RELATIONSHIP BETWEEN CHOLESTEROL CRYSTAL IN LIVE BLOOD ANALYSIS METHOD AND SERUM LIPID PROFILE LEVELS

RUCHADAKORN AYUKARN

MASTER OF SCIENCE
IN
ANTI-AGING AND REGENERATIVE SCIENCE

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
MAE FAH LUANG UNIVERSITY

2013

RELATIONSHIP BETWEEN CHOLESTEROL CRYSTAL IN LIVE BLOOD ANALYSIS METHOD AND SERUM LIPID PROFILE LEVELS

RUCHADAKORN AYUKARN

THIS INDEPENDENT STUDY IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANTI-AGING AND REGENERATIVE SCIENCE

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
MAE FAH LUANG UNIVERSITY

2013

RELATIONSHIP BETWEEN CHOLESTEROL CRYSTAL IN LIVE BLOOD ANALYSIS METHOD AND SERUM LIPID PROFILE LEVELS

RUCHADAKORN AYUKARN

THIS INDEPENDENT STUDY HAS BEEN APPROVED
TO BE A PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
IN
ANTI-AGING AND REGENERATIVE SCIENCE
2013

INDEPENDENT STUDY COMMITTEE

.....CHAIRPERSON
(Prof. Dr. Thamthiwat Nararatwanchai)

.....ADVISOR
(Prof. Dr. Vichit Punyahotra)

.....EXAMINER
(Assoc. Prof. Dr. Anek Hirunraks)

ACKNOWLEDGEMENTS

It is my pleasure to thanks several individual who in one way or another contributed their valuable assistance in my independent study. I would like to thank Mae Fah Luang University giving me an opportunity to study in Master of sciences program in anti-aging and regenerative science**.**

I would like to express my deepest gratitude to my advisor Professor Dr. Vichit Punyahotra, who inspired me to come up with the thesis of my medical technology background and gave such a valuable advice. I would also like to show my gratitude to my committee Professor Dr. Thamthiwat Nararatwanchai and Associate Professor Dr. Anek Hirunraks who gave me a valuable statistical suggestion and constructive comment.

I extend my sincere appreciation to Central lab Suphan Buri which offering me all the data and did laboratory experiment for this research until it finished.

Lastly, I offer my regards and blessing to thanks my beloved family for their love and support, and all my classmates for their pleasant friendship.

Ruchadakorn Ayukarn

Independent Study Title	Relationship Between Cholesterol Crystal in Live Blood Analysis Method and Lipid Profile Levels
Author	Ruchadakorn Ayukarn
Degree	Master of Science (Anti-Aging and Regenerative Science)
Advisor	Prof. Dr. Vichit Punyahotra

ABSTRACT

The relationship between lipid profile level and cholesterol crystal in live blood analysis was studied in 235 healthy subjects, divided into 106 men and 129 women aged between 30 and 60 years, who conducted laboratory check-up. Lipid profiles were measured by using chemical reaction in semi-automated machine while cholesterol crystal in live blood analysis was counted under dark field microscope for 10 oil fields. From the results, there was no correlation among socioeconomic factors, total cholesterol level, triglyceride level, HDL-C level, and LDL-C level. However, the results showed significant correlation between cholesterol crystal and type of food ($p<0.05$). Healthy food was different from unhealthy food ($p<0.05$) and, in the same way, unhealthy food was different from general food ($p<0.05$) whereas general food and healthy food were unaltered. The results also showed that cholesterol crystal was detected in live blood analysis when the subject usually consumed fatty meals or their body had poor digestion of fat. These preliminary data suggested that unhealthy food and cholesterol crystal in blood stream might play the important role in the aging process and chronic and degenerative diseases.

Keywords: Live blood analysis/Cholesterol crystal/Lipid profile/Dark field microscope

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Nowadays, hematology technology advance towards molecular and genetics, but among of many techniques, one of them, which is called live blood analysis or dark field microscope, is non-invasive due to finger stick technique. It is commonly used in anti-aging clinics and holistic medicine applied centers. Live Blood Analysis is a method to examine fresh whole blood cells under a specially adapted microscope that projects the dynamic image onto a computer screen. This presents digestive status, toxic elimination status, and immune functions. It can be assessed as well as the presence of bacteria and other micro-organisms, such as virus and fungus. An evaluation of the blood cells and blood constituents can hints to the presence or cause of many diseases, for example, the bacterial recurrent problems, chronic problems of toxicity (e.g. amalgam disturbances), and fat congestion. From the disquisition, some researchers claim that polluted environment and synthetic foods create disturbance in the delicate acid-alkali balance in our body fluid and tissue. Because of this imbalance, the blood picture can reveal the degree of disturbance. One of the most important and most seen crystals found in live blood analysis is the cholesterol crystal which crystallizes when the body is acidic. Cholesterol and other types of lipid in serum (Triglyceride, LDL-Cholesterol and HDL-Cholesterol) derive from endogenous synthesis by the liver and exogenous dietary sources. Many studies show that high level of lipid profile may increase risk in cardiovascular disease, fatty liver, insulin resistance, and metabolic syndrome. At the same time, low level of lipid profile may affect cell membrane, myelin sheaths, and steroid hormone production. Therefore, this research is initiated to study the relationship of crystallization, cholesterol concentration, triglyceride concentration, HDL-C concentration, and LDL-C concentration in peripheral blood. The researched data will also be used as reference to validate this new technique. It will also be used as the screening test instead of biochemistry analysis method,

which use a large amount of blood by venepuncture technique and required automated machine.

1.2 Objectives

1.2.1 To study the prevalence of hyperlipidaemias in Thai people.

1.2.2 To study the relationship between lipid profile levels in blood and cholesterol crystallization resulting from live blood analysis.

1.3 Hypothesis

There is a correlation of crystallization and lipid profile level in blood.

1.4 Benefits

The result will bring about awareness of wellness in people and convince them to change their lifestyle, avoiding any kinds of hazardous substances. These changes will then affect the behavior of the blood. Eventually, the information will be applied to diagnostic and prevention of people unhealthiness.

1.5 Glossary

1.5.1 Live blood analysis is the method to find out abnormality of red blood cell and blood constituents, for example, nutritional status, digestive status, liver intoxication, immunological status, and parasitic infection.

1.5.2 Crystal is a substance in which the constituent atoms, molecules, or ions are packed in a regularly ordered, repeating three-dimensional pattern. Most crystals are formed by solidification of chemical element.

1.5.3 Lipid profile is a pattern of lipids in the blood. A lipid profile usually includes the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and the low-density lipoprotein (LDL) cholesterol.

1.5.4 Dyslipidemia is the condition of lipid in serum level that presents total cholesterol (TC) more than 200mg/dl, LDL-Cholesterol (LDL-C) more than 100 mg/dl, HDL-Cholesterol (HDL-C) more than 40 mg/dl, and Triglyceride (TG) more than 150 mg/dl, following National Cholesterol Education Program (NCEP) criterion.

CHAPTER 2

LITERATURE REVIEW

Lipid profile is the term used to describe a pattern of lipids in the blood which usually includes the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides.

2.1 Cholesterol

Cholesterol is a chemical compound that is naturally produced by the body and is structurally an amphiphilic molecule containing 27 carbon atoms. The majority of the cholesterol in the body is in the free, unesterified form: it is this form that is the structural component of cell membranes. Cholesteryl esters in normal cells represent a store for future use and appear microscopically as intracellular droplets. Cholesterol is presented in the diet but cholesterol in the body is produced by *de novo* synthesized from acetate. Small amount of the body's cholesterol pool comes from dietary cholesterol which the main sources of dietary cholesterol are meat, poultry, fish, and dairy products. Organ meats, such as liver, are especially high in cholesterol content, while foods of plant origin contain no cholesterol. Liver is the key organ that maintains cholesterol balance by converting it into bile acids, which are also excreted into the bile. Moreover, cholesterol is a building block for cell membranes and precursor for steroid hormones like estrogen and testosterone. Cholesterol is also a multifunctional sterol molecule with a role in regulating protein activity, including signaling protein in the brain, oncogenic G protein, membrane proteins, and enzymes, such as proteases of amyloid protein. Additionally, it is indispensable in maintaining membrane permeability and it is a principle substrate for cholesterol-derived molecules that are essential to mammals' bodies. (Rozner & Garti, 2006) Despite the

importance of cholesterol for normal cell growth and function, high levels of cholesterol and/or low levels of oxidized cholesterol in the serum and other tissues can be dangerous and can cause cytotoxic effects (e.g. necrosis, cell growth inhibition, and cell damage), atherogenesis (reduction in blood vessel caliber, due to deposition of cholesterol and fat on innermost layers of arterial walls), and cardiovascular diseases, including coronary heart diseases. Cholesterol levels can be lowered using drugs like statins (Maggini et al., 2004), which inhibit the rate-limiting step in cholesterol synthetic pathway by converting 3-hydroxy-3-methyl-glutaryl coenzyme A to mevalonate catalysed by enzyme hydroxyl methylglutaryl-CoA reductase (HMG-CoA reductase). Although a small amount of the body cholesterol pool comes from dietatry cholesterol, it has an important role in regulating the rate of *de novo* cholesterol synthesis.

2.2 Triglyceride (Triacylglycerols)

Triglyceride comprises three fatty acid esterified with a glycerol backbone. The majority of triglyceride comes from dietary fat, which is hydrolysed in intestinal tract by lipase to monoglycerides and three fatty acids, and then monoglycerides incorporate into chylomycrons. The endogenous triglyceride synthesis comes from the liver and adipose tissue. In normal condition, hepatic triglyceride is secreted in very low density lipoproteins (VLDL) and triglyceride also accumulates in hepatocytes, leading to hepatic steatosis. Furthermore, adipose tissue triglyceride represents the main energy store of the body.

