. It can cause damage to nervous system, fatigue and irritability (Singh *et al*, 2011). The diet contain some amount of Cr such as in pulses, whole grain products, processed meat, dairy products and most fruits and vegetables (Anderson, 1995). Ingestion of Cr lead to stomach ulcer, nausea, vomiting, damage to liver and kidney and ultimately death. Chromium (VI) is most hazardous and major source of cancer (Durham and Snow, 2006). Chromium is very controversial

metal; some people claim that its supplements are beneficial for human health. It is involved in building of muscles, losing weight without dieting and exercising. The supplements are also involved in preventing diabetes, osteoporosis, heart diseases and aging (Nath, 2000). Major sources of Cr are mine and minerals. It can cause damage to nervous system, fatigue and irritability (Singh *et al*, 2011). The diet contain some amount of Cr such as in pulses, whole grain products, processed meat, dairy products and most fruits and vegetables (Anderson, 1995) Chromium inhalation causes irritation of respiratory tract, rhinitis, bronchospasm, and pneumonia (Dayan *et al*., 2000). It can cause oral squamous cell carcinoma. People working in the welding of stainless steel industries are more exposed to fumes of Cr and it is observed that they are mostly suffering from larynx and pharynx carcinoma (Gustavsson *et al*., 1998). Generally Cr (VI) is reduced to Cr (III) because Cr (III) is more stable form. During this conversion many reactive intermediates are formed which are cytotoxic and genotoxic. Cr (VI) is also responsible for mutations in DNA; it is also involved in chromosomal damage and oxidative changes in proteins (Shrivastava *et al*., 2002). It can also cause skin problems such as allergic contact dermatitis, irritant contact dermatitis and necrotic skin lesion (Rowbotham *et al*., 2000). Exposures to elevated Cr-levels are associated with increased lung cancer risks (Smith and Steinmaus *et al*., 2009).

1.3.4 Cobalt

Cobalt is an essential micro nutrient; it is present in the human diet, especially in fish, vegetables and drinking water. It is also present in vitamin B-12 (Taylor and Marks, 1978). It is a cofactor for many enzymes that are involved in amino acid metabolism and DNA biosynthesis (Schwartz, 1975). Excess of Co exposure may lead to acute intoxification, anorexia and vomiting. It can also be cause of lung related diseases which are major occupational problem for the people working in hard metal industries (Lauwerys and Lison, 1992). Low concentration of Co can trigger allergic diseases and can also affect the metabolism of thyroid hormone (Christensen and Poulsen, 1994). High level exposure may lead to serious problems such as heart failure and bronchial asthma (Gheysens *et al*., 1985; Machado *et al*., 2012; Swennen *et al*., 1993). Cobalt can also be used for treatment of anaemia because it stimulates the formation of red blood cells (Baborik and Dusek, 1972). Excessive occupational exposure to Co powder can lead to progressive hearing loss and atrophy of the optic nerve (Meecham and Humphrey, 1991). Excessive exposure can also lead to pulmonary cancer. The carcinogenic effect of Co can be explained based on its

ability to induce DNA damage, to inhibit DNA repair mechanisms and to generate reactive oxygen species (Lee *et al*., 2012).

1.3.5 Copper

Copper is an essential trace metal which is involved in many vital functions. Cu rich foods include legumes, wholegrain, oyster, nuts and dried fruit (Standstead, 1995). Major anthropogenic sources of Cu include mining, pesticide production, chemical industry and metal piping. The inhalation of Cu mist can be a source of congestion of nasal and mucous membrane. Exposure to its fumes may cause nausea, gastric pain and diarrhoea (Stellman, 1998). It can also cause anaemia, liver and kidney damage. Copper is considered as integral part of many enzymes and is responsible for their proper functioning (Harris, 2001). Its deficiency in diet leads to retardation in the growth, defective keratinization, hypothermia, mental retardation, and pigmentation of hair. It can also cause changes in skeletal system and degenerative changes in aortic elastin (Odell, 1982). Its deficiency can also cause heart diseases (Nath, 2000). Absence of Cu based enzyme named tryosinase leads to Albinism which is characterized by the absence of pigmentation in hair, skin and the eye (Guy, 1999). Deficiency in Cu may lead to defective immune system, by impairing the immune cell number and also disturbing their proper function (Turnlund *et al.*, 2004). It is also involved in oxidative damage; it may result in the formation of hydroxyl radical, which is strong oxidizing radical and is capable of reacting many biological molecules, and initiates the oxidative damage (Buettner, 1993). It is also involved in lipid peroxidation in membranes, direct oxidation of proteins and cleavage of DNA and RNA molecules (Halliwell and Gutteridge, 1984; Tapiero *et al*., 2003). Abnormalities in Cu concentration also lead to Menkes and Wilson disease (Danks *et al*., 1973; Tapiero *et al*., 2003).

1.3.6 Iron

Iron is the major component of red blood cells while some significant contribution was found in liver, bone marrow and muscles. The major Fe containing substance is Heme. It is the part of many enzymes (Vasudevan and Sreekumari, 2007). Iron deficiency is the major reason of anaemia which can lead to heart failure. The major clues of Fe deficiency are tiredness, achlorhydria, impaired attention, irritability, and lowered memory (Prashanth *et al*, 2015). However, elevated exposure of Fe may leads to nausea, vomiting, diarrhoea, hepatic damage, diabetes, testicular atrophy, arthritis, cardiomyopathy,

peripheral neuropathy, and hyperpigmentation (Andrews, 1999). Genetic disorders related to Fe are very few; one of such genetic disorder which is related to abnormal Fe contribution can result in Head and Neck carcinoma. Iron may play a huge contribution in oesophageal carcinogenesis (Rajendran *et al*., 1990; Boult *et al*., 2008). Its contribution is significant in infection and immunity. It also plays role in proper functioning of brain, affecting the visual, auditory, learning and interacting behaviours by doing the proper myelination of the neurons in oligodendrocytes (Lozoff *et al*., 2004). Many behavioural developments such as attention processing, inhibition and extraneous motor movements are sensitive to changes in Fe concentration. There are many chances of absorption of Pb and other toxic metals during the deficiency of Fe (Kwong *et al*., 2004; Bressler *et al*., 2004). Excess of Fe can catalyses many reactions that lead to generation of hydroxyl free radicals through Fenton reaction (Toyokuni, 2009). These free radicals can exert intracellular damage, oxidative damage to DNA, proteins and lipids (Aust *et al*., 1985; Kadiiska *et al*., 1995). Iron also plays an important role in oxidative energy production (Haas and Brownlie, 2001).

1.3.7 Lead

Occupational sources of Pb include refining, mining, pipe fitting, plumbing, glass manufacturing, printers, auto repair, battery manufacturing, construction work and plastic manufacturing. There are also risks of Pb exposure for workers of gas stations. Environmental sources of Pb include paints, lead-core candle wicks, soil or dust near roadways, plastic window blinds and plumbing leachate (Sanborn *et al*., 2002). In children Pb poisoning can adversely affect their intellectual and cognitive behaviour (Rosen, 1995). It can replace Ca thus affecting all the Ca mediated processes (Goldstein, 1992). It can bind to many enzymes or with the part of the enzymes, resulting in lose their activity (Morris *et al*., 1988). If children are more exposed to Pb, their gastrointestinal tract and the central nervous system are the most likely to be affected. (Markowitz *et al*., 1999) Children suffer from hyperactivity even at low level of Pb exposure. Number of studies reveals that Pb workers exhibit increased blood pressure or hypertension (Batuman *et al*., 1983; Weiss *et al*., 1986). It also affects vitamin-D metabolism. As far as carcinogenicity of Pb is concerned, it is not still completely resolved by the International Agency for Research on Cancer (IARC). It can induce liver cell proliferation, but Pb-induced liver cell hyperplasia does not support initiation by liver carcinogens (Columbano *et al*., 1987). However, In smelter workers and battery workers there is excess of malignancies at all

sites especially the lungs (Cooper *et al*., 1985; Goyer, 1990).

1.3.8 Magnesium

Magnesium acts as a cofactor for more than 300 enzymes. It is considered vital for many cellular functions, including protein synthesis, enzymatic reactions, and the regulation of ion channels. It also plays important role in cardiovascular, neurological, and metabolic functions (Ago *et al*., 2016). It plays a significant role in maintaining effective homeostatic regulation, phosphorylation reaction, energy transfer, protein synthesis, lipid and carbohydrate metabolism. Foodstuff from plant origin contains very higher concentration of Mg, and about 50% of the human requirement is fulfilled by these foodstuffs. Excess of blood level lead to nacrosis, hypertension and respiratory paralysis. Immune response to an antigen is mainly controlled by Mg (Guy, 1999). Any disturbance in normal Mg level can result in neuromuscular exutibility, heart dysrhythmia and cardiovascular disease. It is also in involved in blood coagulation process (Seiler and sigel, 1994). Its deficiency is related to increased production of cytokines and reactive oxygen species (Bussiere*et al*., 2002; Song *et al*., 2005). Its deficiency can also cause stress, neuromuscular hyper excitability, hyperirritability and cardiovascular manifestation in human.

1.3.9 Manganese

Manganese is considered as nutritionally essential metal, It acts as activator and also a part of many enzymes (Goyer and Clarkson, 2001; Goyer and Golub, 2004). It can perform many important cellular functions (Sigel, 2000). Manganese is present naturally in rocks, soil and water. It can be added in the environment through welding, fuel addition and ferromanganese production (Singh *et al*., 2011). It can be found in nuts, grains, and cereals. Coffee and tea also contain high level of Mn (Burch *et al*., 1975). The deficiency of Mn can lead to bleeding disorders due to increased prothrombin time, while its excess can cause anorexia, apathy, headache impotence, leg cramps, speech disturbance, and encephalitis and Parkinson disease (Prashanth *et al*., 2015). Manganese exposure can affect the nervous system but there is no hazardous effect at low (Santamaria, 2008; Mergler, 1999). If Mn exposure is very high it will slow down the development in children and their intellectual function is diminished. Consumption of water which has higher Mn concentration will lead to hyperactive and oppositional behaviours (Bouchard *et al*., 2007; Ericson *et al*., 2007; Wasserman *et al*., 2006; Kim *et al*., 2009).

1.3.10 Potassium

Potassium is very important in neurotransmission, muscle and enzymatic activity. It is classified as essential macronutrient. Deficiency of K is termed as hypokalaemia, which results in muscular weakness, increased nervous irritability, disorientation, cardiac irregularities and ultimately paralysis and respiratory failure (Guy, 1999). Osmoregulation and membrane resting potential are controlled by K (Seiler and Sigel, 1994). Higher K concentration is known as hyperkalaemia, which may result in nausea, decreased urine production and cardiac arrest. Due to its deficiency skin become dry and acne start to appear. Deficiency of K also cause muscle weakness and slow reflexes which ultimately lead to nervous disorder, irregular heartbeat and loss of gastrointestinal tone. Potassium plays a major role in cellular biochemical reactions such as carbohydrate metabolism and energy metabolism. It can convert glucose to glucagon, which can be stored in liver for future energy (Sigel, 2013). Higher concentration of K can lower the blood pressure in normotensive and hypertensive people; thus it helps in maintaining the blood pressure at normal level (He and MacGregor 1999; He *et al*., 2012). It also reduces urinary Ca excretion, which in turn reduces the risk of kidney stones and prevents bone demineralization (Lemann *et al*. 1991, 1993). Higher serum concentration of K also decreases the risk of ventricular arrhythmias in patients with Ischaemic heart disease, heart failure, and left ventricular hypertrophy (Yusuf *et al*. 2004; McKee *et al*., 2006; He and MacGregor, 2008).

1.3.11 Sodium

Processed foods, including breads, Cereals and grains, contributed heavily to Na intake (Anderson *et al*., 2010). In human body Na is mostly present in the form of cation acting as an electrolyte. It is important in maintaining osmolality. It is also involved in controlling the water balance. Long term use of Na leads to stroke and coronary heart diseases, while its deficiency leads to enteric, renal and adrenal disease. Excess of Na leads to osteoporosis, gastric cancer and bronchial reactivity. It also plays a major role in cardiovascular issues (Caballero, 2009; Whelton *et al*., 2012). High concentration of Na is termed as hypernatremia, which leads to excessive thirst and lessened urine production. Very low concentration of Na is termed as hyponatremia, which leads to headache, confusion, muscle spasm, nausea, seizure and vomiting (Sigel, 2013). Table salt is the major cause of high blood pressure which can lead to other serious problems so it is

generally recommended to decrease the salt intake in order to control blood pressure. It can be replaced by the mineral salt that is low in Na concentration but contain high levels of K thus reducing the problems of blood pressure and cardiovascular disease (Anderson *et al*., 2010; He and MacGregor., 2012). Excess of Na can be a cause of kidney stones, asthma, osteoporosis (Paul *et al*., 2012; He and MacGregor, 2010).

