A STUDY ON SPECIFICITY OF LIVE BLOOD ANALYSIS FOR HEAVY METAL MEASUREMENT COMPARE WITH URINE HEAVY METAL

NATTHIRA PREECHASILP

MASTER OF SCIENCE
ANTI-AGING AND REGENERATIVE SCIENCE

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
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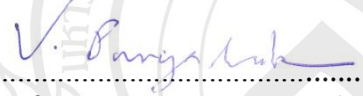

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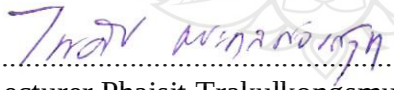

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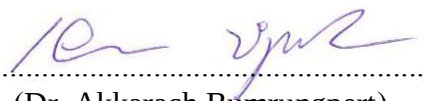

.....EXAMINER
(Dr. Akkarach Bumrungpert)

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Natthira Preechasilp

Thesis Title	A Study on Specificity of Live Blood Analysis for Heavy Metal Measurement Compare with Urine Heavy Metal
Author	Natthira Preechasilp
Degree	Master of Science (Anti-Aging and Regenerative Science)
Advisor	Dr. Werner Kurotschka
Co-Advisor	Dr. Phaisit Trakulkongsmut

ABSTRACT

Background: In human life has many kind of heavy metals are involve such as lead, mercury, cadmium, arsenic .They use in industrial factory such as battery factory, in agriculture use in insecticide and fertilize or use in medical such as dental tools. Human always have risk of heavy metals exposure pass through the body in different way such as inhalation or oral via food consumption, drinking water which has contaminated with heavy metal toxicity. In every year has a reported show in Thailand and in the world can be detect heavy metal toxicity in the environment especially near the industry which drain waste product to the river or soil. Therefore it has to early detect about heavy metal toxicity which a key factor in successful treatment. The screening test is rapidly technique for early detection of disease. Live blood analysis is screening test for many disease such as nutritional deficiencies, immune System, heavy metal. The results present crystal in live blood analysis which can indicate a patient has abnormal symptoms. After that patients come to check in lab testing such as urine testing or hair testing and continue to treatment. In this research use live blood analysis technique which has screening test for heavy metals compare with urine heavy metal.

Objective: A study on specificity of live blood analysis for heavy metal measurement compare with urine heavy metal.

Method: The study design was a retrospective study from secondary data. Collect secondary data from 99 Patients in TRIA Integrative Wellness at Piyavate hospital, male and female, who have to receive assessment for live blood analysis and urine heavy metal. Sixty-two patients who have crystals in live blood analysis and thirty-three patients who have no crystals in live blood analysis. The assessment of results based on clinical features by Physicians and can analyze the collection form result in live blood analysis and the presence of whole urine heavy metal by Statistics analysis.

Result: The statistical analysis of the data for compare between live blood analysis and urine heavy metal, the results shows a group with crystal of five heavy metals such as lead, cadmium, manganese, nickel, arsenic (mean10.14±16.57, 0.56±0.87, 12.80±32.79, 1.73±2.36, 86.28±133.24, respectively) has significantly high level of heavy metals in urine than those without crystal (mean5.42± 6.89, 0.26± 0.25, 1.38± 0.79, 0.63± 0.87, 23.28±16.87, respectively), p value P<0.001, P=0.003, P<0.001, P<0.001, P<0.001, respectively. Second, after the statistical analysis of the data for correlation between crystal in live blood and higher level of heavy metals in urine shows four heavy metals have statistically significant difference such as cadmium, manganese, nickel, arsenic (p = 0.039, p = 0.037, p = 0.008, p = 0.005, respectively). Furthermore the statistical analysis of the age and sex has not statistically significant difference for correlation between live blood analysis and urine heavy metal.

Conclusion: The result from live blood analysis that appears to have crystal could be a good indication that the following five heavy metals are lead, cadmium, manganese, nickel, manganese will be found in urine.

Keywords: Live Blood Analysis/Heavy Metal/Urine Heavy Metal

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

In human life has many kind of heavy metals are involve such as lead, arsenic, cadmium, mercury, chromium, manganese. They use in industry such as produce in plastic, paint, batteries. In agricultures use in insecticide and fertilize. In medicals can use in ingredient in drug, cosmetics, medical devices. Heavy metal also found in nature which have benefit and disadvantages. Human always have risk of heavy metal exposure pass through the body in different way such as inhalation or oral via food consumption, drinking water which has contaminated with heavy metal toxic. In every year can be detect heavy metal toxic in environment especially near the industry which drain waste product to the river or soil. In part of Thailand have heavy metals in high level such as in Kanchanaburi province have lead contaminated in the river or high cadmium level in Tak province

For each year, Bureau of Epidemiology, Department of Disease Control, Ministry of Public health has the surveillance reports from the hospital about patients who have disease from toxic of heavy metals. In year of 2011, about lead poisoning, National Disease Surveillance (Report 506) since 1 January 2011 to 21 July 2011 found 15 patients from 6 provinces, found in male more than female, mostly found in age between 45-54 years (20.00%), also seen in high level of exposure of agriculture (Ministry of Public Health, 2011a). Further, about manganese, mercury, arsenic poisoning that National Disease Surveillance (Report 506) reported since 1 January 2011 to 21 August 2011 found 24 patients from 9 provinces, found in male more than female , mostly found in age between 15-24 years (29.17%), also seen in high level of exposure of labor. (Ministry of Public Health, 2011b).

In year of 2010 About lead poisoning, National Disease Surveillance (Report 506) found 26 patients from 17 provinces, found in male more than female, mostly found in age between 35-44 years (23.08%), also seen in high level of exposure of agriculture (Ministry of Public Health, 2010a). Further, about manganese, mercury, arsenic poisoning that National Disease Surveillance (Report 506) found 109 patients from 17 provinces, found in female more than male, mostly found in age above 65 years (32.11%), also seen in high level of exposure of labor. (Ministry of Public Health, 2010b).

Since year of 2000 to year of 2009 reported in lead poisoning found 397 patients and other heavy metals such as mercury, arsenic has been reported average per year 82 patients. (Ministry of Public Health, 2009).

As mentioned above, it has to early detect about heavy metal toxicity which a key factor in successful treatment. The measurement of heavy metal toxics are urine, blood sample, hair. Lab testing is provides for reducing risk of toxic when it show false negative. It can appropriate for beginning, middle and end of therapy. The screening test is appropriate for early detection of disease for rapidly and effective treatment. The screening test is a procedure that is performed to early detect the presence of a specific disease which have the individual or group of individuals does not present symptoms of the disease. The screening test also has a quick and cheap test which is followed by more accurate test but also more expensive to carry out on the positive reactors to the screening test. The one of screening test is live blood analysis. Live blood analysis is screening test for many disease such as digestive disorder, nutritional deficiencies, immune System, heavy metals and other disease. When result in live blood present that indicate a patient has an abnormal symptom. After that patient has to check in lab testing and continue to treatment.

In this research is interesting in live blood analysis for heavy metal testing. In live blood analysis present crystal which indicate to heavy metals. In this research use live blood analysis technique which has screening test for heavy metals compare with urine heavy metal.

The aim of current study is study specificity of live blood analysis for screening test in heavy metals

1.2 Reasons for Conducting Human Study

1.2.1 In live blood analysis measurement has shown benefit for evaluate about heavy metals in human and one choice of heavy metal measurement in the future and further research.

1.2.2 Measurement of live blood analysis in human has not a serious side effect.

1.3 Research Objectives

A study on specificity of live blood analysis for heavy metal measurement compare with urine heavy metal

1.4 Research Questions

Does the live blood analysis have specificity for heavy metal measurement?

1.5 Research Hypothesis

The live blood analysis has specificity for heavy metal measurement.

1.6 Expected Benefits and Applications

From this study, if the result shows the effectiveness of live blood analysis can have specificity for heavy metal measurement. It can be one of effective screening measurement of heavy metal and apply to use this technique for evaluate heavy metals. This technique can apply to evaluate because cost effectiveness cheaper than other measurement such as urine, hair. It also has benefit for early detection like a screening test because of this technique can assessment at real time which physician and patient can see in result at the moment that effect to rapidly treatment as same as the one of aim of anti aging medicine is prevent disease

1.7 Conceptual Framework

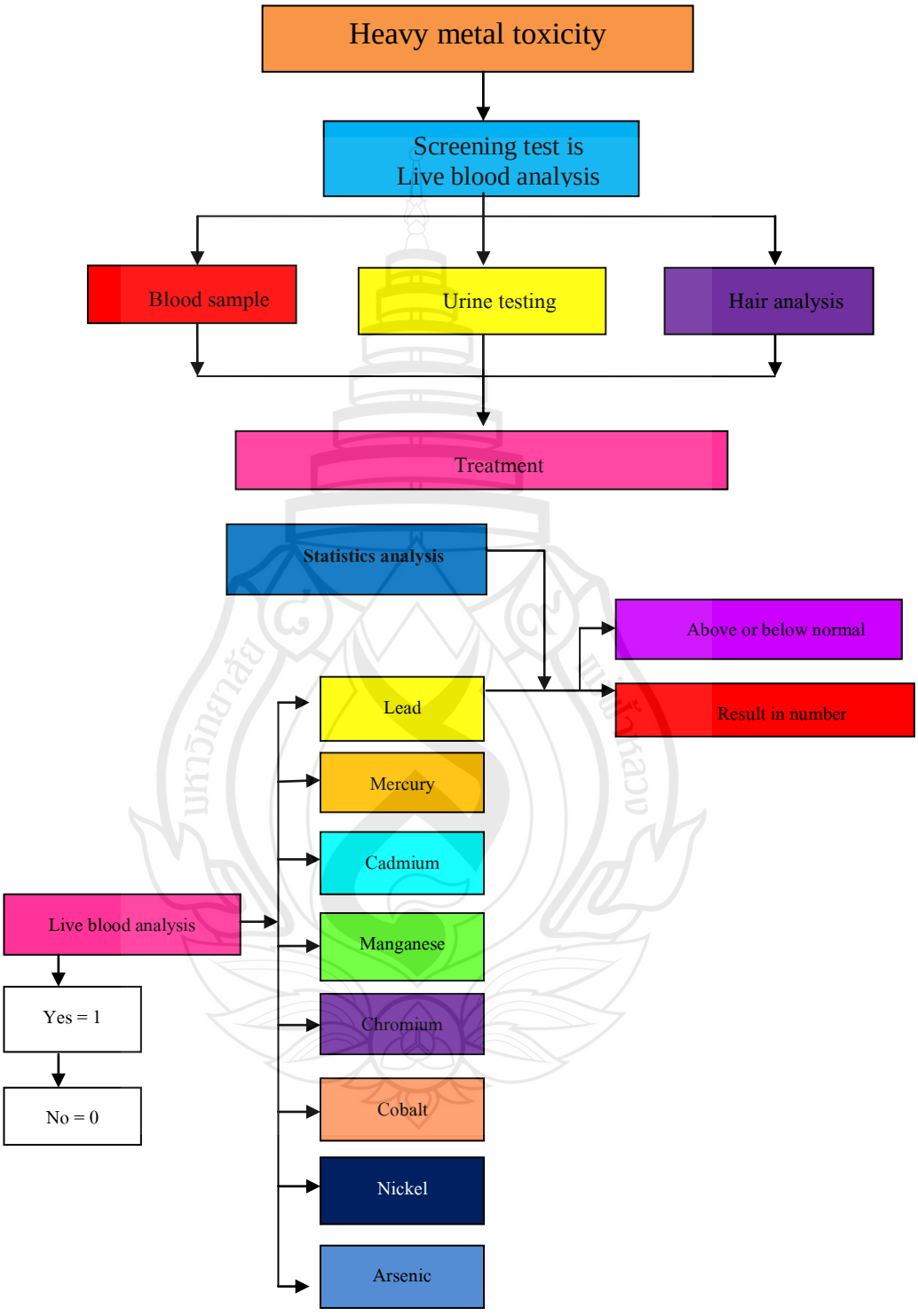


Figure 1.1 Conceptual Framework

1.8 Contribution of the Study

The result of this study can have benefit in anti–aging is effectiveness of live blood analysis can have specificity for heavy metal measurement and also have benefit in human health for early treatment as aim of anti aging is prevent disease

1.9 Scope of Research

1.9.1 Population: Thai population

1.9.2 Sample: People who have result in assessment about live blood analysis and urine heavy metal in TRIA Integrative Wellness at Piyavate hospital

1.10 Terms and Definition

1.10.1 Live blood analysis (LBA),

It is the use of high-resolution dark field microscopy to observe live blood cells in vitro. It can promote by alternative medicine practitioners, who affirm that it can diagnose a range of diseases. Live blood analysis is an "Unestablished diagnostic test" which methods are not generally accepted in laboratory and not validity in laboratory test .There is not scientific research for validity of live blood analysis.

1.10.2 Heavy metal

Metallic compounds, such as aluminum, arsenic, cadmium, lead, mercury, and nickel. Exposure to these metals has been linked to immune systems, kidney, and neurotic disorders. (Jonas, 2005)

1.10.3 Urinalysis

A physical, chemical or microscopic examination of the urine is known as the specimen is physically examined for color, turbidity, and specific gravity. Then it is spun in a centrifuge to allow collection of a small amount of sediment, which is examined microscopically for blood cells, casts, crystals, pus, and bacteria. Chemical analysis may be performed to measure the pH and to identify and measure the levels of ketones, sugar, protein, blood components, and many other substances. (Mosby's medical dictionary, 2009)

1.10.4 Lead

A common soft blue-gray metallic element which has atomic number is 82 and atomic mass is 207.19. In its metallic form, lead is used a protective shielding against x-rays. Lead has poison and a characteristic can reduce in the use of lead compounds as pigments for paints and inks. Normal concentrations in whole blood are 0 to 5μg/dL. The normal amount in urine after 24-hour collection is less than 100 μg. (Mosby's medical dictionary, 2009)

1.10.5 Mercury

Mercury is a chemical element, atomic number is 80. Acute poisoning effect, due to ingestion, is marked by abdominal pain, vomiting, bloody diarrhea with watery stools, anuria, and corrosion and ulceration of the digestive tract; in the chronic form, due to absorption through skin and mucous membranes, inhalation, or ingestion, there is stomatitis, blue line along the gum border, sore hypertrophied gums that bleed easily, loosening of teeth, erethism, tremors, and in coordination. (Saunders, 2007)

1.10.6 Cadmium

Cadmium is a chemical element, atomic number is 48. Cadmium has a poison; inhalation of cadmium fumes or dust causes of pneumoconiosis, and ingestion of foods contaminated by cadmium-plated containers causes of violent gastrointestinal symptoms. (Saunders, 2007)

1.10.7 Manganese

Manganese is a chemical element, atomic number is 25; its salts occur in the body tissue in very small amounts and activate liver arginase or other enzymes. Inhalation of manganese dust can cause poison and can manifested by symptoms including mental disorders accompany a syndrome resembling parkinsonism, and inflammation of the respiratory system. (Saunders, 2007)

1.10.8 Chromium

A lustrous hard metallic element, atomic number is 24.It can resistant to tarnish and corrosion. It found primarily in chromite and used to harden steel alloys, in decorative platings, and as a pigment in glass. (The American Heritage Medical Dictionary, 2007)

1.10.9 Cobalt

Cobalt is a chemical element, atomic number is 27. Inhalation of the dust is cause of pneumoconiosis and exposure to the powder is cause of dermatitis. (The American Heritage Medical Dictionary, 2007)

1.10.10 Nickel

Nickel is a chemical element, atomic number is 28. Long-term exposure of metallic nickel such as jewelry can cause contact dermatitis; nickel fumes can be carcinogenic. (Saunders, 2007)

1.10.11 Arsenic

A poison metallic element, atomic number is 33 which compounds of which are used as antamebics. (The American Heritage Medical Dictionary, 2007)

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

From the Bureau of Epidemiology, Department of Disease Control, Ministry of Public health has been reported about heavy metal

2.1.1 Lead poisoning

Since year of 2000 to year of 2009 reported in lead poisoning found 397 patients, average per year 39.7 patients. In year of 2001, it has highly number of patient about 104 patients and in year of 2005, it has lowest number about 14 patients and trend to decrease in number of patient since 2005 to 2008

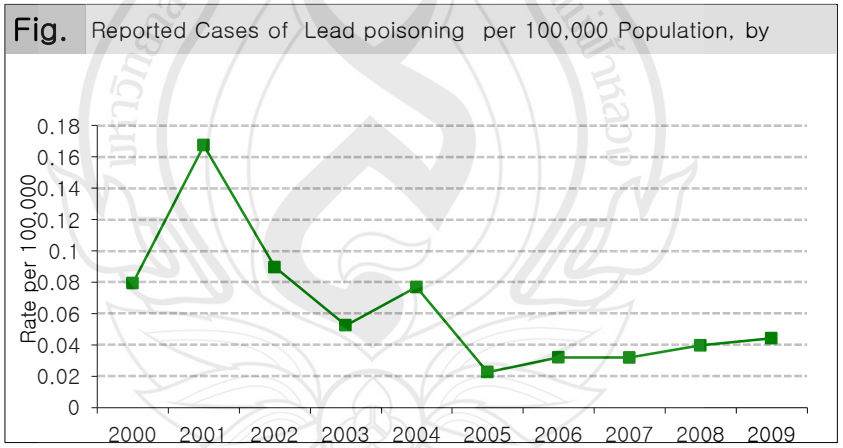


Figure 2.1 Reported cases of lead poisoning per 100,000 population by year (Bureau of Epidemiology, Department of Disease Control, Ministry of Public health)

In year of 2009, it has been reported lead poisoning found 28 patients. In north part has 15 patients, in north-east part has 9 patients, central region has 4 patients.