2.3 Lipoproteins

Lipoproteins are soluble lipid complexes of proteins (apolipoproteins, enzyme) and lipids (cholesterol, triglyceride, phospholipids) held by non-covalent forces that deliver insoluble lipids in the circulation from the synthesized tissues to those that utilize, degrade, or store them. Lipoproteins are synthesized in the liver and the intestines, arise

from metabolic change of precursor lipoproteins, or are assembled at the cell membranes from cellular lipids and exogenous lipoproteins or apolipoproteins. In circulation, they undergo enzymatic reactions of their lipid components, facilitated and spontaneous lipid transfers of soluble apolipoproteins, and conformational changes of the apolipoproteins in response to the compositional changes. Finally, lipoproteins are taken up and catabolized in the liver, kidney, and peripheral tissues via receptor-mediated endocytosis and other mechanisms. (Vance D. E & Vance, J. E, editors, 2008) There are several classes of lipoproteins that were classified on their size, density, or protein composition. The name of lipoproteins is base on their density: Chylomicrons (<0.95g/mL), Very Low Density Lipoproteins (VLDL) (0.95-1.006g/mL), Intermediate Density Lipoproteins (IDL) (1.006-1.019g/mL), Low Density Lipoprotein (LDL) (1.019-1.063g/mL), and High Density Lipoproteins (HDL) (1.063-1.210g/mL). (Marshall & Bangert, editors, 2008)

2.3.1 Chylomicrons

Chylomycrons are newly synthesized intestinal apo B-containing particles. The lipid core consists of rich triglyceride and small cholesterol ester, surrounded by monolayer, which comprices protein, phospholipids, and cholesterol. The cholesterol in chylomicrons is added extracellularly from HDLs. The major protein components in chylomicrons are apo B48, apo A-I, apo A-II, and apo A-IV. They obtain apo E and apo C from HDL.

2.3.2 Very Low Density Lipoproteins (VLDL)

Very low density lipoproteins are the largest lipoproteins that contain apo B100, apo C-I, apo C-II, apo C-III, apo E, and small amount of apo A. These are secreted by liver and acquired apo E and apo A from HDL like chylomicrons. Moreover, the core consists of endogenous triglycerides.

2.3.3 Intermediate Density Lipoproteins (IDL)

IDL particles density lie between that of VLDL and LDL. These particles are produced during the conversion of VLDL to LDL and contain cholesteryl esters and triglycerides in the cores.

2.3.4 Low Density Lipoproteins (LDL-C)

LDL is the major cholesterol-containing lipoproteins. It represents the end product of VLDL catabolized by lipase. The cores of LDL are mainly choleteryl esters; the protein component is apo B-100. (Marshall & Bangert, editors, 2008) Moreover, studies demonstrate that increasing in oxidized LDL increases risk of artherosclerotic artery coronary disease. (บัณลังก์ เอกบัณฑิต, 2546; Fernandes et al., 2004)

2.3.5 High Density Lipoproteins (HDL-C)

HDL is the smallest lipoprotein but has the most density among the lipoproteins because they collect cholesterol and phospholipids from peripheral tissue returned to the liver. HDL particles may contain only apo A-I, both apo A-I and apo A-II, or only apo A-II. HDL has anti-atherogenic properties which can regulate down the expression of molecules adhesion on the surface of the vascular endothelium and inhibit platelet aggregation. Besides, it prevents the inhibition of nitric oxide synthesized by oxidized LDL, regulates up ATP binding cassette protein A1 (ABCA1), and activates intracellular signaling, leading to stimulate cholesterol efflux.

2.4 Hyperlipidemia

Hyperlipidemia is the condition involving abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It is the most common form of dyslipidemia (which also includes any decreased lipid levels). This condition is divided into primary and secondary subtypes.

2.4.1 Primary Hyperlipidemia

It is usually caused by genetic abnormalities. Familial Hyperlipidemias are classified according to The Fredrickson classification, which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation. It was later adopted by the World Health Organization (WHO). It does not directly account for HDL, and it does not

distinguish among the different genes that may be partially responsible for some of these conditions.

2.4.1.1 Familial Combined Hyperlipidemia (FCH)

It is inherited as an autosomal dominant trait. The typical findings are increase in plasma apo B-100 concentration and increase in LDL, VLDL, or both in plasma level.

2.4.1.2 Familial Hypertriglyceridemia (FHTG)

It is apparently autosomal dominant. The majority of affected individuals with FHTG have a moderate elevation in triglyceride concentrations, but only minority may manifest a more severe hypertriglyceridaemia with and elevation in VLDL and presence of chylomicron in fasting sample.

2.4.1.3 Disorders of metabolism of apo B-containing lipoproteins

Chylomicronemia syndrome manifests as eruptive xanthomata, lipaemia retinalis, and recurrent bouts of abdominal pain, including acute pancreatitis and hepatosplenomegaly. The increase in trigyceride concentrations and HDL concentrations is very low and chylomicrons are presented in the fasting state. There are three inherited disorders that have been described as the cause of this condition:

1. Lipoprotein lipase deficiency

This is the rare autosomal recessive condition. (Approximately found 1 in 1,000,000) Presence of this condition usually occurs in childhood. The major mutation is Gly188Glu, which results in an enzymatically non-functional protein and is the main cause of chylomicronaemia in the general people.

2. Apo C-II deficiency

This is the homozygous apo C-II deficiency that tends to present later and somewhat milder than lipoprotein lipase (LPL) deficiency. The protein produced, however, is unable to activate LPL.

3. Familial lipoprotein lipase inhibitor

This is the defect appeared to be inherited in an autosomal dominant fashion.

2.4.1.4 Remnant Heperlipoproteinaemia (RH)

The characteristic feature of RH is cholesterol enrichment of the VLDL fraction. This is due to an accumulation of remnant particle. There is an increasing risk of

premature atherosclerosis involving the peripheral vascular system as well as the coronary arteries.

2.4.1.5 Autosomal dominant hypercholesterolaemia

1. Familial Hypercholesterolemia (FH).

This is the most severe hyperlipidemias. It can develop atherosclerosis if untreated. Heterozygous state manifests high LDL and premature cardiovascular disease. The probability of occurrence of heterozygous FH is about 1/500 in most people. The defect in FH involves the LDL receptor gene.

2. Familial Defective Apolipoprotein B-100 (FDB)

FDB is an autosomal co-dominant disorder, resulting from a mutation in the LDLR-binding domain of apo B-100. This also brings about reduction in binding to the LDLR. It elevates total plasma and LDL cholesterol concentrations in subjects.

3. Autosomal Dominant Hypercholesterolemia 3(AHD3)

This condition is caused by one of function mutations described in the protein covertase subtilisin kexin 9 (PCSK9) genes, which result in reduced receptor expression on the cell surface and, consequently, reduced cellular LDL uptake and increasing plasma LDL concentration.

2.4.1.6 Autosomal Recessive Hypercholesterolemia (ARH)

This is a rare disorder condition, which results from a mutation in the ARH gene.

2.4.1.7 Polygenic hypercholesterolemia

Polygenic hypercholesterolemia is the term used in genetic background of the affected individuals. It will affect the extent to which lipoprotein metabolism will be deranged by any environmental insult.

2.4.1.8 Dysalphalipoproteinemias

The dysalphalipoproteinemias are disorders of the synthesis and secretion of apo A-containing lipoproteins. With the exception of apo A-I_{MILANO}, all other genetic caused by familial hypoalphalipoproteinemia (FHA) are associated with some degree of increased cardiovascular risk. This condition can classify to apo A-I deficiency and abnormal apolipoprotein A.

1. Apo A-I deficiency

There are three types of Apo A-I deficiency, which are associated with corneal clouding and premature coronary heart disease.

2. Abnormal apolipoprotein A

From population screening, the structure of apo A-I has at least 11 variants. The most described structural variation is apo A-I_{MILANO}, which is associated with a low HDL-C concentration. Anyway, there is no increase in incidence of atherosclerosis, and, additionally, LCAT activity is reduced.

2.4.1.9 Disorder of HDL metabolism

1. Tangier disease

This condition is autosomal recessive and rare disease. Total cholesterol is low (<3.2mol/L), and plasma triglyceride levels are normal or increase. Virtually no HDL is presented (HDL-C approximately 0.1 mmol/L). Tangier disease is caused by the loss function of ABCA1 protein, which is the cholesterol-efflux regulatory protein.

2. Familial Lecithin-Cholesterol Acytransferase deficiency (LCAT)

This type is also an autosomal recessive condition, which will result in the accumulation of the free cholesterol in all lipoprotein fractions. The clinical feature is high plasma triglyceride and low HDL-C.

3. Fish eye disease

This is an autosomal recessive disorder resulting from mutations in the LCAT gene. Low HDL concentration and increase in VLDL give the eyes appearance like the dead fish or corneal opacities.

4. Hepatic Triglyceride Lipase deficiency (HTGL)

Several familial cases of HTGL have been reported but the mode of inheritance in uncertain.

5. Cholesterol ester transfer protein deficiency.

The cause of this condition is mutations of the CETP gene. Cholesterol ester transfer protein deficiency is relatively common in Japan. Subjects with this condition have high concentration of HDL-C, apoA-I, apo A-II, and apo E.

2.4.2 Secondary Hyperlipidemia

Secondary hyperlipidemia arises from other underlying causes.

2.4.2.1 Diabetes mellitus

Poorly controlled diabetes mellitus give rise to hypertriglyceridaemia. Insulin activates lipoprotein lipase and there by enhances the rate of clearance of the triglyceride-rich lipoproteins. Therefore, in insulin deficiency, there is an increasing influx of free fatty acid to the liver, which leads to increase in hepatic triglyceride synthesis.

2.4.2.2 Hypothyroidism

Lipoprotein lipase activity may also be impaired in hypothyroidism, which explains the hypertriglyceridaemia. Untreated hypothyroidism will escalate LDL-C level, and LDL receptors will be reduced in consequent. It is imperative that subjects who have subclinical hypothyroidism are found to have hyperlipidaemia.

2.4.2.3 Nephrotic syndrome

The hyperlipidaemia occurring in the nephritic syndrome is the most common hypercholesterolaemia, which is the result of hepatic overproduction of apo B-100 as a part of hepatic protein synthesis. Hydroxymethylglutaryl-CoA (HMG-CoA) reductase activity also increases.

2.4.2.4 Renal transplantation

Impaired renal function accompanies in high concentration of plasma lipid after transplantation. Moreover, immunosuppressant therapy may cause hyperlipidaemia due to corticosteroids.

