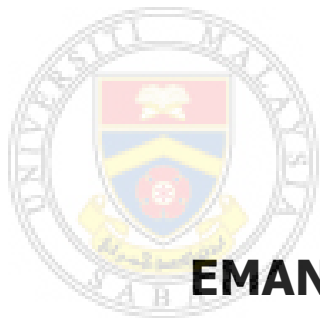


**HIGH SENSITIVITY C-REACTIVE PROTEIN  
AND ALKALINE PHOSPHATASE LEVELS  
IN APPARENTLY HEALTHY ADULTS OF  
BUMIPUTERA AND NON-BUMIPUTERA  
ATTENDING LUYANG HEALTH  
CLINIC IN KOTA KINABALU  
SABAH**

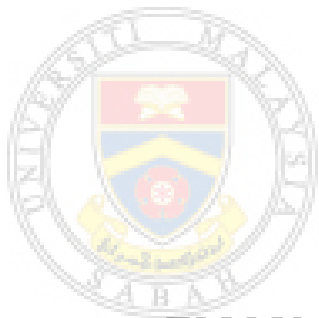


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**FACULTY OF MEDICINE AND HEALTH  
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UNIVERSITI MALAYSIA SABAH  
2016**

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2016**

## DECLARATION

I hereby declare that the work in this thesis is of my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

12 June 2015

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## CERTIFICATION

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MATRIC NO : **PU20108247**

TITLE : **HIGH SENSITIVITY C-REACTIVE PROTEIN AND ALKALINE PHOSPHATASE LEVELS IN APPARENTLY HEALTHY ADULTS OF BUMIPUTERA AND NON-BUMIPUTERA ATTENDING LUYANG HEALTH CLINIC IN KOTA KINABALU SABAH**

DEGREE : **MASTER IN SCIENCE (MEDICAL SCIENCE)**

VIVA DATE : **4 Feb 2016**



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## ACKNOWLEDGMENTS

First and foremost, thanks for Allah, the most gracious, the most merciful for giving me the patience, strength and inner peace to continue this study.

I would like to take this opportunity to thank deeply UMS for funding and supporting this work (UMS/CN1.12/P25/11-1/2/28).

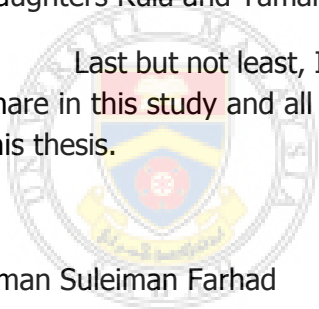
Special thanks and appreciation to my supervisors Assoc. Professor Dr. Tan Tek Song and Assoc. Professor Dr. Shamsul Bahari Bin Shamsudin for their encouragement and guidance.

Similarly, my thanks and gratitude to Mr. Thamrone Keowmani CRC/Queen Elizabeth Hospital for sacrificing his time in guiding me to perform the statistics of this study.

My sincere thanks also extended to my husband Dr.Hassan Fadil, my daughters Rula and Tamara for their unlimited support throughout this period.

Last but not least, I offer my thanks to all those participants whom agreed to share in this study and all those supported me in one way or another in completing this thesis.