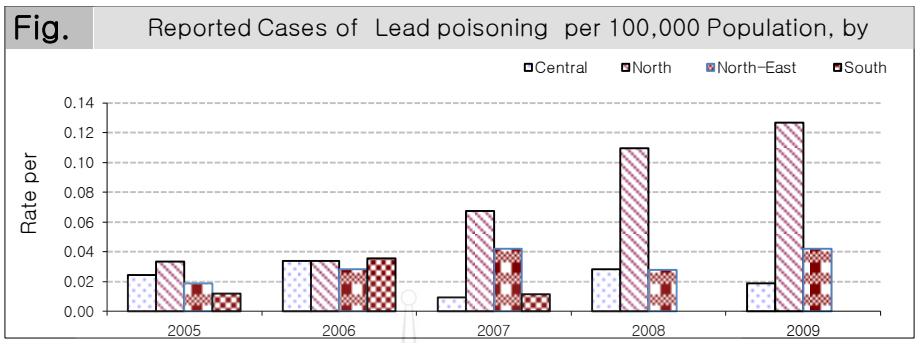


Figure 2.2 Reported Cases of Lead Poisoning per 100,000 Population by Region

Ratio between male and female are 1:1.15. In age between 0-4 years highly found in 0.10, and then 25-34 years, 45-54 years, 35-44 years and 10-14 years in rate 0.07, 0.06, 0.05 and 0.05, in respectively.

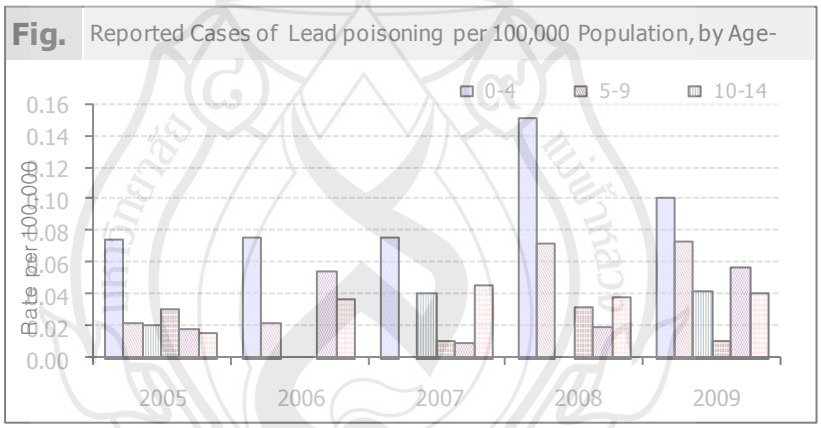
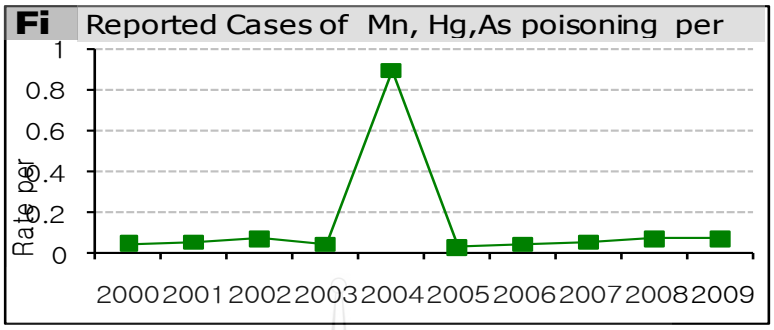


Figure 2.3 Reported Cases of Lead Poisoning per 100,000 Population by Age

2.1.2 Mercury, arsenic, manganese poisoning

Other heavy metals such as mercury, arsenic and manganese that they have been reported average per year 82 patients. In year of 2004, it has highly number of patient in 556 patients and lowest in 14 patients in year of 2005. (Ministry of Public Health, 2009)



From Ministry of Public Health. (2009). **Mn, Hg, As poisoning. National Disease Surveillance (Report 506)**. Bangkok: Bureau of Epidemiology.

Figure 2.4 Reported cases of Mn, Hg, As Poisoning per 100,000 Population by Year

In year of 2009, it has been reported lead poisoning found 42 patients. Rate of illness has 0.12 in north part, in north-east part has 0.07, in central part has 0.05, south part has 0.05.

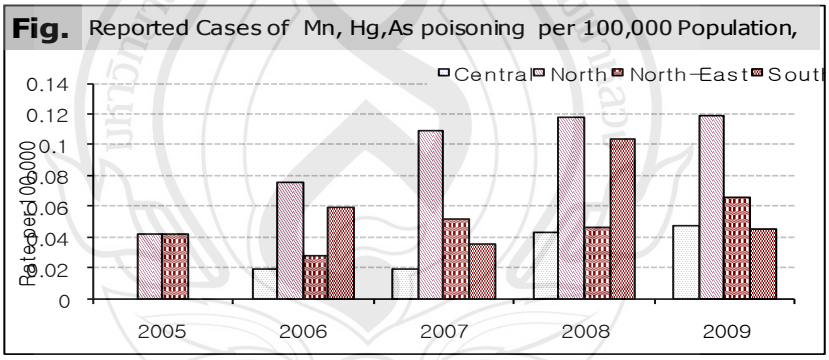


Figure 2.5 Reported cases of Mn, Hg, As Poisoning per 100,000 Population by Region

Ratio between male and female are 1:1.1. In age above 65 years highly found in 0.17, and then 55-64 years, 45-54 years, 15-24 years and 35-44 years in rate 0.12, 0.08, 0.08 and 0.07, in respectively.

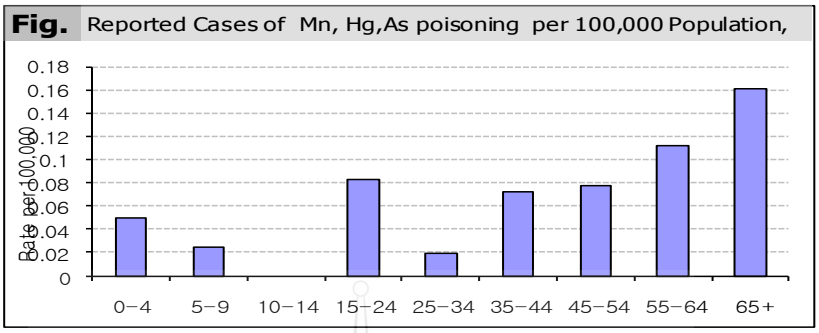


Figure 2.6 Reported cases of Mn, Hg, As poisoning per 100,000 Population by Age

2.1.3 In year of 2011: Lead poisoning

In year of 2011, In National Disease Surveillance (Report 506) from Bureau of Epidemiology, Department of Disease Control, Ministry of Public health has been reported since 1 January 2011 to 21 July 2011 found 15 patients from 6 provinces, found in male more than female, mostly found in age between 45-54 years (20.00%) and then 25-34years (20.00%), 15-24 years (13.33%). It waseen in high level of exposure of agriculture (33.3%). Rate of illness has 0.09 in north part, in north-east part has 0.00, in central part has 0.00, south part has 0.02. (Ministry of Public Health, 2011a,b)

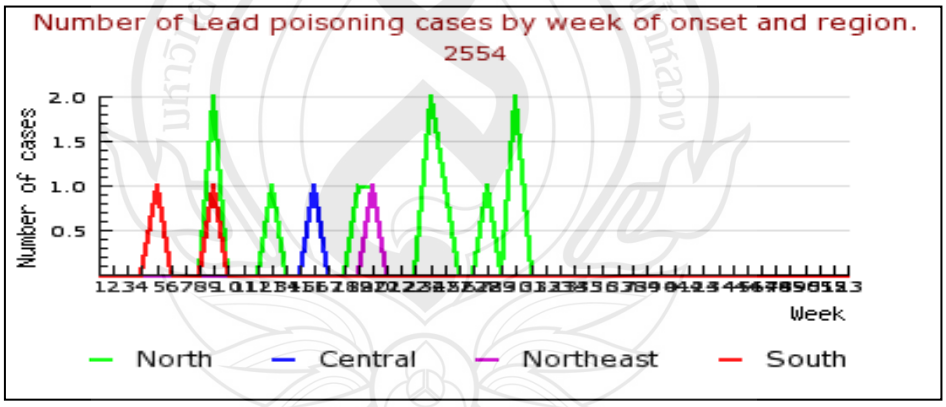
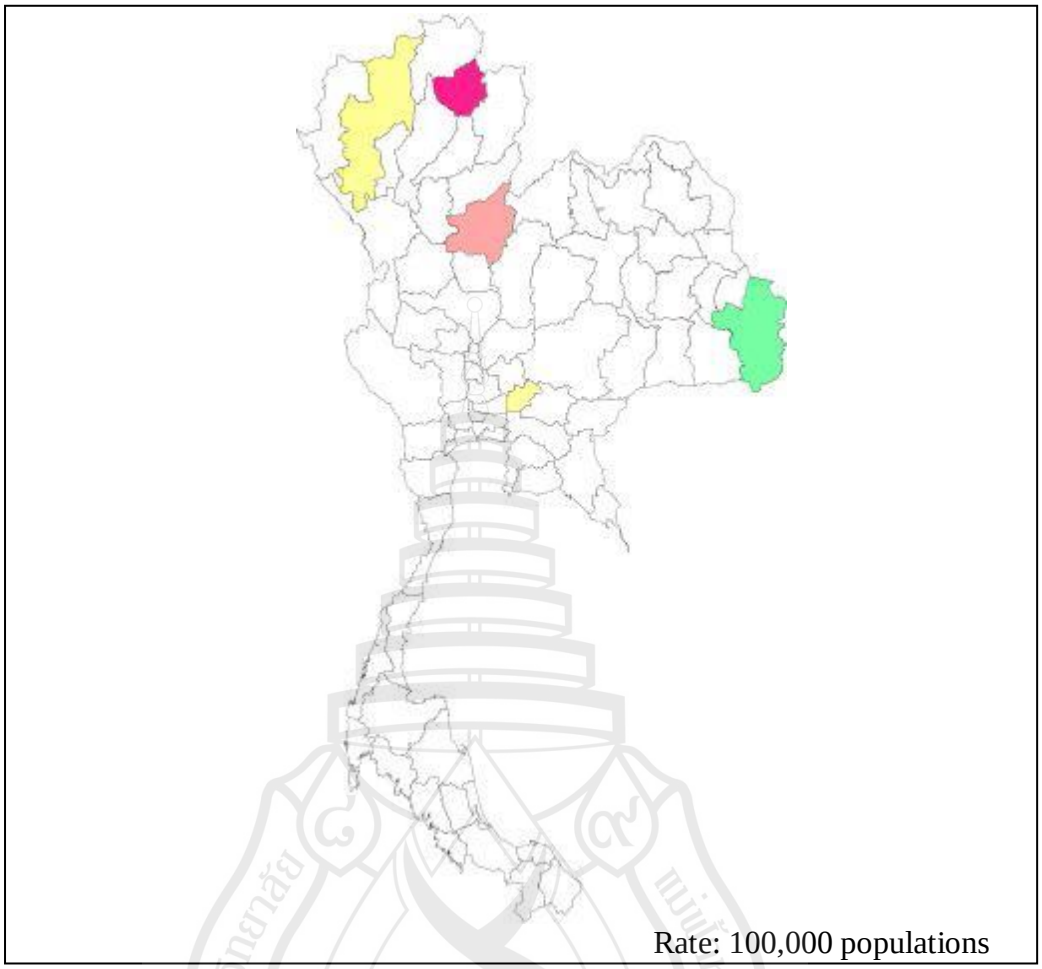


Figure 2.7 Number of Lead Poisoning Case by Week of Onset and Region



Note.

	0
	0.01-0.20
	0.21-0.40
	0.41-0.60
	0.61-0.80
	0.81-1

From Ministry of Public Health. (2011a). **Mn,Hg, As poisoning: National Disease Surveillance (Report 506).** Bangkok: Bureau of Epidemiology; Ministry of Public Health. (2011b). **Lead poisoning: National disease surveillance (Report 506).** Bangkok: Bureau of Epidemiology**.**

Figure 2.8 Rate of 100,000 Populations in Thailand

2.1.4 Mercury, arsenic, manganese poisoning

It has been reported since 1 January 2011 to 21 August 2011 found 24 patients from 9 provinces, found in male more than female, mostly found in age between 15-24 years (29.17%) and then 45-54 years (20.83%), 35-44 years (12.50 %). It seen in high level of exposure of labor (29.2%). Rate of illness has 0.14 in north part, in north-east part has 0.01, in central part has 0.00, south part has 0.06. (Bureau of Epidemiology, 2011)