2.4.2.5 Liver disease

The liver plays a key role in lipoprotein metabolism. Moreover, the body has to excrete cholesterol by hepatic secretion into the bile. Bile acid contains apo C, apo D, albumin, and cholesterol. In liver, disease associates with hypertriglyceridaemia or mixed hyperlipidaemia.

2.4.2.6 Alcohol

Epidermiological studies have shown that moderate alcohol intake is associated with an increase in HDL-C, but alcohol cause hypertriglyceridaemia in susceptible individuals. This results from a combination of increasing production and impaired removal of VLDL.

2.4.2.7 Drug-related hyperlipidaemia

Several drugs can affect lipoprotein concentrations, including antihypertensive drugs, corticosteroids, sex steroids, immunosuppresants, and antiretroviral drugs. Glucocorticoid treatment results in an increase in LDL-C, triglyceride and HDL while estrogens treatment raise hepatic VLDL secretion and HDL. Hormone replacement therapy with progesterone in postmenopausal woman tends to reduce HDL concentration and leverage LDL concentration. On the other hand, tamoxifen, a selective estrogen receptor modulator, can cause massive hypertriglyceridaemia. In addition, anti hypertensive drugs raise plasma triglyceride concentration and lower HDL-C concentration in blood.

2.5 Acquired Hypolipideamia

Acquired or secondary hypolipideamia occurs with cachexia, malabsorbtion, hyperthyroidism, malnutrition, liver failure, and some forms of malignancy.

2.5.1 Investigation of Lipid Disorders

The simple assessment is measurement of plasma cholesterol, triglyceride, HDL-C, and LDL-C after twelve hours fast.

2.5.2 Appearance Sample Analysis

The appearance of serum after standing overnight at 4˚C gives an excellent guide to gross lipoprotein abnormalities. The large size of VLDL and chylomicron particles will lend an opalescent appearance to the sample when the percent increase in amount. Chylomicrons have less density than plasma, and will form a layer on the surface whereas, if the opalescent remains distributed throughout the sample, this indicates an increasing amount of VLDL. An increase in LDL is often accompanied by an orange color of the sample due to the β-carotene contained with it, but it does not cause the plasma or serum to appear opalescent.

2.5.3 Total Cholesterol Analysis

In the diagnostic laboratory, enzymatic (cholesterol oxidase) method

2.5.4 Triglycerides

Triglycerides are enzymatically hydrolyzed by glycerol phosphate oxidase method.

2.5.5 High Density Lipoprotein Cholesterol (HDL-C)

HDL cholesterol is measured by accelerator selective detergent method.

2.5.6 Low Density Lipoprotein Cholesterol

Low density lipoprotein cholesterol can be measured by direct measurement and indirect measurement. Direct measurement uses liquid selective detergent method. For the indirect method, the level of LDL-C is derived by substituting the result of the analysis of total cholesterol, HDL-C, and triglyceride into the Friedewald formular.

2.6 Treatment

Hyperlipidemia can increases mortality and morbidity, depending on cardiovascular events. Lipid lowering therapy is beneficial in patients with dyslipidemias for both primary and secondary prevention of coronary heart disease. Hypertriglyceridemia has now been recognized as another important risk factor for coronary heart disease. Recently, much attention has been focused on the atherogenesis of small, dense LDL, remnant lipoprotein, and isolated hypoalphalipoproteinemia. Hypertriglyceridemia is known to be deeply involved in the pathogenesis of all of these abnormal lipoprotein profiles. (Yoshino) Medical treatment should be performed immediately in order not to be reached optimal serum choresterol level despite controlling weight, physical activity, lifestyle changing, and lipid lowering diet. Lipid altering agents include the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. They represent a major advance in the treatment of hypercholesterolemia in terms of potency and relative safety or statin drugs, fibric acid derivates, bile acid sequestrants, cholesterol absorption inhibitors, and nicotinic acid. These drugs are different in mechanism of action, the degree and type of lipid reduction, and adverse action. Thus, the indications for a particular drug are influenced by the underlying lipid abnormality. (Deslypere, 1995)

2.7 Epidemiology

Lipid and Lipoprotein abnormalities can be found broadly in Thailand. Changes in the shape of pyramid population, which are decrease in birth rate and increase in life expectancy, reflect that people can live longer than they did in the past. Therefore, people will be in trouble with dyslipideamia in the next 40-50 years. Moreover, they are exposed to cardiovascular disease and coronary heart disease because of change in lipoprotein metabolism and its influence on atherosclerosis. From the studies, in rural area in Thailand, women have more abnormalities of lipid percentage than men have. The percentage of female and male who have high cholesterol are 39.9% and 19.9% respectively. The number of LDL concentration more than 150 mg/dl is found 39.9% in female and 10.9% in male. HDL concentration less than 40 mg/dl is found 15.5% in female and 8.3% in male. For Triglyceride, its concentration more than 150 mg/dl is found 41% equally in both male and female. (บัณลังก์ เอกบัณฑิต, 2546)

2.8 Live Blood Analysis or Dark Field Microscope

Darkfield Microscope or Live Blood Analysis is a way of studying live whole blood cells under a specially adapted microscope that projects the dynamic image onto a video screen. This microscope has a special condenser and a diaphragm which light scattered from the object is stopped by, with the result that the object appears bright and the background dark. This examination was developed and described by Prof. Dr. G. Enderlein. With this method, he proved that co-relations exist between blood parasites, symbionts, bacteria, and fungi. The main proven fact is that chronic diseases are created by increasing sickness tendencies of the endobionts and bacteria, viruses, and fungi developed in the human body, or are changed to pathogenic agents of diseases depending upon the inner terrain (determined by acid-base balance, protein content and level of trace elements). The existence of pre-stages, which are not yet able to make one ill but can endanger an illness, can also be found in the darkfield examination. Therefore it is also an important preventative examination. (Crislip, 2009) In 2006, there was a pilot study to

test the interobserver reliability and test-retest reliability of 2 experienced darkfield specialists who had undergone comparable training in Enderlein blood analysis. The result indicates that Enderlein darkfield analysis is very difficult to standardize and that the reliability of the diagnostic test is low. (Teut, Lüdtke & Warning, 2006) However, regarding one of researchers' study, the results showed that Live Blood Analysis is a preliminary screening to determine the mercury in bloodstream. It had similar statistical significance, compared to blood mercury level test as well. (Burusliam, 2011) The other study also applied this technique to monitor rouleau formation and crystal depositions after Chelation Therapy, and it was verified as the acceptable method. (Tiyajinda, 2011)

Enderlein's observations of live blood using darkfield microscopy revealed structures and phenomena that had not yet been described. The concept can be classified by red blood cell abnormality or other constituent abnormality, using dark field microscope method, into three groups.

2.8.1 Digestion Abnormality

2.8.1.1 Normal erythrocytes

The Erythrocytes (cells) are round and separated and move through the capillaries very easily. The average size of healthy red blood cells (RBCs) is 7.2 microns. RBCs lifespan is 120 days

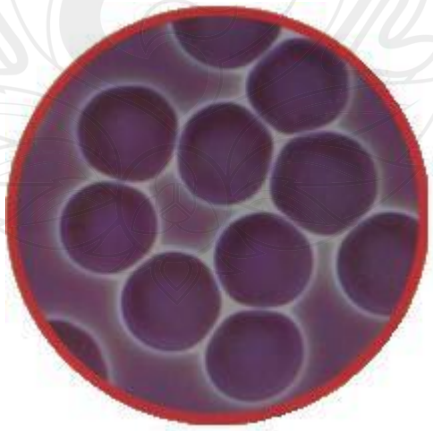

Figure 2.1 Normal Red Blood Cell

2.8.1.2 Protein linkage

This condition is the first sign of cell stickiness and may progress into rouleau if not corrected. Protein linkage is a sign that excessive protein is being consumed or the protein is not being digested completely due to lack of protease. As the cells start sticking together, it becomes harder for the heart to push the blood through the veins and arteries.

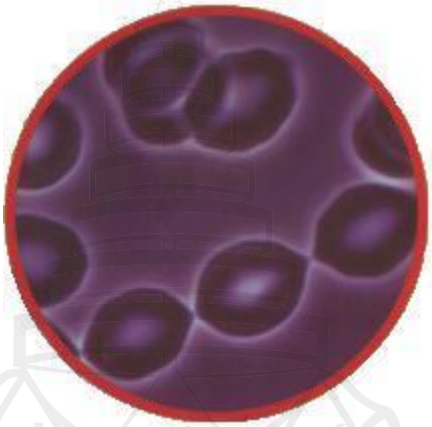

Figure 2.2 Protein Linkage

2.8.1.3 Rouleaux formation

Rouleaux formation is the coin-like formation. It occurs when high fat and protein diets and high acidity exist. This condition will stay that way for at least two hours. When the blood gets to this condition, the amount of oxygen that can be transported is severely diminished. Because cells are not getting oxygen, you will feel tired and have poor digestion, edema, and skin disorders.

Figure 2.3 Rouleaux Formation

2.8.1.4 Poikilocytosis

Poikilocytes or Stomatocyte is caused by free radicals or insufficient antioxidant, bringing about change in RBCs figure. This circumstance results from toxic liver or chemical that leads to abnormal RBCs proliferation. This also lowers the oxygen carrying capacity in blood and shortens the cell lifespan. Moreover, there is free radical damage, signifying that there will also be damage to the nuclei of tissue cells which is the beginning of mutations that lead to cancer.

2.8.1.5 Ovalocytes

This condition represents RBCs in oval shape and reflects overused endocrine, iron insufficiency, folic acid insufficiency, and vitamin B12 insufficiency. This can be found in menopausal stage, pregnancy state, and contraceptive usage.

2.8.1.6 Microcytes and macrocytes

Microcytes are small RBCs having a diameter less than 5 microns. These cells have less hemoglobin than normal cells and are often seen in people with iron deficiency anemia. Macrocytes are cells which greater than 10 microns in size. These cells are often seen in people with hemolytic anemia, vitaminB12 deficiency and folic deficiency.