Eman Suleiman Farhad



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## ABSTRACT

C-reactive protein (CRP) is an inflammatory marker considered as a risk factor of cardiovascular diseases. This inflammatory marker was widely studied in research. It was found to be associated with other chronic conditions like metabolic diseases, diabetes type 2 and certain cancers. CRP levels in healthy people of different populations also were highlighted in many studies. However no study was done about the level of this inflammatory marker in Sabah. This cross-sectional study was conducted in Kota Kinabalu, Sabah to assess the level of this marker in healthy people from different races to identify the factors associated with high levels of this marker for early intervention towards health promotion in the community. A total of 232 randomly selected healthy participants were included in this study. The relations of hs-CRP with alkaline phosphatase (ALP) , among different races, as well as different age groups, genders, waist circumferences, basal metabolic rate (BMI), mean arterial pressure (MAP), smoking, marital status and family history of disease were evaluated using multiple regression model. The median for high sensitivity C-reactive protein (hs-CRP) was 1.2 mg/L and higher in males than females 1.2 mg/L, 1.1 mg/L respectively. There was no significant difference in median hs-CRP levels in different races in the study population. A significant correlation between high levels of hs-CRP and ALP was found  $p < 0.001$ . BMI, MAP, ALP were contributing significantly to the model  $p < 0.001$ . The odds of high risk hs-CRP  $> 3$  mg/L in females with high BMI was almost 23 times higher as compared with females of normal BMI and four times higher in overweight and obese females in comparison to overweight and obese males. Those with ALP level 1 U/L higher had 5% more odds of having high risk hs-CRP compared to those with lower ALP level and those with 1mmHg higher in MAP had 7% more odds of having high risk hs-CRP. The model explained 36% (Nagelkerke  $R^2$ ) of the variance in high hs-CRP ( $>3$  mg/L) and correctly classified 84% of the cases. In conclusion, this study revealed that BMI, MAP, ALP were independent predictors of high levels of hs-CRP. High levels of hs-CRP were found significantly in overweight and obese which was more obvious in females than males rendering them at higher risk of developing chronic conditions like cardiovascular disease. Further epidemiological studies are suggested.

## **ABSTRAK**

**TAHAP HS-CRP YANG TINGGI TELAH DIDAPATI AGAK KETARA  
DIKALANGAN KAUM WANITA YANG OBES ATAU YANG  
MEMPUNYAIBERAT BADAN YANG BERLEBIHAN.  
INI MENYEBABKAN KAUM INI MEMPUNYA  
I RISIKOLEBIH TINGGI DARI LELAKI UNTUK  
MENDAPAT PENYAKIT KRONIK SEPerti  
PENYAKIT KARDIOVASKULAR.  
KAJIAN EPIDEMIOLOGI YANG  
LANJUT DISYORKAN**

*C-reactive protein (CRP) merupakan penanda tumor yang merupakan faktor risiko bagi penyakit kardiovaskular. Penanda ini telah dikaji secara menyeluruh diserata dunia dan kajian menunjukkan ia berkait rapat dengan penyakit-penyakit seperti diabetes mellitus, sesetengah kanser dan penyakit metabolik. Kajian juga telah dijalankan diatas tahap penanda CRP bagi individu yang sihat. Walaubagaimanapun kajian ini belum lagi di buat di Sabah. Kajian telah dibuat di Kota Kinabalu untuk mengkaji tahap penanda CRP untuk individu sihat dari berbagai bangsa di kalangan penduduk Kota Kinabalu. Seramai 232 individu dipilih secara rawak bagi kajian ini. Penanda CRP dan enzim alkaline phosphatase (ALP) di kalangan beberapa kaum, juga dikalangan kumpulan orang-orang yang berlainan umur, ukuran pinggang, taraf kahwin, indeks jisim tubuh (BMI). Tekanan darah, sejarah keluarga dan merokok menggunakan model regresi berganda. Median bagi hs-CRP ialah 1.2mg/L dan ia lebih tinggi bagi kaum lelaki dibandingkan perempuan iaitu 1.2mg/L dan 1.1 mg/L. Tiada perubahan yang ketara diperolehi bagi median ini bagi kes berlainan kaum akan tetapi ada perubahan ketara berkaitan dalam paras hs-CRP dan ALP iaitu  $p < 0.001$ . BMI, tekanan darah dan ALP memberi sumbangan yang amat ketara bagi model tersebut. Kemungkinan risiko tinggi hs-CRP > 3mg/L bagi wanita yang mempunyai BMI yang tinggi adalah 23 kali lebih tinggi. Dibandingkan dengan wanita yang mempunyai BMI normal dan 4 kali lebih tinggi bagi wanita obes dibandingkan dengan lelaki. Bagi mereka dengan tahap ALP 1 IU/L lebih tinggi mempunyai 5% kemungkinan risiko tinggi hs-CRP dibandingkan dengan individu yang mempunyai tahap ALP rendah. Bagi MAP yang mempunyai bacaan 1mmHG lebih tinggi ada risiko 7% lebih tinggi untuk dapat hs-CRP. Model ini menunjukkan bahawa 33.5% (Nagelkerke  $R^2$ ) varians bagi penanda hs-CRP yang tinggi (>3mg/L) diklasifikasikan dengan tepat 82.8% daripada kes-kes tersebut. Kesimpulannya, kajian ini mendedahkan bahawa BMI, MAP, ALP dapat meramalkan tahap h-CRP. Wanita yang berlebihan berat badan dan obes akan mendapat manfaat kesihatan apabila intervensi awal dibuat dengan tahap hs-CRP tinggi. Kajian lanjut diperlukan dalam bidang ini untuk menyiasat lebih penanda yang berkaitan dengan hs-CRP.*