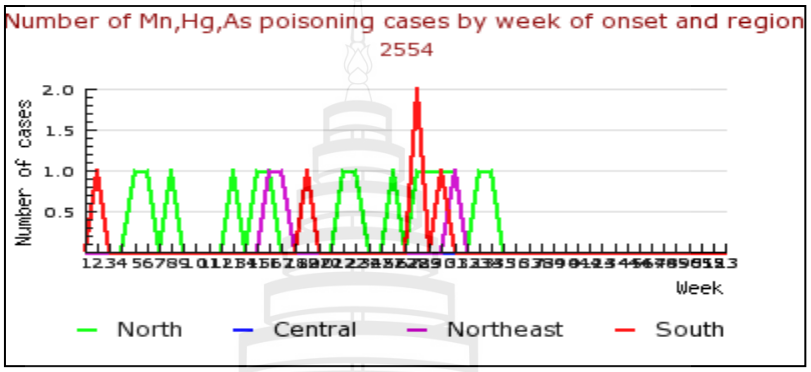
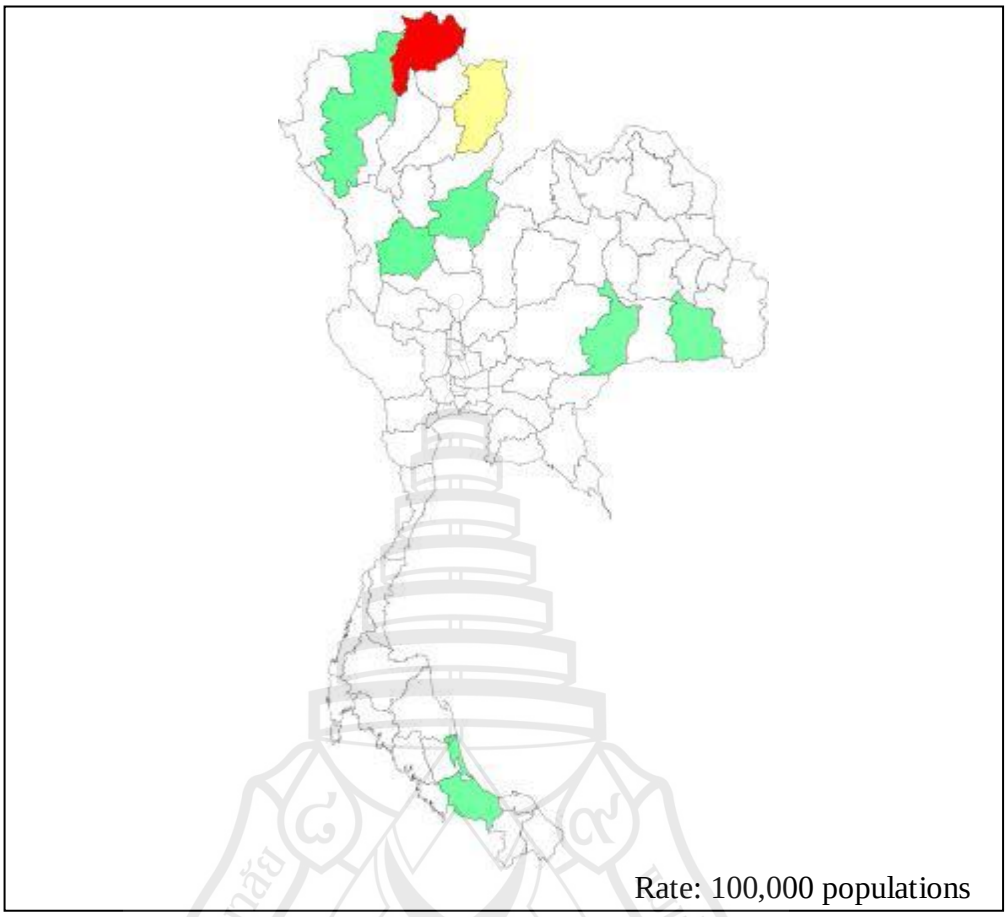


Figure 2.9 Number of Mn, Hg, As Poisoning Case by Week of Onset and Region

Table 2.1 Number and Percent of Heavy Metal in Population

Population	Number	Percent
Arsenic poisoning(As)	2	8.3
Other	3	12.5
Unknown	19	79.2



Note.

	0
	0.01-0.20
	0.21-0.40
	0.41-0.60
	0.61-0.80
	0.81-1

From Ministry of Public Health. (2011a). **Mn, Hg, As poisoning: National Disease Surveillance (Report 506).** Bangkok: Bureau of Epidemiology; Ministry of Public Health. (2011b). **Lead poisoning: National disease surveillance (Report 506).** Bangkok: Bureau of Epidemiology

Figure 2.10 Rate of 100,000 Populations in Thailand

Heavy Metals (HMs) can affect to human health and on the environment. Toxicology is generally well known that should be taken consideration in this topic. Human population in the world increases unprecedented at a rate of 1 billion in every 11 years, which occur in developing countries. (Miller, Monin & Prentice, 2000).

It leads to replacement of natural systems by large chemical, psychical effects on human beings. One million populations have contamination of atmosphere can be seen in the table;

Table 2.2 Output of Contaminants to Atmosphere (Tons per year)

Contaminants	1000 tons/year
Water as vapor	11.000
CO2+CO	1.200+240
SO2+N2O5	240+60
Hydrocarbons	108
Organics	8
Cl2 and HCl	5
HMs	-
Pb oxides	0.5
V oxides	0.05
Ni hydroxides	0.04
Hg, Cd, Be	0.0015

From Davydova, S. (2004). Heavy metals as toxicants in big cities. **Microchemical Journal, 79**(1-2), 13-136.

It presents in every area of modern consumerism-cosmetics, healthcare, medications, energy, transportation, and construction. Heavy-metal toxicity has complication about the effects of heavy metals are often delayed because the accumulation of heavy metal in the body, which have half lives of many years, although heavy metal present for long time, it is frequently misdiagnosed and miss about cause of disease. (Michael & Olmstead, 2000). Heavy metals toxicity caused by chemicals in industry and pollution can destroy health. Effects of long term exposure of heavy metals, and can damage caused by their accumulation in the body, it can identify of chronic heavy-metal including a level and duration of exposure, individual health can define the nature and severity of symptoms. (Dean, 2001). When human body has accumulation of heavy metal and also deposit in brain can affect to developmental and neurological damage. (The Great Plains Laboratory, Inc) The worse effects of heavy metals, including arsenic, cadmium, Iron, Aluminum, lead, mercury, are of great concern to the all people such as lead can cause of Parkinson, cognitive decline, and low intelligence quotient (IQ). Also mercury can cause of mood problems, hypertension and immune dysfunction. Some heavy metals exposure can destroy multiple tissues, organs and system that are exposed to heavy metals when the organ was young and occur development of permanent damage that is not reversible with current therapy, also increase the heart's susceptibility to infective myocarditis, change the mineral balance in heart muscle tissue. Some study show Eggelstone have shown effect with nickel and mercury which can affect to kidneys that has heavy metal concentrate rich in urine for remove, on the liver that

organ of detoxification. Person who has toxic heavy metals may experience no symptoms or have combination of symptoms of physiologic dysfunction. The presenting of symptoms is not the primary result of metal toxicity but secondary and tertiary results of the disturbances associated with high titers of heavy metals. For example, a patient has chronic fatigue or food allergies can be fell combined with inadequate absorption of essential nutrient through a leaky gut, overgrowth of intestinal flora by pathogenic microbes, and impaired function of liver enzymes which remove metals and harmful chemicals from the body. Heavy metals toxic remove is one of treatment measures such a patient requires. Practitioners have to identify and confirm that patients have unusually heavy toxic metal loads. It is important to report that "acute and chronic heavy metal toxicity" has a well-defined set of symptoms.

2.1.5 Routes of Entry

Toxic substances may be gases, liquids, solids, or vapors which has several ways to enter into the body. It enter pass through the lungs by inhalation, skin, mucous membranes, eyes by absorption, and gastrointestinal tract by ingestion.

2.1.6 Respiratory System

The respiratory system is consisted of the nose, pharynx, larynx, trachea, bronchi, and lungs and has function is supply oxygen to the cells of the body and expel carbon dioxide from the body in process called respiration. The action of breathing is brings air into and out of the lungs. External respiration is transfer of oxygen and carbon dioxide between the atmosphere and blood and internal respiration is transfer of gases between blood and cells. Air can pass through nasal hair into nasopharyngeal area at the vestibule. Nerve endings in nasal are stimulate to sneeze reflex for help to remove the mucous.

Air enters the trachea-bronchial area by inhalation. It has bronchi branch into bronchioles with smaller diameters until they stop at thin-walled of alveoli which gas exchange takes place. Mucociliary streaming is a process which remove dust and pollen of 10 µm or larger particle by the constant mucous propelled from the bronchial and tracheal passages by the cilia beating at over 1300 times per minute.

Bronchi and trachea have large tube surrounded by smooth muscle layer that has response about irritate allergens. Capillaries have alveoli surrounding them which red blood cells give up carbon dioxide and receive oxygen in exchange. Some particle in the air greater than five µm can be removed within the nasopharyngeal areas by mucociliary streaming, swallowed, sneeze. Some particles are in range of one µm, alveoli will submerge by macrophages or moved respiratory tree in a mucous sheet, store it in the interstitial tissues of the lung and enter the lymphatic system to blood circulation. Some inorganic particles cannot be digestion by macrophages.

2.1.7 The Skin

The skin is commonly barrier to chemicals and biological agents. The skin is largest organ which compost of tissues covering an area about 3,000 in adult. The skin has an outer layer call epidermis and inner layer call dermis under this layer is subcutaneous layer of glandular and adipose tissues. Function of skin are regulate body temperature through sweat glands, provide a physical barrier for dehydration, excrete salts, water, and organic compounds, a sensory organ for touch, temperature, pressure, and pain, immune. The skin can effective in keep water and water soluble (hydrophilic)

which absorption can be increased as sweating increases, moisturizers and oily. About fat soluble (lipophilic) such as organic solvents, petroleum compounds can be absorbed by passive diffusion through the skin.

2.1.8 The Gastrointestinal Tract

The gastrointestinal tract is about 30 feet in length from the mouth to the anus. Components of the digestive tract include the mouth, pharynx, esophagus, stomach, small intestine, large intestine, and anus. The digestive system is function for ingest and take food for digestion and nutrient absorption. The system also tract is a major route of absorption which occurs between the stomach and the upper part of the intestine for heavy metal toxic including mercury, lead, and cadmium which appear in food and water and can be transfer from hands to mouth. Toxic can pass through epithelial cells of the villus into the blood and lymph vessels. Carbohydrates, proteins, electrolytes and other nutrients and are absorbed by facilitated and active transport. The absorption can be influenced by physical health such as parasites, viruses, infectious bacteria, ulcers, cancer and it is affected by nutritional status, chemical and physical substances in the intestinal tract, age, physical activity.

2.1.9 Factors governing toxicity

Toxic exposure depends on a number of factors that include:

2.1.9.1 The chemical substance
2.1.9.2 The concentration of chemical substance
2.1.9.3 The duration of heavy metal toxic exposure
2.1.9.4 The route of exposure
2.1.9.5 Bio-transformed in the body
2.1.9.6 The interaction with other pollutants
2.1.9.7 Other factors including age, level of activity or stress, and health status.

2.1.10 Chemical Properties

Chemicals variable about toxicity to mammals such as oral dose of sodium chloride require 3,000 mg/kg to achieve an LD50 in rats whereas strychnine require less than 2.5 mg/kg. The LD50 is defined the dose of a chemical which cause of death in 50 percent in animal population.

2.1.11 Concentration

The concentration of the chemical substance is important because of concentration and duration of the exposure determines the dose. Dose-response has relationship between toxin and health effect. Adverse effects of toxic exposure are a slight irritation, itchy, red eyes to rapid death. Large doses have dangerous more than small dose in the same toxin. In acute illness, symptom effects are occurs immediately. Chronic illness is affect in long term exposure to small dose of toxins where illness presents in slowly and insidiously. The effective dose is the amount that reaches in general circulation and eventually the location which effect can be seen or felt. This dose, with the speed, it can be determines the pharmacologic effect. For example, drinking five beers in an hour are clearly different effect from drinking five beers in four hours. In the general circulation, the toxic element

can be absorption, store, bio-transformed and excrete. The route of entry is determine exposure of toxic such as lead and mercury can absorption pass through the lungs more than the intestines. Chemical substances are stored in body compartments characterized by structures and functions such as lead tend to be stored in long bones. If it stored at levels above normal that this is termed bioaccumulation. Metabolic processes change in structure and characteristics of a chemical that this is called biotransformation.

Absorbed toxic substances may also be excreted. The major organs which can excreted about toxic are the kidney, liver, and lungs.

2.1.12 Interactions

The effects of substances are additive that two substances taken together are results in the two substances taken separate. If you expose to chemical A which produces response of a 5 percent decrease in red blood cells (RBCs), and chemical B produces a reduction of 3 percent in RBCs, when they taken together (A+B) can be produce an 8 percents reduction. It produces in effect called synergistic. When chemical A and chemical B are taken together which effect is produce a decrease only 2 percent in RBCs, the effect is less than results in two acting called antagonistic.

2.1.13 Age

Age is a major factor. Young and elderly tend to be increased risk of toxic substances. The young are sensitive to toxic as a following

2.1.13.1 Inhalation or ingestion more toxic per pound of body weight than adults

2.1.13.2 Put substances into their mouths

2.1.13.3 Rate of absorption about toxic into the tissues is higher than adults

2.1.13.4 Tend to keep a large proportion of toxic than adults

2.1.13.5 Nervous systems and detoxify systems of children are not fully developed cause of disproportionate effects from toxic.

The elderly also have a risk of toxic, they tend to have pre-existing respiratory and cardiovascular diseases, depressed immune systems, and stored toxic agents in higher level in the body than younger people.

2.1.14 Exercise and Physical Stress

Athletes always aware that shouldn't run in hot, humid, heavily polluted air because can be reduce the performance, and have pain in the area of the lungs. There is tends to take in large amounts of air pass through the mouth. Then the pollutants bypass the nasopharyngeal area and enter directly to tissues of the lungs.

2.1.15 Health Status

The health status is more important in result in toxic chemical exposure. Persons who have asthma and always smoke also induce sensitive to the pollutants. Genetic polymorphism, or variations of gene can be produce slight or dramatic differences to response of toxic chemicals. The P450 genes produce enzymes which detoxify some substance in tobacco smoke. Some people has variations of P450 genes can increase the risk of lung cancer. Weaken diseases, poor nutrition, and lack of exercise all lead to increase risk of adverse effects from toxin.

2.1.16 Lead

Lead is a naturally bluish-gray metal and no special taste or smell. It can be found in environment and from mining and burning of fossil fuels. It is used in storage batteries, alloys, solder, ceramics and plastics. It also used in pigment manufacture, tetraethyl lead, ammunition, atomic radiation and x-ray protection. Lead is used in aircraft manufacture, building construction materials, insulated cables and wiring, household utensils, laboratory equipment, reflectors, paper industry, inks, glass industry, water purification and waterproof in the textile industry. It does not break down but can change by sunlight, air, and water. When it enter into the air, it will stays in air about 10 days and then fall into the ground. Lead does not move from soil to underground water or drinking water unless the water is acidic or "soft" and will stay a long time in both soil and water. Environmental Protection Agency (EPA) limit in the air not to over than 1.5 micrograms/m^3 averaged more than 3 months and drinking water is limit in15micrograms/L. OSHA limit in a workroom air is 50 micrograms/m3 for an 8-hour workday. If they have a blood lead level of 40 micrograms/dL, OSHA suggested they should be move out from the workroom air. Lead poisoning is one of the most occupational diseases and can move from industry to environment, although it will decrease in recent years. Inorganic lead is not significantly absorbed through the skin but can absorb by the gastrointestinal tract which through swallowing of inhaled particles or food and tobacco Lead dust, fumes can absorbed through respiratory tract then move to the liver and kidneys, then it can stored in the bones and can affect to red blood cells.