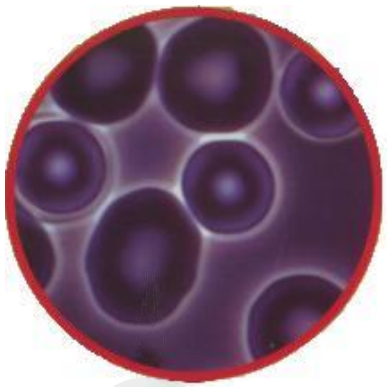

Figure 2.4 Microcytes and Macrocytes

2.8.1.7 Target cell

These RBCs are deficient in iron and therefore hemoglobin, which is the part that carries oxygen. The symptoms produced in the body are tiredness, poor digestion, and anemia.

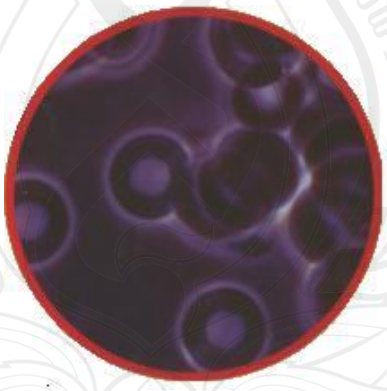

Figure 2.5 Target Cell

2.8.2 Liver Intoxication

2.8.2.1 Thrombocytic aggregation

Platelets or thrombocytes are small disk shaped components of blood that have an important role in blood coagulation. Normally, when a blood vessel is injured, the platelets adhere to each other and to the injury to form a plug which stops the bleeding. Platelets count

approximately 200,000 to 300,000/cu.mm. Anyway, thrombocytes aggregate when there is no injury or very dangerous situation. The aggregated platelets can form a clot which blocks an artery, causing a stroke or heart attack. This clot is called a thrombus. Diets which have high fats and proteins, or high sugar consumption result in this situation.

Figure 2.6 Thrombocytic Aggregation

2.8.2.2 Spicules (Fibrin)

Fibrin are platelets that have change in shape, forming a net-like substance in which blood clots are formed by the entrapment of red and white cells and platelets. They are formed in the blood when there is no injury or there is an imminent danger of a blood clot that can cause a heart attack or stroke. This is caused by liver stress due to incomplete digestion of proteins and fats and heavy metal intoxication of liver.

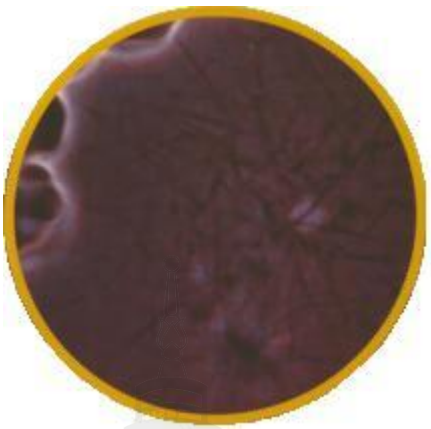

Figure 2.7 Spicules

2.8.2.3 Chylous

Chylomicrons are small particles of fats in the blood after the digestion and assimilation of fat in food. The presence of chyomicrons in the blood after a 12 hour fast indicates a condition known as hyperlipo-proteinemia, and almost all lipids are triglycerides. This can lead to atheriosclerosis, coronary artery disease, and enlargement of the liver and spleen. This condition can be found in hyperthyroidism and lipase deficiency.

Figure 2.8 Chylous

2.8.2.4 Plaque

Atherosclerotic plaque is one of the most dangerous conditions in the blood. It can adhere to the artery walls, narrowing and hardening them. These crystals are formed when the system becomes acidic and fibrin accumulates with calcium and collagen. The fatty acids are crystallized because of protease and lipase deficiency.

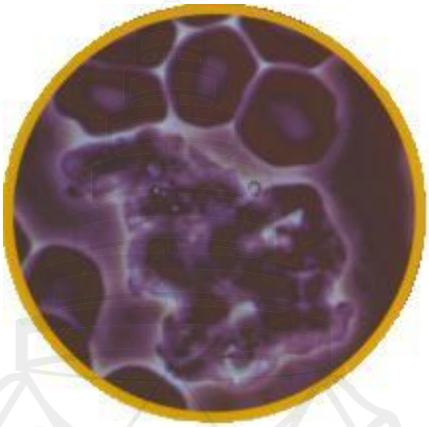

Figure 2.9 Plaque

2.8.2.5 Crystal

Crystal is probably the most seen condition in live blood analysis. This can be classified into uric acid crystal and cholesterol crystal. Uric Acid Crystal is a by-product of protein metabolization and urea. When the body becomes acidic, the urea forms crystals that can lodge in the joints or in the tissues. Uric acid is the cause of gout and one of the causes of fibromyalgia. These crystals are shaped like knives. This is the reason why it can cause so much pain for people with either of these disorders.

Cholesterol is an important sterol in the body. Cholesterol is the precursor for many important hormones. Only when the body is acidic does the cholesterol crystallize and becomes a problem.

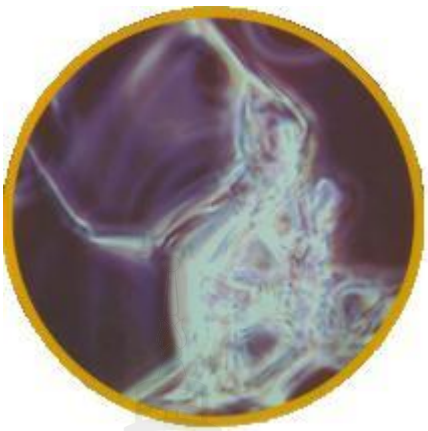

Figure 2.10 Cholesterol Crystal

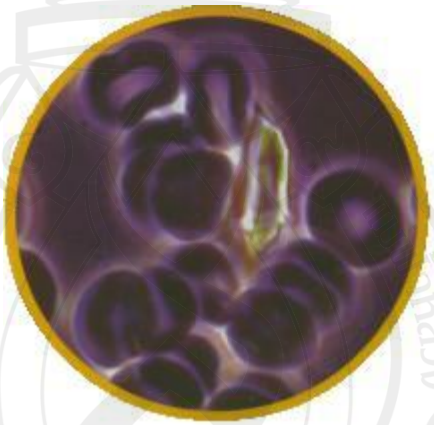

Figure 2.11 Uric Acid Crystal

2.8.2.6 Echinocyte

These are red blood cells that have a thorny appearance. This is usually indicative of kidney stress and crenation (the shrinking of the cell by dehydration).

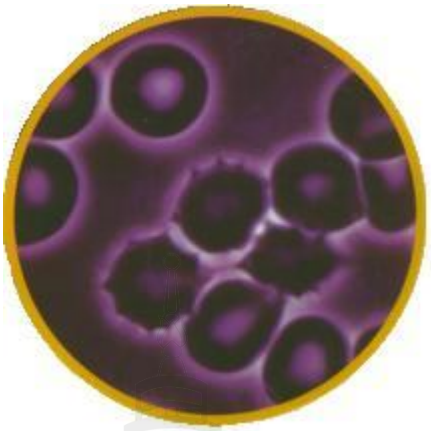

Figure 2.12 Echinocyte

2.8.3 Immune System Abnormality, Parasites, Bacteria, and Fungal Infection

2.8.3.1 L-Form Bacteria

This is a bacterial infection that is shaped like a butterfly. L-Form or measosome usually signifies a condition of low immunity and high blood sugar.

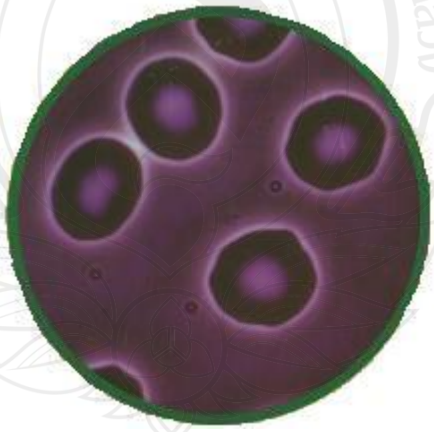

Figure 2.13 L-Form Bacteria

2.8.3.2 Fungal forms

Fungi can spread throughout your body through the blood and develop colonies because of long term anti-biotics usage. They usually develop slowly and are hard to diagnose and usually resistant to treatment. They are seldom fatal and, most of the time, go unnoticed. This is a sign of poor assimilation of nutrients and an acidic condition in the body fluid.

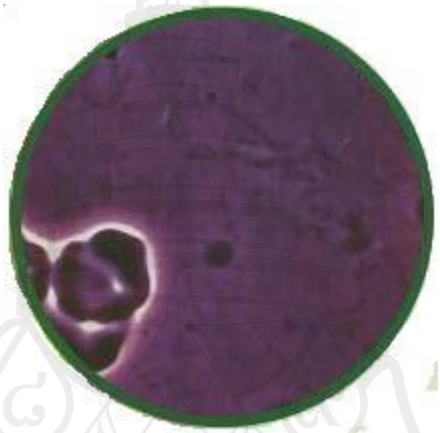

Figure 2.14 Fungal Form

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Methodology

In this study, the correlation between lipid profile levels and cholesterol crystallization was studied in both Thai male and Thai female without gestation. There was no cancer history of all participants while they were conducting laboratory check-up at the Central lab in Suphan Buri province, starting from February 1st, 2013 to March 31st, 2013.

3.1.1 Population and Sample

Sample will be chosen from patients who have blood test result of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride, along with socio-economic history and health examination that includes age, gender, diabetes history, hyperlipideamia history, medication history, for example, smoking history, and alternative supplements.

3.1.2 Inclusion Criteria

3.1.2.1 Patient age 30-60 years

3.1.2.2 Any genders

3.1.2.3 Patients who have blood test after fasting 12 hours at Central lab in Suphanburi province.

3.1.3 Exclusion Criteria

3.1.3.1 Patients who don't have result of lipid profile.

3.1.3.2 Patients who fast less than 12 hours.

3.1.4 Sample Size

$$N = \frac{Z_{\alpha}^{2}P(1-P)}{d^{2}}$$

$$N = \frac{(1.96)^{2}\ (0.154)\ (0.846)}{(0.0462)^{2}} = 234.25$$

N = Number of sample

Z_{α} = confidence level (95%)

P = Expected proportion

(1-P) = unexpected proportion

d = Margin of error in estimating mean, (30%P)

3.2 Research Design

Experimental research; correlation study

3.3 Procedure

Collect the information from selected population.