1.3.12 Strontium

Strontium plays a major role in endocrinology, and it mimics the action of Ca in many cases (Nielsen, 2004). It is effective for bones and enhances the bone health. Absorption of both Ca and Sr is stimulated by vitamin-D, and they also share same physical characteristics (Holick, 2004). It reduces the risk of osteoporosis. Excessive intake of Sr may result in nausea, diarrhoea, skin irritation and headache. However, it is very effective in reducing vertebral and non-vertebral fractures especially in women. It increases the bone formation and bone mineral density by decreasing bone re-sorption (Marie *et al*., 2001; Meunier *et al*., 2004; Reginster *et al*., 2005). One of the causes of lung cancer is strontium chromate especially the occupational exposure in industries. Any disturbance in Sr concentration leads to a disease called rickets. It is also used for the treatment of osteoporosis or any kind of bone mineralization. Some of its salts can cause coughing and shortness of breath (Watts and Howe, 2010).

1.3.13 Zinc

Zinc is an essential metal for Human. It is involved in three main functions; catalytic, structural and regulatory functions (Cousins *et al*., 1996). Major sources of Zn are cereal, pulses, dairy products, meat, fish, fruits, cakes, poultry, whole grains, leafy grains, root vegetables, shell fish, oysters and lobster (Deshpande *et al*., 2013). Occupational sources of Zn include refineries, metal plating, plumbing and brass manufacturing units. Its deficiency is mainly observed in vegetarian diet because the plant constituent such as dietary fibres and lignin can bind the Zn, thus inhibiting its absorption (Singh *et al*., 2011; Nriagu, 2007). Deficiency of Zn can affect epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems (Hambidge and Walravens, 1982). Differentiation, proliferation and metabolic activity of cell are mainly controlled by Zn (Prashanth *et al*., 2015). It is important constituent of DNA binding protein and reproductive tract (Seiler and Sigel, 1994). Zinc supplements are used to cure childhood diarrhoea, treatment of malaria, in the postsurgical wounds and pneumonia (Sigel, 2013;

Bhutta *et al*., 1999; Roohani *et al*., 2013). Its deficiency can lead to respiratory and skin allergies, asthma, chronic diarrhoea, abnormal neurosensory changes, poor appetite, mental lethargy, fertility problems, birth defects; growth failure, growth retardation, premature aging, vision problems, loss of taste, joint pain, hypertension, angina pectoris, delayed wound healing, scleroderma, systemic scleroderma, loss of hair colour, anaemia, night blindness, acne, defective connective tissue, macular degeneration, apathy and irritability (Nriagu, 2007). Zinc also act as an antioxidant and involve in stabilization of membranes. It can prevent the free radical induced injury during inflammatory processes. It also decreases the oxidative stress and inflammatory cytokines (Prasad, 2008). Excess of Zn can also prove toxic and lead to impaired immune response, hypocupraemia, microcytosis, neutropenia, nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea and headaches (Deshpande at al., 2013).

1.4 Biological Specimen used for Analysis

Due to industrialization, toxic pollutants increase significantly, thus it became a major concern to assess these pollutants which are deleterious for human health. For this purpose, various biological specimens have been used in past; some of the most commonly used specimens are blood, plasma, serum, hair, nails, urine, saliva and sweat (Agahian *et al*., 1990; Zaprianov, 1992). Monitoring the exposure of the essential and toxic metal has critical importance in human health (Angerer *et al*., 2007; Parsons and Barbosa, 2007). The choice of biological specimen depends on various factors such as toxicokinetics, the convenience or invasiveness of the specimen collection procedure, and the potential for specimen contamination (Wilhelm *et al*., 1994; Nowak and Chmielnicka, 2000; Wilhelm *et al*., 2002; Pereira *et al*., 2004; Barbosa *et al*., 2006a,b; Slotnick and Nriagu, 2006).

Scalp hair can be used for monitoring environmental and occupational exposures of different metals and pollutant. Its sampling is non-invasive. Moreover it is easy to store and transport. Handling of the hair sample is less hazardous (Wang *et al*., 2009). It is an effective tool for evaluation of metals and other pollutants because it reflects exposure from a long-term period. Many metals are present in high concentrations in the hair strands thus it can be used for analysis of trace metals on large cohort (Rodrigues *et al*., 2008; Morton *et al*., 2002; Pereira *et al*., 2004). Nevertheless, hair analysis has certain limitation such as the occurrence of exogenous contamination, which can interfere with

analysis making it less reliable (Bencze, 1990; Miekeley *et al*., 1998; Frisch and Schwartz, 2002). Another disadvantage of hair analysis is the lack of scientific knowledge about the kinetics of trace metal incorporation in it and there is insufficient epidemiological data that can support the predictions concerning health effect of a particular metal concentration (Bozsai, 1992; Seidel *et al*., 2001; Harkins and Susten, 2003)

Human nail is recognized as a valuable tissue for the analysis of human environmental exposure. It provides a good indication of exposure to various toxic and essential metals over a period of time (Nowak and Chmielnicka, 2002; Samatha *et al*., 2004). Concentration of trace metals in the nail tissue are higher than those of body fluids and other accessible tissues (Rodushkin and Axelsson, 2000; Sukumar and Subramanian, 2007). Growth of the nail is a continuous process throughout the life, nails reflect the total metal body burden and it can provide a continuous record of trace metal concentrations (Barbosa *et al*., 2005; Were *et al*., 2008; Wilhelm and Hafner 1991). Sampling and analysis of the nail is easy but it requires prewashing (Mehra and Juneja, 2005).

Blood is slightly basic having pH in the range of 7.35 to 7.45 (Marieb, 1998). It is actually a connective tissue, which consists of plasma and formed elements. Plasma is extracellular fluid while formed elements include erythrocytes (known as red blood cells), leukocytes and platelets. These are called formed elements because they are enclosed in a plasma membrane which give them definite shape and structure (Saladin, 2004). It is a transport medium for various gases, hormones, nutrients as well as toxic substances in the biological systems. It also transports the waste products to liver and kidney so that they can be detoxified or removed from the body (Sherwood, 2004). Metals in the blood are mostly bound to proteins and red blood cells, in order to perform specific transport functions such as transferring metallothionein and haemoglobin. Blood is an ideal matrix for trace metal research because of its significance and ease of sampling. It is in contact with all tissue and organs where metals are deposited. It provides the information about the materials which are recently absorbed by the body (Pasha *et al*., 2010b; Ilyas and Shah, 2015). As hair and nails samples require washing but blood does not need any washing prior to analysis thus it is mostly free of external contamination. Blood should be stored below freezing point and the stored blood can be infectious, therefore should be carefully handled (Cocciardi, 2012).

1.5 Cancer Incidence in Pakistan

Pakistan, like other developing countries faces a double burden of diseases with a significant incidence of cancers and a rising trend in risk factors' profile and incidence itself (Ferlay *et al*., 2004; Bhurgri *et al*., 2000). Gallbladder cancer cases are more common in females than in males; these are actually related to gall bladder stones. Breast cancer is the more common type of the cancer in females all over the country (Bhurgri *et al*., 2005; Bhurgri *et al*., 2002). However, in Quetta oesophageal cancer is more common type, and breast cancer is second most common cancer. Ovary cancer is the sixth most common type of cancer while prostate cancers are very less in number (Bhurgri *et al*., 2003; Bhurgri *et al*., 2000). The most common malignancies in males are oral cavity, lung, urinary bladder, and larynx and in females the common types are oral cavity, breast, and ovary. There has been an increase in cancer incidence for lung, larynx, and urinary bladder in males and breast, oesophagus, and cervix in females (Bhuegri *et al*., 2006). The main risk factors of oral cancer in Pakistan are smoking, areca nut, betel quid or paan, tobacco chewing, naswar, paan masala, gutka and poor nutrition (Bhurgri *et al*., 2006). Pakistan has one of the highest rates of consanguinity in the world (Hashmi, 1997). Inbreeding is known to increase the risk of diseases caused by homozygosity of deleterious recessive genes. Parental consanguinity has been implicated in 60% of mortality and severe morbidity in Pakistani children born in Britain. An excess of childhood cancers are reported among the children of consanguineous marriages (Powell, *et al*., 1995). There is little information on the possible role that recessive genes play in adult cancers. One study from Pakistan has described an association between consanguinity and risk of breast cancer (Shami *et al*., 1989).

The study of thyroid cancer in Agha Khan Hospital, Karachi showed that out of 8541 tumour cases, 1.2% are of thyroid cancer. Different types of thyroid cancer were prevailing as papillary carcinoma, follicular carcinoma, medullary and anaplastic carcinoma (Shah *et al*., 1999). Papillary carcinoma is the major type in the thyroid cancer incidents (Zuberi *et al*., 2004).

There are many chances that multinodular goitre is converted into thyroid carcinoma (Memon *et al*., 2010). Globally, thyroid cancer is relatively uncommon (Zuberi *et al*., 2004; DeGroot and Jameson, 2001).

1.6 Aims and Objectives

The aim of the present study is to evaluate the concentrations of selected essential and toxic metals in thyroid cancer patients and their counterpart healthy donors with comparable age, gender, socioeconomic status, smoking habits and food habitats. Following are the major objectives of the study.

- To assess the comparative distribution of selected essential and toxic metals in the blood of thyroid patients and healthy donors.
- To find out the mutual relationship among the essential and toxic metals in cancer patients and healthy donors using correlation coefficients.
- To explore the multivariate apportionment among the metal in the blood of the patients and controls by multivariate methods.
- To compare the concentrations of selected metals in different stages/types of thyroid cancer
- To explore the role of selected metals towards the thyroid cancer so that it may be helpful in treatment of thyroid cancer in near future.
- To compare the measured metal levels with the reported levels in literature.

Chapter 2

EXPERIMENTAL METHODOLOGY

2.1 Study Population

Subjects included in the present study were thyroid cancer patients and healthy donors. There was no compelltion on the subjects to be a part of this research. All the participants were selected on volunteer basis and written consent was obtained from all subjects before sample collection. It was assured to every subject that the information collected from them will remain confidential. The blood samples were collected from both the patients and healthy counterparts. A questionnaire was filled in by the patients and healthy donors to record information such as gender, age, residence, occupation, smoking habits, diet, disease type, duration, medication and any other disease. The patients and their counterpart control were selected on the basis of similar gender, socioeconomic status, food habits, smoking habits and almost same age (Kazi *et al*., 2014).

2.1.1 Cancer Patients

Thyroid cancer patients of different ages, gender, food habits, smoking habits and adobe were included in the present study. Blood samples were collected from the patients admitted in Nuclear Oncology and Radiotherapy Institute (NORI), Islamabad. Before the sample collection protocol of the study was approved by ethical review committee of the institute. The selected donors were newly diagnosed thyroid cancer patients without any treatment such as radiotherapy or chemotherapy. They were not taking any kind of mineral supplements as well (Khuder *et al*., 2014; Afridi *et al*., 2014; Pasha *et al*., 2010). Blood samples were collected from a total of 70 thyroid cancer patients on volunteer basis. Clinical diagnosis was confirmed in the institute by histopathological examinations of all the patients.

2.1.2 Controls

The controls were not suffering from any type of cancer. They were not taking any kind of mineral supplements on regular basis. Mostly the controls had close relationship with the patient, thus they had similar socioeconomic status and environmental exposure. Most of the controls were matched with the patients regarding age, gender and food habits.

A total of 70 blood samples were collected from the controls (Qayyum *et al*., 2014; Pasha *et al*., 2010). A Proforma filled to record the information regarding the donors name, age, sex, social and general health status, nutrition habits, job description, socioeconomic status past diseases etc. at the time of sample collection. The demographic details related to patients and controls are shown in Table 1.