In the United States lead presents in residents of the northeastern region, inner-city residents, low-income individuals. Lead can pass through maternal into fetal by placenta and lactation that fetal can sensitive to toxic. When the body has to exposure toxic from environmental can lead to neurobehavioral disorders such as Parkinson's disease, of permanently physically and emotionally handicapped. Lead can destroy DNA, damage kidney functional, decrease the immune system, anemia, lung fibrosis, emphysema, hypertension, increased tooth decay, decrease intelligence quotients (IQ) and learning disabilities. The worse effect has been study too long for past 100 years. It can stay in human body for long cause a half-life in bone of 62 years. Past of study found in people who exposure to lead increase in dental caries by 40% and decrease in parotid gland functioning by 30% in people who had been exposed to lead and another study show lead activate from bone of mothers who lactating, resulting in milk lead levels more than serum. It affect to baby is apparent. Lead exposures in mother relate to birth of low-birth weight children with decrease neurobehavioral development, induce aggressive behavior. In the guidelines of the centers for disease control and prevention in Baltimore, 50% of children was screened had blood lead levels, In the United States, Blood levels of lead and lead poisoning still have affect and some study indicate DNA damage. Reduce enzyme in brain, Alzheimer's disease (Michael & Olmstead, 2000) Blood sample test is available for assessment the amount of lead and the amount of your exposure to lead. It can screen children for chronic lead poisoning. The Centers for Disease Control and Prevention (CDC) determine children have an elevated level of lead if the amount of lead in the blood is at least 10 micrograms/dL. Lead in teeth and bones can be measured with X-rays

2.1.17 Mercury

Each year of North Americans in human body found mercury embed in the form of mercury-silver fillings. In the U.S. Bureau of Mines 1991 Minerals Yearbook that show in dental use average in 50 tons of mercury. Governments of Sweden and Germany limit in use of dental amalgams that it has mercury because of increasing risk of glioblastoma reported in Swedish dental workers due to factor such as amalgam, chloroform or radiography. Mercury can effects on the central nervous system (CNS) which inhibition of the binding of guanosine triphosphate to tubulin in the brain like lesion of Alzheimer's disease, the renal and reproductive systems and depress immune systems. Forms of absorption is various forms such as, organic or methylated (most common organic is methyl mercury, which produced by organisms in the water and soil), Inorganic which compounds are white powders or crystals or salts that mercury salt use in skin-lightening creams and as antiseptic creams, Metallic mercury can produce chlorine gas and caustic soda in thermometers, amalgams (dental fillings), and batteries. Metallic and inorganic mercury enters in the air from burning coal and waste, and manufacturing plants. In the water or soil can enter from natural deposits or wastes which in forms of bacteria. It has different metabolic pathway in each form. First elimination passes through liver and biliary avenues and occurs in urinary. In one study present result in neuropsychologic and motor control effects in dentist compare with non-dentists are significant results. In rat mercury can increase autoimmunity, depression immune system, disturbance of human lymphocyte functions including proliferation, and cytokine production. The study in monkey, sheep, humans show result in kidney concentration of mercury after amalgam placement. The worse effect of mercury exposure are brain development which affect to young people more than adults such as a irritability, shyness, tremors, changes in vision or hearing, and memory problems, menstrual disturbances, spontaneous abortion, premature deliveries, infertility, pregnancy outcome and reproductive dysfunction, autism (Michael & Olmstead, 2000). Acute toxic depend on route of exposure. Ingestion is largely without effects. By inhalation cause severe respiratory irritation, acute pulmonary edema, coughing, trouble in digestion system and kidney damage. Mercury can cause “Kawasaki” disease which is a immunological mediated. The World Health Organization (WHO) reported no evidence in carcinogenic from inorganic mercury regarding mutagenicity. In the study of chloralkali plants workers who exposure of metallic mercury show no significant increases in chromosome aberrations in peripheral. Available testing can measure mercury levels in the body are blood or urine which test for exposure to metallic mercury and forms of inorganic mercury. Measurement in whole blood or in scalp hair is used to test for result in exposure to methylmercury. (Heavy Metal Toxicity, n.d)

2.1.18 Cadmium

Cadmium is a natural element and normally found combine with another elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide). In the industry uses cadmium for extraction of production of metals like zinc, lead, and copper, and also used in process engraving, in electrodes of vapor lamps, photoelectric cells, photometry of ultraviolet sun-rays. In the industry products use cadmium for batteries (Ni-Cd batteries of mobile phones), pigments, metal coatings, and plastics. It used a powder in dentist, as an amalgam. Cadmium enters in the air from industry, burning coal and waste products and can travel in the air for long distances before fall into the ground or water. Also enters into soil from waste product and binds with soil so strong. In the water cadmium can disappears but cannot break down still changes to other forms, fish, plants, and animals got cadmium from the environment and will stays in the body for long time and progress from many years of exposure to low levels. Environmental Protection Agency (EPA) drinking water is limit to 5 ppb and has limits how much cadmium can enter lakes, rivers, waste sites, and in pesticides. Food and Drug Administration (FDA) limit in food colors: 15 ppm. Occupational Safety and Health Administration (OSHA) workplace air limit: 100 microgram/m3 as fumes of cadmium and 200 microgram/m3 as dust of cadmium. (http://tuberose.com). OSHA also limit cadmium compounds to 1 or 5 microgram/m3. It has low levels in all foods (highest in shellfish, liver, and kidney meats), breathing cadmium in smoke, breathing contaminated air near the burning fuels, drinking water.

Cadmium has a long half-life of 15-20 years in human and can lead to chronic disease such as liver damage, kidney damage, renal tubular dysfunction, pulmonary emphysema, immune-system depression myocarditis and distorted calcium metabolism with attendant osseous effects and osteoporosis and also interaction with calcium in the skeletal system can cause osteodystrophy. When you breathing high cadmium level such as dust or fumes can occur acute effects of cadmium which cause throat dryness, coughing, headache, nausea and vomiting, chest pain, e restlessness and irritability and bronchopneumonia or consume food or drinking water with very high levels can induce salivate, irritates the stomach and vomiting and diarrhea. It also can classify a carcinogen in human in 1993 by the International Agency for Research on Cancer. In study of the George Washington University School of Medicine, it show that occupational and environmental cadmium exposure can cause to prostate cancer and male infertility which has indicate male reproductive is target organ for accumulation of toxic heavy-metal and risk of lung cancer. It affects to neurological disorders in body human , also confirm by laboratory test in animals shown low-level, multigenerational exposure to inorganic cadmium can affect the nervous system because can pass through placental barrier and show in mother milk. Body burden can pass from generation to generation. Some study in Belgium in 1997 said cadmium is neurotoxic to the peripheral nervous system. The major target organ of cadmium is liver and Metallothione, a cysteine-rich metal-binding protein can resist cadmium induced liver damage. Though cadmium is not increase liver enzyme levels, but can affect to hepatic affect such as granulomatous inflammation; apoptosis, of preneoplastic nodules and nonspecific chronic inflammation. (Michael & Olmstead, 2000). The measurement of cadmium is in the blood which can show recent exposure, urine which show recent, early exposure and can indicate kidney

damage, hair, or nails. But reliability for cadmium levels in hair or nails testing is unknown.

2.1.19 Manganese

Manganese is occur in natural and has silver color with no taste or smell. Manganese is the element compound with oxygen, sulfur, or chlorine. It has benefit in human health, you intake adequate with a balanced diet. Manganese is two forms of manganese that inorganic and organic manganese. Inorganic manganese compounds use in steel production, batteries, ceramics, and dietary supplements. Organic manganese compounds used in pesticides, fertilizers, and in a gasoline additive called methlcyclopentadienyl manganese tricarbonyl (MMT). Manganese presents in the air and can be dissolves in ground water or drinking water. Infants and children can be exposed pass through breast milk, diet, air, drinking water. High concentrations in hair or blood of children are associated with learning disability, neuromuscular effects and choleastatic liver disease (with decreased liver function). The primary targets of manganese are in the brain and central nervous system. It can accumulate in the brain and destroy them which have a symptom such as impaired neurological and neuromuscular control, mental and emotional disturbances, muscle spasm, lack of coordination, tremors, difficult with breathing or swallowing, and neuromuscular problems. About high exposure has symptom of impaired male fertility, impaired bone development, anemia and other. (EPA's, 2007a, b). In human has report manganese can induce in Parkinson's Diseases at least ten years after exposure. Route of exposure are in ingestion, inhalation pass through gut and enter into the brain in two ways by olfactory to brain tissue and by lung uptake or other route.**:** The U.S. EPA and The World Health Organization International Agency for Research on Cancer (IARC) determine "manganese is not classifiable as a human carcinogen" because about studies were inadequate to assessment the carcinogenicity of manganese.

Table 2.3 Manganese Exposure

Exposure Media	Relative Potential for Children's Exposure	Basis
Diet	Higher	Manganese can found in infant formulas and breast milk, some cereals, leafy vegetables, fruits, and fruit juices. Elevated intake of manganese above recommended amounts may be of concern in some dietary situations.
Ambient Air	Medium	Manganese uptake by inhalation way is greater concern for toxicity than uptake pass through ingestion. Manganese can be found in ambient air. Air concentrations can be high concern near industries processing or using manganese (e.g., mining operations, metal processing plants, coke ovens, power plants, and certain pesticide producers). Use of the manganese-containing additive MMT in gasoline contributes to ambient air contamination, though MMT use in the U.S. is uncommon.

Table 2.3 (Continue)

Exposure Media	Relative Potential for Children's Exposure	Basis
Ground Water	Lower	Manganese is a natural in ground water. Elevated concentrations of manganese may exist in some ground water due to certain bedrock formations or pollution sources.
Drinking Water	Lower	Elevated concentrations of manganese may exist in some drinking water due to certain bedrock formations or pollution sources.
Surface Water	Lower	Manganese is not generally found at elevated concentrations in surface water.
Indoor Air	Lower	Manganese is not generally found in indoor air.
Soil	Lower	Manganese can be found in soil, but is not likely to be a significant contributor to exposure for most children

From EPA's. (2007a). **Manganese TEACH Chemical Summary**. Retrieved Sepetmber 20, 2011, From http://www.epa.gov/teach/chem_summ/manganese_summary.pdf

2.1.20 Chromium

Chromium is element in environment and has three forms as forms chromium (0) which not occur in naturally, chromium (III) which occur in nature and more stable and it is nutrient in diet, and chromium (VI) which occur very rare. It is also no odor and taste like other element. Chromium found in soil, plants, animals, volcanic dust and gases in natural. Chromium is more important in life beginning which has a process of cell duplication by division (mitosis) over and over again that need more energy which was establish from adenosine triphosphate (ATP). ATP is more important in cell duplication because it provides energy to cells. ATP produces from glucose (blood sugar) and oxygen. Function of chromium is to bring glucose into the cells for *glycoloysis*--the first step in ATP production. Chromium chloride is salts of chromium are absorbed at a level of 0.05 percent less than 20 and 50 times of chromium in food which can absorbed form 10-25percent. Chromium by itself has no found about toxic at any level of usage and can safe at levels up to 10,000 micrograms. In manufacture use chromium for chrome-steel or chrome-nickel-steel alloys and other alloys, furnaces, dyes and pigments, leather tanning, and wood preserving. When chromium enters into the air, it can settle less than 10 days then falling into the ground and can stay in soil and can move to groundwater or water.EPA determined maximum level for chromium(III) and chromium(VI) in drinking water: 100 micrograms/L. Occupational Safety and Health Administration (OSHA) limit for an 8-hour work per day, 40-hour work per week: 500 micrograms/m^3 for water-soluble chromic (chromium (III)) or chromous (chromium (II)) salts and 1,000 micrograms/m^3 for metallic chromium(chromium(0)). Chromic acid and chromium (VI) compounds in the workplace air should not be higher than 100 micrograms/m^3 for any period of time. National Institute for Occupational Safety and Health (NIOSH) exposure

limit: 500 micrograms/m³ for chromium (0), chromium (II), and chromium (III) for a 10-hour work per day, 40-hour work per week that considers all chromium (VI) compounds to be carcinogens, and suggests limit of exposure in 1 microgram/m³ for a 10-hour work per day, 40-hour work per week. National Research Council (NRC) dietary limit for intake chromium (III) is 50-200 micrograms/day. All forms of chromium can be toxic in high levels, but chromium (VI) is more toxic than chromium (III). Acute effects of toxic show when breathing in high levels of chromium (VI) in the air, and can irritate and destroy a nose, lungs, stomach, and intestines, asthma attacks when breathing at high levels of r chromium (VI) or (III). For Long term exposures to moderate or high levels can damage nose (bleeding, itching, sores) and lungs, and increasing risk of non-cancer lung diseases, stomach upsets and ulcers when ingestion of large amounts of chromium, kidney and liver damage, and death. Liquid can lead to skin ulcers and have allergic reactions such as redness and swelling. The Department of Health and Human Services has determined that certain chromium (VI) compounds are known carcinogens, it increase lung cancer in people who work to exposure to chromium. The measurement of chromium is urine, blood, hair and red blood cell. It useful for people who exposure at high level. Allergy reaction of chromium can be detected by skin patch test.

2.1.21 Co - Cobalt

Cobalt is occurs in naturally and present in different chemical forms. In industry use cobalt in import or obtain by recycle scrap metal which contains cobalt and make alloy ,colored pigments, for paint and porcelain enamel used on steel bathroom fixtures, and kitchen wares. In food can found in small amount and Vitamin B12 is a compound with cobalt that is essential for health. Cobalt can use in anemia treatment causes can produce red blood cell. Sources of cobalt from natural are soil, dust, and seawater. It can liberate from burning coal and oil, car and truck exhaust. When cobalt enters to the air, it can stay in a few days. Cobalt does not liquate in water but can stay in water and soil for years. Also it move to underground water from soil and take into plants. OSHA exposure limit: 0.1 0.1mg/m³ for cobalt in workplace air for an 8-hour workday, 40-hour work week. American Conference on General and Industrial Hygiene (ACGIH) occupational determine limit of exposure at 0.02 mg/m³ for cobalt for an 8 hour work per day, 40 hour work per week. National Institute for Occupational Safety and Health (NIOSH) occupational has limit of exposure at 0.05 mg/m³ for cobalt for a 10-hour workday, 40-hour workweek. Acute toxic effect of cobalt can observed in lungs, asthma, pneumonia, and wheezing. It found in high level in people breathe cobalt in the air. In people who more drinking beer has symptom of nausea, vomiting, and effects on the heart. The International Agency for Research on Cancer (USA) present that in the human, cobalt is possible to carcinogen. In the animals studies shown that cobalt causes cancer when direct contact to the skin and into the muscle. Human studies are not conclusion about cobalt and cancer. The cobalt testing are urine and blood level and can accurate for up to a few days after exposure because cobalt can leaves from the body so quickly.

2.1.22 Nickel

Nickel is a very plentiful element and can found nickel combine with oxygen or sulfur. Nickel can be forms alloyed with are iron, copper, chromium, and zinc that alloys can use to making coins and jewelry, also use for nickel plating, color ceramics, batteries.

It is not to odor or taste. Nickel can distribute in the air and then falling to the ground or are taken out of the air in rain. In environment can found nickel in soil because nickel particles containing in iron or manganese which present in soil and sediments. Environmental Protection Agency (EPA) presents children drinking water limited 0.04 mg/L; 1–10 days of exposure. Occupational exposure limited 1 mg/m3; 8-hour workday; 40-hour work per week. *Nickel Carbonyl: Lethal concentration inhaled by rat 35 ppm (240 mg/m3; 30 min. American Congress on General and Industrial Hygiene (ACGIH) Limit Value (TLV-TWA) is 0.05 mg/m3. Major sources of exposure are: tobacco, auto exhaust, fertilizers, superphosphate, food processing, hydrogenated-fats-oils, industrial waste, stainless steel cookware, testing of nuclear devices, baking powder, fuel oil, dental work and bridges. The most common adverse health effect of nickel in humans is an allergic reaction from nickel alloys which have 15 and 30 percent of the population (Michael & Olmstead, 2000), asthma attacks, chronic bronchitis and reduced lung function, depressed growth, altered serum lipids and glucose levels, lowered reproductive rates, rhinitis and sinusitis from nickel aerosols;, nasal sinus cancer, nephrotoxic effect. People can become sensitive to nickel when things containing it are in direct contact with the skin which effect on skin rash at the site of contact, eating food, drinking water which If people drink water containing 100,000 times more nickel than in normal drinking water had stomach aches and effects to blood and kidneys, or breath dust containing nickel, acute pneumonitis from inhalation. Acute toxic effects present in two stages, immediate and delayed. Symptom of immediate nickel exposures are headache, dizziness, shortness of breath, vomiting, and nausea. The delayed effects (10 to 36 h) are chest pain, coughing, shortness of breath, bluish discoloration of the skin, and in severe cases, delirium, convulsion nickel may be anticipated to be carcinogens. The test is available for evaluates the amount of nickel exposure are in the blood, feces, and urine.