3.3.1 Patient Information

3.3.1.1 General information

1. Age
2. Gender
3. Education
4. Diabetes history
5. Hyperlipidemia history
6. Medication history, for example, diabetes, drug and smoking history
7. Alternative supplements

3.3.1.2 Laboratory information

1. Live blood analysis

Researcher examines and asses live blood analysis, collect the picture by shooting the photograph. Count the number of cholesterol crystal in 10 fields working with the highest power objective lenses and record the result.

2. Total cholesterol concentration detected by Colorimetric method (Cholesterol oxidase test)

3. Triglyceride concentration detected by

4. High density lipoprotein cholesterol (HDL-C) concentration detected by Colorimetric method (Phosphotungstic acid method)

5. Low density lipoprotein cholesterol (LDL-C) concentration detected by Colorimetric method

3.3.2 Data Acquisition and Correlation Study

Collect all general data and laboratory data and correlate each other by SPSS 17.0 for window. For general information; analyze by descriptive statistic, conduct statistic analysis by Pearson product moment correlation, t-test and one way ANOVA.

3.3.2.1 Independent variable is cholesterol crystal in blood

3.3.2.2 Dependent variables are age, gender, education, diabetes history, hyperlipidemia history, medication history, smoking history, alternative supplements, total cholesterol, Triglyceride, LDL-C, and HDL-C

3.4 Equipment

3.4.1 Questionnaire and Consent Letter

3.4.2 Recorder

3.4.3 Dark Field Microscope

3.4.4 Automated Machine for Chemistry Analysis

3.5 Location

This study will be operated at Central lab in Suphan Buri province.

3.6 Duration of Research

February 2013–March 2013

Table 3.1 Research Operation Plan

Month/Plan	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Literature Review	←	—	—	→								
Proposal writing		←	—	—	→							
Data collection									←	—	—	→
Sample collection									←	—	—	→
Data analysis										←	—	— →
Proposal defense							←	→				
Manuscript writing and submission										←	—	— →
Thesis defense											←	→

3.7 Experimental Design

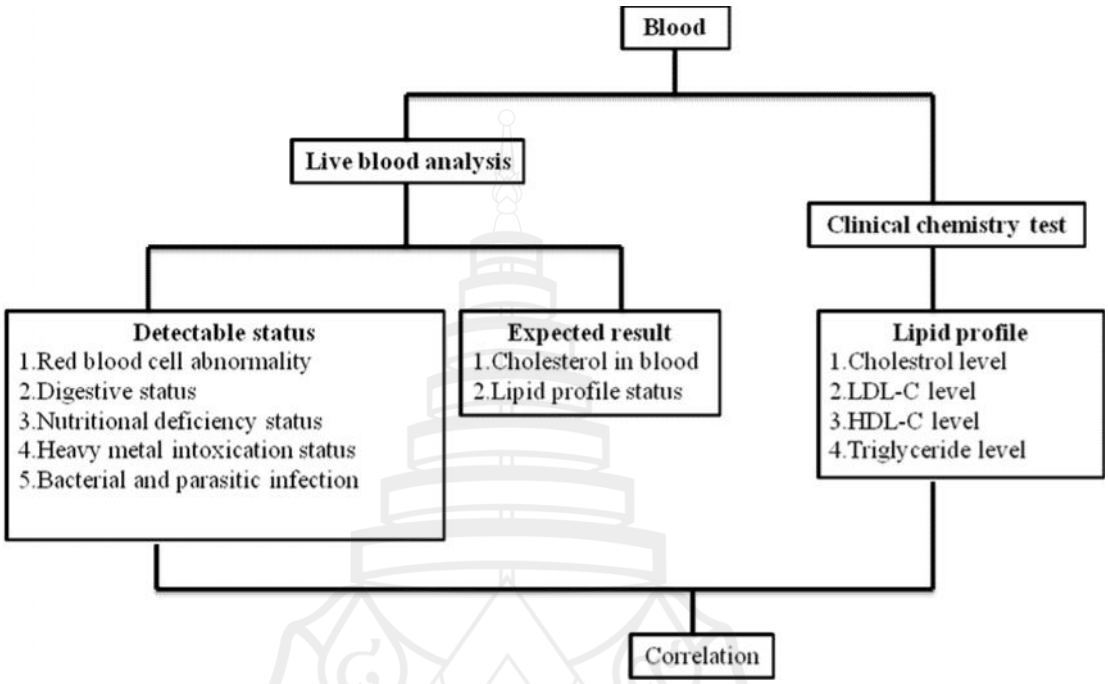

Figure 3.1 Experimental Design

3.8 Expected Result and Application

The result from live blood analysis in this research can evaluate abnormality of blood constituents in participants and correlate with lipid profile level detected by biochemistry method with efficiency, precision, rapidity, and reliability.

The data can be basic information for preventive treatment in alternative study.

3.9 Expected Cost

Live blood analysis 45,000 Baht

Lipid profile analysis 30,000 Baht

Total 75,000 Baht

CHAPTER 4

RESULT

In this study, the correlation between lipid profile levels and cholesterol crystallization was studied in both Thai male and Thai female without gestation. There was no cancer history in all participants while they were conducting laboratory check-up at the Central Lab in Suphan Buri province, Thailand. This study enrolled 235 subjects who were randomly selected. The results were described below.

4.1 Demographic Data

Age of two hundred and thirty five participants are between 30 to 60 years, which the average age was 49.47 years (s = 8.617 years). The group aged 55-60 years included 87 participants, which was the majority. The second group aged between 42 years to 48 years had 52 participants, followed by the group of age between 49 years to 54 years with 49 participants, the group of aged 36 years to 41 years with 29 participants, and the group aged between 30 years to 35 years with 18 participants, which was the minority. The number of subjects in the each group above can be displayed in the form of percentage as 37, 22.1, 20.9, 12.3, and 7.7 percent respectively. There were 106 males (45.1%) and 129 females (54.9%) who attended this study. The data were described in table 4.1 and 4.2.

Table 4.1 Frequency and Percentage of Gender

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	106	45.1	45.1	45.1
	Female	129	54.9	54.9	100.0
	Total	235	100.0	100.0	

Table 4.2 Frequency and Percentage of Age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	30-35	18	7.7	7.7	7.7
	36-41	29	12.3	12.3	20.0
	42-48	52	22.1	22.1	42.1
	49-54	49	20.9	20.9	63.0
	55-60	87	37.0	37.0	100.0
	Total	235	100.0	100.0	

Table 4.3 shows the data of highest education and occupation of participants. They are 32.8% self employed, 20.9% employee, 18.3% unemployed, 16.6% farmer, and 11.5% government officer. Education levels of participants are 76.6% below than bachelor's degree, 19.1% bachelor's degree, 3.8% master's degree, and only 0.4% doctor's degree. All of the data were provided by the participants.

Table 4.3 Frequency and Percentage of Education and Occupation

		Frequency	Percent	Valid Percent	Cumulative Percent
Occupation					
Valid	Government officer	27	11.5	11.5	11.5
	Employee	49	20.9	20.9	32.3
	Self employed	77	32.8	32.8	65.1
	Farmer	39	16.6	16.6	81.7
	Unemployed	43	18.3	18.3	100.0
	Total	235	100.0	100.0	
Education					
Valid	< Bachelor's degree	180	76.6	76.6	76.6
	Bachelor's degree	45	19.1	19.1	95.7
	Master's degree	9	3.8	3.8	99.6
	Doctor's degree	1	0.4	0.4	100.0
	Total	235	100.0	100.0	

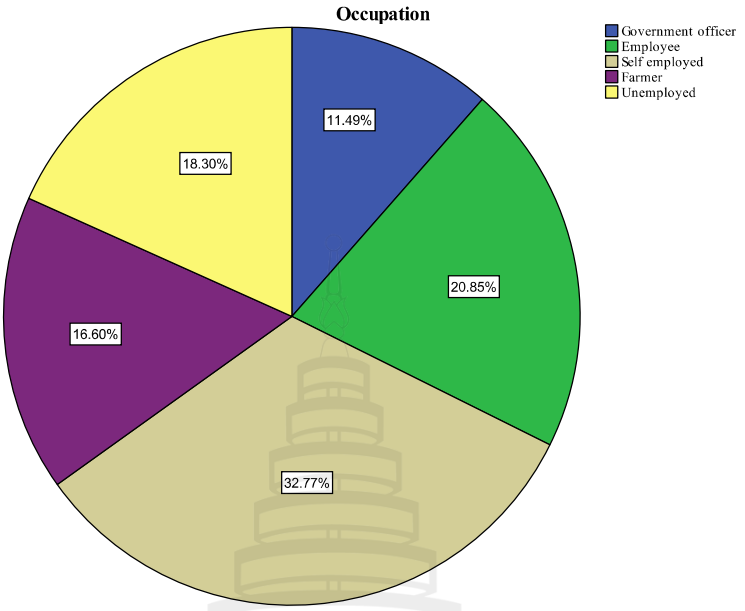

Figure 4.1 Occupation Pie Chart

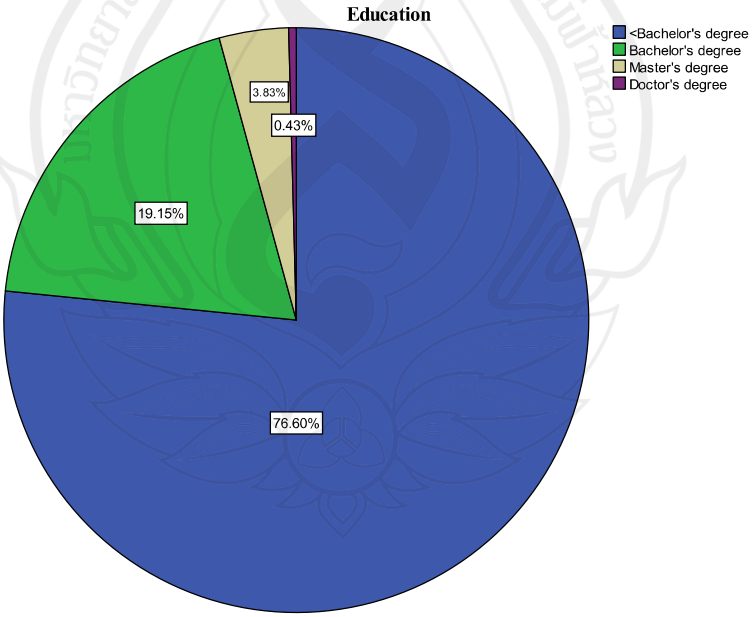

Figure 4.2 Education Pie Chart

4.2 Physical Status, Personal Data and Laboratory Data

Table 4.4 Descriptive Statistics of Weight

	N	Minimum	Maximum	Mean	Std. Deviation
Weight (Kg.)	235	40	117	65.22	12.742
Male (Kg.)	106	45	117	70.28	12.725
Female (Kg.)	129	40	100	61.06	11.193
Valid N (listwise)	235				