Table 1. Demographic characteristics of the subjects

Characteristics	Thyroid Cancer Patients	Healthy Donors/Controls
n	70	70
Age (years)		
Range	15-82	18-78
Mean	48.5	48.0
Gender		
Female	39 (56%)	39 (56%)
Male	31 (44%)	31 (44%)
Diet		
Vegetarian	46 (66%)	42 (60%)
Non-vegetarian	24 (34%)	28 (40%)
Habitat		
Urban	40 (57%)	37 (53%)
Rural	30 (43%)	33 (47%)
Tobacco Use (Smoking)		
No use	28 (40%)	46 (66%)
Use	42 (60%)	24 (34%)
Stages of the Cancer		
Stage I	21 (30%)	--
Stage II	16 (23%)	--
Stage III	14 (20%)	--
Stage IV	19 (27%)	--

2.2 Collection/Storage of the Samples

There are many chances of contamination during blood collection; it may originate from the skin of the subject or from the hands of the person who is collecting the sample. There are also chances of contamination during storage or processing of these samples .So in order to avoid any external contamination, especially designed evacuated tubes are used for the collection of blood. Before collecting the blood sample from the subjects their skin was properly cleaned. Separate syringes should be used for each subject. The blood sample is collected from forearm antecubital vein. Needle is carefully inserted into the vein and the blood sample is drawn into the plastic syringe (Gonzalez *et al*., 2008). Almost 3 mL of blood should be collected and transferred to the blood collection tube. Some of the method reported in literature for the collection and storage of the blood samples are discussed below:

- About 5 ml blood sample was withdrawn from the antecubital vein by vein puncture method. It was then transferred to the blood collecting tube containing lithium-heparin. The samples collected were then stored at -80°F until the further processing (Ladon *et al*., 2004).
- The blood sample was drawn by vein puncture under contamination controlled conditions. The Vacuette system was used for collection of blood. The blood collection tubes were made up of polyethylene terephthalate (PET) plastic. These tubes contained EDTA as an anticoagulant. Samples were analyzed within twelve hours after the collection (Banks *et al*., 2005).
- About 9 mL of blood sample was withdrawn from the subjects and transferred to lithium heparin monovettes. To avoid any contamination, blood in the first monovette were always removed and the blood in a second monovette was used for further analysis. About 20 monovettes were leaches with deionized water and other 20 with 3% (V/V) nitric acid solution for 48 hours, so that any contamination from the tube and anticoagulant agent can be removed. These blood samples were stored at 4°C and then analysed after 2 days (Heitland and Koster, 2006).
- Whole blood samples were collected from the adults in a blood bag that contain an anticoagulant agent citrate phosphate dextrose adenine solution (14%, w/v). Almost 5 mL of the blood was transferred to polyethylene centrifugal tubes, and properly weighed. The samples were spiked with the appropriate volume of standard multi element working solutions and left to equilibrate in a water bath for

1 h at 37°C. After that, a small amount of water (about 2 mL) was used to quantitatively transfer the spiked samples into PTFE tubes for the digestion (Daftsisa and Zachariadis, 2007).

- Blood samples were taken from an antecubital vein. Proper precautions were taken during this process so that to avoid contamination with exogenous metals (Aitio *et al*., 1994). Approximately 3 mL venous blood was withdrawn and transferred to the evacuated blood collection tubes. Each sample was gently shaken by hand and centrifuged at 2000 rpm for 15 minutes. The serum was separated carefully by using Finn pipette into another clean polyethylene vial duly labelled with relevant codes related to the donor's name, age, eating and drinking habits, adobe, social and general health status, all recorded and compiled on regular Proforma at the time of sampling. Samples were stored at −70°C before further processing (Chappuis *et al*., 1994; Subramanian *et al*., 1995).
- Venous blood samples (5 mL) were collected by using metal-free vacationer EDTA tubes. Then, 2 mL of venous blood sample of each study subjects was stored at −4°C until required for analysis, while the remaining (3 mL) was used for separating the sera (Arain *et al*., 2014).
- The blood samples were collected from the median cubital vein without anticoagulants and placed in vacationer tubes. Serum was separated by centrifugation at 14000 rpm for 10 min. The serum samples were stored at −20°C until digestion (Golasik *et al*., 2015).
- Venous blood sample (10 mL) was collected using heparinized vacutainer tube and the sample was stored at -20°C until elemental analysis. The blood was allowed to clot at room temperature for 15-30 min. When the blood was clotted completely then centrifuged for 5-10 min at 2500 rpm. The supernatant fluid was then separated by a Pasteur pipette, labelled and stored at - 20°C until analysis (Kolachi *et al*., 2012; Kazi *et al*., 2008; Tim *et al*., 2006).

The sample collection method used in the present study is described below:

Proper precautions were taken in order to avoid any kind of external contamination. For the collection of blood, specially designed evacuated tubes were used. Skin of subjects was properly cleaned with 70% isopropyl alcohol. Blood sample was collected from the antecubital vein by vein puncture method. Approximately 2 to 3 mL blood was taken from each subject. BD syringes (5 mL) were used for the blood collection. The samples were stored at -15°C until further analysis.

2.3 Digestion of the Sample

Digestion is done to remove the organic content of the blood so that it should not be problematic during analysis. Some of the methods reported in literature for the digestion of blood are listed below:

- Blood samples were transferred to 50 mL closed polytetrafluoroethylene (PTFE) tube. Approximately 5 mL of concentrated HNO_3 was added to each sample. For the decomposition of organic matter these samples were allowed to heat at 120 ± 1 °C for 1 h. The tubes were cooled and opened when the internal temperature was under 30°C. These digested samples were then transferred to 25 mL volumetric flasks and diluted with deionized water (Daftsisa and Zachariadis, 2007).
- Sample of whole blood (0.5 mL) was directly taken into PTFE flasks. Then, 3 mL of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) was added and kept at room temperature for 10 min. Then, placed the flasks in covered PTFE container and heated at 80 % of total power (900 W) for 3– 4 min. The digested samples were diluted up to 10 mL with 0.1 M HNO_3. A blank extraction was carried out through the complete procedure (Blazweic*et al*., 2010; Prystupa *et al*., 2016; Arain *et al*., 2014).
- Blood was directly taken into Pyrex flasks. 3 mL of a freshly prepared mixture of concentrated HNO_3-H_2O_2 (2:1, v/v) was added to each flask and stood for 10 min, then the flasks was covered with watch glass and digested at 60-70°C for 1-2 h. The digests was then treated with 2 mL HNO_3 and few drops of H_2O_2, heated on hot plate about 80°C until the clear digested solution was obtained. Evaporated the excess of acid mixture to semidried mass, cooled and diluted up to 10 mL with 0.1 M HNO_3. For validation of methodology certified reference materials of whole blood were also analysed (Kolachi *et al*., 2012).
- Polyethylene bottles were used for the blood collection and the sample was then stored in refrigerator. For the digestion of sample, sulphuric acid and nitric acid were added in 1 mL of blood sample. These samples are then heated nearly to dryness. Distilled water was used for dilution of the residue. It was then filtered through Whatman filter paper. A 30 µL sample solution was used for the determination trace metals (Shrivas and Patel, 2009).
- The blood sample (1 mL) was mineralized with 5 ml concentrated HNO_3 and 1 mL

of 30% H_2O_2 in a microwave oven. After mineralization, the solution was evaporated to the volume of 0.1 ml and diluted with deionised water to a final volume of 5 mL (Memon *et al*., 2007; Batariova *et al*., 2006).

The method used for the digestion of blood sample in the present study is as follows:

For the digestion of the blood sample, it was transferred from the storage tube to a digestion flask. Sample was correctly weighed on weighing balance, and then digested with nitric acid and perchloric acid (10:1, v/v) mixture. Samples were heated on hot plate at 80°C to a soft boil until white dense fumes evolved. The fumes marked the completion of the digestion. Samples were then cooled to room temperature and diluted to proper volume (50 mL) with 0.1 N HNO_3. Blanks containing all the reagents in the same sequence (without blood sample) were also processed with each batch of the samples.

2.4 Quantification of the Metals

Atomic absorption spectrometry (AAS) is an analytical technique used to quantify the metals at trace and ultra-trace level. It has many applications including clinical analysis, environmental analysis and biological analysis. Moreover it is used in pharmaceuticals, industry and also in mining. In the present study, selected essential and toxic metals including Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Pb, Sr, Na, K and Zn were measured on flame atomic absorption spectrophotometer (Shimadzu AA-670, Japan). Various analytical conditions such as detection wavelength, hollow cathode lamp current, slit width, flame type and fuel/oxidant flow rates were optimized for the analysis of each element independently as shown in Table 2. The instrument has many advantages; high speed, dual frequency and simultaneous photometric system. It has the capacity to operate automatically in background compensation mode thus correcting all fluctuations in the observed signal arising from sources other than the sample. It has automatic operation conditions. Calibration lines are automatically recorded, thus ensuring the high analytical precision and accuracy. Lamp position, detector gain and beam balance, are all adjusted automatically with convenience to exclude deviant data in repeated analytical modes. The digitalized output can be held for transient signals in the memory storage for further reproduction. Other techniques that can be used for analysis of metals are atomic emission or fluorescence spectroscopy and inductively coupled plasma emission or mass spectrometry (Helaluddin *et al*., 2016).

Table 2. Optimum analytical conditions maintained on AAS (Shimadzu AA-670, Japan) for the analysis of selected essential and toxic metals using air-acetylene flame

Metal	Wavelength (nm)	HC lamp current (mA)	Slit width (nm)	Fuel-gas flow rate (L/min)	1% Absorption concentration (ppm)
Ca	422.7	6.0	0.5	2.0	0.08
Cd	228.8	4.0	0.3	1.8	0.02
Co	240.7	6.0	0.2	2.2	0.20
Cr	357.9	5.0	0.5	2.6	0.09
Cu	324.8	3.0	0.5	1.8	0.09
Fe	248.3	8.0	0.2	2.0	0.10
K	766.5	5.0	0.5	1.9	0.04
Mg	285.2	4.0	0.5	1.6	0.007
Mn	279.5	5.0	0.4	1.9	0.05
Na	589.0	6.0	0.5	1.6	0.02
Pb	217.0	7.0	0.3	1.8	0.20
Sr	460.7	4.0	0.5	1.6	0.10
Zn	213.9	4.0	0.5	2.0	0.02

2.5 Statistical Analysis

The quantified results of the metal levels were subjected to both univariate and multivariate statistical analysis, which was performed using MS Excel and STATISTICA software. Basic statistical parameter, including minimum, maximum, median, standard deviation (SD), standard error (SE), kurtosis and skewness were computed alongwith the correlation analysis. They can provide us information about the relative distribution pattern and mutual relationships of the metal levels in the analysed samples. Multiple relationships between different metals present in the blood samples of thyroid cancer patient and healthy donor was also performed using multivariate statistical methods, including principal component analysis (PCA) and cluster analysis (CA). One can classify the relationship among the measured variables by using multivariate statistical methods (Hua *et al*., 2006). Multivariate techniques have many advantages. It has been successfully applied to levels of trace elements in human hair to distinguish between healthy people and cancer patients (Qayyum and Shah, 2014). Recently applied multivariate statistical

techniques have been used on hair metal data to distinguish between drug-free subjects and drug abusers (Boumba *et al*., 2006). Other notable examples of their use in environmental chemistry are found in (Lopes *et al*., 2009) the trace metal analysis in water, soil and sediments (Zimmerman and Weindorf, 2010). The multivariate techniques have also been used for source identification and apportionment of trace metals in analysis of scalp hairs (Shah *et al*., 2006). The two procedures used in the present investigation are outlined below.

2.5.1 Principal Component Analysis

Principal component analysis (PCA) rotates the data-set such that maximum variables are projected onto the axes and it is a multivariate procedure. Essentially, a set of correlated variables is transformed into a set of uncorrelated variables which are ordered by reducing variability. The uncorrelated variables are linear combinations of the original variables, and the last of these variables can be removed with minimum loss of real data. The main use of PCA is to reduce the dimensionality of a data-set while retaining as much information as possible. It computes a compact and optimal description of the data-set. The first principle component (PC) is the combination of the variables that explains the greatest amount of the variance. The second PC defines the next largest amount of the variation and it is independent to the first PC. There can be as many possible PCs as there are variables. It can be viewed as a rotation of the existing axes to new positions in the space defines by the original variables. In this new rotation, there will be no correlation between the new variables defined by the rotation. The first new variable contains the maximum amount of the variations unexplained by the rest.