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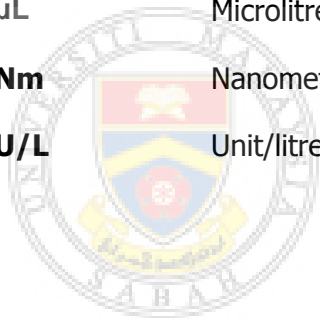
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## LIST OF ABBREVIATIONS

<b>ALP</b>	Alkaline phosphatase
<b>BMI</b>	Body mass index
<b>CRP</b>	C- reactive protein
<b>hs-CRP</b>	high sensitivity C- reactive protein
<b>IQR</b>	Inter quartile ratio
<b>IL-1</b>	Interleukin 1
<b>IL-6</b>	Interleukin 6
<b>IQR</b>	Interquartile range
<b>LDL</b>	Low density lipoprotein
<b>MAP</b>	Mean arterial pressure
<b>pH</b>	Chemical Scale measure of how alkaline or acidic is a substance or solution
<b>R1</b>	Reagent 1
<b>R2</b>	Reagent 2
<b>OD</b>	Odd ratio
<b>TNF<math>\alpha</math></b>	Tumour necrosis factor $\alpha$

## LIST OF UNITS

<b>°C</b>	Celsius
<b>Cc</b>	Cubic centimetre
<b>Cm</b>	Centimetre
<b>kDa</b>	Kilodelton
<b>Kg</b>	Kilogram
<b>M</b>	Metre
<b>m<sup>2</sup></b>	Square metre
<b>mg/L</b>	Milligram/litre
<b>mmHg</b>	Millilitre mercury
<b>µL</b>	Microlitre
<b>Nm</b>	Nanometre
<b>U/L</b>	Unit/litre



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# CHAPTER 1

## INTRDUCTION

### 1.1 Background of the Study

Prevalence of chronic diseases like cardiovascular diseases, metabolic disease, diabetes, cancers are in increasing trend worldwide. There is growing evidence that those conditions are sharing in common a low grade chronic inflammatory process in their pathogenesis (Jeemon *et al.*, 2011; Nayeem *et al.*, 2010; Nirmitha and Suresh, 2010; Monteiro and Azevedo, 2010; Ridker, 2003; Pradhan *et al.*, 2001; Visser *et al.*, 1999).

CRP is an acute inflammatory marker. Levels of CRP are found to be increased in human serum after any inflammatory state initiated by stimuli. Most of the CRP in blood is released by liver hepatocytes. This release is under control of proinflammatory cytokines including interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) (Kritchevsky *et al.*, 2004).