2.1.23 As – Arsenic

Arsenic is found in environment at low levels and combines with oxygen, chlorine, and sulfur (inorganic arsenic) but in plants and animals combines with carbon and hydrogen (organic arsenic). Inorganic arsenic is more harmful than organic arsenic. It is no specific smell or taste. Inorganic arsenic used to make insecticides, weed killers, fungicides, antifouling paints, drugs, war gases and weapon. Other compounds are alloys, manufacture and certain glass. Arsenic does not evaporate in the environment, dissolve in water, enters into the air when has burning of material and then falling to the ground which cannot break down but can change in another form. Environmental Protection Agency (EPA) drinking water limit: 0.05 ppm and stop for use in pesticides. Occupational Safety and Health Administration (OSHA) limit exposure for workplace airborne arsenic: 10 micrograms/m³ breathing or smoke from wood containing arsenic, contaminated water, soil, and air. High levels of inorganic arsenic in food or water can be mortal. Arsenic can destroy many tissues including nerve, stomach and intestines, skin, sore throat and irritated lungs when you breaths in high levels. Also inhibit sulfhydryl enzyme which for cell metabolism except arsine and the potency depends on atom of arsenic. It effect on hemoglobin to strongly form of hemolytic poison inorganic arsenic exposure at low level can cause nausea, vomiting, and diarrhea, reducing of red blood cells and white blood cells, unusual heart rhythm, blood vessel damage, a “pins and needles” sensation in hands and feet, painful, shock, coma, convulsions and

death, irritation, inflammation, kidney damage. When the skin contacts to direct can shows in redness and swelling. Arsenic can plays a key role in the pathogenesis of vascular endothelial dysfunction as it inactivates endothelial nitric oxide synthase and lead to reduction in the generation and bioavailability of nitric oxide. Arsenic can cause arrhythmia by increasing the QT interval and accelerating the cellular calcium overload. The chronic exposure to arsenic up regulates the expression of tumor necrosis factor-α, interleukin-1, vascular cell adhesion molecule and vascular endothelial growth factor to induce cardiovascular pathogenesis. Excess manganese interferes with the absorption of dietary iron. Long-term exposure to excess levels may result in iron-deficiency anemia. Chronic effects also are fatigue, loss of energy, induce pigmentation of skin, dermatitis, rashes, muscular paralyses and atrophy, sensory and visual disturbances and also blindness, liver dysfunction, hypertension. The Department of Health and Human Services (DHHS) present that arsenic is a known carcinogen and also increases the risk of lung cancer and respiratory tract. Inorganic arsenic induces risk of skin cancer and tumors of the bladder. The measurements are available for assessment arsenic exposure level is urine which the most reliable test for arsenic exposure. It will stay in the body in short time, test must be done soon after exposure. In hair or fingernails test can evaluate high level of arsenic exposure over the past 6-12 months, but not helpful for low exposure levels. Tests do not predict harmful health effects due to toxicity of arsenic.

2.2 Heavy Metal Testing

In polluted areas, such as cities and industrial areas which has been reported in higher incidence of chronic illnesses in people. Mercury, aluminum, cadmium, nickel, cobalt, uranium, lead, thallium, arsenic, etc. exist in our water, air, food, amalgam dental fillings and also absorbed into the body's cells which cause destroy in tissue , functional disturbances and affect to body weakness that can result in severe illness. After heavy metal was into body and can live in body's cell but not strong present in the blood, urine or hair samples. Chronic disease must be concern, if it cause by heavy metals, you have to remove it by appropriate detoxification. The detection of heavy metal toxicity can be a key factor to a successful in treatment of acute or chronic disease. Heavy Metal lab testing is appropriate for beginning, middle and end of therapy. Heavy metal lab testing provide for detection of toxic metal and reducing risk of toxic when it show false negative.

2.2.1 Urinary Toxic Elements

Urine heavy metal testing provide for evaluates urinary excretion of toxic heavy metal acquired acute or chronic exposure. It can determine from baseline test when heavy metal shows. This test can do by administrating a 'challenge agent' such as DMSA or DMPS which metal and collect urine 2-6 hours after oral administered then sent it to lab after 5-7 day , you will receive a results. If the results show higher than 5ppm of toxic metal, you have to remove by detoxification. If it not, liver and kidney have to integrated before retesting. This test also shows the progress of detoxification regimens and nutrition status during treatment.

2.2.2 Limitations for Urine testing:

It is not present body burden of heavy metals. . End point of detox can have a 'layered' effect in lab results that things are changed in a person's environment. For example: patient show result in lab testing below 5 ppm of heavy metal for several months upon completing an wide heavy metal and basic health program. After she stopped smoking and Urinary output of mercury, cadmium, and aluminum show result between 17 and 20 ppm of toxic metals. Smoking kept these deeper levels 'cloaked' until patient was ready to quit and receive detoxification program

2.2.3 Hair Analysis

It presents toxic heavy metals and mineral transport to hair by blood circulation. Hair can reflect change before abnormalities are obvious and show concentration of toxic 200-300 time more than blood and urine. The Centers for Disease Control (CDC) present the hair mercury levels as a maternal and infant marker for exposure to neurotoxic methylmercury from fish.

2.2.4 Limitations for Hair testing

It is not present body burden of heavy metals**.** Heavy metal toxic level in body will be influenced by ability to eliminate toxin from the body. Also show substance of external contamination such as perm, dyes. It can show toxic exposure in recently but not the present.

2.2.5 Whole Blood Element

This test is assessment for deficiencies, excesses and imbalances of elements as well as recent or continuing of toxic metal exposure. It evaluates total portion level that circulates extra-cellular (serum/plasma) as well as intra-cellular (function within blood cells).

2.2.6 Limitations of Blood testing

It is not present body burden of heavy metals for accurately such as heavy metal in blood circulation is accumulation of tissue and bind very tightly. For example, when body exposure to lead which appear to peak 4 to 5 hours in blood and decrease with a half-life of about 27 days. Then blood lead level is limited to detection in recent or continuing exposure

2.2.7 Fecal Metal Test

This test is assessment for toxic metal burden. Fecal is a one way for remove primary natural but can irritate in people who have problem with GI or colon. It indicates to dietary exposure to heavy metal toxicity. The primary process of body eliminates sulfhydryl reactive metals through metal-glutathione complex which more than 90% are remove by the bile. Dental amalgams in mouth is high relate to fecal mercury level, also have 10 times more than people who have not mercury amalgam

2.2.8 Limitations for fecal testing

It is not present body burden of heavy metals. The end point of detoxification difficult to re-absorption with the irregularity of bowel movements compared with heavy metal exposure level. (BioRay, Inc., n.d.)

2.3 Heavy Metal Urine Testing

This test is evaluates any heavy metal in your body and then remove it through kidneys into your urine.

2.3.1 Precautions

There are two major for concern about heavy metal urine testing. First of all, if you have kidney disease this test is not appropriate because it can load on your kidneys. Second, this test can chelate all heavy metals, minerals and it also temporarily chelate out calcium and iron. If these minerals are important for you and when it decrease that can dangerous, the test should not be done.

You should tell your physician about your kidney disorder.

2.3.2 Preparation:

2.3.2.1 Do not take any supplements for 72 hours before you start test. If you have any concerns about stopping supplements.

2.3.2.2 Do not stop prescription medications.

2.3.2.3 Do not collect sample while menstruate.

2.3.3 Procedure

2.3.3.1 Begin by urination. This will act as a flush before you started with the urine collection.

2.3.3.2 After urine take Captomer capsules (ALL the capsules you were dispensed in the envelope at 30 mg of DMSA/kg body weight) with a small amount of food-and start at time 6 hours. This is when the urine collection begins from this point on, collect all your urine in the plastic cup provided and pour it into the large 3000 ml orange container to store.

2.3.3.3 Continue to collect your urine for the next 6 hours, and make sure to drink at least 30oz of filtered water.

2.3.3.4 The end of the urine collection. Place the 3000-ml container on a level surface and read the volume from the markings on the container. Record this testing and the number of hours collected (6) on the submit form. The total dosage of Captomer must be recorded.

2.3.3.5 Mix urine gently by tilting the collection container. Pour the urine into the small plastic 60 ml bottle until it is nearly full.

2.3.3.6 Follow the instructions in the kit for recording and shipping. (\\Granite\ecn_data\Doc\ECN Internal Documents\Patient Handouts\Heavy Metal Urine Testing 6 hour.doc Revised 05/0)

Table 2.4 Reference Range of Toxic Metals Profile from CMS Lab (Comprehensive Medical System lab) ACGIH = American Conference of Governmental Industrial Hygienists

S261-Blood			
Metal	**Reference Range**	**Reference**	
Lead (Pb)	Less than 30.00 ug/dL	ACGIH, 2009	Occupational Safety and Health Bureau Department of Labour Protection and Welfare Normal person < 40 ug/dL Exposed person < 60 ug/dL
Mercury (Hg)	Less than 15.00 ug/L	ACGIH, 2009	Occupational Safety and Health Bureau Department of Labour Protection and Welfare Normal person <20µg/L Exposed person < 40µg/L
Cadmium (Cd)	Less than 5.00 ug/L	ACGIH, 2009	
Manganese (Mn)	Normal person < 10.00 ug/L Exposed person < 100.00 ug/L	Occupational Safety and Health Bureau Department of Labour Protection and Welfare	
Chromium (Cr)	Less than 5.00 ug/L	Occupational Safety and Health Bureau Department of Labour Protection and Welfare	
Cobalt (Co)	Less than 1.00 ug/L	ACGIH, 2009	
Nickel (Ni)	Normal person < 2.00 ug/L Exposed person < 10.00 ug/L	Occupational Safety and Health Bureau Department of Labour Protection and Welfare	

Table 2.5 Reference Range of Toxic Metals Profile from CMS Lab (Comprehensive Medical System Lab)

S262-Drinking Water		
Metal	**Reference Range**	**Reference**
Lead (Pb)	Less than 10.000 ug/L	World Health Organization (WHO) Guidelines for drinking-water quality, third edition, incorporating first and second addenda (2006)
Mercury (Hg)	Less than 6.000 ug/L	
Cadmium (Cd)	Less than 3.000 ug/L	
Manganese (Mn)	Less than 400.000 ug/L	
Chromium (Cr)	Less than 50.000 ug/L	
Cobalt (Co)	N/A	
Nickel (Ni)	Less than 70.000 ug/L	
Arsenic (As)	Less than 10.000 ug/L	

Table 2.6 Reference Range of Toxic Metals Profile from CMS Lab (Comprehensive Medical System lab) ACGIH = American Conference of Governmental Industrial Hygienists

S260-Urine			
Metal	**Reference Range**	**Reference**	
Lead (Pb)	Normal person < 65.00 ug/g Creatinine Exposed person < 150.00 ug/g Creatinine	Occupational Safety and Health Bureau Department of Labour Protection and Welfare	
Mercury (Hg)	Less than 35.00 ug/g Creatinine	ACGIH, 2009	Occupational Safety and Health Bureau Department of Labour Protection and Welfare Normal person <20µg/L Exposed person <50µg/L
Cadmium (Cd)	Less than 5.00 ug/g Creatinine	ACGIH, 2009	
Manganese (Mn)	Less than 3.00 ug/g Creatinine	Occupational Safety and Health Bureau Department of Labour Protection and Welfare	
Chromium (Cr)	Less than 25.00 ug/L	ACGIH, 2009	
Cobalt (Co)	Less than 15.00 ug/L	ACGIH, 2009	
Nickel (Ni)	Normal person < 5.00 ug/L Exposed person <70.00 ug/L	Occupational Safety and Health Bureau Department of Labour Protection and Welfare	
Arsenic (As)	Normal person: < 50.00 ug/L Exposed person: < 300.00 ug/L	Occupational Safety and Health Bureau Department of Labour Protection and Welfare	(ACGIH, 2009) ACGIH recommends Inorganic arsenic < 35 ug As/L

Note. Data in 13/05/2011

2.4 Live blood Analysis

In human body has liquid about 90% and have blood about 7.5% of body weight. Men has about 5.5 liters (1 ½ gallons) of blood, while a woman has about 3.25 liters (1 gallon)

The cell of blood are of three type:

1. Red blood cells (Erythrocytes)
2. White blood cells (Leukocytes)
3. Platelets (Thrombocytes)

The three element of whole blood float in plasma, which straw color liquid about 90 % water. In plasma also contain organic acids, glucose, hormones and salts for nutrients as a following

1. Circulating blood components such as a nutrient throughout the body's network of artery, vein, and capillaries
2. Delivery a nutrient to the tissue and organs
3. Carrying the vitamins , mineral , hormone and antibody
4. Remove waste product
5. Recycle a nutrient which a important for reinforce health

Blood cycle use time about 20 second which start travel from the heart to lungs through the pulmonary artery , It pick up oxygen and flow it back to the heart after that pump it out to the human body . When it release oxygen to cells and also takes carbon dioxide which produce from cell back to the lung and then remove waste product from the body. When blood travel through the body, they also pick up hormone from adrenal gland, thyroid gland and other gland to specific organ.

The advantages of blood as a following:

1. Help maintain homeostasis of internal environment
2. Take oxygen to cell and collect waste product
3. Regulate function involve nutrient of cells
4. Regulate body temperatures
5. Defense mechanisms
6. Adaptation the body for various environment such as climate , stress or dietary habit
7. Combat to injury and infection organism

2.4.1 Microscope

An equipment use to enlarge image of small object and show a detail of structure. A binocular microscope is one or two eyepieces for use both eyes. Lens of microscope is one or two lens systems which image of the object is magnify by lens nearer to the eye.

2.4.2 Compound light microscope

Use two sets of lenses, known as the ocular and objective, and light as its source of illumination which light rays from illuminator are pass through a condenser which redirect then through the specimen under observation. The light rays pass into the objective lens and are enlarge again by the ocular lens or eyepiece. The microscope is shorter wavelengths of light produce.

2.4.3 Resolution

Ability of lens to discriminate a fine detail .The resolution of microscope relate to the ability of an optical system to distinguish two closely spaced particle as two distinct images. This aspect of lens performance can call the depth of field. The object is closer, the angle is large which is the greater of resolution.

The light which use in compound of microscope cannot determine detail smaller than 0.3 micrometers. The best light of microscope is magnified image only about 1500 times. The Dark field mode of illumination is solve the problem

2.4.4 DARKFIELD MICROSCOPE

A Dark field microscope is constructed that illumination is receive from side of field and a detail or structure appear as light images against a dark background . A condenser lens illuminates the specimen from side rather than by direct transmission. The background or field appears dark while the specimen is reveals by reflect light. The illumination is increase contrast in light intensity between areas of high and low light absorption when the contrast increase may perform electronically through the use of a video camera and monitor. When it pass through video, the difference between light and dark areas may be more prominent about detail of a specimen

Form of illuminations is bright field, dark field and phase contrast.

1. Bright field: Light pass directly through the specimen from illumination source into eye. About the detail or structure that originate from light absorption

2. Dark field: Light pass through optical device known as condenser which provide light intensity over the entire field of view This microscope does not allow light pass direct through the specimen into eye but the specimen is illuminate perpendicular to the barrel of the microscope that the light pass into eye is scatter by the specimen. The specimen appear in bright on dark background.

3. Phase contrast: The object is more visible as various shade of gray by transparent from microscope. The shade of the objects can decide by slow velocity of light pass through it.

About your condition of blood can seen in the dark field microscope by monitoring and then give good dietary and lifestyle recommendation for balancing of the blood.

The dark field microscope can evaluate shape and property of blood cells which can see about digestive and nutrition disorders by train health professionals. This microscope can see some disorder which cannot see easily in traditional method of blood analysis which found chemical change in the blood by centrifuging and separating the blood. The advantage of microscope can found digestive and nutrition disorder before standard blood test that can found chemical change which effect to successful of treatment because of the problems are discover where it is still in infant stages. The dark field microscope can evaluate present blood and nutrition condition.

About background of dark field microscope is known as another name is live cell analysis which start in 20^{th} century for scientific research. They see reaction of live blood cell to chemical and stimuli. It use in worldwide but Germans were the forerunners in publishing the results of their finding. This technique use to during World War II for check soldiers for gonorrhea that provide a quick diagnosis after doctors could suddenly give medical attention in the field.