There are several algorithms for calculating the principal components. Given the same starting data they produce the same results with one exception; which is that, if at some point, there are two or more possible rotations that contain the same “maximum” variations, and then which one is used is indeterminate. In two dimensions, the data cloud would look like a circle, instead of an ellipse. In a circle, any rotation would be equivalent. In an elliptical data cloud, the first component would be parallel to the major axis of the ellipse. It can be viewed as finding a projection of the observations onto orthogonal axes contained in the space defined by the original variables. The criteria being that the first axis “contains” the maximum variation, or “accounts” for the maximum amount of the variation. The second axis contains the maximum amount of variation orthogonal to the first and the third axis contains the maximum variation orthogonal to the first and second

axis and so on until one has the last new axis which is the last amount of variation left. PCA was applied to assist the identification of sources of trace metals, and it can show the multiple relationships among the analyzed metals (Yongming *et al*. 2006).

2.5.2 Cluster Analysis

Cluster analysis (CA) classifies a set of observations into groups based upon combinations of internal variables in the form of dendrogram. This technique is a classification procedure that involves a measurement of the similarity between the variables. The purpose of cluster analysis is to discover a system of organizing observation where member of the groups/variables share the properties in common. The variables are grouped in the cluster in terms of their nearness or similarity. The measurement of similarity is based on Pearson-r distance. The clustering method used in present study was Ward's method, which considers the heterogeneity or deviance (sum of square of distance of variables from the baricenter of the cluster) of every possible cluster that can be created by linking two existing clusters. Therefore, it is cognitively easier to predict the mutual properties based on overall group membership (Prystupa *et al*., 2016; Pasha *et al*., 2010).

Chapter 3

RESULTS AND DISCUSSION

3.1 Distribution of Selected Metals

Basic statistical parameters for the distribution of selected essential and toxic metal levels (µg/g, wet weight) in the blood samples of thyroid cancer patients are shown in Table 3. A wide range of concentrations as shown by the minimum and maximum levels were exhibited by most of the metals. Considerably higher mean levels were observed for Na (1404 µg/g) and K (1322 µg/g), followed by moderately higher levels of Fe (309.6 µg/g), Ca (35.6 µg/g), Mg (25.94 µg/g), Co (25.67 µg/g), Pb (18.06 µg/g), Cr (14.68 µg/g), Zn (11.18 µg/g), Sr (10.38 µg/g) and Cd (9.304 µg/g), while the least concentrations were found for Mn (3.473 µg/g) and Cu (2.050 µg/g) in the blood of thyroid cancer patients. Overall the average concentrations of metals showed following decreasing order: Na > K > Fe > Ca > Mg > Co > Pb > Cr > Zn > Sr > Cd > Mn > Cu.

Table 3. Statistical distribution parameters for the concentrations of selected essential and toxic metals (µg/g) in the blood of thyroid cancer patients

	Min	Max	Mean	Median	SD	SE	Kurtosis	Skewness
Ca	10.39	90.01	35.65	31.35	16.57	1.981	0.695	0.877
Cd	0.048	60.03	9.304	5.987	11.41	1.416	7.605	2.541
Co	0.780	76.45	25.67	17.21	21.37	2.651	-0.090	0.960
Cr	0.042	72.35	14.68	7.055	17.70	2.247	1.895	1.644
Cu	0.027	11.15	2.050	1.494	1.818	0.226	8.961	2.384
Fe	168.6	884.1	309.6	288.1	139.9	16.72	5.848	2.219
K	285.0	3243	1322	1026	728.7	87.10	0.710	1.251
Mg	3.601	52.06	25.94	27.18	10.28	1.229	-0.196	-0.028
Mn	0.022	15.36	3.473	2.186	3.654	0.476	2.012	1.494
Na	681.7	2877	1404	1349	424.7	50.76	2.733	1.408
Pb	0.110	62.54	18.06	11.80	16.56	2.233	-0.185	0.986
Sr	0.026	47.21	10.38	6.839	9.806	1.299	2.338	1.406
Zn	0.616	47.97	11.18	6.847	10.41	1.254	2.071	1.547

A random distribution pattern was exhibited by the most of the metals as demonstrated by relatively higher SD and SE values on one hand and distinctly dissimilar mean and median levels on the other hand. Relatively large spread of the concentration was observed in the case of Na, whereas some of the metals (Cu, Mg, Mn, Pb, Sr and Zn) exhibited moderately normal distribution as supported by relatively lower SE and SD values. Large skewness and kurtosis values for Cd, Cu, Fe, Na, Sr, Zn and Mn indicated their significant asymmetric distribution while modest skewness values of Ca, Co, K, Mg and Pb showed moderately symmetrical distribution of these metals in the blood of thyroid cancer patients.

Table 4. Statistical distribution parameters for the concentrations of selected essential and toxic metals (µg/g) in the blood of healthy subjects

	Min	Max	Mean	Median	SD	SE	Kurtosis	Skewness
Ca	10.77	85.13	29.40	25.80	15.87	1.911	3.695	1.877
Cd	0.189	37.73	10.29	7.946	9.764	1.230	-0.233	0.886
Co	3.329	74.49	38.36	38.73	18.28	2.304	-0.966	-0.081
Cr	0.080	46.22	13.66	11.930	11.73	1.582	0.510	1.077
Cu	0.170	6.96	2.239	1.949	1.409	0.179	1.257	1.133
Fe	101.3	490.0	255.0	211.1	107.3	13.21	-0.875	0.647
K	136.0	3094	1003	871.0	645.9	77.76	1.966	1.448
Mg	9.354	42.93	23.89	23.79	7.828	0.942	-0.735	0.144
Mn	0.115	18.38	7.445	6.698	5.369	0.671	-1.171	0.306
Na	144.0	2361	1325	1346	432.8	52.10	0.004	-0.176
Pb	0.108	56.01	21.04	14.41	15.73	1.982	-1.028	0.600
Sr	0.175	49.49	12.85	9.082	11.26	1.441	1.962	1.511
Zn	0.201	47.90	13.13	8.834	12.69	1.562	1.103	1.362

Basic statistical parameters for the distribution of selected essential and toxic metal levels (µg/g, wet weight) in the blood of the controls/healthy subjects are shown in the Table 4. Very broad range of concentrations was exhibited by most of the metals as shown by their minimum and maximum levels. On the average basis, significantly higher concentrations were observed for Na (1325 µg/g), and K (1003 µg/g), followed by moderately higher levels of Fe (255 µg/g), Co (38.36 µg/g), Ca (29.40 µg/g), Mg (23.89

µg/g), Pb (21.04 µg/g), Cr (13.66), Zn (13.13 µg/g), Sr (12.85 µg/g) and Cd (10.29 µg/g). However, Mn (7.445 µg/g) and Cu (2.239 µg/g) were found at the lowest levels. The selected metals in the blood of normal donors showed following decreasing order in their average concentrations: Na > K > Fe > Co > Ca > Mg > Pb > Cr > Zn > Sr > Cd > Mn > Cu. A random distribution pattern was displayed by the most of the metals as established by their elevated SD and SE values as well as markedly dissimilar mean and median levels in the blood of controls. Comparatively large dispersion in the concentration was observed for K, Na and Fe while some of the metals (Cu, Mn, Mg and Cd) exhibited relatively normal distribution pattern supported by somewhat lower SE and SD values. Large skewness and kurtosis values for Ca, K and Sr indicated their predominantly asymmetric distribution while Cd, Co, Mg and Na showed relatively symmetrical distribution in the blood of healthy subjects. Overall, most of the metals showed non-Gaussian distribution in the blood of healthy subjects but the extent of randomness was found to be comparatively less than those of the patients which showed reasonably higher randomness in their concentrations.

The quartile distribution of selected essential and toxic metals (in the form of box-whisker plot) in the blood of thyroid cancer patients and healthy donors is shown in Figures 1 and 2, respectively. Most of the metals demonstrated relatively broad and asymmetrical distribution in the blood of both donor groups; nevertheless, relatively narrow distribution was observed for Na, Fe, Mg, K and Ca in the blood samples of cancer patients. Conversely, Pb, Co and Zn exhibited rather broad variations in the blood samples of cancer patients. Interestingly, Cu, Cr, Cd, Mn and Sr showed overlapping of outer quartiles thus exhibiting rather lower extremum variations and consistent concentrations towards higher and lower ranges in the cancer patients. Such imbalances in the metal levels may be ascribed to the disproportions of the essential nutrients and toxic metals in the thyroid cancer patients. In the case of healthy subjects, very broad range and asymmetric variations were noted for Pb, Cr, Sr, Zn, Mn and Cd while least variations were observed for Mg, Fe, Ca and Na. Relatively narrow and somewhat symmetric distribution was noted for Cu, K and Co in the blood of healthy donors (Figure 2).

The average metal concentrations in the blood of thyroid cancer patients and healthy donors were also compared as shown by bar-graph in Figure 3. On comparative basis, average concentrations of Mn, Co, Sr, Zn, Pb, Cd and Cu were found to be evidently higher in the blood of healthy donors than the patients which showed relatively higher contributions of Ca, Fe, K and Mg in their blood samples. Nonetheless, average levels of

Cr and Na were marginally higher in the blood of the patients than the controls but the differences were not statistically significant. The comparative study thus indicated an imbalance of the metal levels in the blood of thyroid cancer patients.

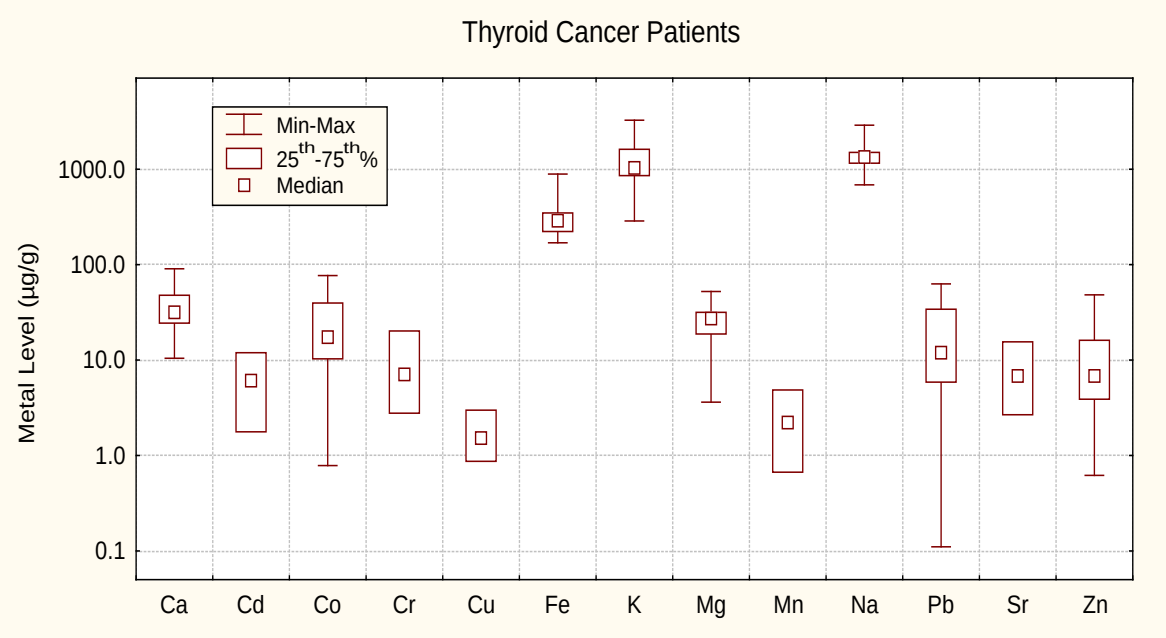

Figure 1. Quartile distribution of selected essential and toxic metal levels (µg/g) in the blood of thyroid cancer patients