The elevation in CRP levels has been linked to pathogenesis of many diseases like cardiovascular diseases, metabolic syndrome, diabetes and cancers. In the last three decades, researcher has been studying this biomarker extensively and collecting evidence about its role since there is growing evidence that these conditions are sharing in common their pathogenesis, which is a low grade chronic inflammatory process. Whether CRP is a non-specific marker or cause in these diseases, many studies worldwide were carried out including mechanistic studies, epidemiological studies, genetic studies, experimental trials to find out this (Anand and Yusuf, 2010).

A high sensitive assay was developed to measure levels of CRP to detect levels near to zero and was called high sensitivity C-reactive protein (hs-CRP).The



high sensitivity method is standard for all commercial tests, this ensure a common analytic structure for the investigators on CRP (Ridker, 2009).

CRP as inflammatory marker with other markers and biochemical tests were investigated in different studies as a try from researcher to look for a test that can in a way expect the appearance of conditions like cardiovascular diseases, metabolic syndrome, diabetes and others in the preclinical period. Researcher studied and investigated many factors associated with this marker for example basal metabolic rate (BMI), waist circumference, smoking which found to be strongest predictors, as well as other biochemical tests including hormones, fasting lipid profile, fasting blood sugar and others of interest to researchers.

Alkaline phosphatase (ALP) is another biomarker that studied in research with C-reactive protein but not in many studies. ALP are group of enzymes that are produced to the circulation mainly from liver and bones mainly but still can produced by intestine, kidney and placenta. It is a useful diagnostic marker for liver function and bone diseases. The association of high levels of ALP with risk of cardiovascular, metabolic syndrome, hypertension was mentioned in studies (Kim *et al.*, 2013; Wannamethee and Shaper, 2010; Webber *et al.*, 2010; Kunutsor *et al.*, 2014).

There are baseline references of CRP for populations that have been studied in research. Researchers reported a mean and/or median values for the study populations recruited. In communities with diversity, the mean and/or median were divergent in different races and also for the same population, different means in varies cities were observed as well (Xingwang *et al.*, 2007; Shah *et al.*, 2010).