2.4.5 Healthy and Unhealthy Blood

Health blood will shoe red blood cell is round shape and free floating in plasma. If the plasma is clear may be with few fat globules or toxins, it is no sign of stress, bacteria, fungal and other opportunistic and our immune will slow lymphocytes and active WBC's. About Unhealthy blood, If we understand in function of healthy blood in circulating system, we will know about unhealthy blood which blood in human body has 90%liquid, blood is 7.5of body weight. Men has about 5.5 liters (1 ½ gallons) of blood, while a woman has about 3.25 liters (1 gallon)

2.4.6 DARK FIELD MICROSCOPE PART

2.4.6.1 Base: The bottom portion on which the microscope rests

2.4.6.2 Body tube: The portion that receives the ocular (eyepiece)

2.4.6.3 Arm: Angular part of the frame that supports the body tube and stage

2.4.6.4 Stage: Platform which support the slides or object to study and the opening center aloe light to pass from below source through object being examined. It has a knob below the stage that move forward, backward or from side to side which slide and stage will move together.

2.4.6.5 Stage spring clips: Clip mounted on the stages that hold the slide securely in place

2.4.6.6 Diaphragm: Devices below the stage that regulate light intensity through condenser and lenses to the observer

2.4.6.7 Condenser: Lens below the stage opening that concentrate the light beam on the specimen.

2.4.6.8 Adjustment Knob: Knob to raise and lower condenser

2.4.6.9 Coarse adjustment knob: Knob to raise and lower the body tube for focusing the microscope

2.4.6.10 Fine adjustment knob: Smaller knob attached to coarse adjustment knob for focusing as fine

2.4.6.11 Nosepiece: A circular plate at the bottom of the body tube which can carry a objective and can rotate 360 degrees

2.4.6.12 Low power objective: A dry lens, mark 10x

2.4.6.13 High -power objective: A dry lens, mark 40x

2.4.6.14 Oil immersion objective (with iris): A lens, marked 100x

2.4.6.15 Ocular (eyepiece): A removable lens at the top of the body tube, offer 10x 0r 50x magnification

2.4.6.16 Camera or video camera body

2.4.6.17 Trinoclar camera tube: A tube for hold two eyepiece and has a third space for attaching the camera tube

About the light illumination is oblique angle not direct from the light source into the object lens. Only light that strikes the cells directly under objective in use is reflected to image the cells you are viewing. It has oil on top of condenser lens that make a contact in the bottom of the slide but the contact will made when condenser will slow raise up until light illuminate the whole slide. Oil from an immersion oil dispenser tube is use before the slide set on the stage. Then put the slide on the stage above the condenser , so blood on the slide will see directly over it, turn condenser focusing knob and raise condenser slowly until oil meet the bottom of the slide. When it uses 10x objectives, you will see cell in sharp focus and dark circle form by dark field condenser. The 10x objective and 40x objective use dry no oil, except between the bottom of slide and condenser because if use oil then a image may be fuzzy and you cannot seen it but 100x objective need immersion oil between cover slip and objective lens.

The microscope must be clean and cover when not in use for effectiveness. When you use oil on slide do not squeeze so quickly it make a air bubbles affect to reflect and refract. You should do step by step for efficiency. Before you begin, you should make sure all of part of the microscope is clean. After the finger has been punctured, wipe the

first three drop of blood in gentle and place the cover glass which have drop of blood over the slide, put cover slip and slide come together.

Always use low objective first to focus

Use 10x objectives, See whole detail of live blood on the slide. It should be analysis form thinnest area of slide

Use 40x objectives for thrombocyte aggregations. Observe proportion of red blood cell and white blood cell but cannot count in number of white blood cell but can see when it increase

100x objective, before you change to 100x, please drop oil on top of cover slide, this is final analysis of live blood.

The Dark field microscope is not a diagnostic tool but rather a nutritional assessment. Health professional should know an information about digestion of protein, carbohydrate and fat, toxicity of blood, red blood cell (equal size, round, flowing) or white blood cell.

Procedure of live blood analysis

1. Clean slide, set cover glass, clean finger of patient (left hand, ring finger).
2. Take blood sample, Bleed and wipe finger 3 times and then touch cover slip to bead of blood. Then put cover glass on top of slide
3. Drop oil on the light source and quick scan sample for center location
4. The most analysis is 40x that can use
5. It can see blood sample no longer than 10-15 minutes after that should turn off light , monitor and remove slide.
6. Give information for the patient

About group of disorder can found from the dark field microscope can divide into three group

First: Digestive disorder and Nutritional deficiencies

Second: Immune System, Parasites, Bacteria, and Fungal Forms

Third: Fat, Protein, and Liver Congestion

(Enzymology Reasearch Center and women's health by Dr. DicQie Fuller)

1. First: Digestive disorder and Nutritional deficiencies
   1) Macrocyte

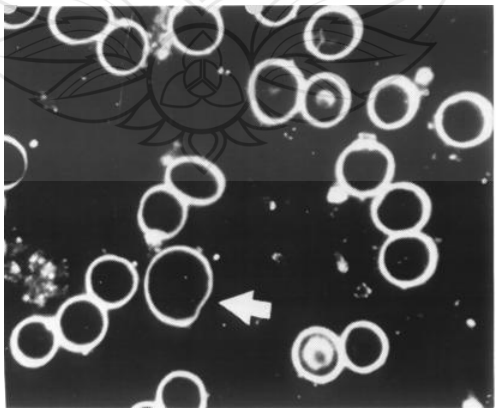


Figure 2.11 Macrocyte

The large red blood cell greater than 9 microns (average is 7.2)**.** It was seen in folic and B_{12} deficiency as result from food allergy, poor absorption, worm infestation. It occur after eating a meal and cannot digest due to food allergy that recommend to take digestive enzymes with meal which increase according to amount of food eaten

2) Microcyte

Figure 2.12 Microcyte

These are small RBCs having a diameter of less than 5 microns. It cannot develop because of nutrient deficiency such as iron deficiency. It is also seen in people with fatigue, anemia, migraine. The recommendation is High chlorophyll diet such as chlorella, spirulina, alfalfa tablets, Hcl, Vitamin C, B12, Folic acid.

3) Ovalcyte

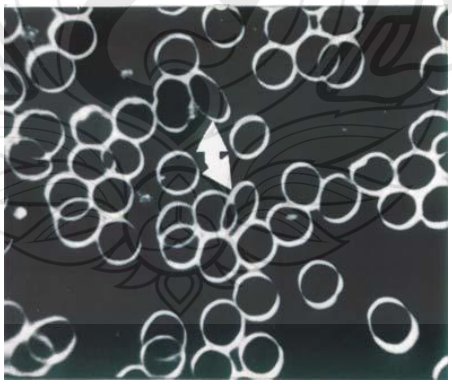


Figure 2.13 Ovalcyte

The shape of red blood cell is oval like egg results from iron, folic and B12 deficiency that can show mal-absorption and endocrine stress. Recommendation is a diet low in fat with lipase add with meal

4) Erythrocyte

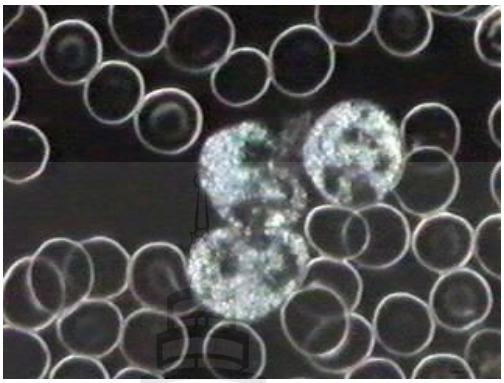


Figure 2.14 Erythrocyte

This condition is red cell aggregation, stick that one step worse than rouleau. It show drawn together at the center This is caused by undigested saturated fats and degeneration of tissue cause by low oxygen and acidity affect to precede a blood clot which can occur a stroke or heart attack.

5) Poikilocytosis

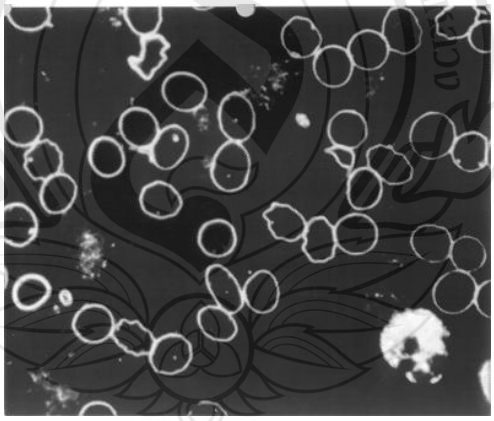


Figure 2.15 Poikilocytosis

It is a deform red blood cell cause by free radicals, fermentation of toxin in the liver chemical agent include arsenic, lead, benzene and nitrate can destroy erythrocyte. Also junk food or vegetable poison can damage red blood cell. It can tendency to hemolyze since cell membrane have unsaturated fat and other fat affect to shape of blood cell is odd shape.

6) Protein linkage

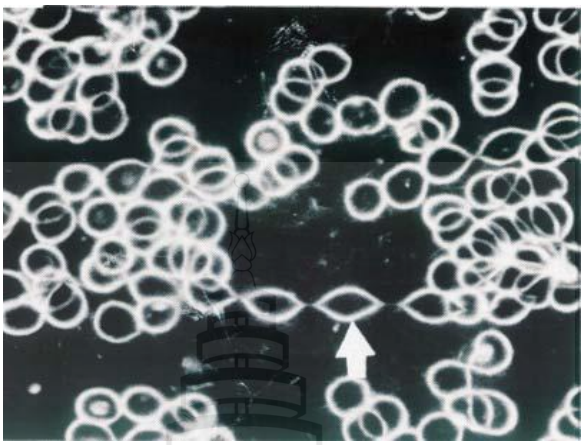


Figure 2.16 Protein Linkage

This condition is excess of protein in blood cause by undigested completely protein. It has cell stickiness and become harder for heart to push blood through the veins and arteries and may progress into rouleau. It was seen in high protein diet, vegetarians who have created a protease deficiency.

7) Rouleau

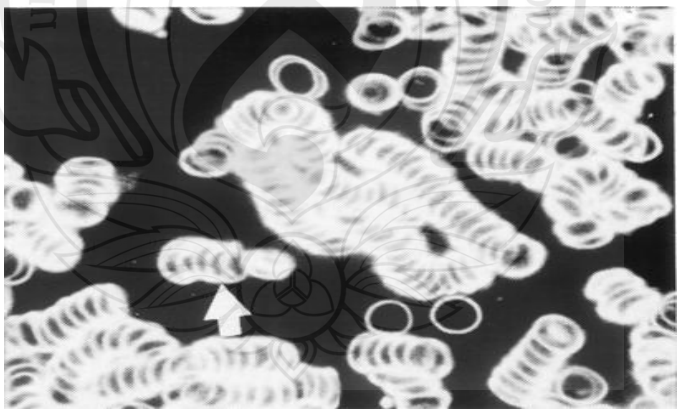


Figure 2.17 Rouleau

This condition presents a roll of red blood cell resembling a pile of coin. The amount of oxygen can be decrease in transported caused by high fat and protein diets and high acidity. Patient has symptom like fatigue, poor digestion and skin disorder.

8) Target cell

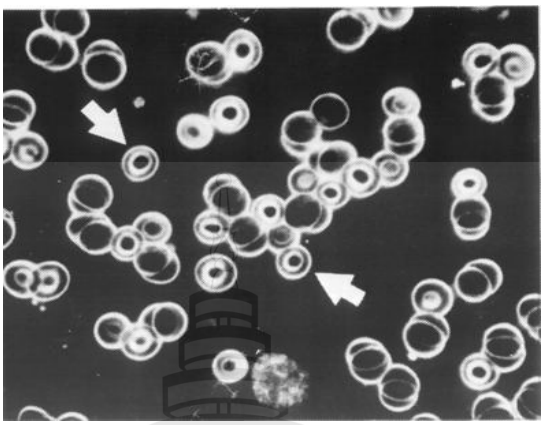


Figure 2.18 Target Cell

This condition is red blood cell with bull's eye cause by deficient in iron and therefore hemoglobin, which unable to carry oxygen and potential energy. Also cause from meal high carbohydrate or fat and alcohol. It is often seen in people with tiredness, poor digestion, and anemia.

2. Second: Immune System, Parasites, Bacteria, and Fungal Forms

1) Mesasomes

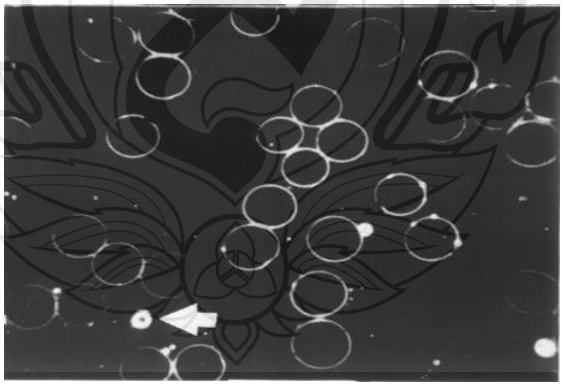


Figure 2.19 Mesasomes

Mesasomes are embryonic bacteria in blood which have round donut shape. It is prominent from chylous in blood which are smaller and faster than L form and mesasomes

2) Parasite

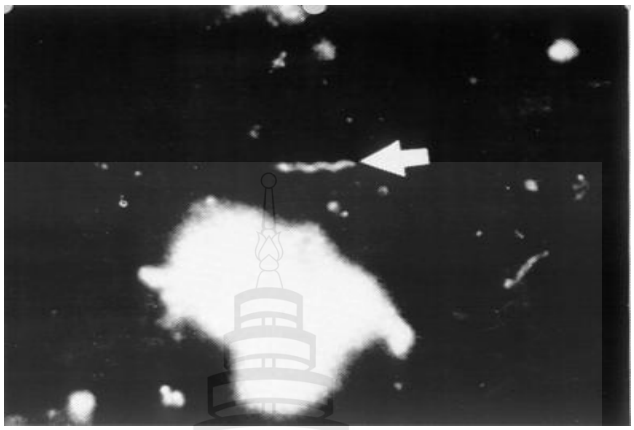


Figure 2.20 Parasite

This condition has bacteria or parasites that get inside the cells affect to cell will die. The parasites will stay permanent with host and unless they are stopped by the immune system, they will continue to attack other cells.

3) Protoplast

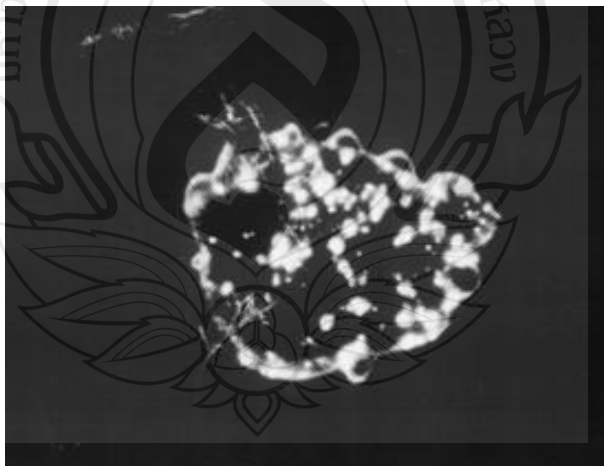


Figure 2.21 Protoplast

This condition has large structure in blood which contain bacteria that can show in body has toxic and sometime also seen red crystal is toxic actinomycin. Symptoms have fatigue, low immune system and decay teeth.

4) L- form

Figure 2.22 L-form

This is a bacterial infection in blood that is shaped like round, bright, sparkling and moving forms. It has hole in them look like tiny donuts. This usually signifies a condition of low immunity and high blood sugar.