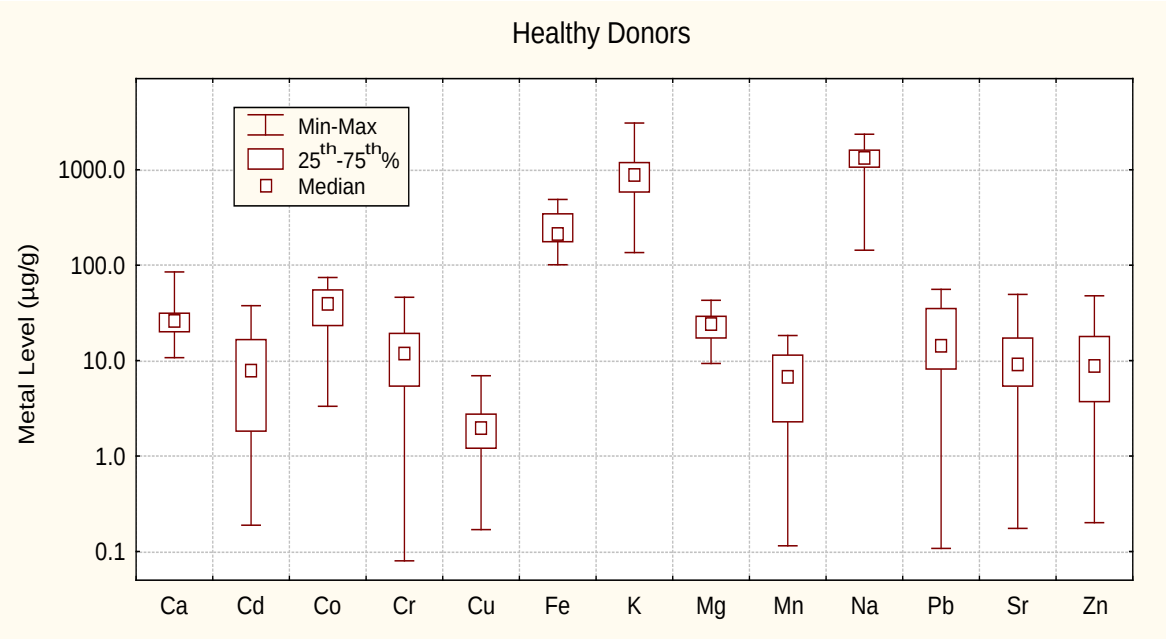

Figure 2. Quartile distribution of selected essential and toxic metal levels (µg/g) in the blood of healthy subjects

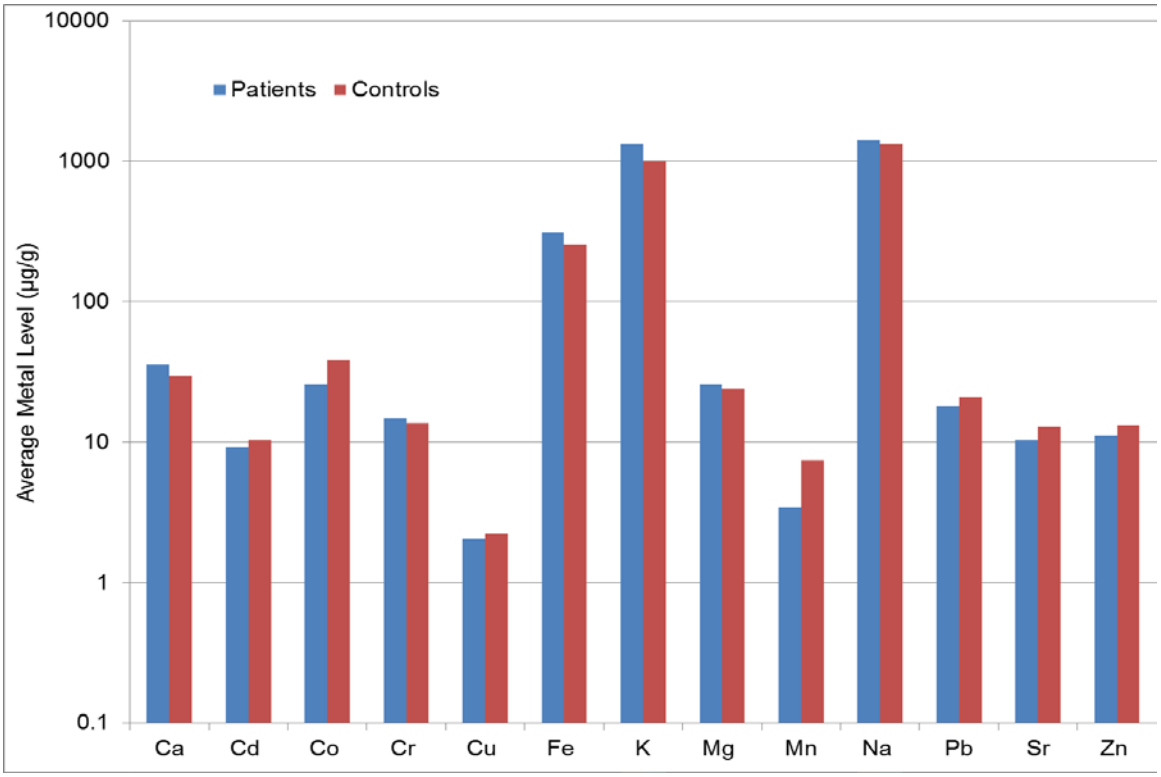

Figure 3. Comparison of the average essential and toxic metal concentrations (µg/g) in the blood of thyroid cancer patients and healthy subjects

3.2 Correlation Study of Selected Metals

The data on metal-to-metal correlations in the blood of thyroid cancer patients are shown in Table 5, wherein the bold *r*-values are significant at $p < 0.05$. Among the selected metals, Zn-Cd ($r = 0.651$), Mn-Cd ($r = 0.563$), Mg-Fe ($r = 0.516$), Mg-Cd ($r = 0.498$), Sr-Pb ($r = 0.491$), Fe-Cd ($r = 0.489$), Sr-Co ($r = 0.470$), Mg-Ca ($r = 0.459$), Zn-Sr ($r = 0.446$), Mg-Cu ($r = 0.425$), Zn-Mn ($r = 0.389$), Mn-Mg ($r = 0.378$), Pb-Mg ($r = 0.361$) and Pb-Cr ($r = 0.304$) showed strong and significant positive correlations. Some of the metal pairs such as Mg-K ($r = -0.453$), Sr-K ($r = -0.398$), K-Ca ($r = -0.349$), K-Co ($r = -0.347$), Na-K ($r = -0.337$), Pb-K ($r = -0.310$) exhibited inverse relationships and opposing distributions in the blood of thyroid cancer patients. Rest of the metal pairs exhibited either weak positive or negative relationships. The correlation study showed mutual association among Zn, Cd, Mn, Mg, Fe, Sr and Pb in the blood of the thyroid cancer patients while one of the major electrolytes (K) revealed contrasting variations with most of the metals thus demonstrating the disproportional distribution of the metals in the blood of cancer patients.

Table 5. Correlation coefficient (r)* matrix for selected essential and toxic metals in the blood of thyroid cancer patients

	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Pb	Sr	Zn
Ca	1.000												
Cd	-0.041	1.000											
Co	0.102	0.197	1.000										
Cr	0.059	0.033	0.293	1.000									
Cu	0.296	0.114	-0.010	-0.113	1.000								
Fe	0.287	**0.489**	0.225	0.206	0.115	1.000							
K	**-0.349**	-0.169	**-0.307**	-0.277	-0.221	-0.129	1.000						
Mg	**0.454**	**0.498**	0.164	0.216	**0.425**	**0.516**	**-0.453**	1.000					
Mn	0.053	**0.563**	-0.044	0.282	0.026	0.284	-0.130	**0.378**	1.000				
Na	0.228	-0.043	0.244	-0.015	0.021	-0.040	**-0.337**	0.187	0.037	1.000			
Pb	0.137	0.157	0.196	**0.304**	0.140	0.132	**-0.310**	**0.361**	0.034	-0.103	1.000		
Sr	-0.076	0.196	**0.470**	0.266	-0.018	0.213	**-0.398**	0.094	-0.068	-0.045	**0.491**	1.000	
Zn	-0.162	**0.651**	0.290	0.289	-0.089	0.283	-0.202	0.237	**0.389**	-0.059	0.148	**0.446**	1.000

*bold *r*-values are significant at $p < 0.05$

Table 6. Correlation coefficient (r)* matrix for selected essential and toxic metals in the blood of healthy subjects

	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Pb	Sr	Zn
Ca	1.000												
Cd	**0.430**	1.000											
Co	0.067	**0.358**	1.000										
Cr	0.226	0.121	0.053	1.000									
Cu	**0.348**	**0.465**	0.220	0.258	1.000								
Fe	-0.004	0.293	0.036	-0.035	0.078	1.000							
K	-0.158	-0.207	-0.098	-0.161	-0.120	0.173	1.000						
Mg	**0.460**	**0.387**	0.238	0.299	0.084	0.283	0.170	1.000					
Mn	0.232	0.269	0.122	**0.643**	0.226	**-0.326**	-0.277	**0.309**	1.000				
Na	-0.082	-0.175	-0.031	0.149	0.002	-0.069	**0.523**	0.237	0.114	1.000			
Pb	-0.014	0.245	0.196	**0.324**	0.202	-0.082	0.062	0.264	**0.352**	0.136	1.000		
Sr	0.126	**0.570**	0.200	**0.388**	0.184	0.106	-0.065	**0.336**	0.265	0.050	0.239	1.000	
Zn	0.159	0.204	0.258	**0.306**	0.158	-0.014	-0.035	**0.502**	0.253	0.162	**0.346**	0.220	1.000

*bold *r*-values are significant at $p < 0.05$

The correlation coefficient matrix for trace metals pertaining to the blood of healthy subjects is shown in Table 6, wherein the significant *r*-values are shown in bold at $p < 0.05$. Significantly strong positive correlation was observed between Mn-Cr (r = 0.643), Sr-Cd (r = 0.570), Na-K (r = 0.523), Zn-Mg (r = 0.502), Cu-Cd (r = 0.465), Mg-Ca (r = 0.460), Cd-Ca (r = 0.430), Sr-Cr (r = 0.388), Mg-Cd (r = 0.387), Cd-Co (r = 0.358), Pb-Mn (r = 0.352), Cu-Ca (r = 0.348), Sr-Mg (r = 0.336), Pb-Cr (r = 0.324), Mn-Mg (r = 0.309) and Zn-Cr (r = 0.306). Nevertheless, Mn-Fe (r = -0.326) exhibited inverse relationship and opposing distributions in the blood of healthy donors. In comparison to the patients, correlation study demonstrated mutual associations among most of the essential metals while toxic/trace metals showed separate grouping in the blood of controls. Consequently the correlation study pointed out considerably diverse associations among the metals in the cancerous patients and healthy subjects.

3.3 Comparative Evaluation of Selected Metals in the Blood based on Demographic Characteristics of the Subjects

Gender-based comparison in the average concentrations of selected metals in the blood of thyroid cancer patients and controls is shown in the Figure 4. The comparative evaluation revealed elevated average concentration of Sr and Zn in the male patients than the female patients, while average levels of Cr, Ca, Fe, K, Mg and Na were more or less comparable in both male and female patients. Nevertheless, mean levels of Cd, Co, Cu, Mn and Pb were found to be higher in the female patients than the male subjects. In the case of controls, mean levels of Cd, K and Pb were found to be higher in the male donors, while average concentrations of Ca, Co, Cr, Cu, Mn and Zn were found to be higher in the blood of female controls. Nonetheless, average concentrations of Fe, Mg, Na and Sr were almost comparable in both male and female controls (Figure 4).

Comparative average metal levels in the blood based on habitat of the subjects are shown in Figure 5, which revealed comparatively high concentrations of Cd, Mg, Mn, Pb and Zn in the urban patients than the rural counterparts which exhibited elevated levels of Co, Cr and Sr. Nevertheless, average levels of Ca, Cu, Fe, K and Na were found to be comparable in both patient groups. In the case of controls, mean contents of Cu and Fe were higher in the blood of rural than urban controls, whereas mean levels of Co, K, Mg and Na were nearly comparable in the blood of both control groups. Average levels of Ca, Cd, Cr, Mn, Pb, Sr and Zn were found to be higher in urban than rural controls.