Table 4.5 Frequency and Percentage Distribution of Weight

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	40-54	49	20.9	20.9	20.9
	55-69	102	43.4	43.4	64.3
	70-84	67	28.5	28.5	92.8
	85-99	12	5.1	5.1	97.9
	100-114	4	1.7	1.7	99.6
	>114	1	0.4	0.4	100.0
	Total	235	100.0	100.0	

From table 4.4 and 4.5, weight of participants ranged from 40 kg to 117 kg, and the average was 65.22 kg (sd = 12.742 kg). 43.4% of the group had weight between 55 kg to 69 kg, 28.5% of the group had weight between 70 kg to 84 kg, 20.9% of the group had weight between 40 kg to 54 kg, 5.1% of the group had weight between 85 kg to 99 kg, 1.7% of the group had weight between 100 kg to 114 kg, and 0.4% of the group had weight higher than 114 kg.

Table 4.6 Descriptive Statistics of Height

	N	Minimum	Maximum	Mean	Std. Deviation
Height (cm.)	235	145	180	161.66	7.807
Male (cm.)	106	150	180	166.94	12.725
Female (cm.)	129	145	175	157.33	5.987
Valid N (listwise)	235				

Table 4.7 Frequency and Percentage Distribution of Height

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	145-151	27	11.5	11.5	11.5
	152-158	55	23.4	23.4	34.9
	159-165	90	38.3	38.3	73.2
	166-172	40	17.0	17.0	90.2
	173-179	21	8.9	8.9	99.1
	>180	2	0.9	0.9	100.0
	Total	235	100.0	100.0	

Refer to the data in Table 4.6 and 4.7 on height. The height ranged from 145 cm up to 245 cm, and average height was 161.66 cm (s = 7.8 cm). The majority was the participants with height ranged between 159 cm and 165 cm, the other participants were categorized into groups with height between 152 cm to 158 cm, 166 cm to172 cm, 145 cm to 151 cm, 173 cm to 179 cm, and higher than 180 cm. The percentage of each group was 38.3%, 23.4%, 17.0%, 11.5%, and 0.9% respectively.

Table 4.8 Frequency of Cardiovascular Disease History and Diabetes History

		Frequency	Percent	Valid Percent	Cumulative Percent
Cardiovascular					
Valid	Non-CVS	228	97.0	97.0	97.0
	CVS	7	3.0	3.0	100.0
	Total	235	100.0	100.0	
Diabetes					
Valid	Non-DM	212	90.2	90.2	90.2
	DM	23	9.8	9.8	100.0
	Total	235	100.0	100.0	

Concurrent medical conditions were also self-disclosed by all participants. Cardiovascular disease history was identified in 7 persons. Diabetes was identified by 23 participants and hyperlipidemia history was identified by 123 participants. All were indicate in table 4.8.

Table 4.9 Descriptive Statistics Total Cholesterol Level

	N	Minimum	Maximum	Mean	Std. Deviation
Total cholesterol	235	98.80	420.90	225.966	47.63537
Male	106	98.8	378.90	222.604	48.68190
Female	129	110.9	420.90	228.727	46.76671
Valid N (listwise)	235				

Table 4.10 Frequency and Percentage Distribution of Total Cholesterol Level

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<200	70	29.8	29.8	29.8
	200-239	88	37.4	37.4	67.2
	≥240	77	32.8	32.8	100.0
	Total	235	100.0	100.0	

From laboratory data in table 4.9 and 4.10, total cholesterol level ranged from 98.8 mg/dl to 420.9 mg/dl, and average level was 225.966 mg/dl (s = 47.64 mg/dl). 29.8% of participants had total cholesterol level below than 200mg/dl, while 37.4% had total cholesterol level 200-239 mg/dl, and 32.8% had total cholesterol level equal to or higher than 240 mg/dl.

Table 4.11 Descriptive Statistics of Triglyceride Level

	N	Minimum	Maximum	Mean	Std. Deviation
Triglyceride	235	35.30	585.20	146.8170	90.47360
Male	106	49.80	554.50	155.4274	86.86946
Female	129	35.30	585.20	139.7419	93.6930
Valid N (listwise)	235				

Table 4.12 Frequency and Percentage Distribution of Triglyceride Level

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<150	152	64.7	64.7	64.7
	150-199	41	17.4	17.4	82.1
	200-499	40	17.0	17.0	99.1
	≥500	2	0.9	0.9	100.0
	Total	235	100.0	100.0	

Laboratory data indicated in table 4.11 and 4.12 shows serum triglyceride level in participants. The minimum level of triglyceride was 35.3 mg/dl, the maximum level was 585.2 mg/dl, and the average level was 146.817 mg/dl (s = 90.47 mg/dl). 64.7% of participants had triglyceride level below 150mg/dl, 17.4% had triglyceride level ranged from 150 mg/dl to 199 mg/dl, 17% had triglyceride level between 200 mg/dl and 499 mg/dl, and 0.9% had triglyceride level equal to or higher than 500 mg/dl.

Table 4.13 Descriptive Statistics of HDL-Cholesterol Level

	N	Minimum	Maximum	Mean	Std. Deviation
HDL-C	235	29.30	130.50	58.9728	17.26422
Male	106	29.30	130.50	53.2255	14.94611
Female	129	33.60	127.60	63.6953	17.65788
Valid N (listwise)	235				

Table 4.14 Frequency and Percentage Distribution of HDL-Cholesterol Level

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<40	21	8.9	8.9	8.9
	40-59	122	51.9	51.9	60.9
	≥60	92	39.1	39.1	100.0
	Total	235	100.0	100.0	

From table 4.13 and 4.14, the lowest and highest HDL-Cholesterol level was 29.3 mg/dl and 130.5 mg/d respectively, and the average level was 58.97 mg/dl (s = 17.26 mg/dl). The majority, 52.9% of all participants, had HDL-Cholesterol level between 40 mg/dl and 59 mg/dl. The group with the lowest percent (8.6%) was the group that had HDL-Cholesterol level lower than 40 mg/dl while the group that had HDL-Cholesterol higher than 60 mg/dl was accounted for 39.1%.

Table 4.15 Descriptive Statistics of LDL-Cholesterol Level

	N	Minimum	Maximum	Mean	Std. Deviation
LDL-C	235	43.40	245.80	128.5523	39.69234
Male	106	44.40	231.70	127.8783	37.50309
Female	129	43.40	245.80	129.1062	41.54199
Valid N (listwise)	235				

Table 4.16 Frequency and Percentage Distribution of LDL-Cholesterol Level

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<100	49	20.9	20.9	20.9
	100-129	81	34.5	34.5	55.3
	130-159	54	23.0	23.0	78.3
	160-189	31	13.2	13.2	91.5
	≥190	20	8.5	8.5	100.0
	Total	235	100.0	100.0	

Refer to the data in Table 4.15 and 4.16 on LDL-Cholesterol, LDL-cholesterol level ranged from minimum value at 43.4 mg/dl up to maximum value at 245.8 mg/l while the average level of LDL-Cholesterol was 128.55 mg/dl (s = 39.69 mg/dl). The highest percentage of participants fell into the group that LDL-Cholesterol ranged between 100 mg/dl and 129 mg/dl, followed by the group with LDL-Cholesterol ranged from 130mg/dl to 159 mg/dl, level lower than 100 mg/dl, level ranged from 160mg/dl up to 189 mg/dl, and equal to or higher than 190 mg/dl. The percentage of each group above was 34.5%, 23.0%, 20.9%, 13.2%, and 8.5% respectively.

Table 4.17 Frequency and Percentage Distribution of Smoking and Alcohol Experience

		Frequency	Percent	Valid Percent	Cumulative Percent
Smoking					
Valid	Non-smoking	213	90.6	90.6	90.6
	Smoking	17	7.2	7.2	97.9
	Cessation	5	2.1	2.1	100.0
	Total	235	100.0	100.0	

Table 4.17 (continued)

		Frequency	Percent	Valid Percent	Cumulative Percent
Alcohol consumption					
Valid	No	192	81.7	81.7	81.7
	Yes	24	10.2	10.2	91.9
	Occasion	19	8.1	8.1	100.0
	Total	235	100.0	100.0	

Smoking and alcohol drinking conditions were informed by all participants. There were 213 non-smoking participants (90.6%) while 5 participants had already quit smoking and 17 participants had had smoked for 2 years at least. The most participants in the group of smoker had smoked for more than 20 years (data not shown). In the same way, there were192 participants (81.7%) who didn't drink while the other 24 participants (10.2%) had drunk every day with at least 1 glass up to 10 glasses per day. The last group, including 19 participants (8.1%), had occasionally drunk. All data were shown in table 4.17.