5) Fungal form

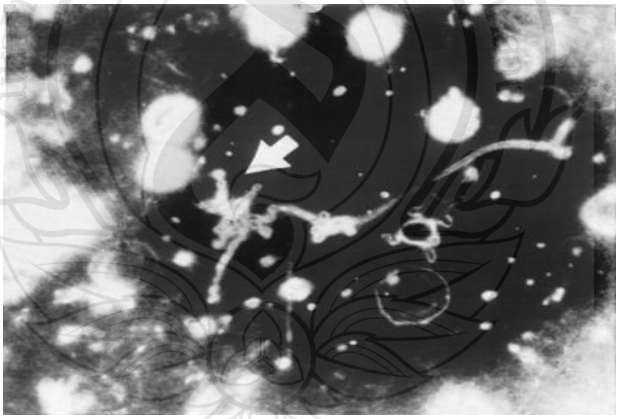


Figure 2.23 Fungal Form

This condition is skin disease cause by Fungi can spread throughout your body through the blood and develop colonies. It can cause asthma and allergic alveolitis. It can slow develop and difficult to diagnosis and treatment. It is seldom fatal and most of the time go unnoticed and has sign of poor assimilation of nutrients and an acidic condition in the body fluids

3. Third: Fat, Protein, and Liver Congestion
   1) Echinocyte

Figure 2.24 Echinocyte

This condition has red blood cell with have a thorny appearance. It occurs deteriorates or die of cells. Also It has worse of oxygen change in cellular level. It indicate kidney stress and crenation (the shrinking of the cell by dehydration) when has excessive amount of echinocyte with tubules present.

   2) Plaque

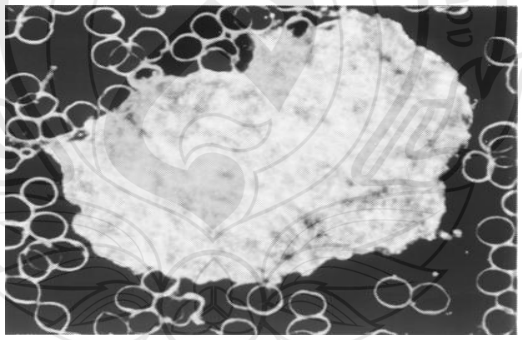


Figure 2.25 Plaque

This condition is one of the most dangerous conditions in the blood. It appear as granular “chunks” and have sharp edge and no brighter in the dark field. It must be distinguish from protoplast which more translucent and small size. Plaque can adhere to the artery walls narrowing and hardening them. When system becomes acidic and fatty acid can have form of crystal such as red crystal.

3) Spicules

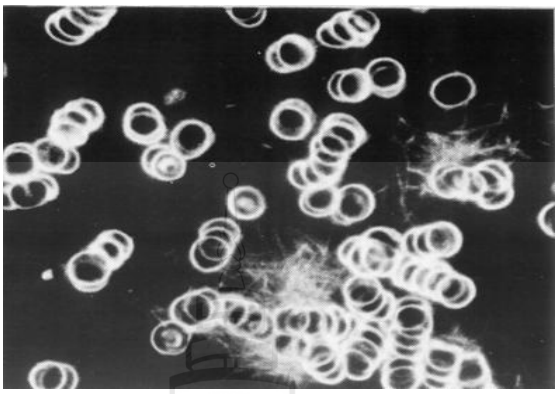


Figure 2.26 Spicules

There are fibrin or fibrinogen strands. It appears like a cracked glass. They have form a net-like substance in which blood clots are formed by the entrapment of red and white cells and platelets. This is caused by heavy proteins in blood and seen liver stress due to incomplete digestion. It often seen symptom like headache, fatigue, should be aware of poor circulation.

4) Thrombocytic aggregation

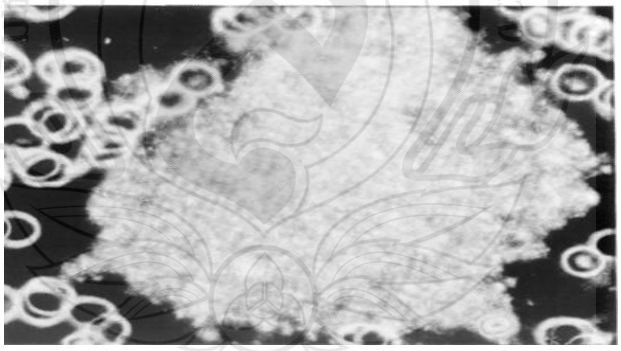


Figure 2.27 Thrombocytic Aggregation

This is platelet clumpling. It presents small form element in blood. It appears in small disk shape and can form a clot which can block an artery causing a stroke or heart attack. This clot is called a thrombus. Diets high in fats and proteins or high sugar consumption can create this situation. Animal fat will increase a problem and will see in people who have craving sugar or diabetics.

5) Chylous

Figure 2.28 Chylous

Chyle is the milky fluid taken up by lacteal from intestine during digestion. It consist of lymph and triglyceride fat in emulsion. A chylomicron is a stable droplet contains triglyceride fat, cholesterol, phospholipids and protein. They are found in blood and intestinal lymphatic after eating meal. The presence of chylomicrons in the blood after a 12 hour fast indicates a condition known as hyperlipo-proteinemia, which can lead to atherosclerosis, coronary artery disease, and enlargement of the liver and spleen.

6) Crystal

Figure 2.29 Crystal

Crystals are sclerotics (inflammation or hardening) or pseudocrystaline (abnormal crystals) chunks. The color of crystal is red, yellow or bright, glowing chunks. They can remove from blood and often return if it has a condition. Some condition such

as arthritis, gout, joint problem fungus or bacteria, poor digestion in colon and also found in chronic condition and stress in immune system.

Red crystals: it indicates airborne toxins, proteolytic enzyme and antioxidant are very helpful.

Yellow crystals: It indicates bowel toxicity which has bacteria in bowel produce by their waste product and put it back to the bloodstream for kidney and liver to detoxify. It cause by poor digestive food such as fat turn rancid, protein putrefy and carbohydrate ferment.

Yellow-orange / brown crystals: It indicates heavy metal.

Yellow-blue-green indicates a preneoplastic stage.

Steel-blue with small red rims indicates tuberculosis.

Blue reflection indicates a metabolic disturbance or aspergillus

Crystal is highly resistant to competitors in their environment and often related to elevated blood pressure, plaque, build up and clogged artery. Trapezoidal shapes are caused by poor digestion of fat that good sign for EDTA or herbal chelation. Triglyceride crystal appears look like broken glass. Square crystal indicates neurological problem and immune disorder and symptoms are headaches, migraines, nervousness, depression. Bright color orange / red crystal indicates uric acid which is a byproduct of protein metabolization and urea. When the body has acidic the urea forms crystal can lodge into joint or tissues which can lead to gout disease. It has shape like knives. (BRiA Health Center, 2004)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Population and Sample Size

Study population

People (patients) in TRIA Integrative Wellness at Piyavate hospital, male and female, who have to receive assessment for live blood analysis and urine heavy metal in TRIA Integrative Wellness at Piyavate hospital.

3.2 Sample Size Calculation

$$n = \frac{Z^2\delta^2}{d^2}$$

n = Sample size
Z = Confident interval at 95 % (Z = 1.96)
δ^2 = Variance of live blood analysis
δ^2 = 0.927172
d = Allowable error in estimating (20%)
d = 0.2

$$N = \frac{(1.96)^2(0.927172)^2}{0.2^2}$$

= 82.560586
= 83

3.3 Research Design

Retrospective study from secondary data
Study variables
Independent variables: live blood analysis
Dependent variable: urine heavy metal

3.4 Selection of Sample

People who have result in assessment about live blood analysis and urine heavy metal in TRIA Integrative Wellness at Piyavate hospital.

3.4.1 Inclusion criteria

3.4.1.1 Thai people (patients) in TRIA Integrative Wellness at Piyavate hospital including male and female.

3.4.1.2 Thai people (patients) who have assessment about live blood analysis and urine heavy metal in period since 17 December 2010 until 27 August 2011.

3.4.1.3 Thai people (patients) who have assessment about live blood analysis and urine heavy metal in TRIA Integrative Wellness at Piyavate hospital.

3.4.2 Exclusion criteria

3.4.1.1 People who are not Thai people.

3.4.1.2 The place except in TRIA Integrative Wellness at Piyavate hospital

3.5 Research Tools

3.5.1 Data collection form
3.5.2 Dark field microscope
3.5.3 Urine heavy metal
3.5.4 Computer
3.5.5 Statistical sofeware spss 2011

3.6 Research Procedure

3.6.1 Collected secondary data about from live blood analysis and urine heavy metal in TRIA integrative Wellness at Piyavate hospital.

3.6.2 Analyzed the collection form result in live blood analysis and the presence of whole urine heavy metal.

3.7 Data Collection

Data collection was performed evaluate from by Investigator

3.8 Statistics used for Data Analysis

3.8.1 Use descriptive statistics to demonstrate characteristics demography

3.8.2 Compare result in blood analysis and result in urine heavy metal which characteristic in number that could be indicate each heavy metal level by using t-test analysis.

3.8.3 Correlation between crystal in live blood and high level of heavy metal in urine by using Pearson correlation test

3.9 Ethical Consideration

Information from this research would be present in overview information but not reveal personal information.

3.10 Administration and Time schedule

Table 3.1 Administration and Time Schedule

Activities	2011							2012				
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1. Literature Review	←	→										
2. Develop proposal			←	→								
3. Conduct research					←	→						
4. Data analysis								←	→			
5. Produce Report									←	→		
6. Present Thesis										←	→	
7. Publish result											←	→

3.11 Budget of this Study

Table 3.2 Budget of this Study

Financial plan	Budget (Bath)
1. Live blood analysis	-
2. Urine heavy metal	-
3. Other, Xerox, data analysis etc.	20,000
Total	**20,000**

Acknowledgement this study was supported about information for research by Dr. Aunyawut chuaiwongyat at TRIA integrative Wellness at Piyavate hospital.

CHAPTER 4

RESULTS

This study is retrospective study from secondary data from TRIA Integrative Wellness at Piyavate hospital. The research objectives are study on specificity of live blood analysis for heavy metal measurement compare with urine heavy metal. The results from the study are categorized into demography, clinical characteristics of patients who have results in live blood analysis which can divide into two results that have crystal and no crystal and results in urine heavy metal

4.1 Demography

Ninety-nine patients in TRIA Integrative Wellness at Piyavate hospital, male and female, who have to receive assessment for live blood analysis and urine heavy metals, sixty-two patients who have crystal in blood analysis and thirty-three patients who have no crystal in blood analysis. The assessment of results based on clinical features by Physicians.

Table 4.1 Demographic Data

Variables	Patients	
	Sample	**percentage**
Age (years)		
0-20	19	19.19
21-40	28	28.28
41-60	42	42.42
61-80	9	9.09
>80	1	1.01
Mean±S.D = 40±18.18, Min = 3, Max = 87		
Gender		
Female	54	54.5
Male	45	45.5
Crystals in Live Blood		
Yes	62	62.6
No	37	37.4

From Table 4.1, In this study has 99 patients in TRIA Integrative Wellness at Piyavate hospital were included 62 patients with have crystals and 37 patients with have no crystals in live blood analysis.

1. The mean age of 99 patients (±S.D.) was 40 ± 18.18 years (range, 3-87 years).
2. The range of age
    1) from 0-20 years have 19 patients as a percentage is 19.19
    2) from 21-40 years have 28 patients as a percentage is 28.28
    3) from 41-60 years have 42 patients as a percentage is 42.42
    4) from 61-80 years have 9 patients as a percentage is 9.09
    5) more than 80 years have 1 patients as a percentage is 1.01
3. In 99 patients have 45 male and 54 female as a percentage is 45.5% and 54.5%, respectively.
4. In 99 patients have 62 patients who have crystals and 37 patients who have no crystals as a percentage is 62.6% and 37.4%, respectively.

Table 4.2 Heavy Metals in Urine

Heavy Metal	N	Range	Min	Max	Mean	SD
LEAD	99	71.73	0.18	71.91	8.37	13.92
MERCURY	99	21.23	0.42	21.65	4.13	3.61
CADMIUM	99	3.86	0.01	3.87	0.45	0.72
MANGANESE	99	191.19	0.21	191.40	8.53	26.46
CHROMIUM	99	7.93	0.66	8.59	1.24	1.42
COBALT	99	3.56	0.01	3.57	0.25	0.49
NICKEL	99	8.87	0.11	8.98	1.32	2.00
ARSENIC	99	941.05	1.18	942.23	62.75	109.97

From Table 4.2, Shows results in urine have 8 types of heavy metals.

1. Lead: The mean lead of 99 patients (±S.D.) was 8.37 ± 13.92
    1) Minimum of lead level in urine is 0.18
    2) Maximum of lead level in urine is 71.91
2. Mercury: The mean mercury of 99 patients (±S.D.) was 4.13 ± 3.61
    1) Minimum of mercury level in urine is 0.42
    2) Maximum of mercury level in urine is 21.65
3. Cadmium: The mean Cadmium of 99 patients (±S.D.) was 0.45 ± 0.72
    1) Minimum of Cadmium level in urine is 0.01
    2) Maximum of Cadmium level in urine is 3.87
4. Manganese: The mean Manganese of 99 patients (±S.D.) was 8.53 ± 26.46
    1) Minimum of Manganese level in urine is 0.21
    2) Maximum of Manganese level in urine is 191.4
5. Chromium: The mean Chromium of 99 patients (±S.D.) was 1.24 ± 1.42
    1) Minimum of Chromium level in urine is 0.66
    2) Maximum of Chromium level in urine is 8.59

6. Cobalt: The mean Cobalt of 99 patients (±S.D.) was 0.25 ± 0.49
   1) Minimum of Cobalt level in urine is 0.01
   2) Maximum of Cobalt level in urine is 3.57
7. Nickel: The mean Nickel of 99 patients (±S.D.) was 1.32 ± 2.00
   1) Minimum of Nickel level in urine is 0.11
   2) Maximum of Nickel level in urine is 8.98
8. Arsenic: The mean Arsenic of 99 patients (±S.D.) was 62.75 ± 109.97
   1) Minimum of Arsenic level in urine is 1.18
   2) Maximum of Arsenic level in urine is 942.23

4.2 Compare Between Live Blood Analysis and Urine Heavy Metal

Table 4.3 Lead

Crystal in Live Blood	Samples (n)	LEAD		t test	p-value
		Mean	SD		
No Crystal	37	5.42	6.89	11.09	0.001
Crystal	62	10.14	16.57		

Note. * The difference between the means is statistically significant (P<0.001)

From Table 4.3 shows compare between Live blood analysis and Lead in urine level were using independent T-test that shows a group with crystal (mean 10.14±16.57) has significantly high level of lead in urine than those without crystal (mean 5.42± 6.89), P value < 0.001 denotes significant difference.

Table 4.4 Mercury

Crystal in Live Blood	Samples (n)	MERCURY		t test	p-value
		Mean	SD		
No Crystal	37	3.54	2.88	1.58	0.212
Crystal	62	4.48	3.96		

Note. * The difference between the means is not statistically significant P = 0.212

From Table 4.4 shows compare between live blood analysis and mercury in urine level were using independent T-test that shows a group with crystal (mean4.48±3.96) has significantly high level of mercury in urine than those without crystal (mean3.54±2.88), P value = 0.212 denotes no significant difference.

Table 4.5 Cadmium

Crystal in Live Blood	Samples (n)	CADMIUM		t test	p-value
		Mean	SD		
No Crystal	37	0.26	0.25	9.03	0.003
Crystal	62	0.56	0.87		

Note. * The difference between the means is statistically significant P=0.003

From Table 4.5 shows compare between live blood analysis and cadmium in urine level were using independent T-test that shows a group with crystal (mean 0.56±0.87) has significantly high level of cadmium in urine than those without crystal (mean0.26± 0.25), P value =0.003 denotes significant difference.

Table 4.6 Manganese

Crystal in Live Blood	Samples (n)	MANGANESE		t test	p-value
		Mean	SD		
No Crystal	37	1.38	0.79	13.53	0.000
Crystal	62	12.80	32.79		

Note. * The difference between the means is statistically significant P<0.001

From Table 4.6 shows compare between live blood analysis and manganese in urine level were using independent T-test that shows a group with crystal (mean 12.80±32.79) has significantly high level of manganese in urine than those without crystal (mean 1.38± 0.79), P value <0.001 denotes significant difference.