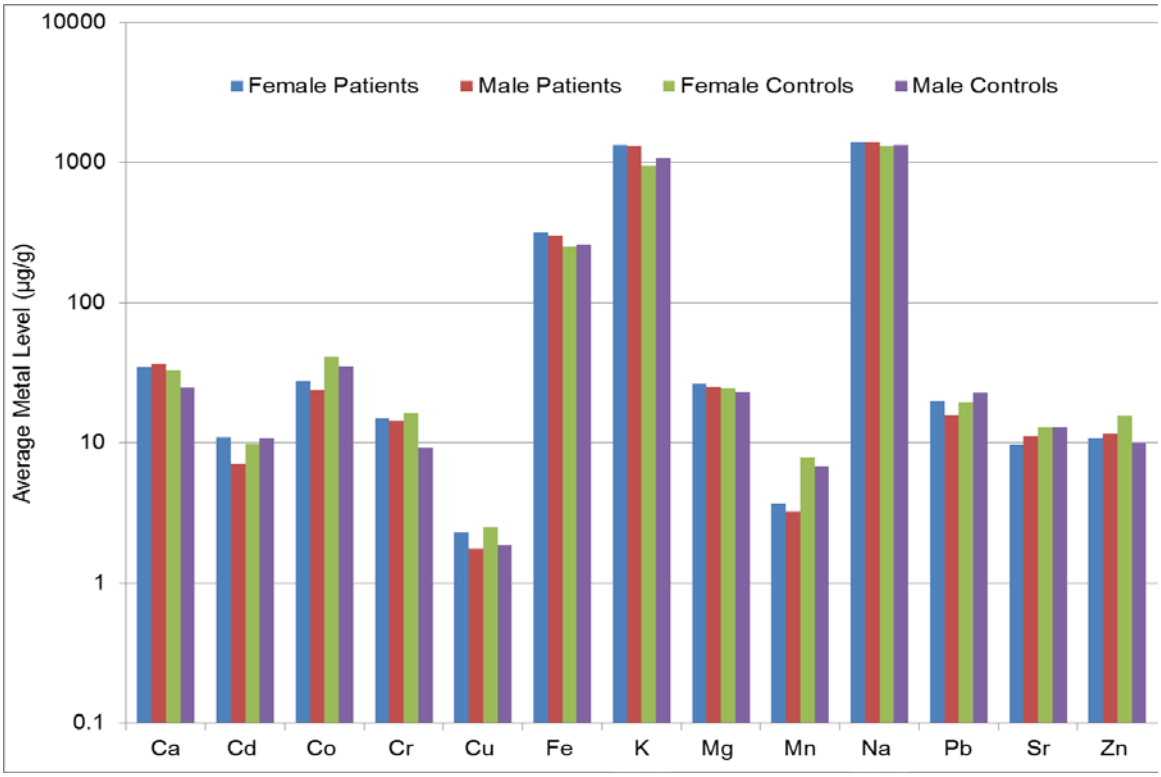

Figure 4. Comparison of the average levels of selected essential and toxic metals (µg/g) in the blood of thyroid cancer patients and controls based on their gender

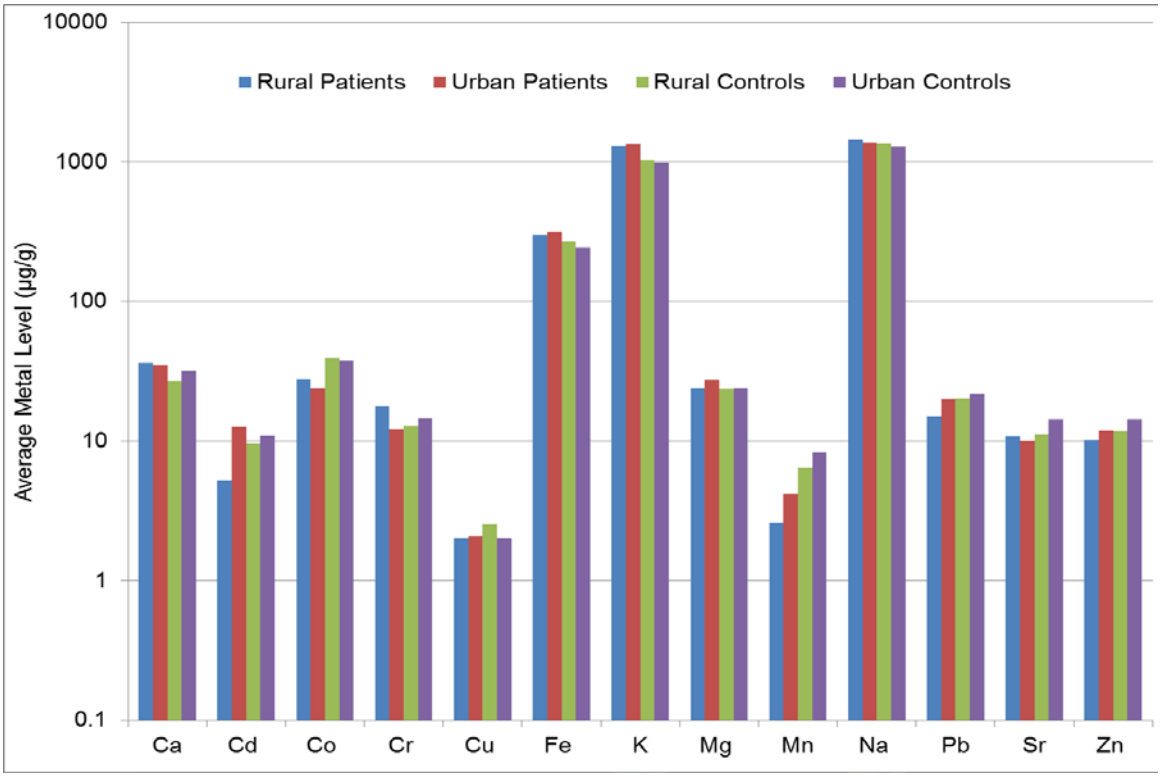

Figure 5. Comparison of the average levels of selected essential and toxic metals (µg/g) in the blood of thyroid cancer patients and controls based on their habitat

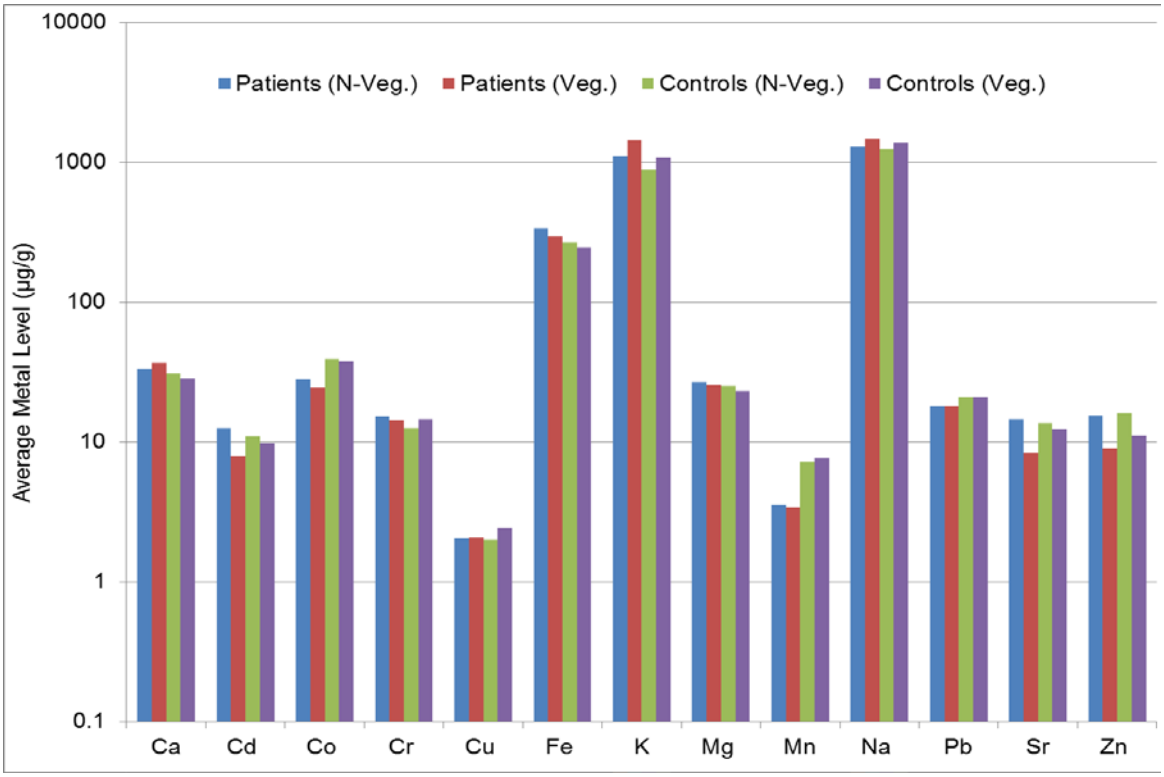

Figure 6. Comparison of the average levels of selected essential and toxic metals (µg/g) in the blood of thyroid cancer patients and controls based on their food habits

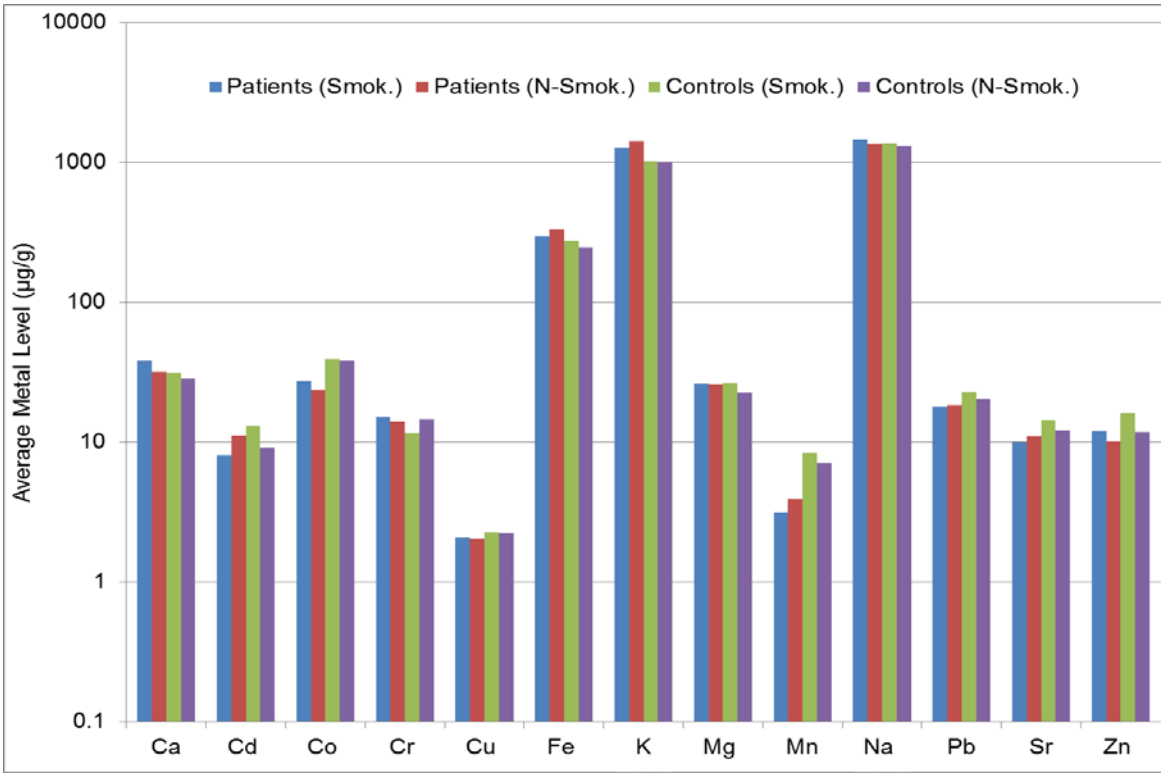

Figure 7. Comparison of the average levels of selected essential and toxic metals (µg/g) in the blood of thyroid cancer patients and controls based on their smoking habits

Average concentrations of selected metals in the blood of the patients and controls based on their food-habits are depicted in the Figure 6. Comparative assessment of the metals showed approximately equivalent levels of Cu, Mn and Pb in the blood of vegetarian and non-vegetarian patients. However, mean contents of Ca, K and Na showed higher contribution in the blood of vegetarian patients, while Cd, Co, Cr, Fe, Mg, Sr and Zn were found to be comparatively higher in the non-vegetarian patients than the vegetarian patients. On the other hand, in the case of controls relatively high concentrations of Ca, Cd, Fe, Mg, Sr and Zn were observed in the blood of non-vegetarian subjects compared with the vegetarian donors. However, mean levels of Cr, Cu, K and Mn were relatively higher in the blood of vegetarian controls than the non-vegetarian donors. Average concentration of Co and Pb was found to be almost comparable in the blood of both control groups.