Table 4.18 Frequency and Percentage Distribution of Supplement

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Non-supplement	184	78.3	78.3	78.3
	Supplement	51	21.7	21.7	100.0
	Total	235	100.0	100.0	

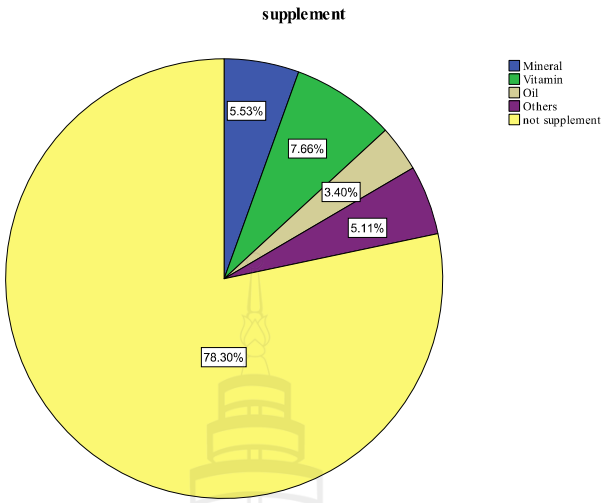

Figure 4.3 Supplementary Food Pie Chart

Based on the information presented in the pie chart and table 4.18, the largest group with 51 participants (78.3%) did not take supplementary food. The other participants who had vitamins, minerals, and others supplementations included 7.66%, 5.53%, and 5.11% of all participants respectively while the group that took oil as supplementary food were only at 3.4%.

Table 4.19 Frequency and Percentage Distribution of Food Type Consumption

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Not answer	3	1.3	1.3	1.3
	Healthy foods	112	47.7	47.7	48.9
	Not healthy foods	60	25.5	25.5	74.5
	General foods	60	25.5	25.5	100.0
	Total	235	100.0	100.0	

Table 4.19 shows the data of foods that participants always took. 47.7% of participants consumed healthy foods, which have low fat, high fiber, low sugar, white meat, contain a lot of vegetables and healthy fat. 25.5% of participants took general foods, which referred to non-specific foods, and the other 25.5% took non-healthy foods which are saturated fat and/or trans-fat containing food and fatty meal. The last 3% of participants were non-respondents.

Table 4.20 Frequency and Percentage Distribution of Cholesterol Crystal Finding in Microscope

Crystal	Frequency	Percent	Cumulative Percent
0	116	49.4	49.4
1	54	23.0	72.3
2	33	14.0	86.4
3	15	6.4	92.8
4	7	3.0	95.7
5	7	3.0	98.7
6	2	0.9	99.6
7	1	0.4	100.0
Total	235	100.0	

Table 4.20 shows the frequency and number of cholesterol crystal(s) found in microscope. There are 49.4% no cholesterol crystal found, 23.0% found 1 crystal per 10 oil fields, 14.0% found 2 crystals, 6.4% found 3 crystals, 3% found both 4 crystals and 5 crystals, 0.9% found 6 crystals, and 0.4% found 7 crystals which was maximum finding.

4.3 Correlation Between Cholesterol Crystal and Factors

The factors involved in this study were age, gender, education, medical history, total cholesterol level, triglyceride level, HDL-C level, LDL-C level, alternative supplements, smoking and alcohol drinking habit. All of them correlated with cholesterol crystal.

Table 4.21 Correlation Between Cholesterol Crystal and Factors

Variable	p-value
Gender	1.00
Hyperlipidemia history	0.489
CVS history	0.0735
Diabetes history	0.103
Food supplementary	0.3524
Smoking	0.461
Alcohol consumption	0.722
Type of food	0.001*
Occupation	0.69
Total cholesterol	0.357
Triglyceride	0.548
HDL-C	0.759
LDL-C	0.405

Note. * The mean difference is significant at the 0.05 level.

From the table 4.22 and 4.23, comparisons of statistical difference between parameters were performed, utilizing t-test and ANOVA. The result indicated no significant differences between cholesterol crystal and all factors in study; hyperlipidemia

history, cardiovascular history, diabetes history, alternative food supplementary, and alcohol consumption. More details can be found in appendices.

Table 4.22 Pair Comparisons of Cholesterol Crystal Among Type of Foods

Pair of criteria	Mean Difference	F	Sig.
ealthy food compare with Unhealthy food	-1.789*	45.337	0.000
Healthy food compare with General food	0.222		0.252
General food compare with Unhealthy food	-1.567*		0.000

Note. * The mean difference is significant at the 0.05 level.

Healthy food was different from unhealthy food (P<0.05) while unhealthy food was different from general food (P<0.05) and general food was not significantly different from healthy food.

.

CHAPTER 5

DISCUSSION

5.1 Discussion and Conclusion

From this study, the results suggest that cholesterol crystal has no statistically significant relation to socioeconomic factor and lipid profile. However, cholesterol crystal has significant relation to the types of food.

There is no relationship between cholesterol crystal that was found in live blood analysis method and serum lipid profile level, which do not support hypothesis. The causation could be that the cholesterol found in serum passed through metabolic and transport system pathway. It was carried by lipoprotein, which made the molecule water-soluble and conformation change, then it didn't form to be crystal and polarized under microscope. In the same time, cholesterol from food that was processed by heat might change in structure and accumulate with other molecules and appeared to be crystal and polarized under microscope. Moreover, some types of lipid-lowering medicine and/or agent might also affect the cholesterol, for example, one distinct feature of phytosterol that competitive solubilization and co-crystallization called physico-chemical effect (Rozner & Garti, 2006) determine effectiveness of reduction of cholesterol absorption in intestinal. In co-crystallization feature that takes place in small intestine, precipitation of dietary sterols takes place during food digestion and, as a result, crystallization is in the oil–water interface. All of these are possibly the reasons the cholesterol crystal and serum lipid profile did not correlate to each other.

Without previous scientific evidence and research to support, however, this study showed significant relationship between cholesterol crystal and food types. Conforming to Enderlein's original hypothesis, this research found that cholesterol crystal identified the status when the body had poor digestion of fats or after fatty meal. Consumption of

food rich in unhealthy fat is always found cholesterol crystal(s) anyway, cholesterol crystal will not be found in food rich in healthy fat consumption, This condition might lead to the accumulation of crystal and certain proteins and/or molecules, causing cholesterol crystal deposition within the arterial wall and influence atherosclerosis plaque (Abela). It can be concluded that by using easier method, less time, and less amount of blood, live blood analysis can separate healthy fat from unhealthy fat and cholesterol crystal was the most likely culprit in triggering inflammation, leading to thrombosis, cardiovascular diseases, and other metabolic syndromes. Although one previous research indicated that dark field analysis was very difficult to be standardized and had low reliability (Teut, Lüdtke, & Warning, 2006), some parts of this study can be applied as a screening and preventive test. The finding will bring about awareness of wellness in people and convince them to change their lifestyle, avoiding any kinds of unhealthy foods even though this was only the pilot study, considering the advanced direct study of live blood analysis in the future.

5.2 Recommendation for the Coming Research

5.2.1 This study didn't focus on some factors that would affect cholesterol crystal and didn't finely classify the types of food which all participants ate every day. In addition, examining duration of consumption unhealthy food before withdrawing blood, following by adding the part of questionnaire about what type of hypolipidaemic agents that some participants took, can be the better choice to rule out interference.

5.2.2 The prospective further study should investigate how the cholesterol crystal changes after alteration of participant's attitude and substitution of unhealthy food by healthy food in a long term.

5.2.3 In the next research, it should study thoroughly in details about the role of cholesterol crystal in the body as well.

REFERENCES

REFERENCES

บัณลังก์ เอกบัณฑิต. (2546). *ความชุกของภาวะระดับไขมันในเลือดผิดปกติในผู้ป่วยโรคข้ออักเสบรูมาทอยด์ที่โรงพยาบาลจุฬาลงกรณ์*. วิทยานิพนธ์วิทยาศาสตร์มหาบัณฑิต สาขาวิชาอายุรศาสตร์. จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพฯ.

Burusliam, R. (2011). *Detection of mercury in blood between live blood analysis and blood mercury level.* Master's Independent Study of Science in Anti-Aging and Regenerative Science. Mea Fah Luang University, Chiang Rai.

Crislip, M. (2009). *Live blood analysis: The modern auguries*. Retrieved August 13, 2012, from http://www.sciencebasedmedicine.org/index.php/live-blood-analysis-the-modern-auguries/

Deslypere, J. P. (1995). The role of HMG-CoA reductase inhibitors in the treatment of hyperlipidemia: a review of fluvastatin. *Current Therapeutic Research, 56*(2), 111-128. doi: 10.1016/0011-393x(95)85036-8

Fernandes, J. L., Orford, J. L., Garcia, C., Coelho, O. R., Gidlund, M. & Blotta, M. H. (2004). Differences in human antioxidized LDL autoantibodies in patients with stable and unstable angina. *Journal of Autoimmunity, 23*(4), 345-352. doi: 10.1016/j.jaut.2004.08.004

Maggini, M., Raschetti, R., Traversa, G., Bianchi, C., Caffari, B., Da Cas, R. & Panei, P. (2004). The cerivastatin withdrawal crisis: A "post-mortem" analysis. *Health Policy, 69*(2), 151-157. doi: 10.1016/j.healthpol.2004.04.001

Marshall, W. J. & Bangert, S. K. (Eds.). (2008). *Clinacal Biochemistry Metabolic and clinical aspects* (2nd ed.). Edinburgh: Churchill Livingstone Elsevier.

Rozner, S. & Garti, N. (2006). The activity and absorption relationship of cholesterol and phytosterols. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 282-283*, 435-456. doi: 10.1016/j.colsurfa.2005.12.032

Teut, M., Lüdtke, R. &Warning, A. (2006). Reliabilityof Enderlein's darkfield analysis of live blood. *Altern Ther Health Med., 12*(4), 36-41.

Tiyajinda, P. (2011). *The study of result of chelation therapy: Monitoring rouleau formation and crystal depositions by looking at live blood analysis in Villa Medica clinic.* Master's Independent Study of Science in Anti-Aging and Regenerative Medicine. Mae Fah Luang University, Chiang Rai.

Vance, D. E & Vance, J. E. (Eds.). (2008). *Biochemistry of Lipids, Lipoproteins and Membranes* (5th ed.). Alberta: Elsevier.