## **1.2 Problem Statement**

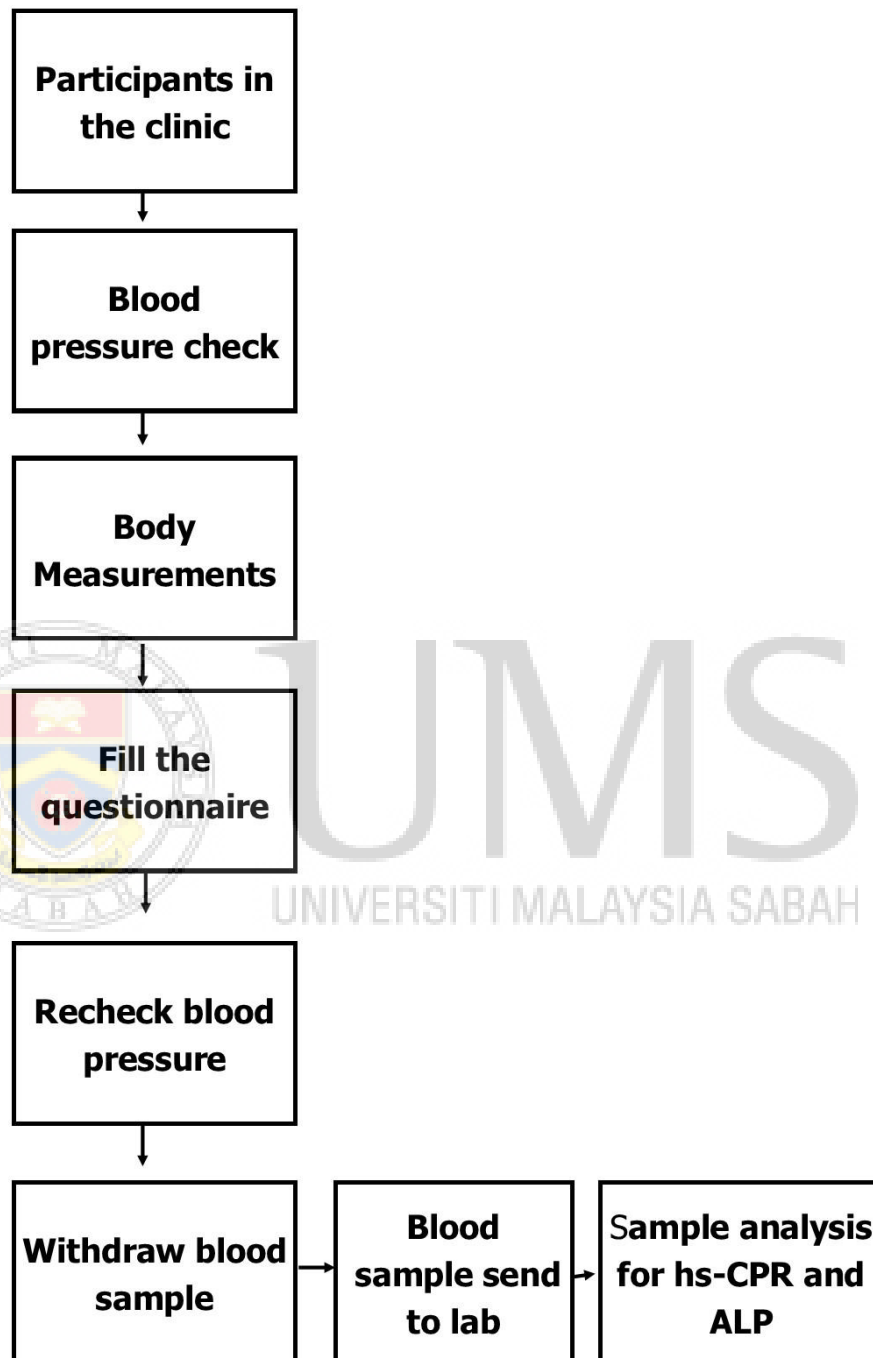
CRP is inflammatory marker which is considered as risk marker for some diseases like cardiovascular, metabolic syndrome and few kinds of cancers (Ridker, 2003; Ko *et al.*, 2012; Xingwang *et al.*, 2007; Nirmitha and Suresh, 2010).

It is found in healthy people in very low levels. There are many factors may lead to the increase in level of this marker. In long term studies, it was found that healthy people with high level hs-CRP baseline are at risk of developing some chronic diseases in future (Ridker, 2009; Hage, 2014; Blake *et al.*, 2003; Nanri *et al.*, 2007). In addition level of ALP was investigated in studies and the relation of this increase to many conditions like pathophysiology of vascular calcification (Wannamethee and Shaper, 2010), the prediction of metabolic syndrome (Kim *et al.*, 2013) was studied. Studies about these two marker in healthy individuals and the relationship of them with some risk factors are lacking in Kota Kinabalu. The lack of this piece of information was encouraged us to carry out this study to highlight this area and identify the group at high risk for early intervention.

### **1.3 Research Rationale**

The information about levels of hs-CRP in healthy adult people in Malaysia was not studied before. The relation of hs-CRP levels with ALP levels and some risk factors related to the study population need to be identified in order to obtain more knowledge about this association. The strength of association and how much those factors could predict high risk levels of hs-CRP in healthy individuals need to be explored which may be different from other populations in the world.

#### 1.4 Conception Framework



## 1.5 Concept Definition

CRP is an acute inflammatory marker, the level in the human body has no seasonal or circadian variation and easily measured by standardized high sensitivity commercial assays (Albert and Ridkar, 2006). It is produced by liver (Foody *et al.*, 2006). The elevation in hs-CRP levels has been linked to pathogenesis of few diseases like cardiovascular diseases, metabolic syndrome, diabetes type 2 and cancers (Anand and Yusuf, 2010; Nanri *et al.*, 2007; Santos *et al.*, 2005; Ko *et al.*, 2012).