Smoking-based comparison of the selected metals in blood of the patients and controls is shown in Figure 7, which showed that relatively high concentrations of Ca, Co, Cr, Na and Zn were found for smoking patients than the non-smoking patients. Average contents of Cu, Mg and Pb were almost comparable in both smoking and non-smoking patients, while average levels of Cd, Fe, K, Mn and Sr were found to be relatively higher in the blood of non-smoking patients. In the case of controls, mean levels of Ca, Cd, Fe, Mg, Mn, Pb, Sr and Zn showed relatively higher contribution in smoking subjects while mean level of Co, Cu, K and Na showed comparable levels in both donor groups. Besides, average concentrations of Cr were found to be higher in the blood of non-smoking controls than the smoking controls.

Selected metal levels in the blood of both donor groups were also compared to explore the age-based variations and for this purpose the donors were classified into four age groups; < 30 years, 31-40 years, 41-50 years and > 50 years. Age-based comparison among the patients is shown in Figure 8, which revealed high concentrations of Cu, Fe, Ca, Sr ad Mg in the patients of < 30 years; while 31-40 years age group showed higher contents of K. Patients of age group 41-50 years showed higher levels of Cr. Nonetheless, relatively higher levels of Zn, Cd, Co, Pb and Mn were shown by the patients of >51 years age. Age-based comparison for the controls is shown in Figure 9. Comparatively higher contributions of Pb, Sr, Zn, Na, K, Cr and Cd were observed in < 30 years of controls, while those of 41-50 years showed higher concentration of Co and Fe. However, relatively higher levels of Mn and Ca were shown by >51 years controls (Figure 9).

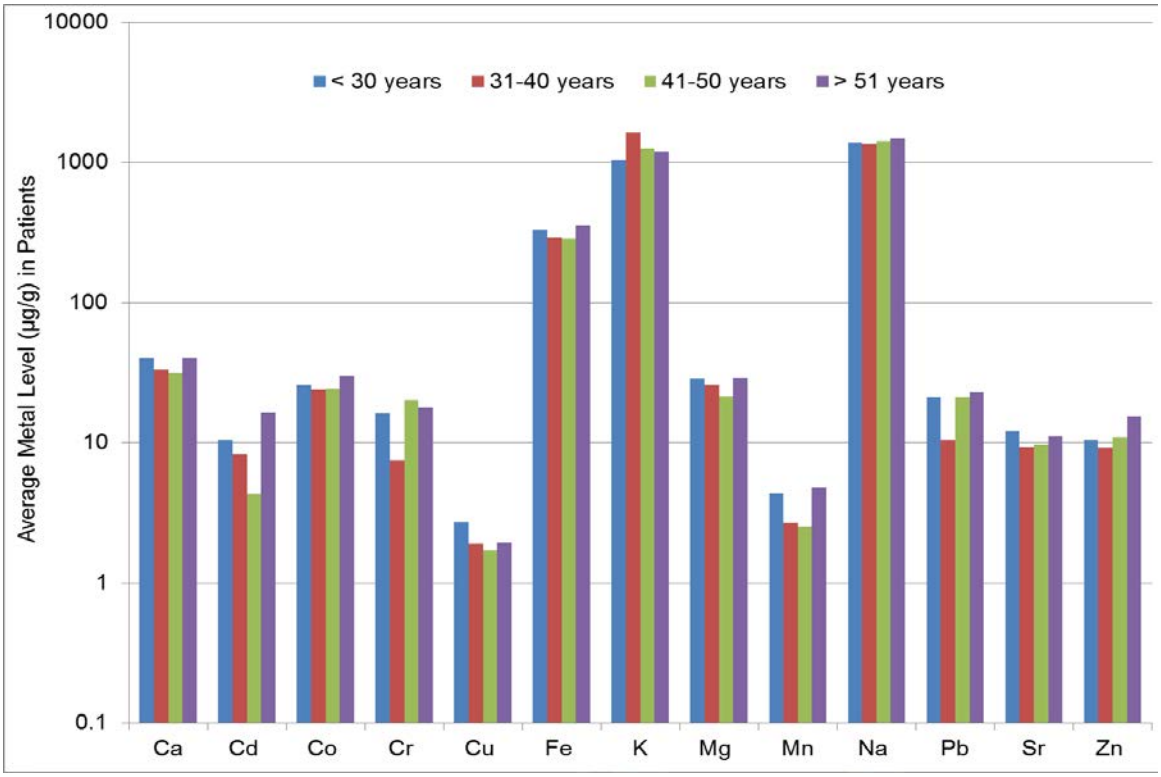

Figure 8. Comparison of the average concentrations of essential and toxic metals (µg/g) in the blood of various age groups of thyroid cancer patients

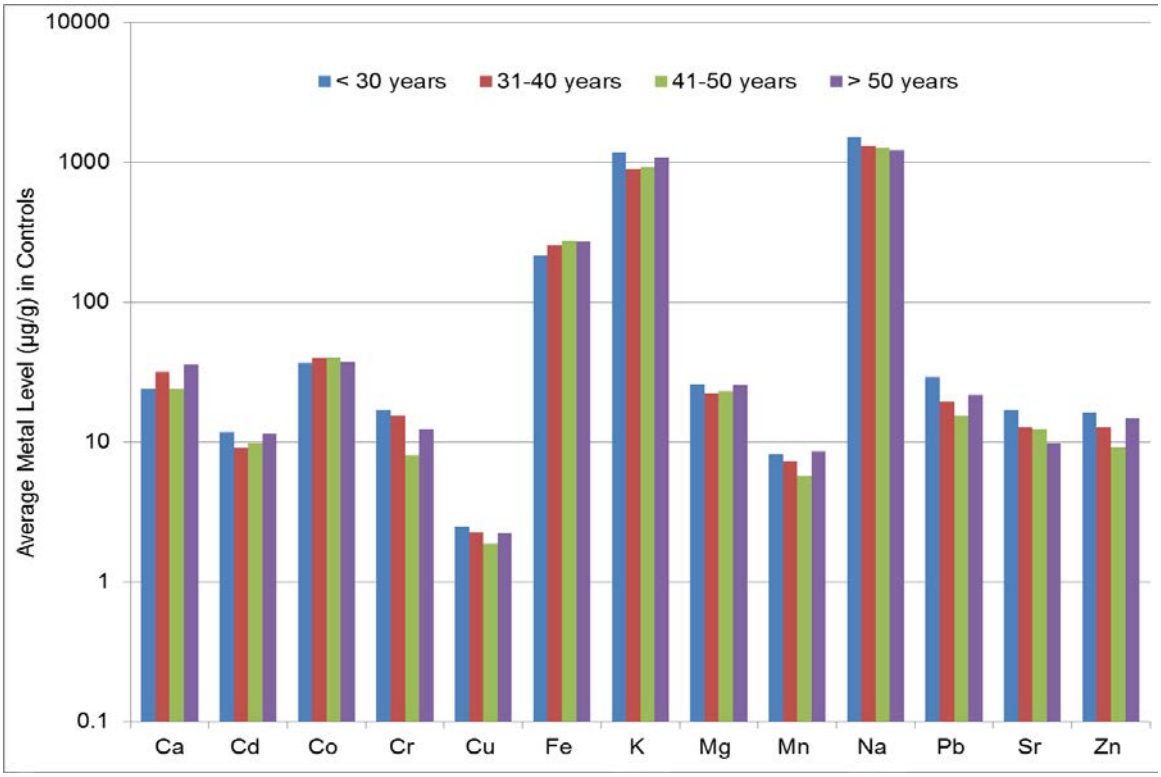

Figure 9. Comparison of the average concentrations of essential and toxic metals (µg/g) in the blood of various age groups of healthy donors

3.4 Comparative Evaluation of Selected Metals in the Blood based on Cancer Stages

In the present study, selected metal levels measured in the blood of the cancer patients were also compared based on the cancer stages and the comparison of average metal levels at different cancer stages is shown in Figure 10. A comparative evaluation indicated that the average levels of Cd, Pb, Fe, Mg and Zn were found to be significantly elevated at stage-II, while moderately elevated levels of Sr and Mn were found at stage-I and stage-III, respectively. Significant increase in the concentrations of Co, Cr, Cu and Ca were observed in the blood of patients at stage-IV whereas mean levels of Na and K were almost comparable at all four stages. Overall, the comparative study revealed significant variations in the metal levels at various cancer stages (Figure 10).

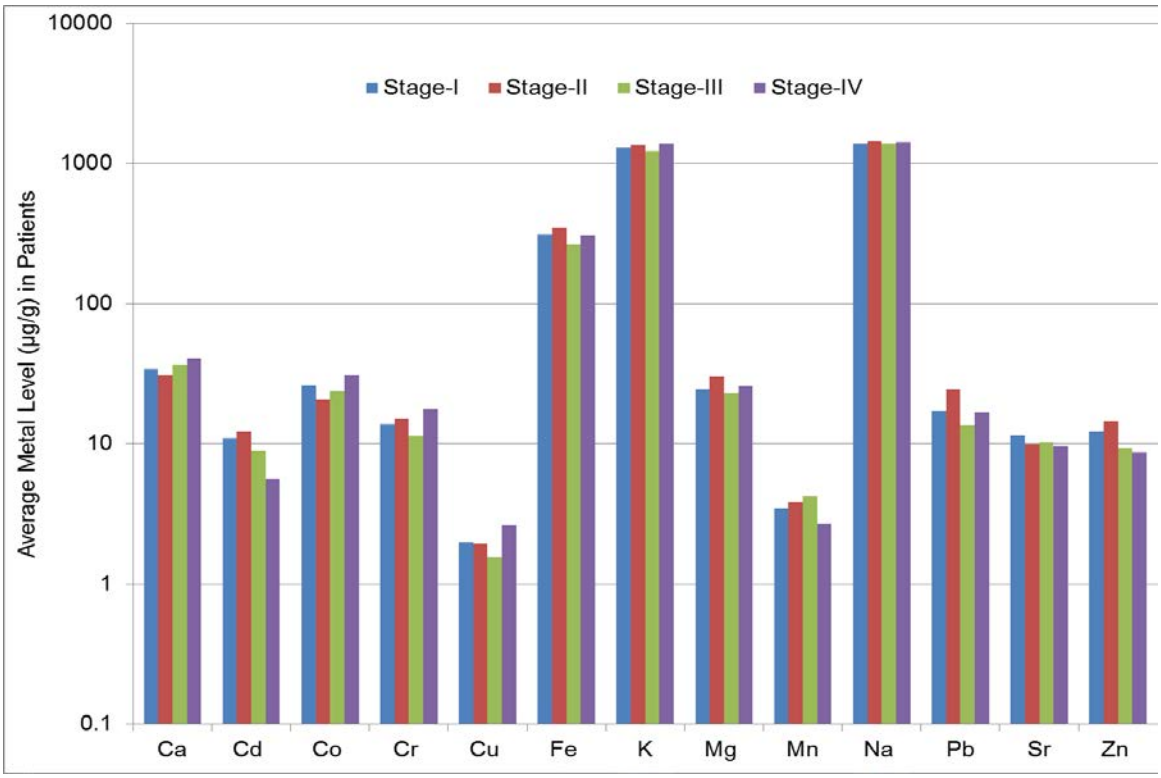

Figure 10. Comparison of the average concentrations of essential and toxic metals (µg/g) in the blood of thyroid cancer patients at various stages

3.5 Multivariate Analysis of Selected Metals

Another fascinating aspect of the present study was the multivariate apportionment of the metal levels in the blood of thyroid cancer patients and controls using PCA and CA. The PC loadings extracted by varimax-normalized rotation on the metals data for the

patients and healthy donors are shown in Tables 7 and 8, respectively. In case of the patients, PCA yielded five significant PCs with eigen value greater than 1, commutatively explaining approximately 74% of the total variance of data (Table 7). The CA of metal data pertaining to the cancer patients is shown in Figure 11. PC 1 showed higher loading for Cd, Fe and Zn with a similar cluster of the metals in CA. PC 2 showed maximum loadings for Cu, Pb and Mg with a parallel cluster of metals in CA. These two PCs showed the interference of toxic metals (Pb and Cd) with the essential metals in the patients and they were believed to be mainly contributed by anthropogenic sources. PC 3 indicated higher loading for Co and Sr along with a similar cluster in CA. PC 4 shows higher loadings of Na and Ca while PC 5 shows higher loadings of Mn and Cr with a similar cluster in CA. These metals are mostly derived from the nutritional habits of the subjects. The PCA and CA results were in very good agreement with each other.