APPENDICES

APPENDIX A

PATIENT DATA SHEET

Date…….

Name-Surname.....Age..... Gender □ Male □ Female

Education

□ Below Bachelor's □ Bachelor's degree □ Master's degree □ Doctoral's degree

Occupation.....Weight.....Height.....

Do you have hyperlipidaemia? □ Yes □ No

Total cholesterol…….mg/dl

Triglyceride…….mg/dl

HDL-C……mg/dl

LDL-C……mg/dl

Do you have cardiovascular disease? □ Yes □ No

Do you have diabetes mellitus □ Yes □ No

Do you smoke? □ No □Yes…..Yrs ……..piece/day □ Cessasion

Do you drink alcohol?□ No □ Yes.....glass/day □ Occasion

Food that you always eat

□Healthy food □Unhealthy food □ General food

Do you take vitamin or other supplements? □ No □ Yes

Please specify.....

Live Blood Analysis

Number of Cholesterol

Crystal……

Other.....

.....

APPENDIX B

STATISTICAL TABLES

Table B1 Independent Samples Test of Cholesterol Crystal and Heperlipidemia and Cholesterol Crystal, CVS History and Cholesterol Crystal

	F	Sig.	t	df	Sig. (2-tailed)
Hyperlipidemia history	0.081	0.777	0.026	233	0.979
CVS history	3.612	0.059	1.455	233	0.147
Diabetes history	1.431	0.233	1.269	233	0.206
Food supplementary	0.000	0.999	-0.379	233	0.705

Table B2 Independent Samples Test of Cholesterol Crystal and Smoking, Cholesterol Crystal and Alcohol Consumption

	Levene Statistic	df1	df2	Sig.
Smoking	1.733	2	232	0.179
Alcohol consumption	2.801	2	232	0.063

Table B3 Independent Samples Test of Cholesterol Crystal and Heperlipidemia and Cholesterol Crystal, CVS History and Cholesterol Crystal

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Smoking					
Between Groups	3.127	2	1.564	0.778	0.461
Within Groups	466.260	232	2.010		
Total	**469.387**	**234**			
Alcohol					
Between Groups	1.318	2	0.659	0.327	0.722
Within Groups	468.069	232	2.018		
Total	**469.387**	**234**			

Table B4 Relationship Between Cholesterol Crystal and Food

		N	**Mean**	**Std. Deviation**	**Std. Error**
Cholesterol crystal	Healthy food	112	0.54	1.047[a]	0.099
	Not healthy food	60	2.33	1.580[b]	0.204
	General food	60	0.77	1.047[a]	0.135
	Total	232	1.06	1.420	0.093

Note. The different superscript alphabet is the different couple at 95%confident interval

Table B5 Correlations Between Cholesterol Crystal and Lipid Profile

		Cholesterol crystal	Food	Total cholesterol	Triglyceride	HDL-C	LDL-C
Cholesterol crystal	Pearson Correlation	1	0.148*	0.060	0.039	0.020	0.055
	Sig. (2-tailed)		0.025	0.357	0.548	0.759	0.405
	N	235	232	235	235	235	235

APPENDIX C

BLOOD PICTURE

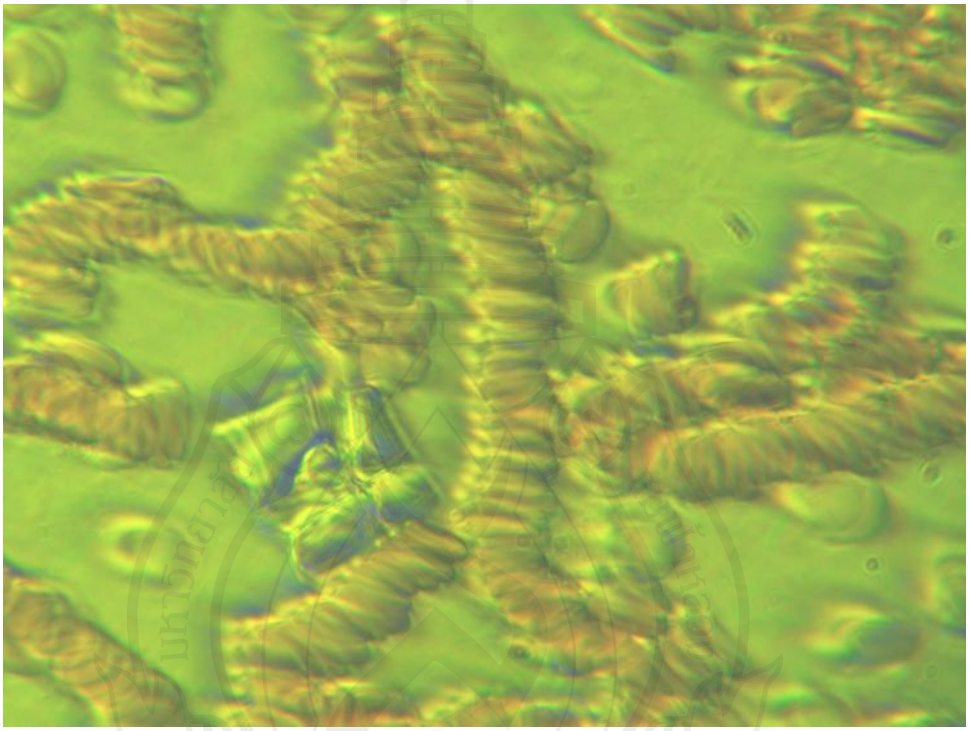

Figure C1 Cholesterol Crystal Found in People Who had General Diet

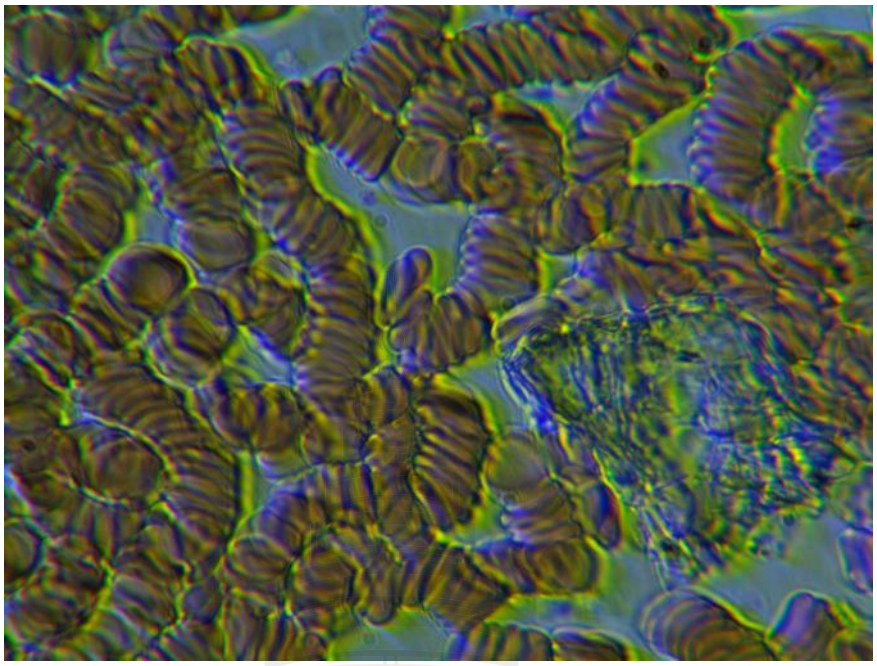

Figure C2 Cholesterol Crystal in Live Blood Found in People Who had Unhealthy diet

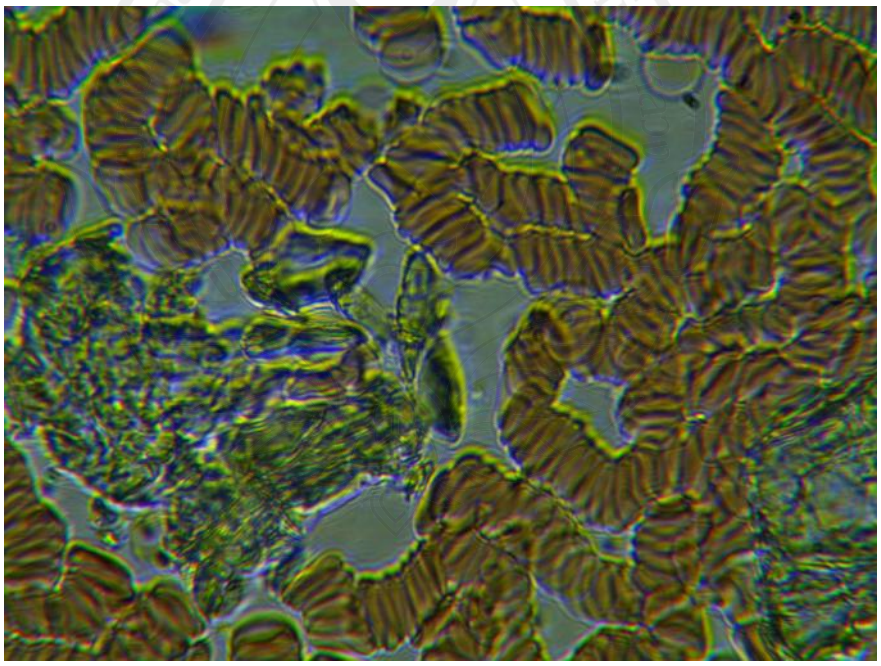

Figure C3 Cholesterol Crystal in Live Blood Found in Participant Who had Unhealthy Diet

CURRICULUM VITAE

CURRICULUM VITAE

NAME	Miss Ruchadakorn Ayukarn
DATE OF BIRTH	1 May 1985
ADDRESS	15, Nangbuakli Road Thapeeleang District, Muang, Suphan Buri, Thailand 72000

EDUCATIONAL BACKGROUND

2006	Bachelor of Science Medical Technology Chiang Mai University

WORK EXPERIENCE

2010-Present	Medical Technologist Central lab, Suphan Buri
2008-2010	Marketing Support Bio-Rad Laboratories, Bangkok
2006-2010	Medical Technologist N Health Sathorn, Bangkok