**ALP:** are a group of enzymes encoded at separate genetic loci associated with membrane and cell surface (Burtis *et al.*, 2008). In healthy people ALP mainly produced by liver and bones. It is measured as indicator for liver diseases.

**Health:** is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (WHO).

The concept definition of the other factors included in the questionnaire:

**Race:** A group of people sharing the same culture, history, language (Oxford University Press, 2015).

**Age:** The length of time that a person has lived (Oxford University Press, 2015).

**Gender:** The state of being male or female (Oxford University Press, 2015).

**Family history:** Family health history of diseases and health conditions present in your family (CDC, 2014).

**Smoking status:** A person who smokes tobacco regularly (Oxford University Press, 2015).

**BMI:** Is defined as the body mass divided by the square of the body height, and is universally expressed in units of  $\text{kg/m}^2$  (Sharples *et al.*, 2012).

**Waist circumference:** is measured at a level midway between the lowest rib and the iliac crest (WHO, 1999)

**Weight:** A body's relative mass or the quantity of matter contained by it (Oxford University Press, 2015).

**Height:** The measurement of someone or something from head to foot (Oxford University Press, 2015).

**Blood Pressure:** The pressure in the aorta and in the brachial and other large arteries in adult human rises to a peak value (systolic pressure) during each cycle

and falls to a minimum value (diastolic pressure).The arterial pressure is conventionally written as systolic over diastolic in mmHg (Ganong, 1995)

These two marker were measured in healthy adults to investigate the association with some selected factors of interest to find the group at high risk of developing chronic diseases in future.

### **1.6 Operational Definition**

Venous blood sample was taken from healthy people to find levels of hs-CRP using high sensitivity immunoturbidmetric assay, a light was allowed to pass through the prepared solution. The transmitted light was proportional to the levels of hs-CRP in the solution. ALP measured by kinetic method using spectrophotometric technique by adding the n-paranitrophenyl phosphate which is colorless in the alkaline solution, presence of ALP in the solution removes the phosphate group leaving the yellow paranitrophenol. The rate of absorbent is proportional to the enzyme activity.

The participants in the study were the healthy people after doing medical checkup not having acute or chronic diseases, not operated on in the last six month, not on medication or supplement, ladies not using contraceptive pills, injections, implants or intrauterine devices. Pregnant ladies excluded also.

The association of these two markers then was analyzed with information of each participant from the questionnaire. The questionnaire includes information about gender, age, marital status, race, smoking status, family history in first and second degree relative of Hypertension, Myocardial infarction, Diabetes, stroke, years of residence in urban and/or rural areas. Measurement of height in cm by stadiometer (Seca) bear feet with cloths on, weight in kg by scale (Seca) placed on a hard surface, waist circumference in cm measured by placing tape measure midway between the lowest rib and the iliac crest of hip (WHO, 1999). BMI was calculated following this equation: (Sharples *et al.*, 2012).

$$\text{Weight (kg) / Square of height (m}^2\text{)}$$

Blood pressure is checked at the beginning of the session using a calibrated mercury sphygmomanometer (ACCOSON) and stethoscope (LITMANN) and is recorded as systolic and diastolic in sitting position, another blood pressure is checked in sitting position also at the end of the session, the average is documented. Mean arterial pressure was calculated following this equation: (Razminia *et al.*, 2004)

$$\text{MAP} = 2/3 \text{ Diastolic} + 1/3 \text{ Systolic}$$

### 1.7 Objectives

The general objective of this study to assess the risk of the two inflammatory markers hs-CRP and ALP in healthy adults and their association with some risk factors of interest. Under this general objective, five specific objectives are set:

- a. Determine level of hs-CRP in the study population.
- b. Determine level in the ALP in the study population.
- c. Investigate the association of hs-CRP and ALP levels in the study population.
- d. Investigate the association of hs-CRP with race, gender, BMI, MAP in the study population.
- e. Investigate the association of ALP with race, gender, BMI, MAP in the study population.