Table 7. Principal component loadings for selected essential and toxic metals in the blood of thyroid cancer patients

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen value	3.635	1.996	1.748	1.186	1.015
Total Variance (%)	27.96	15.36	13.44	9.126	7.806
Cumulative Eigen value	3.635	5.632	7.379	8.566	9.580
Cumulative Variance (%)	27.96	43.32	56.76	65.89	73.70
Ca	-0.215	0.561	-0.110	-0.518	0.109
Cd	0.907	0.056	0.143	0.034	0.027
Co	0.228	-0.123	0.780	0.016	-0.047
Cr	-0.005	0.056	0.294	0.080	0.784
Cu	0.107	0.677	-0.114	0.060	-0.347
Fe	0.841	-0.095	0.031	-0.202	0.011
K	-0.216	0.341	0.587	-0.346	0.136
Mg	0.591	0.583	0.112	-0.312	0.071
Mn	0.500	-0.033	-0.160	-0.071	0.693
Na	-0.228	0.059	-0.054	0.901	0.096
Pb	-0.038	0.717	0.255	0.099	0.377
Sr	0.230	0.070	0.816	0.049	0.238
Zn	0.712	0.044	0.384	0.108	0.230

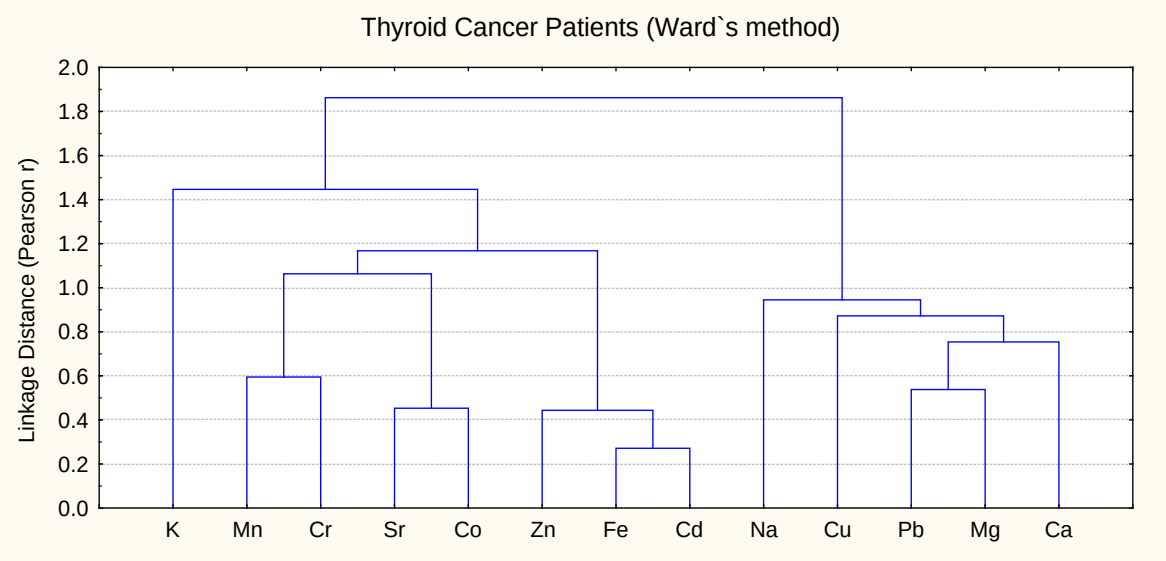

Figure 11. Cluster analysis of selected essential and toxic metals in the blood of thyroid cancer patients

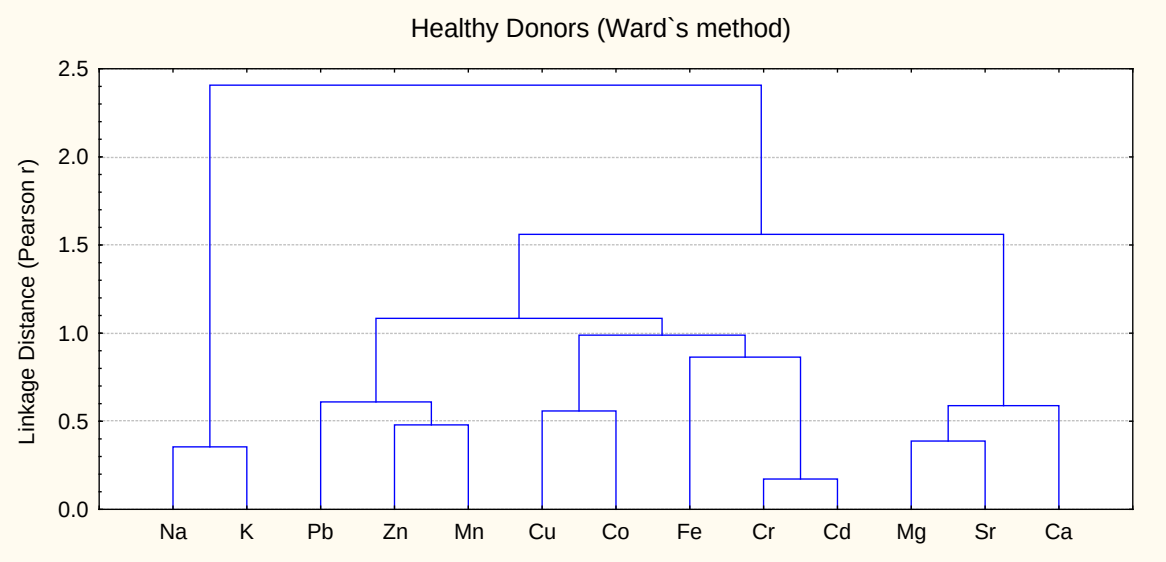

Figure 12. Cluster analysis of selected essential and toxic metals in the blood of healthy subjects

In the case of controls, PCA of the metal data yielded four major PCs with eigen value >1, commutatively explaining approximately 69% of the total variance of data (Table 8). The CA of metal data pertaining to the blood of controls is shown as dendrogram in Figure 12. PC 1 showed higher loadings for Fe, Mn, Cr and Cd whereas PC 2 showed higher loadings for Na and K with a similar cluster of the metals in CA. These metals were mostly associated with food habits and also significantly affected by the anthropogenic contamination. PC 3 showed higher loadings for Ca, Mg, and Sr while PC 4

showed elevated loadings for Co, Cu, Pb and Zn. The CA also revealed strong clusters of these metals in the blood of controls. These metals were mostly regulated by internal body metabolism in healthy donors. It is important to note that in the case of controls, the toxic metals were not primarily associated with the essential metals as was the case in the cancer patients thus indicating a disproportion among the metals in cancer patients. Overall, PCA and CA showed significantly diverse apportionment of the essential and toxic metals in the blood of cancer patients and healthy subjects which may be ascribed to the imbalances of trace metals in the cancer patients. Consequently, the multivariate methods can be employed for diagnostic and prognostic purpose in clinical studies but they required further validation by considering more variables on larger population groups from different geographical areas around the world.

Table 8. Principal component loadings for selected essential and toxic metals in the blood of healthy subjects

	PC 1	PC 2	PC 3	PC 4
Eigen value	4.115	2.028	1.673	1.118
Total Variance (%)	31.65	15.60	12.87	8.603
Cumulative Eigen value	4.115	6.144	7.817	8.935
Cumulative Variance (%)	31.65	47.26	60.13	68.73
Ca	0.184	0.341	0.683	-0.055
Cd	0.745	0.261	0.249	0.422
Co	0.235	0.245	-0.052	0.714
Cr	0.685	-0.007	0.261	0.242
Cu	0.026	0.249	0.051	0.716
Fe	0.799	-0.041	-0.373	-0.055
K	-0.045	0.754	0.268	0.235
Mg	-0.001	-0.330	0.715	0.192
Mn	0.933	0.110	0.411	0.071
Na	0.103	0.874	0.064	0.041
Pb	0.143	-0.409	0.301	0.637
Sr	0.062	0.209	0.665	0.298
Zn	0.277	-0.163	0.295	0.472

3.6 Comparison of Selected Metals in the blood of Cancer Patients with the Reported Studies

Average metal levels found in the blood of thyroid cancer patients in the present study were compared with other types of cancer reported from various regions around the world as shown in Table 9. Generally, most of the metal levels observed in the present study were considerably higher than those reported for other cancer types. Mean levels of Cd, Co, Cr, K, Mn, Pb and Sr were found to be comparatively higher in the blood of thyroid cancer patients than those reported for other types of cancer. However, average concentrations of Ca and Mg in the blood of thyroid cancer patients were more or less comparable with most of the reported levels in the Table except significantly elevated Ca levels were reported for lung cancer patients from Pakistan and China. Similarly, mean level of Cu in the blood of thyroid cancer patients was almost comparable with the reported blood levels for lung cancer patients from Spain and prostate cancer patients from Pakistan but the present Cu levels was lower than the reported level for breast cancer patients from England and higher than rest of the levels given in Table. Likewise, average level of Fe in the present investigation was found to be higher compared with the ovary cancer patients from Pakistan but lower than those of prostate cancer patients from Turkey and Pakistan. However, the mean level was not significantly different than those of lung cancer patients from Spain and Pakistan. Mean concentration of Na in the blood of thyroid cancer patients measured in the present study was relatively lower than the reported levels for lung, ovary and prostate cancer patients from Pakistan as well as liver cancer patients. Average level of Zn in the present study was found to be lower than the reported levels for liver cancer patients and breast cancer patients from England but the present Zn level was higher compared to the lung, ovary and prostate cancer patients from Pakistan, lung cancer patients from China and Spain, prostate cancer patients from Turkey, oral cancer patients from Taiwan as well as head and neck cancer patients (Table 9). Overall, the comparative study showed elevated levels for most of the metals in the blood of thyroid cancer patients than the reported levels for other cancer types which may be concomitant with the disproportions of the trace metals due to carcinogenesis.

Table 9. Comparison of the average metal levels (µg/g) in the blood of thyroid cancer patients with the reported levels for other types of cancer

Type/Country	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Pb	Sr	Zn	Ref.
Thyroid cancer/Pakistan	35.65	9.304	25.67	14.68	2.050	309.6	1322	25.94	3.473	1404	18.06	10.38	11.18	Present study
Ovary cancer/Pakistan	35.63	0.301	2.383	2.200	1.029	184.6	277.5	33.46	0.156	1822	2.735	1.943	6.318	Qayyum and Shah, 2016
Prostate cancer/Pakistan	36.22	1.084	2.190	1.230	1.903	850.8	165.2	29.43	1.524	1529	3.658	1.301	4.283	Qayyum and Shah, 2014
Lung cancer/Pakistan	54.31	0.425	2.737	1.080	1.647	353.9	708.7	36.59	0.278	1662	5.275	1.267	6.501	Qayyum and Shah, 2014
Lung cancer/China	70.03				1.31			17.92					0.41	Chen *et al*., 2011
Lung cancer/Spain	31.20				2.087	300.3		39.21					6.783	Diez *et al*., 1989
Prostate cancer/Turkey					0.456	623							4.375	Ozmen *et al*., 2006
Breast cancer/England	35.21				3.312			32.21	2.102				17.25	Capel *et al*., 1982
Oral cancer/Taiwan		1.000		0.83	0.88						3.34		6.73	Yuan *et al*.,2011
Head and neck cancer	37.34	2.340		1.480			872.2		1.932				0.0613	Abdullah *et al*., 1979
Liver cancer		7.603			0.3339			31.24		1775			42.75	Khlifi *et al*., 2013

3.7 Salient Findings of the Present Study

Based on the deliberations stated in foregoing sections, following salient findings emerged from the present study:

- Most of the metals showed random distribution in the blood of both donor groups, however the dispersion and asymmetry was higher in the patient group.
- Average levels of Sr, Mn, Cu, Co, Cd and Zn were found to be higher in the blood of controls than the patients.
- Comparative variations in the quartile distribution were considerably divergent in the patients than the controls.
- Correlation study showed diverse associations among the metals in the blood of the patients and controls.
- Significant variations in the metal levels were observed with the gender, habitat, food habits and smoking habits of both donor groups.
- Metal levels exhibited considerable differences with the age of the donors and stages of thyroid cancer.
- PCA and CA revealed considerably divergent apportionment of the metals in the patients and controls; it may be considered as a diagnostic tool in clinical studies.
- In comparison with the reported levels for other types of cancer, average levels for most of the metals in thyroid cancer patients were found to be higher.

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