Table 4.7 Chromium

Crystal in Live Blood	Samples (n)	CHROMIUM		t test	p-value
		Mean	SD		
No Crystal	37	1.10	0.97	1.60	0.209
Crystal	62	1.32	1.63		

Note. * The difference between the means is not statistically significant P=0.209

From Table 4.7 shows compare between live blood analysis and chromium in urine level were using independent T-test that shows a group with crystal (mean 1.32±1.63) has significantly high level of chromium in urine than those without crystal (mean 1.10± 0.97), P value =0.209 denotes no significant difference.

Table 4.8 Cobalt

Crystal in Live Blood	Samples (n)	COBALT		t test	p-value
		Mean	SD		
No Crystal	37	0.24	0.64	0.56	0.456
Crystal	62	0.26	0.37		

Note. * The difference between the means is not statistically significant P=0.456

From Table 4.8 shows compare between live blood analysis and cobalt in urine level were using independent T-test that shows a group with crystal (mean 0.26±0.37) has significantly high level of cobalt in urine than those without crystal (mean 0.24±0.64), P value =0.456 denotes no significant difference.

Table 4.9 Nickel

Crystal in Live Blood	Samples (n)	NICKEL		t test	p-value
		Mean	SD		
No Crystal	37	0.63	0.87	20.30	0.000
Crystal	62	1.73	2.36		

Note. * The difference between the means is statistically significant P<0.001

From Table 4.9 shows compare between live blood analysis and nickel in urine level were using independent T-test that shows a group with crystal (mean 1.73±2.36) has significantly high level of nickel in urine than those without crystal (mean 0.63± 0.87), P value <0.001 denotes significant difference.

Table 4.10 Arsenic

Crystal in Live Blood	Samples (n)	ARSENIC		t test	p-value
		Mean	SD		
No Crystal	37	23.28	16.87	11.40	0.001
Crystal	62	86.28	133.24		

Note. * The difference between the means is statistically significant P<0.001

From Table 4.10 shows compare between live blood analysis and arsenic in urine level were using independent T-test that shows a group with crystal (mean 86.28±133.24) has significantly high level of arsenic in urine than those without crystal (mean 23.28±16.87), P value <0.001 denotes significant difference.

Table 4.11 Age

Crystal in Live Blood	Samples (n)	AGE		t test	p-value
		Mean	SD		
No Crystal	37	40.54	18.00	0.03	0.870
Crystal	62	39.03	18.42		

Note. * The difference between the means is not statistically significant P=0.870

From Table 4.11 shows compare between live blood analysis and age were using independent T-test that shows a group with crystal (mean 39.03±18.42) and group without crystal (mean 40.54± 18.00) has not significantly with age P value=0.870 denotes significant difference.

4.3 Correlation Between Crystal in Live Blood and High Level of Heavy Metal in Urine

Table 4.12 Correlation Between crystal in Live Blood and High level of Heavy Metal in Urine

Variable	Crystal Live Blood	
	r	p-value
LEAD	0.16	0.103
MERCURY	0.13	0.211
CADMIUM	0.21	0.039
MANGANESE	0.21	0.037
CHROMIUM	0.08	0.443
COBALT	0.01	0.880
NICKEL	0.27	0.008
ARSENIC	0.28	0.005
AGE	0.04	0.692
SEX	0.03	0.736

Note. The correlation for parametric variables was analyzed by using Pearson correlation test.

From Table 4.12 Shows correlation between results crystal in live blood analysis and high level of heavy metal in urine.

1. Lead: There was a non-significant difference between crystal in live blood and high level of lead in urine (r= 0.16, p value = 0.103). There was no correlation.

2. Mercury: There was non-significant difference between crystal in live blood and high level of mercury in urine (r=0.13, p value = 0.211). There was no correlation.

3. Cadmium: There was significant difference between crystal in live blood and high level of cadmium in urine (r=0.21, p value = 0.039) that shows a group with crystal has positive relation with high level of cadmium in urine.

4. Manganese: There was significant difference between crystal in live blood and high level of manganese in urine (r=0.21, p value = 0.037) that shows a group with crystal has positive relation with high level of manganese in urine.

5. Chromium: There was a non-significant difference between crystals in live blood and high level of chromium in urine (r=0.08, p value = 0.443). There was no correlation.

6. Cobalt: There was a non-significant difference between crystals in live blood and high level of cobalt in urine (r=0.01, p value = 0.880). There was no correlation.

7. Nickel: There was significant difference between crystal in live blood and high level of nickel in urine (r=0.27, p value = 0.008) that shows a group with crystal has positive relation with high level of nickel in urine.

8. Arsenic: There was significant difference between crystal in live blood and high level of arsenic in urine (r=0.28, p value = 0.005) that shows a group with crystal has positive relation with high level of arsenic in urine.

9. Age: There was a non-significant difference between crystals in live blood and Age (r=0.04, p value = 0.692). There was no correlation.

10. Sex: There was a non-significant difference between crystals in live blood and sex (r=0.03, p value = 0.736). There was no correlation.

CHAPTER 5

CONCLUSION, DISCUSSION AND COMMENT

5.1 Conclusions

In this study have ninety-nine patients in TRIA Integrative Wellness at Piyavate hospital, have 45 male (percentage of male is 45.5) and 54 female (percentage of female is 54.5), who have to receive assessment for live blood analysis and urine heavy metal, sixty-two patients (percentage of patients is 62.6) who have crystals in blood analysis and thirty-three patients (percentage of patients is 37.4) who have no crystals in blood analysis. The assessment of results based on clinical features by Physicians.

The research hypothesis is the live blood analysis has specificity for heavy metal measurement. First, after the statistical analysis of the data for compare between live blood analysis and urine heavy metal, the results shows five heavy metals have statistically significant difference such as lead, cadmium, manganese, nickel, arsenic and three heavy metals have not statistically significant difference such as mercury, chromium, cobalt. Second, after the statistical analysis of the data for correlation between crystal in live blood and higher level of heavy metals in urine shows four heavy metals have statistically significant difference such as cadmium, manganese, nickel, arsenic and four heavy metals have not statistically significant difference such as lead, mercury, chromium, cobalt. Furthermore the statistical analysis of the age and sex has not statistically significant difference for correlation between live blood analysis and urine heavy metal.

5.2 Discussion

From the statistical analysis of the data for compare between live blood analysis and urine heavy metal, the results shows five heavy metals have statistically significant difference such as lead, cadmium, manganese, nickel, arsenic. The result shows the effectiveness of live blood analysis can have specificity for heavy metal measurement. When you use live blood analysis technique and its present crystal which indicate to heavy metal, you can find about five heavy metals. Why you can found them because heavy metals can be found in general environment and in daily life such as in food, drinking water as following:

5.2.1 Lead

Lead is a metal in the order of 2 in top 10 Hazardous substances ATSDR (Agency for toxic substances and disease registry) has ranked them. You can found them in manufacture such as storage batteries, ceramics, plastics, water pipes, pesticides, lead based paint, ammunition (Janine, 2005). In general environment such as all people have the opportunity to lead exposure from food and drinking water, people who live in areas with heavy traffic or near the road, people who living near lead smelting plant manufacturers or near factories that use lead, low-quality toys with lead contamination, family members of workers exposed to occupational lead, because dust can lead to skin under clothing or hair of workers from work to home. It can be absorbed through the skin, gastrointestinal tract.

5.2.2 Cadmium

Cadmium is a metal in the order of 7 in top 10 Hazardous substances ATSDR (Agency for toxic substances and disease registry) has ranked them. It is present in the batteries manufacture and can be exposures in general industry, construction industry, and agricultural industry, workers paint on the car and motorcycle. (Janine, 2005) Food contaminated with cadmium including oysters, crabs, squid and in liver's animals. Plants with high cadmium and grains such as rice, wheat bran, especially the green leafy vegetables such as carrots, potatoes.

5.2.3 Manganese

It can be found in battery factory because of use as a component in a battery, chemical production plants such as potassium (potassium permanganate). Usually people get manganese from natural food such as whole grains, Green leafy vegetables, beans and tea (http://www.lenntech.com) which has a huge amount of manganese. The average person get manganese into the body from drinking water and food sources.

5.2.4 Nickel

Nickel is silver-white. It can be found in jewelry, necklace, the watch and power compact made from metal, bracelet, the lipstick metal boxes, earring, jeans button, buckle, zip, eyelash, dental tools, scissors, knob. And also found in food such as oysters, shrimp, cabbage, onion, lettuce, oat, runes, raspberry, chocolate, almonds

5.2.5 Arsenic

Arsenic is a metal in the order of 1 in top 10 Hazardous substances ATSDR (Agency for toxic substances and disease registry) has ranked them. Arsenic contamination is usually found in Vegetables, fruit, Water, food, cosmetics, traditional medicines and use in agriculture make an alloy or ceramics factory. (Graeme & Pollack,1998). It also found in seafood because of in the oceans found about 0.5-50 mg/kg. For plants on the ground can found 0-20 mg/kg depending on the area of cultivation especially rice can found 150-250 mg/kg and mushrooms (National Food Institute Thailand, 2004).

As mentioned above heavy metals which found in research data correlates with the Top 10 priority list of hazardous substance of Agency for toxic substances and disease registry.

Table 5.1 The ATSDR 2011 Substance Priority List

2011 RANK	SUBSTANCE NAME	TOTAL POINTS	2007 RANK	CAS RN
1	ARSENIC	1665.5	1	007440-38-2
2	LEAD	1529.1	2	007439-92-1
3	MERCURY	1460.9	3	007439-97-6
4	VINYL CHLORIDE	1361.1	4	000075-01-4
5	POLYCHLORINATED BIPHENYLS	1344.1	5	001336-36-3
6	BENZENE	1332.0	6	000071-43-2
7	CADMIUM	1318.7	7	007440-43-9
8	BENZO(A)PYRENE	1305.7	9	000050-32-8
9	POLYCYCLIC AROMATIC HYDROCARBONS	1282.3	8	130498-29-2
10	BENZO(B)FLUORANTHENE	1252.4	10	000205-99-2

In Thailand, From Bureau of Epidemiology, Department of Disease Control, Ministry of Public health has the surveillance reported from the hospital about patients who have disease from toxic of heavy metal. In year of 2012, about lead poisoning, National Disease Surveillance (Report 506) since 1 January 2012 to 26 April 2012 found 17 patients from 5 provinces, found in male more than female, mostly found in age 3 years (23.53%), 2 years (23.53%),4 years (11.76%), respectively. (Bureau of Epidemiology, 2012a). Furthermore, about manganese, mercury, arsenic poisoning that National Disease Surveillance (Report 506) reported since 1 January 2012 to 9 May 2012 found 7 patients from 6 provinces, found in male more than female, mostly found in age between 10-14 years (28.57%), 55-64 years (14.29%), 35-44years (14.29%), respectively.

The results of this study shows three heavy metals have not statistically significant difference such as mercury, chromium, cobalt because

1. 99 patients in this study who have to receive assessment for live blood analysis and urine heavy metal, in TRIA Integrative Wellness at Piyavate hospital, found in small amount of mercury, chromium, cobalt.

2. They have general occupations and live in city areas, not the people who work and live in industrial factory areas.

3. Some people cannot found high level of heavy metal because they already did chelation technique before. Chelation technique can be use chelating agent such as EDTA (Ethylene Diamine Tetra-acetic Acid) or oral DMSA (Dimercaptosuccinic acid). DMSA is a sulfhydryl-containing, water-soluble, low-toxicity, orally adminishtered metal chelator. (Miller,1998).The efficacy of DMSA can remove lead which studying in human by use DMSA 30 mg/kg/day and results show significantly increases urine lead elimination in lead poison patients (Liu, Heitz & Bradberry, 2009) . For mercury, some study shown it can increase excretion of mercury though urine about 6o% efficient. (Juresa, 2005) For cadmium, animal studies have shown removing cadmium from the kidneys. Finally, animal research has shown removing arsenic from not only the blood

but also the brain. (Flora, Bhadauria, Pant & Dhaked, 2005) Some study in human research has shown removing arsenic more effective in cases of acute poisoning than in those of long-term exposure. Clinical studies indicate DMSA is a safe and effective of decreasing these metals such as lead, arsenic, mercury, cadmium (Miller,1998) but cannot remove too much about mineral such as calcium, magnesium, copper, iron (Berthon,1995)

EDTA (Ethylene Diamine Tetra-acetic Acid) is a type of chelating agent which consists of two amino acids and four acetic acid binding with oxygen and nitrogen atoms which has ability to binding with heavy metals such as lead, mercury, arsenic and excreted them from the body by kidneys. It can use by orally or intravenously and treat acute and chronic lead poisoning by pulling toxins (including heavy metals such as lead, cadmium, and mercury) from the bloodstream.

Some author said that chelation technique for patients, oral chelation with oral dimercaptosuccinic acid (DMSA) supported by nutrients even though slower than DMPS or EDTA through IV administration, is better abandon and a safe for practitioners without considerable training in IV chelation. (Pizzorno, 2010)

4. Another reason for mercury cannot found in this study because the most commonly accepted methods for measure mercury exposure are urine or blood testing. Both tests usually measure levels of total mercury (elemental, inorganic and organic).

1) Mercury urine testing: The elevated level of mercury in urine can be indicates exposure to an elemental or inorganic source of mercury, such as from a job that uses mercury. Urine-mercury levels represent the chronic, steady state, exposure to the body of mercury

2) Mercury blood testing : The elevated level of mercury in blood can be indicates exposure to all three type of mercury (organic, elemental and inorganic mercury), such as eating fish and other seafood because fish (mostly deep sea fish) may containing methyl-mercury or recent exposure to high level of mercury

Some author said that hair testing are not an accurate for mercury testing because Mercury can be accurate assessing in blood and urine, which is a distillate of blood serum. Hair is the outer layer of the skin and has no blood supply. Thus amount of mercury in hair does not reflect the concentration in the body and hair is subject to washing, shampoos, sun exposure, swimming and bathing, hair dryers. Substances can be removed from the hair by these treatments, but the amounts removed are not known and also changes. Similarly heavy metals can be added by some of these processes. It can be changes constantly and uncertain. (Baratz, 2005)

From mentioned above, patients in this study live in the city and not to work with mercury so that why it cannot found in urine. But they probability eat fish or seafood, if we change to blood testing, it may be found. But another author said that measurements of blood and urine from thousands of people have never shown high levels of mercury in the general population but shown only workers with high work exposure have shown abnormal levels in blood and urine (Baratz, 2005)

5.3 Comments

5.3.1 Next study, it should be assessment in industrial areas or factory areas and should be increase the number of patients for found various number of heavy metals.

5.3.2 It should be increase number of heavy metals because actually have a lot of heavy metals in environment but in this study select 8 heavy metals. Although live blood analysis may not specific for some heavy metals in this study but it may specific in other heavy metals

5.3.3 It should be use hair testing because it is more accuracy and can assessment for chronic disease and should be use teeth testing for assessment about amalgam which can indicate for mercury.

5.3.4 It should be to do the experiment research, not retrospective study. In the further study should be grading crystal in live blood analysis. It may count amount of crystals in live blood for indicate to level of heavy metals or type of heavy metals

5.3.5 It should be to analyze about specificity and sensitivity for live blood analysis

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APPENDIX

APPENDIX

Collection Data Form

ลำดับคนไข้	Live blood	Lead	Mercury	Cadmium	Manganese	Chromium	Cobalt	Nickel	Arsenic	Age	Sex
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
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19											

CURRICULUM VITAE

CURRICULUM VITAE

NAME Miss Natthira Preechasilp

DATE OF BIRTH 29 October 1981

ADDRESS 299/146 Soi 12 Nakhonsawantok,
Nakhonsawan
Thailand 60000

EDUCATIONAL BACKGROUND

2003 Bachelor of Physical therapy
Mahidol University, Thailand

WORK EXPERIENCE

2010-Present Free lance of physiotherapy

2003-2010 Physiotherapist
Kanya physical therapy clinic