### 1.8 Hypothesis

- a. There is significant association between hs-CRP and ALP in the study population.
- b. Levels of hs-CRP can be influenced significantly by race, gender, BMI, MAP.
- c. Levels of ALP can be influenced significantly by race, gender, BMI, MAP.

## CHAPTER 2

### LITRETURE REVIEW

#### 2.1 Inflammation

Inflammation is well known as one of the defense mechanisms of human body. The living tissues respond to the injurious agents at the time irritation is initiated with the involvement of vascular and immune systems. This response is considered acute when it is of short time and the cells predominant in the injured tissue are neutrophils, polymorphonuclear cells and some macrophages. This process ends by healing. The response is considered chronic when continues for long time, months or more and the cells recognized mainly plasma cells, lymphocytes, macrophages mononuclear cells and fibroblast. Tissue injury is the dominant in situation here. It can happen that chronic inflammation maybe the primary event and not a sequel to acute stage (Biswas, 1997). In Low-grade chronic inflammation condition like obesity, the inflammatory mediators are increased in blood although there is no clear injurious agent (van Greevenbroek *et al.*, 2013).

Inflammation is not a disease, as observed by Hunter but a response to tissue injury and body reactions to repair when sometimes more harmful than the original disease (Biswas, 1997). In attempt to understand inflammation, (Mohan, 2000) defines inflammation as local response of living mammalian tissues to injury due to any agent. It is body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosal cells and tissue. MacSween and whaley, (1992) offer more broad definition to inflammation as a physiological response to injury which is essentially a protective response, there are complicated interactions involving the vascular system, the immune system and repair mechanisms. The response, however may itself injurious effects for the organ involved or for the host.

On the other hand, still there is a belief that inflammatory process is complex and incompletely understood (Kritchevsky *et al.*, 2004).

## **2.2 Acute Phase Proteins**

In the inflammatory process, in addition to cellular response there will be systemic response which is the acute phase response. The result of this response is change in plasma proteins levels (Kritchevsky *et al.*, 2004; Biswas, 1997). The liver is stimulated to produce many proteins which are called acute phase proteins during inflammatory process includes C-reactive proteins, fibrinogen, and serum amyloid protein A. These changes are mediated by low molecular weight proteins called cytokines like IL-1, IL-6 and TNF $\alpha$  which are produced by many kinds of cells mainly macrophages and lymphocytes as result of any tissue injury or bacterial toxins. These cytokines have the capability to influence the function of other cells in the body (Kritchevsky *et al.*, 2004).

## **2.3 CRP**

CRP is an acute phase protein, was discovered more than 80 years ago by Tillet and Francis in 1930 at Rockefeller University while studying the inflammatory response of adult patients infected with acute pneumococcal pneumonia. (Ridker, 2003).

CRP level in the human body has no seasonal or circadian variation and easily measured by standardized high sensitivity commercial assays (Albert and Ridkar, 2006). It is stable after freezing and thawing (Localzo, 2005). The level of CRP in the circulation rises within 4-6 hours after exposure to injury or infection (Biswas, 1997) and has a half-life of 18-20 hours (Ridker, 2003; Kao *et al.*, 2006). The main function of CRP is defense mechanism against bacterial infection and removal of necrotic cells.

### **2.3.1 Chemical Structure of CRP**

CRP is a pentraxin proteins with symmetrical ring molecule that consists of five non-covalent but associated identical protomers 23-kDa. Each protomer has two calcium binding sites and one Phosphocholine binding site. CRP is acute phase proteins which is produced by liver (Foody *et al.* 2006) and smooth muscles of coronary arteries (Ko *et al.*, 2012) and also can be released by peripheral blood mononuclear cells as proved by one experimental study (Haider *et al.* 2006).